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Poster session

Abstract booklet

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NAMs for chemical risk assessment

Poster #1: ASPIS chemical selection working group

Author(s): Jonathan Freedman, Mathieu Vinken & Chemical Selection WG

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Project: PrecisionTox & ONTOX

Abstract:

The ASPIS Chemical Selection Working Group (CSWG), co-chaired by Drs. Mathieu Vinken (ONTOX) and Jonathan Freedman (PrecisionTox) has the goal of coordinating chemical selection and chemical-focused activities in the ASPIS consortia. To accomplish these goals, the CSWG began collecting information on chemical nominees selected by ONTOX, PrecisionTox and RISK-HUNT3R. To most efficiently use this information, a Database/Artificial Intelligence sub-working group was established. The database/AI sub-group, co-chaired by Drs. Barry Hardy (RISK-HUNT3R) and Marc Teunis (ONTOX), is responsible for creating an ASPIS-wide chemical database and developing cutting-edge, AI-based technologies to mine the literature for toxicological information. This database initially contained physicochemical characteristics and toxicological information on chemicals selected by ASPIS. In the future, it will expand to include chemical information from ASPIS collaborators including PARC, ECHA, JRC and the US NTP. As database needs of ASPIS expanded, this subgroup evolved into the new ASPIS Database Working Group (DbWG). The goal of the DbWG is to address database needs beyond chemical selection/chemoinformation and to develop and deploy related biological and toxicological knowledge. Additionally, it aims to expand the ASPIS database to form an international chemical knowledge resource that will support predictive toxicology, risk assessment guidance, policy development, communication and computational approaches. The second major activity of the CSWG is to support ASPIS-wide projects including the Steatosis Case Study. Activity on the Steatosis Case Study currently involves the three consortia and most of the ASPIS working Groups. The next case study being discussed by the CSWG will focus on developmental neurotoxicants. Chemical selection can be an essential component in the development of NAMs and NGRA. For this reason, the CSWG is working closely with the ASPIS Risk Assessment, Computational Approaches and qAOP working groups. Additionally, it has strong interactions with the JRC Regulatory Forum.

Poster #2: The ASPIS risk assessment working group

Author(s): Stefan Scholz & Risk Assessment WG team

Affiliation: Helmholtz Centre for Environmental Research

Project: PrecisionTox

Abstract:

The development of a Next Generation Risk Assessment (NGRA) faces two major challenges, (1) the identification of opportunities to apply NAMs (new approach methods) and replace animal testing already for a transition period under existing legal frameworks for the regulation of chemicals and (2) the need to change the current paradigms in hazard and risk assessment towards a system that allows for an increased use of NAMs.

The three ASPIS projects – ONTOX, PrecisionTox and RISK-HUNT3R – have complementary approaches on how to use NAMs for the hazard and risk assessment of chemicals including also prioritisation, grouping/read-across and hazard characterisation. The ASPIS working group on risk assessment intends to share and link the different approaches, coordinate joint activities and support the development of an ASPIS-wide framework for NGRA. The mission of the RA working group is to (i) critically compare ASPIS research results to previous activities for promoting NAMs (ii) benchmark the ASPIS approaches and results with other similar initiatives, (iii) plan for joint/coordinated activities, (iv) connect research activities in the ASPIS cluster, (v) consider the perspective of end-users and stakeholders and (vi) link and ensure complementarity of ASPIS PARC (Partnership for the Assessment of Risk from Chemicals) to support EU and national chemical risk assessment and risk management bodies for the transition to a next generation risk assessment. Therefore, the ASPIS RA group has started to review various current RA frameworks, contributed to the ASPIS discussion on NGRA (e.g. via meetings, workshops on an NGRA template and the ASPIS Open Science Symposium). Furthermore, we will use ASPIS-wide or project-specific case studies to demonstrate how NAMs can be used in RA, identify gaps or needs for improvement and compare results with the results of traditional animal test-based approaches.

Poster #3: Human peripheral neurons with enhanced nociceptor features for the study of pain-related dysfunctions

Author(s): Anna-Katharina Holzer

Affiliation: Universität Konstanz

Project: RISK-HUNT3R

Abstract:

In vitro models of the peripheral nervous system would benefit from further refinements to better support studies on neuropathies. In particular, the assessment of pain-related signals is still difficult in human cell cultures. Here, we harnessed induced pluripotent stem cells (iPSCs) to generate peripheral sensory neurons enriched in nociceptors. The objective was to generate a culture system with signaling endpoints suitable for pharmacological and toxicological studies. Neurons generated by conventional differentiation protocols expressed moderate levels of P2X3 purinergic receptors and only low levels of TRPV1 capsaicin receptors, when maturation time was kept to the upper practically-useful limit of 6 weeks. As alternative approach, we generated cells with an inducible NGN1 transgene. Ectopic expression of this transcription factor during a defined time window of differentiation resulted in highly-enriched nociceptor cultures, as determined by functional (P2X3 and TRPV1 receptors) and immunocytochemical phenotyping, complemented by extensive transcriptome profiling. Single cell recordings of Ca2+-indicator fluorescence from >9,000 cells were used to establish the "fraction of reactive cells" in a stimulated population as experimental endpoint, that appeared robust, transparent and quantifiable. To provide an example of application to biomedical studies, functional consequences of prolonged exposure to chemotherapeutic drugs were examined at non-cytotoxic concentrations. Oxaliplatin was found to induce (i) neuronal (allodynia-like) hypersensitivity to otherwise non-activating mechanical stimulation that could be blocked by modulators of voltage-gated sodium channels; (ii) hyper-responsiveness to TRPV1 receptor stimulation. Moreover, proteasome inhibitors, such as bortezomib and carfilzomib, exhibited a distinct pattern of toxicant-induced alterations in the neurons. Attenuation of P2X3 signaling, increased levels of resting intracellular [Ca2+], and a reorganization of tubulin to dense structures around the cell somata were characteristic of proteasome inhibitor-induced cell stress. These findings indicate that the model is suitable for pharmacological and toxicological studies related to peripheral neuropathies..

Poster #4: Use of Alamar Blue test for High-Throughput Energy Expenditure Monitoring in Daphnia magna

Author(s): Rubén Martínez

Affiliation: LEITAT

Project: PrecisionTox

Abstract:

Alamar Blue (AB) test is based on the reduction of the resazurin (oxidized form) to resorufin (reduced form) by NADH produced by living cells. It has been widely used during years to measure in vitro cell viability and recently it has been adapted to measure oxidative metabolism - related energy expenditure in whole zebrafish (Danio rerio) embryos and juveniles.

Our goal is to perform a new optimization of this test in Daphnia magna, since as far as we know, it has not been reported before and it could be an interesting phenotyping for Daphnia in PrecisionTox.

With this objective, AB LC50 value was determined in Daphnia. Afterwards, using 3 different appropriate AB concentrations, tests were performed, exposing Daphnia juvelines during 48 h (from 4 to 6 days) to DMSO and caffeine (3 different concentrations: LC10/5; LC10/2, and LC10), measuring the absorbance differences.

DMSO decreased energy expenditure while caffeine increased it, aligning with the expected results (considering that energy expenditure should be directly related with the locomotion, and that DMSO decrease the movement of Daphnia at high doses, and taking into consideration the stimulant effect of the caffeine).

As a conclusion, AB test could be suitable for a fast and 'high-throughput' Daphnia magna energy-expenditure monitoring test. Variability of results reduces, and its quality improves significatively when fluorescence is used, instead of absorbance.

Poster #5: The vibration/startle assay: a semi-automated behavioural assay to assess toxicity in zebrafish embryos

Author(s): Gaëlle Hayot

Affiliation: Karlsruher Institut für Technologie

Project: PrecisionTox

Abstract:

Zebrafish (Danio rerio) are small freshwater fish that can be found in small streams of northern India. Adult zebrafish measure between 2 and 5 cm and can live up to 5 years in laboratory conditions. As vertebrates sharing 70 % of their coding genes with humans, zebrafish are widely used in research for modelling the effects of drugs on human health. Their small size, external development, transparent embryos, easy husbandry and breeding, and the large number of eggs they lay make them a valuable model for drug screening.

We developed a system to rapidly and reliably screen chemicals based on a behavioural phenotype: the startle response to a vibration stimulus. This behaviour depends on the lateral line, an organ homologous to the inner ear in humans. In zebrafish, the lateral line enables sensing of water movement and vibration, which allows the fish to respond to its environment: for example, by eliciting an escape response from a predator and in prey capture.

Motility in response to a vibration is a broad phenotype: it depends on the sensing system (the lateral line and the central nervous system) and the reacting organs (the muscles and the motoneurons). Absence of motility can be due to the death of the embryos or to a compromised motor reaction induced by a drug. Thus, this phenotype allows us to screen for chemicals that impact different organs and pathways: for example, we are able to detect neurotoxicants and drugs affecting the muscles, but also compounds targeting gills or liver, which can lead to the death of embryos.

Our new modular, low-cost and open source vibration / startle system will be very helpful to screen chemicals for various purposes, in a high throughput fashion, and can also be used to detect behavioural phenotypes in mutant zebrafish lines.

Poster #6: A cross-species comparative look at the toxicity of acrylamides and imidazoles

Author(s): Gaëlle Hayot

Affiliation: Karlsruher Institut für Technologie

Project: PrecisionTox

Abstract:

The REACH regulation aims at improving the protection of human health and the environment from the risks that can be posed by chemicals. However, the high number of compounds to be tested to fulfill REACH goals can make this look like an impossible task. To accelerate the testing of chemicals, one strategy could be to group compounds that share structural similarities and to test only a subset of each group. This strategy relies on the assumption that compounds with similar structures will have similar toxicity. To test this hypothesis, the Precision Tox consortium tested two different sets of compounds, acrylamides and imidazoles, to assess whether the toxicity was similar within each group. In a first step, we evaluated toxicity by examining the test compound effects on cell proliferation of human liver cells (HepG2) and on the zebrafish embryo startle response, a behavioural assay. Our preliminary results show that even slight modifications of a molecule can lead to substantial changes in toxicity. Moreover, compounds from the imidazole group showed pH-dependent toxicity. Further work carried out within the Precision Tox consortium will examine how acrylamides and imidazoles impact metabolism and gene transcription across a diverse set of model organisms, and anchor these OMICS data by performing further assays targeting various toxic endpoints. By this “phylotoxicology” approach, we aim to provide a rich data set for the prediction of adverse chemical effects on humans. We expect that our results can help regulators to make informed decisions on appropriate strategies to abide by the REACH regulation.

Poster #7: Differential Cadmium Chloride Toxicity Across Twenty Daphnia magna Clones

Author(s): Marianne Barnard

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Daphnia magna is recommended by the OECD as a test species for acute ecotoxicity testing. Although EC50 responses to toxicants can vary across different clonal lines, test guidelines do not specify a particular D. magna genotype. Not all laboratories use the same clonal lines when conducting toxicity tests. The genetic variation between the various clones may contribute to significant variation in reported EC50 values for chemicals.

Poster #8: Quantitative and qualitative detection of cytotoxicity by fluorescence microscopy in human cells.

Author(s): Schwab Marian

Affiliation: Karlsruhe Institute of Technology

Project: PrecisionTox

Abstract:

Cell-based assays are a popular choice for and an important component of risk assessment.

The epithelial–like human hepatocyte cell line HepG2 is an established model to study metabolism, cytotoxicity and genotoxicity. Utilizing the Automated High-throughput Microscopy Assay (AHM), chemical libraries, such as the one established for the Precision Toxicology Project, can be screened in 96-well assay plates cost efficiently, quickly and reliably.

In the AHM assay, a combination of different DNA stains provides information on diverse cellular endpoints, such as altered cell proliferation, cell viability and mode of cell death. The morphological assessment on the single cell level allows for further identification of adverse phenotypes in cells.

Robust dose-range data allow for subsequent investigation of interesting compounds with regard to cell signalling, oxidative stress, reactive oxygen species and markers of DNA damage.

Poster #9: Advanced in vitro model for drug induced kidney injury assessment - generation of kidney organoid for safety assessment purposes

Author(s): Lukas Wijaya

Affiliation: LACDR

Project: RISK-HUNT3R

Abstract:

Developing in vitro kidney injury model remains a challenge due to the complex architecture of the whole organ. Here, we adapted the Takasato IPSC-derived kidney organoid protocol to generate an advanced high throughput in vitro test method for chemical-induced kidney injury model. This adapted protocol allows us to create larger quantities of kidney organoids with higher compatibility to live cell confocal imaging. The newly generated kidney organoids exhibited a coherent formation of nephron segments including glomerulus, proximal tubule, and distal tubule. Moreover, the kidney organoids also showed clear cellular responses that reflected our understanding of mechanisms of cisplatin-induced kidney injury in vivo. We have established a panel of CRISPR-engineered GFP reporters for various cellular stress response pathways. As a proof-of-concept for application of these reporters in kidney organoids, we first evaluated a DNA damage response iPSC reporter line, iPSC-CRISPR-GFP-p21. Cisplatin-induced GFP-p21 induction was observed in proximal tubular regions of the organoids, but not in glomerular cells. We anticipate that these kidney organoids can be used as an in vitro high throughput test systems to monitor chemical-induced nephrotoxicity.

Poster #10: Physiological maps and chemical-induced disease ontologies: tools to support NAMs development for next-generation risk assessment

Author(s): Luiz Ladeira

Affiliation: Liège Université

Project: ONTOX

Abstract:

Physiological maps (PM) can be defined as a graphical representation of cellular and molecular processes associated to specific organ functions (Vinken et al. 2021). Within the ONTOX project, we designed a total of 6 PMs describing physiological processes in the liver, the kidney and the brain. These PMs are then used as a tool to assess relevant mechanistic coverage and linkage between a specific organ function and a toxicological endpoint. Based on the Disease Maps project (Mazein et al. 2018) pipeline, we developed the first version of 6 PMs describing the following physiological processes: bile secretion & lipid metabolism (liver), vitamin D metabolism & urine composition (kidney), neural tube closure (update of the work of Heusinkveld et al. 2021) & brain development (brain). Our workflow included: (i) data collection from expert curated literature, (ii) identification of the relevant biological mechanisms, (iii) screening of online databases (e.g. Wikipathways, Reactome, and KEGG) for previously described pathways, (iv) manual curation and integration of the data into a PM using CellDesigner, and (v) visualization on the MINERVA platform (Hoksza et al. 2019). These qualitative PMs represent an important tool for exploring curated literature, analyzing networks and benchmarking the development of new adverse outcome pathways (AOPs). These PMs provide the basis for developing quantitative disease ontologies, integrating different layers of pathological and toxicological information, chemical information (drug-induced pathways) and kinetic data. The resulting chemical-induced disease ontologies will provide a multi-layered platform for integration and visualization of such information. The ontologies will contribute to improving understanding of organ/disease related pathways in response to chemicals, visualize omics datasets, develop quantitative methods for computational disease modeling and for predicting toxicity, set up an in vitro & in silico test battery to detect a specific type of toxicity, and develop new animal-free approaches for next generation risk assessment.

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References
Heusinkveld, Harm J., 2021 https://doi.org/10.1016/j.reprotox.2020.09.002.
Hoksza, David, 2019 https://doi.org/10.1093/bib/bbz067.
Mazein, Alexander 2018 https://doi.org/10.1038/s41540-018-0059-y.
Vinken, Mathieu, 2021https://doi.org/10.1016/j.tox.2021.152846.

Poster #11: Re-thinking the concept of spheroids: a new way to generate high-throughput 3D complex liver model

Author(s): Mostafa Kiamehr

Affiliation: KU Leuven

Project: RISK-HUNT3R

Abstract:

Here we introduce a simple, fast, and robust method to generate mid to high-throughput 3D cultures in a soft hydrogel for iPSC-derived hepatocytes (HC3X) alone or together with iPSC-derived non-parenchymal cells. 3D Heps expressed significantly higher levels of key hepatic markers (e.g. CYP3A4, HNF4α, and PEPCK) compared to 2D Heps, HepG2 cells and conventional spheroids. 3D Heps also stained positive for CK18, PEPCK, CYP3A4, MRP2, HNF4α, and ALB. Co-culture of HC3x-heps with iPSC-ETV2-SPI1 endothelial cells in 3D resulted in formation of interconnected vascular networks where the highly polarised (showed by positive MRP2 staining) hepatocytes were in close proximity of endothelial cells. The tri-culture of HC3x-heps, iETV2/SPI1-ECs, and iPSC-macrophages (M□) resulted in an even more physiologically relevant model. Treatments with Lipopolysaccharide (LPS) significantly upregulated L1-α, IL6, and TNFα and the secretion levels of IL6 and TNFα was increased by ± 100-fold. When exposed to Rifampicin, significantly higher expression of CYP3A4 was observed. Last but not least, treatment of the 3D models with excess fatty acids resulted in substantial uptake of fatty acids and deposition into lipid droplets resembling the steatosis stage in the liver. Taken together, we developed methods to generate functional 3D cultures encompassing iPSC-heps and NPCs in robust, consistent and high throughput manner making it a great candidate model for drug screening, toxicity and/or disease modelling and specifically hepatic steatosis.

Poster #12: The rosette formation assay as a method to identify DNT hazard due to disruption of the RAR/RXR pathway (endocrine signaling)

Author(s): Nadine Dreser

Affiliation: University of Konstanz

Project: RISK-HUNT3R

Abstract:

Retinoic acid is an important endocrine signal, and humans are exposed to a large variety of compounds that affect retinoid signaling. Due to the many retinoid receptors and also metabolic steps of interconversion of metabolites, an adverse outcome pathway network needs to be established and verified. This is an important addition to an overall NGRA strategy for DNT. Here we use the neural rosette formation assay (UKN1; RoFA) to assess retinoid analogues causing neural tube toxicity. By analysis of the acute transcriptome response to retinoid treatment we aim to causally link RA signaling to disturbance of neural tube formation as well as to classify DNT compounds according to a retinoid signature.

Nadine Dreser, Marion Kapitza, Christiaan Karreman, Jonathan Blum, Marcel Leist

In vitro Toxicology and Biomedicine, Dept inaugurated by the Doerenkamp Zbinden Foundation, University of Konstanz

Omics approaches

Poster #13: ASPIS Omics Working Group

Author(s): Florian Caiment and Omics WG team

Affiliation: Maastricht University

Project: ONTOX

Abstract:

The ASPIS Omics Working Group, co-chaired by Dr. Florian Caiment (ONTOX) and Prof. John Colbourne (PrecisionTox), has the first goal of promoting the application of the initiative recently published to render the use of omics in regulatory risk assessment possible: the OECD omics reporting framework (Harrill JA et al., 2021, PMCID: PMC8808338) and the R-ODAF (Omics Data Analysis Framework for regulatory application, Verheijen et al, 2022 PMID: 35247516.). The omics reporting framework provide a set of specialized modules allowing any transcriptomics or metabolomics experiments to be described in view of regulatory assessment. The R-ODAF is a framework proposing a pipeline to analysis raw transcriptomics datasets, from the raw data to stringent statistical thresholds to be applied for selecting a list of differently expressed genes.

The second major activity of the Omics WG is to contribute to the steatosis Case Study by assembling a list of omics dataset involving compounds identified by the chemical selection WG to be leading to steatosis. The target datasets will initially be selected from the main publicly accessible omics repositories and will be later expended with private datasets generated within the ASPIS cluster by the three consortia. Transcriptomics will be de facto the main source of omics datasets, but proteomics and metabolomics will also be considered if available. The selected datasets will be re-analyzed with a common framework to minimize pipeline dependent variation and will be used to perform several meta-analyses with the ultimate goal to highlight the capacity of omics generated dataset to identify the steatosis potential of a compound based on its expression data. For this, both supervised and unsupervised methodologies will be applied, using notably the resource and expertise assembled by other ASPIS WGs on steatosis.

Poster #14: An introduction to the semi-automated, robotics-based sample extraction workflow facilitating PrecisionTox omics analyses

Author(s): Martin Robert Jones

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Chemical pollution poses significant risks to animal and human health, and has been linked to the premature death of millions of people each year, globally. PrecisionTox, an EU Horizon 2020-funded research project, aims to tackle the scourge of chemical pollution by developing, and advocating for the adoption of, New Approach Methodologies (NAMs) for improved chemical safety assessment. To fulfil this goal, PrecisionTox will generate ca. 10,000 toxicological test samples through exposure of 3Rs-compliant model systems, including Daphnia magna, Drosophila melanogaster, Danio rerio, Caenorhabditis elegans, Xenopus laevis and a human cell line, to 250 independent chemicals at multiple distinct time points. Each will undergo extraction and subsequent transcriptomic and untargeted metabolomics analyses, yielding information-rich molecular datasets from which, for example, predictive toxicological models might be built concerning the relationship between adverse outcomes, toxicological mechanisms of action, compound class / structure and genetics. In the work presented here, we introduce the semi-automated extraction workflow developed to underpin this mission, which provides a seamless link between upstream sample collection procedures and downstream omics analysis pipelines. The workflow can extract up to 96 samples in a single batch, with each sample being homogenised in the tube in which it was collected. Homogenates are transferred to independent wells of a 96-well deep well plate, wherefrom aliquots are taken and quenched on liquid nitrogen for subsequent RNA purification. Uniquely, the remainder of each homogenate undergoes a Bligh and Dyer-like biphasic extraction in 96-well plate format (2:2:1.8 v/v/v methanol:chloroform:water), yielding for each sample, independent polar and lipophilic metabolite pools. With up to two semi-automated extraction batches possible each day, the semi-automated workflow is approximately 4-5 times faster than comparable manual procedures. By virtue of the workflow being largely automated, it is also anticipated that technical variance will be minimised, leading to more reproducible omics datasets.

Poster #15: The renal proximal tubule TXG-MAPR: safety assessment based on quantitative gene network analysis

Author(s): Hugo van Kessel

Affiliation: Leiden University

Project: RISK-HUNT3R

Abstract:

Scientific advances in -omics technologies and ever-increasing knowledge on human biology render pre-clinical in vivo testing not sustainable in the future. In the kidneys, proximal tubule epithelial cells are the primary target for xenobiotic-induced injury due to increased exposure levels, bilateral transporter-mediated uptake and high oxygen consumption. Through concentration and time course chemical exposure of RPTEC-TERT1 cells using >50 nephrotoxicants and reference compounds that cover a wide range of mechanisms of action, and subsequent TempO-Seq whole genome transcriptomics and weighted correlation network analysis, we have established a human RPTEC/TERT1 in vitro kidney TXG-MAPr tool. The TXG-MAPr tool allows user friendly interactive toxicogenomics data interpretation on mechanisms of action and compound activity correlation. Interspecies network preservation analysis using the in vivo rat kidney TXG-MAPr based on TG-GATEs has revealed preserved cellular processes relevant in kidney toxicity. Identification of co-regulated gene networks using high throughput whole genome transcriptomics will provide mechanistic insight in the cellular stress response which can provide mode-of-action formulation based on quantitative gene network analysis and support hazard characterization for NGRA-based safety assessment.

qAOP approaches

Poster #16: ASPIS quantitative Adverse Outcome Pathway Working Group

Author(s): Mark Cronin and qAOP WG team

Affiliation: Liverpool John Moores University

Project: RISK-HUNT3R

Abstract:

The ASPIS quantitative Adverse Outcome Pathway Working Group (qAOP WG), co-chaired by Dr Huan Yang (ONTOX) and Mark Cronin (RISK-HUNT3R), aims to investigate models that (semi-)quantify molecular initiating events (MIEs) or key event relationships (KERs) within existing AOPs using non-confidential data, as well as identifying and sharing good practice. Specific objectives include adding value by developing common ideas for qAOP development; sharing knowledge of dose-responses, data and models between ASPIS partners; and facilitation integration of qAOPs with knowledge of MIEs and physiologically-based kinetics (PBK) modelling to enable quantitative systems toxicology. The outputs from the APSIS qAOP WG aim to increase understanding of how risk assessors would use qAOPs in Next-Generation Risk Assessment (NGRA). This will be achieved through the development of one or more common qAOPs (linear and networks). Initial work has been on a qAOP for liver steatosis with common data and modelling approaches being shared within ASPIS. This will assist in the determination of outputs from qAOPs and degree of confidence needed for NGRA. Further work will identify obstacles and concerns for assessors to use qAOPs in NGRA. A particular focus of the qAOP WG is the development of a framework for validation of qAOPs through the identification of uncertainties. The work presented in this poster was performed as part of the ASPIS Cluster.

Poster #17: In vitro New Approach Methodologies (NAMs) for assessing effects of chemicals leading to cognitive function defects in children – the contributions of the ONTOX project

Author(s): Eliska Kuchovska

Affiliation: Leibniz Research Institute for Environmental Medicine

Project: ONTOX

Abstract:

The current regulatory developmental neurotoxicity (DNT) guidelines (OECD TG 426 & EPA OPPTS 870.630) are not sufficient for the hazard assessment of the vast chemical universe and only a handful of chemicals have been assessed up to date. The main reasons are the high time and cost consumption of the testing according to the current guidelines and the necessity to use animals with difficult extrapolation and limited predictivity potential for the human risk assessment. Thus, more reliable, efficient, and better predictive new approach methodologies (NAMs) for DNT testing are needed.

This work focuses on two DNT-related adverse outcomes (AO): decreased cognition and impaired learning and memory caused by prenatal exposure to chemicals. An adverse outcome pathway (AOP) network was compiled using existing and novel AOPs. Essential key events (KEs) were selected to be monitored to reliably predict the effects of chemicals leading to the AOs. For this purpose, an in vitro battery has been set up at IUF and NIPH laboratories. The measured endpoints cover generic KEs such as cell death and oxidative stress but also specific KEs covering key neurodevelopmental processes vital for normal brain development i.e. proliferation, migration, differentiation, synaptogenesis, and neural network formation of respective cell types (neurons, oligodendrocytes, radial glia, astrocytes) including the cell morphology and number. The used human cell models are iPSC-derived neural progenitor cells and primary neuroprogenitor cells representing embryonic and fetal developmental stages, respectively.

This battery of in vitro assays is being extensively characterized and will be coupled with in silico models in the framework of the ONTOX project. This approach will serve to create a combined NAM in order to predict systemic repeated dose toxicity effects that, in combination with exposure assessment, will advance human risk assessment in line with Next Generation Risk Assessment principles and without the use of animals.

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Poster #18: Update and optimization of an adverse outcome pathway network of chemical-induced cholestasis

Author(s): Jonas van Ertvelde

Affiliation: Vrije Universiteit Brussel

Project: ONTOX

Abstract:

Background and Objectives: Cholestasis denotes any situation of impaired bile secretion with concomitant accumulation of bile acids in the liver or in the blood circulation and may be induced by various chemicals. Our group previously introduced an adverse outcome pathway (AOP) network mechanistically describing key events (KEs) and their relationships driving chemical-induced cholestatic liver injury. The aim of the present work was to update and optimize this AOP network in line with guidelines issued by the Organization for Economic Co-operation and Development (OECD).
Material and methods: PubMed was queried for studies of chemical-induced cholestasis using a list of predefined key words and several known KE-related terms. SysRev, a newly developed computational tool for systematic reviewing and data extraction, was employed during the abstract screening and full-text screening. The tailored Bradford-Hill criteria, described by the OECD guidelines, were used in the weight-of-evidence assessment of the KEs and KE relationships.
Results: A total of 6572 articles was retrieved from PubMed and uploaded to SysRev. An initial abstract-screening resulted in a total of 544 papers eligible for data extraction in the full-text screening process.
Discussion and Conclusion: Extracted data are used for the assessment of already defined KEs and KE relationships, but also for the identification of potential new KEs, resulting in an updated AOP network on chemical-induced cholestatic liver injury. The fully assessed AOP network will serve as the conceptual basis for setting up an in vitro test battery to identify cholestatic chemicals, consisting of a series of assays that each monitor an individual KE.

Poster #19: Development of an adverse outcome pathway for kidney tubular necrosis

Author(s): Devon Barnes

Affiliation: Utrecht University

Project: ONTOX

Abstract:

Tubular necrosis (TN) occurs in response to proximal tubular injury, obstruction and vasoconstriction and is prevalent within hospitalised AKI and CKD populations. Although characterized morphologically, contributory mechanisms of TN remain ill-defined. Adverse outcome pathways (AOPs) provide analytical constructs to describe a sequential chain of causally linked key events, from the molecular initiating event (MIE) to the adverse outcome (AO) throughout key events (KE) across different levels of biological organization.

This study aims to examine and expand upon described connections between the exposure of the kidney to nephrotoxicants, perturbations to its function and cellular responses observed during kidney TN for the development of an AOP. Initially, a literature search identified existing research utilizing terms relevant to clinical biochemistry, urinary biomarkers, histology and clinical presentations in TN. A list of chemically applicable, data-rich nephrotoxic compounds was also established to support the search. Simultaneously, physiological maps of the kidney were designed to establish physiological mechanisms contributing to TN. Currently reported AOPs regarding nephrotoxicity were also systemically mapped to form networks and identify relevant MIEs and KEs using existing AOPs from the AOP-Wiki to contribute toward producing a mechanistic framework for the identification, development and implementation of in vitro endpoints for TN.

To assess chemical disruption, a custom-designed battery of in vitro assays was developed using conditionally immortalized human proximal epithelial tubule cells to gather relevant information of nephrotoxic effects within TN-related MIEs and KEs throughout the spectrum of mechanisms depicted in the linear AOP. This battery will be utilized in combination with in silico models to identify data gaps toward further testing. These results will form the initial steps toward the development of a combined approach to generate, evaluate and apply AOPs for the advancement of next generation, human risk assessment in TN-related kidney failure in the framework of the ONTOX project.

Poster #20: An adverse outcome pathway network for liver steatosis induced by chemicals

Author(s): Anouk Verhoeven

Affiliation: Vrije Universiteit Brussel (VUB)

Project: ONTOX

Abstract:

Background and Objectives: Adverse outcome pathways (AOP) are frameworks depicting existing information on causal linkages (i.e., key event relationships (KER)) between measurable biological changes (i.e., key events (KE)) leading to an adverse outcome (AO). To better represent complex interactions within organisms, different AOPs sharing one or more KEs are brought together in an "AOP network".

The aim of this research was to update the current AOP network on liver steatosis, with a focus on chemical-induced liver steatosis. Furthermore, to weigh the evidence between KEs, the updated AOP network was also assessed in accordance with the specific guidelines from the Organization for Economic Co-operation and Development.

Material and methods: PubMed was used to collect publications on chemical-induced liver steatosis published after 2016. The key search terms included steatosis, specific nuclear receptors as molecular initiating events of the AO as well as KE-associated keywords. A first title/abstract screening of all collected papers was performed with SysRev (i.e., a computational tool for systematic reviewing and data extraction) using a labelling strategy to include/exclude papers. With the application of a second labelling strategy, data regarding essentiality, biological plausibility/applicability and empirical support of KEs and KERs were manually extracted during full-text screening of included papers. Subsequently, data was used to assess the level of confidence in the updated AOP network on liver steatosis according to the tailored Bradford-Hill Criteria.

Results: The PubMed search resulted in 12,478 papers. The title/abstract screening resulted in 1,626 papers eligible for data extraction in the full-text screening phase.

Discussion and Conclusion: Extracted data was used to assess the level of confidence in previous described KEs and KER. In addition, data was used to identify potential novel KEs. The updated AOP network on liver steatosis will serve as a basis for the development of animal-free methods for toxicity testing purposes.

Poster #21: Computation and visualisation of a Mitochondrial Toxicity qAOP PBK-TD model using Simcyp Designer

Author(s): Elias Zgheib

Affiliation: CERTARA

Project: RISK-HUNT3R

Abstract:

Over the last decade, the use of quantitative adverse outcome pathways (qAOPs) in regulatory dossiers and risk assessment pipelines, has spread quickly. However, the toxicodynamic (TD) models behind these tools often remain dependent on the compounds used for their development. Applicability of qAOPs, could thus be widely improved by linking them to chemical-specific physiologically based kinetic (PBK) models. Simcyp Designer, a new product of the Simcyp® simulator provides an intuitive and powerful graphical interface for building custom PBK models and their visualisation. Here, we propose a novel approach using this tool to develop, amend and visualise a PBK-TD sequential model in a qAOP context. First, a calibrated and validated qAOP TD model is added to a new compartment in Simcyp Designer, that is then linked through a PBK-workflow to the target compartment of the study. Finally, the full model's design is adjusted in a fluid and transferrable fashion as the used tool permits.

Based on the Tebby et al. 2022 work on the quantitation of a generic qAOP, we have developed a PBK-TD model for the mitochondrial toxicity AOP (AOP #3, aopwiki.org). This new model was calibrated with data on Rotenone and Deguelin neurotoxicity in LHUMES neuronal cells. After the identification of data gaps, we recalibrated the model with new Tebufenpyrad and Tolfenpyrad data. The variety of test conditions explored here are meant to challenge the fundamental principles of qAOPs such as wide applicability and chemical agnosticism. Since the in vitro cell concentrations were measured in neurons, the chosen target compartment in Simcyp Designer was set to be the brain.

The next steps will include additional model refinements accounting for assay variability and extensions to liver (HepG2) and kidney (RPTEC/TERT1). Regular follow up with users/stakeholders and regulators will help us account for their concerns, needs, and expectations in this work.

Computational approaches

Poster #22: The activities related to the computational approaches within ASPIS

Author(s): Gerhard Ecker, Emilio Benfenati & Computational Approaches WG team

Affiliation: University of Vienna & Istituto di Ricerche Farmacologiche Mario Negri

Project: RISK-HUNT3R & ONTOX

Abstract:

The Computational Approaches Working Group (WG) organizes the activities related to in silico methods in a broad sense, including the QSAR models, docking studies, read-across, and interacting with several Working Groups within ASPIS. These activities can largely benefit from a networking attitude, since most of the modelling activities are based on collections of data, address specific toxicological and toxicokinetics aspects, and apply multiple algorithms and chemical descriptors. Data derive from multiple sources and projects, the toxicological expertise comes from other Working Groups, and the algorithms and descriptors from one laboratory are very probably useful for a second one. Thus, there is a strong opportunity to establish networking activities, and since the content of this specific WG deals with files, it is also quite simple to establish collaborations.

In general terms the collaboration relates to make available data and specific software components, with the purpose to achieve better results within a synergistic approach.

The first application of this collaboration is focused on steatosis. In this case, the computational approaches include the study on the role of descriptors with a particular physico-chemical meaning, the development of in silico models related to the AOP for steatosis, docking studies investigating the tridimensional aspects of the mechanism, and read-across tools.

In the future, other endpoints will be addressed, such as developmental neurotoxicity.

Poster #23: Combining gene expressions and imaged-based morphological features for chemical-phenotype profiles

Author(s): Natacha Cerisier

Affiliation: INSERM

Project: RISK-HUNT3R

Abstract:

As part of the RISK-HUNT3R project, this computational study was intended to integrate molecules that induced transcriptomic perturbations and cellular morphological changes into a biological network in order to assess chemical-phenotypic relationships. Such a network was enriched with existing disease information in an effort to suggest molecular and cellular mechanisms of action leading to diseases.

Two datasets were used for this study. Firstly, we used the "Cell Painting morphological profiling assay" dataset, composed of 30,000 compounds tested on osteosarcoma cells (U-2 OS). Secondly, we used the "L1000 mRNA profiling assay" (LINCS) dataset, a collection of transcriptional expression data from cultured human cells treated with approximately 20,000 bioactive small molecules. Furthermore, pathways, GO terms and disease enrichment were performed on the transcriptomics data.

Our study allowed us to develop a biological network combining chemical-genes-pathways-morphological perturbations and disease relationships. It contains an ensemble of 9,989 chemicals, 732 significant morphological features and 12,328 genes. We found that some sets of drugs shared similar genes and morphological perturbation which suggest possible links between molecular and cellular perturbations.

Although the study is based on the assumption that the cellular behaviour in presence of a chemical is similar independently of the cell type, some extrapolation about genes-cellular features and diseases relationships can be performed. Our network could be enriched with other types of phenotypic screening, transcriptomic information, based for example on the RNAseq technologies, and chemical-disease or toxicity annotations.

Poster #24: Chemical Effect Predictor: A tool to predict chemical toxicity using biological network properties

Author(s): Jordi Valls Margarit

Affiliation: MedBioInformatics Solutions

Project: RISK-HUNT3R

Abstract:

Characterizing the toxicity of chemicals and drugs in humans is not only important in the field of drug discovery but also for the development of new methods for risk assessment. Currently, most of the predictive methods are addressed to a specific side effect or toxicity endpoint or require features that restrict their applicability in particular endpoints. Moreover, these methods are “black box”, providing limited insights into the mechanisms of action of chemicals. System and network-based methods are suitable approaches to analyse and explain how chemicals and drugs perturb biological systems. In this context, we present the Chemical Effect Predictor (CEP), a machine learning model that leverages properties of biological networks for the prediction of toxicity of compounds, and the proposal of a mechanistic hypothesis for their mode of action. The model is based on a multi-scale, heterogeneous network that integrates different layers of information, such as associations among drugs, proteins, diseases, and biological processes. For each drug and disease, we compute network diffusion profiles, which identify the key proteins and biological functions involved in a given drug adverse reaction. By comparing the similarity of both compound and disease diffusion profiles through the multiscale network we can identify the most likely disease elicited by the compound and identify the potential mechanism of action. Different variables are extracted from the network and incorporated into the model such as the importance of particular biological processes between one chemical and side-effect, or their distance into the network. We present the performance of the model on a benchmark of drug adverse reactions and its application in the context of RISKHUNT3R case studies. CEP is a novel approach that leverages information on biological networks and can be used to support the development of new in-silico risk assessment methods.

Poster #25: A KNIME Workflow for Consensus Target Prediction

Author(s): Palle Steen Helmke

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Public data on compound-target interactions are extremely sparse and could be enriched by respective prediction tools. In order to support several case studies in Riskhunt3r and ASPIS, we evaluated a set of target prediction tools (SwissTargetPrediction, SEA, PASS, ChEMBL-Docker) and created a KNIME workflow for consensus scoring and visualization as heatmap.

The output data extracted from these prediction tools were manipulated and sorted in order to list the predicted targets per compound. Hence, the compound-ChEMBL-IDs and target-ChEMBL-IDs were used for identifier mapping across the different tools. Subsequently, the respective tables were concatenated in order to visualise the compound/predicted target interactions. This combined table shows the number of prediction tools predicting a distinct compound-target pair. For further visualisation, a heatmap was created using KNIME, displaying these compound/predicted target interactions.

As a result, mifepristrone, a synthetical steroid inducing abortion, was predicted by three prediction tools as active for the progesterone and the glucocorticoid receptor for instance. In addition, the compound carbofuran, an insecticide utilised as a feeding protection, was predicted as active for the acetylcholinesterase by three prediction tools, which resembles one of its known mechanisms of action.

Poster #26: Structure-based predictions for MIEs

Author(s): Karin Grillberger and Claudia Immacolata Trivisani

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Due to increased interest in the field of predictive toxicology to focus on non-animal testing and incorporate in vitro and in silico testing approaches for regulatory risk assessment of chemicals, the concept of adverse outcome pathway (AOP) frameworks was recently introduced. This concept comprises a molecular initiating event (MIE) as an initial point of chemical-biological interaction that starts the pathway, more specifically an interaction of a chemical with a toxicologically relevant human target. The MIE is followed by several measurable key events (KE) which eventually lead to the adverse outcome. Two main AOPs of interest in the Riskhunt3r project are developmental neurotoxicity (DNT), and steatosis.

Concerning DNT, there are several targets involved, like the nicotinic acetylcholine receptors (nAChRs), thyroid and mitochondrial systems. Therefore, reliable predictions for MIEs are crucial. In order to achieve this goal, we extract available data about interacting compounds and collect protein structures that are suitable for in silico approaches like molecular docking.

Docking represents a structure-based tool for binding mode elucidation, or for screening a library of potentially toxic compounds. Several docking methods are explored to cover the specific question of interest, i.e. ensemble docking (docking the same set of ligands to multiple protein structures), reversed docking, or induced fit docking (allowing the protein to adapt to a certain degree to the binding of the ligands).

Poster #27: UNIVIE Jupyter Notebooks (JNs) for Data Curation & Machine Learning (ML) model building for Transporters & Off-target predictions

Author(s): Gerhard Ecker

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Machine learning (ML) models require qualitative curated data sets in order to learn from chemical structures to predict activities with high reliability. However, publicly available data have often to be preprocessed before being applied to ML training. Therefore, we propose semi-automated frameworks, one for data retrieval from the ChEMBL database with additional data filtration, data standardization, data classification steps and another one for ML model building including featurization and parameterization. This workflow enables the user to retrieve curated datasets by uploading a CSV-file to the JN. The obtained data sets can be applied to the JN for ML model building where four different classifiers, Logistic Regression, Support Vector Machine (SVM), Random Forest (RF) and k-nearest Neighbor (knn) are used.

Poster #28: Computational modelling of neural tube closure defects

Author(s): Job Berkhout

Affiliation: National Institute for Public Health and the Environment

Project: ONTOX

Abstract:

Closure of the caudal neural tube is a critical event that occurs early in development, around day 27 of human gestation. Failure of neural tube closure results in severe birth defects, such as spina bifida. These neural tube defects (NTD) are among the most prevalent human congenital malformations, which warrants specific attention in chemical safety assessment. Computational models of biological processes are likely to revolutionize chemical safety assessment in the near future. Such models can be used to predict the effect of chemical-induced gene expression changes and provide a template for establishing quantitative adverse outcome pathway networks. This study aims to develop an in silico model of the human neural tube closure, which will be applied to predict chemical-induced NTDs.

By extensively mining the developmental biology and toxicology literature, we first created a physiological map of human neural tube closure. Based on the physiological map, we built a multicellular agent-based model using CompuCell3D.

The constructed physiological map depicts the all-trans-retinoic acid (ATRA) related molecular pathways linked to the various cell types in which they occur, and their morphogenetic consequences, that lead to closure of the neural tube. The morphogenetic events driven by gene expression changes are visualized by the computational model.

We simulated in silico the complex biological process of neural tube closure, in order to demonstrate the feasibility of this approach. At a later stage in the project, the computational model will be applied to predict chemical-induced changes in gene expression and cell characteristics. The predictions of the model will be validated using a set of dedicated in vitro assays in conjunction with existing knowledge on in vivo developmental neurotoxicity. Such computational models may ultimately provide an alternative in silico approach for chemical safety assessment without the use of animals.

Poster #29: Next generation target organ toxicity risk assessment: endogenously tagged human stem cell reporters for high-content screening of oxidative stress response.

Author(s): Tamara Danilyuk

Affiliation: Leiden University

Project: RISK-HUNT3R

Abstract:

Development of in vitro assays for early detection of liabilities to chemical adversity is crucial for the prediction of liver toxicity. Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) are an attractive in vitro model to study mechanism-based xenobiotic toxicity. We set out to build a panel of fluorescent hiPSC reporters, suitable for high-content-screening of cellular stress response activation, upon compound exposure. We established a pipeline for efficient CRISPR/Cas9-mediated reporter generation and reporter's functional characterization upon differentiation to relevant lineages, including HLCs. Here, we present the generation and application of fluorescent hiPSCs reporter lines for sulfiredoxin-1 (SRXN1) and pirin (PIR), which are shown to be inducible and sensitive biomarkers for the oxidative stress response. Oxidative stress induced by diethyl maleate (DEM), sulforaphane and nitrofurantoin was monitored using live-cell confocal imaging of iPSC reporter lines in HLCs differentiation state. Endogenous levels of eGFP-tagged biomarkers accumulated in the cytoplasm of HLCs over 24 hours window. Newly established isogenic fluorescent reporter lines will be used i) as a tool in understanding and quantifying target organ specific oxidative stress response, ii) point of departure modelling to further capture specific lineage sensitivities towards oxidative stress and iii) ultimately, used for the hazard characterization and IATAs for target organ toxicity in next generation risk assessment.

Poster #30: Evaluation of state-of-the-art in silico testing methods to fill physicochemical and pharmacokinetic data gaps within the ONTOX project

Author(s): Rita Ortega-Vallbona

Affiliation: ProtoQSAR

Project: ONTOX

Abstract:

The ONTOX project aims at developing new approach methodologies (NAMs) to address systemic, repeated dose toxicity effects related to six adversities in the liver (steatosis and cholestasis), kidney (tubular necrosis and crystallopathy) and developing brain (neural tube closure and cognitive function defects). NAMs will be based on AI-based systems fed by available data. One of the objectives of the "Chemical Domain" work package is to study the correlations between adversities and kinetic/physicochemical properties of the compounds to find properties able to discriminate between active and inactive chemicals for each of the six ontologies. To do so, we first collected experimental data for 27 physicochemical and pharmacokinetic properties. Secondly, we identified and validated up to 20 available computational tools to predict the most relevant parameters and used them for data gap-filling when experimental data was unavailable.

Relevant kinetic/physicochemical properties for the steatosis case study were identified in this final dataset using two different approaches: The first approach used three statistical analyses (Kolmogórov-Smirnov, t-Student and Wilcoxon) to evaluate the selected properties for near 60 compounds. Among the evaluated properties, four were statistically significant and can help to characterise a compound as steatotic, while two presented a non-significant trend. The second approach consisted of developing a machine-learning model to predict steatosis from these properties, including molecular weight and the number of halogens in the analysis. This approach was performed with a larger dataset (https://doi.org/10.1021/acs.chemrestox.0c00511). Eleven features showed to be relevant, and interestingly, there was a good fit with the more significative properties from the previous analysis with the ONTOX chemicals. Combining both approaches, we found some kinetic/physicochemical properties that significatively correlate significantly with steatosis. These properties may help in the discrimination of pro-steatotic compounds and unravel the underlying mechanism of action of these chemicals.

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Exposure and kinetics

Poster #31: Exposure and kinetics research activities in ASPIS: Moving from hazard identification to risk characterisation in next generation risk assessment

Author(s): Nynke Kramer, Sylvia Escher and exposure and kinetics WG team

Affiliation: Wageningen University and Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

Project: ONTOX and RISK-HUNT3R

Abstract:

Unlike in traditional chemical safety assessment, which focuses on hazard characterization using toxicity tests on animals, next generation risk assessment is an exposure-led, hypothesis-driven approach relying on non-animal test methods. The European Commission strongly supports the development of these non-animal approaches in risk assessment, by funding, amongst others, the three ASPIS cluster projects, ONTOX, PrecisionTox and RiskHunt3R. All three projects aim to develop and apply novel in vitro toxicity assays to identify perturbations in biomolecular toxicity pathways. However, on their own, these assays identify hazards and does not meet the 'exposure-led' criterium of next generation risk assessment. Each of the three projects therefore include activities defining chemical exposure levels in the environment, human populations, target organs and in vitro assays. The ASPIS working group on kinetics and exposure has been setup to align these research activities in the field of exposure and toxicokinetics assessment. The aim of the working group is to integrate the complementary approaches for kinetics and exposure assessment into a common, pragmatic guideline for risk assessors wishing to use exposure, in vitro ADME, in vitro distribution kinetics, physiologically based kinetic (PBK), and in vitro-in vivo extrapolation (IVIVE) models for next generation risk assessment. This guideline details a tiered approach to exposure and kinetics assessment, which is to be evaluated with case study chemicals. In this presentation, the exposure and toxicokinetics tools under development in each of the three projects are compared and mapped along an exposure-effect continuum. The activities planned for the first case study chemicals, triazole fungicides and liver steatosis, are outlined.

Poster #32: A high-throughput analytical workflow to determine internal concentrations of xenobiotics in zebrafish larvae

Author(s): Nico Grasse

Affiliation: Helmholtz Centre for Environmental Research - UFZ

Project: PrecisionTox

Abstract:

Submission of toxicokinetic (TK) data for interpretation of biological effects on 250 chemicals in terms of internal concentrations in zebrafish larvae. This deliverable will also include the predicted internal concentrations of the test chemicals using partition models between water and the embryos. For significantly deviations from the model, a detailed time resolved analysis of internal concentrations will be conducted and reported, including the determination of metabolites. These TK information is essential to estimate the specifity of the effect caused by a specific class of compounds and to estimate to which extent the observed effects are solely related to baseline toxicity (BT). A preliminary efficient workflow was established to screen internal concentrations of 30 chemicals in zebrafish larvae at 96 hours post fertilization (hpf). Quality control experiments were done to demonstrate chemical's stability over the exposure.

Poster #33: Kinetic modelling and quantitative in vitro-in vivo extrapolation strategies for next generation risk assessment in the H2020 ONTOX project

Author(s): René Geci

Affiliation: esqLABS

Project: ONTOX

Abstract:

The EU funded ONTOX project aims to deliver a generic strategy to create innovative new approach methodologies (NAMs) in order to predict systemic repeated dose toxicity effects that, upon combination with tailored exposure assessment, will enable human risk assessment with focus on 6 ontologies for three different organs (Liver, Kidney, and Developing Brain). One of the pillars in the ontologies is a thorough understanding of the toxicokinetics of case study chemicals. Toxicokinetics and chemical exposures are addressed in work package four (WP4) of the project.

Research in WP4 focuses on developing and applying several types of in silico models: i. generic physiologically based kinetics (PBK) model frameworks (chemical specific and generic) for simulating in vivo distribution kinetics and characterising the ADME properties to predict tissue exposure for the selected chemicals and their systemic repeated dose toxicity effects; ii. in vitro distribution kinetics models for estimating free and cell-associated in vitro effect concentrations to perform accurate hazard characterization. These biokinetic models are necessary to integrate information from the exposure to an adverse outcome by using the plethora of NAM data generated in the ONTOX project, to allow quantitative in vitro-in vivo extrapolations (QIVIVE). In parallel, links with quantitative adverse outcome pathways (qAOP) are explored to link kinetics to specific MIE and/or KE. To allow all these integrations, a gap analysis and data mining is ongoing to evaluate and extend the definition of the chemical and biological applicability domains of the biokinetic models. Uncertainty, sensitivity analyses and model validation will be discussed in order to gain confidence in the application of such models by risk assessors and decision makers.

Authors: René Geci1*, Susana Proenca2, Alicia Paini1, Huan Yang1, Stephan Schaller1, Nynke Kramer2,

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Poster #34: Primary human enterocytes for the determination of intestinal metabolism

Author(s): Patrik Lundquist

Affiliation: Uppsala University

Project: RISK-HUNT3R

Abstract:

In RISK-HUNT3R, we investigate external exposure to chemicals via three routes: lungs, skin and intestine. For this purpose, we develop new approach methodologies (NAM) that better reflect human absorption barriers. The more in vivo-like parameters generated in these models are then used to provide data to predictive toxicokinetic models of human exposure. The determination of human exposure via the oral route usually begins with measurements of the permeability of the compound in question over monolayers of intestinal epithelial cells. The permeability gives an approximation of the fraction absorbed from an oral dose. To improve predictions we also need a value for the fraction metabolized by the enterocytes of the small intestinal epithelium. Permeability is commonly measured in the Caco-2 cell model. A weakness of the Caco-2 cells is that they lacks expression of the major metabolizing enzymes found in the human intestine. To measure the intestinal metabolism of compounds we isolate primary human jejunal enterocytes that in theory should retain metabolizing enzymes. Enterocytes are isolated from human jejunal mucosa samples from patients undergoing gastric bypass surgery at Uppsala University Hospital. Enterocytes are purified using a gentle enzyme-free method based on EDTA containing buffers and gentle shaking followed by Percoll fractionation to remove unwanted cell fractions. This results in a high yield of highly viable (> 90 %) enterocytes where a fraction of 25-35 % of cells were caspase-8 positive and had entered early apoptosis. Preliminary results indicate that major intestinal cytochrome P450 enzymes are detected in the isolated enterocytes. Optimization of methods to determine the intrinsic clearance for phase I and II metabolism substrates is ongoing.

Poster #35: Parameterisation and Verification of IVIVE-PB(P)K models for Risk Assessment

Author(s): Barira Islam

Affiliation: Certara

Project: RISK-HUNT3R

Abstract:

The physicochemical, absorption, binding, distribution, and in vitro elimination parameters of a compound are the primary inputs required for building a physiologically based kinetic (PBK) model. These parameters can be obtained from literature, generated experimentally, or predicted by in silico tools using models based on quantitative structure activity relationship (QSAR) and/or quantitative property-property relationship (QPPR). However, parameters predicted by in silico methods are not always reliable model inputs, making it imperative to understand the applicability domain of any QSAR/QPPR models before using them for building PBK models. In the Riskhunt3R project, we developed a case study to understand the impact of using predicted or measured in vitro input parameters on kinetic predictions of PBK models. 28 case study compounds were selected to cover a range of physicochemical properties and elimination pathways. All types of compounds, acidic, basic, neutral and ampholytes were included and log P (octanol-water partition coefficient) varied from 0 to 8 to test both hydrophilic and hydrophobic compounds. The case study compounds were selected to incorporate action of variable metabolic enzymes such as cytochrome P450s (CYP) including CYP1A2, CYP3A4, CYP2D6, CYP2C19, UDP-glucuronosyltransferases and cytosolic enzymes including monoamine oxidase A and aldehyde oxidase. Additionally, compounds with biologically active metabolites have been included to understand the data requirements for the prediction of both parent and metabolite concentrations. As a test case, PBK models for atomoxetine, itraconazole, montelukast, fluticasone furoate and diclofenac have been built (in Certara's Simcyp® Discovery and Simcyp® Simulator) using in silico and in vitro data. The prediction accuracy of the two approaches will be investigated by comparison with human in vivo observations.

Poster #36: Assessment of aggregate exposure in RISKHUNT3R

Author(s): Wouter Fransman

Affiliation: TNO

Project: RISK-HUNT3R

Abstract:

The RISK-HUNT3R project considers the assessment of the total or aggregate exposure of (members of) a population to a single chemical substance from multiple sources, via multiple pathways and routes. The aggregate exposure can be differentiated into aggregation of (a) different routes (for example inhalation and dermal of the same compound during the same use), (b) occupational and consumer exposure, and (c) exposure during daily use of different articles with the same compounds within consumer settings.

The overall objective in RISKHUNT3R is that health-based limit values will be delivered for internal exposures (e.g., absorbed doses). Estimates of the external exposure for each pathway and source will be converted to an internal exposure value before aggregation. Evaluation of risk can be done, for example using a Risk Characterisation Ratio (RCR) approach. Evaluating the aggregate risk for various sources and routes in combination would be done by adding individual RCRi's and requiring that their sum be less than 1. Here we propose a tiered approach for the assessment of the aggregate exposure from multiple sources and routes.

Poster #37: Aggregate exposure model

Author(s): Max Spänig

Affiliation: ITEM Fraunhofer

Project: RISK-HUNT3R

Abstract:

The main use of N-methyl-2-pyrrolidone (NMP) is as a polar aprotic solvent in industrial applications. Occupational exposure may result from its use as a solvent for paint and graffiti removal and indoors from its use in paints and inks as well as rugs and carpets. Dermal route plays a significant role in the total uptake of vaporized NMP, as shown by exposure and biomonitoring studies.

Aggregation of exposure can occur by several pathways or exposure scenarios. In the case study of NMP, various activities within a work shift and the associated dermal and inhalation exposures add up to a total exposure that must be properly assessed. For this approach, exposure models such as ART and ECETOC TRA are used to provide estimates of exposure scenarios.

The model output and its linkage to the internal concentration needs to be investigated as well. Fraunhofer ITEM has already developed a physiology-based kinetic model (PBK) for humans to estimate inhalation of gases, vapors, and liquid and solid aerosols. In order to provide a risk assessment of aggregate exposure, an extension of the PBK model to include dermal uptake is necessary. Human skin is divided into several compartments based on its physiological properties, taking into account skin properties such as hair follicles and water content. A fitted model for NMP is not the main focus of this work, but a generic model that can estimate dermal uptake for a variety of substances and scenarios.

Communication and dissemination strategies for NGRA

Poster #38: A joint voice on the side of NAMs-based strategies for chemical risk assessment: an overview of ASPIS communication activities

Author(s): Giorgia Pallocca, Francois Busqúet, Lucia Milec, Helena Kandarova, Aurelia Boige, Jeanne Laperrouze, Martijn Moné, Agata Ormanin, Simon Perera, Julia D. Zajac, Cyrille Krul, Elisabeth Graf, Jonathan Freedman & Dissemination and Communication WG team

Affiliation: CAAT-Europe, ALTERTOX, CEM, ALTERTOX, Leiden University, Birmingham University, protoQSAR, Vrije Universiteit Brussel, ARTTIC, Hogeschool Utrecht, University of North Carolina at Chapel Hill

Project: RISK-HUNT3R, ONTOX, PrecisionTox

Abstract:

ASPIS's communication and dissemination activities are coordinated by the cluster projects, RISK-HUNT3R, PrecisionTox, and ONTOX, with the shared mission to unbiasedly inform on how New Approach Methodologies (NAMs)-based strategies can rapidly accelerate and improve chemical risk assessment in the EU.

The communication teams of the three projects work side by side to synergize the dissemination efforts and positively impact the cluster visibility and the outreach of its key messages. In particular, the objective of the communication and dissemination working group is to build, beyond projects' specificities, one cluster identity to have a joint stronger voice to vehicle its outcome to regulatory stakeholders, policy-makers, NGO and lay public.

Different actions have been pursued to achieve these goals, such as creating a common visual identity, a joint website (aspis-cluster.eu), and sharing descriptive material, leaflets, and factsheets, to promote the common goals and activities.

The cluster speaks jointly online via a coherent social media presence to facilitate message amplification. The communication teams of each individual projects echo news and outcome of each other and disseminate those via their channels. The cluster also jointly communicates via official press declarations and policy briefs, as support statement of policy legislative initiatives or involvement in public consultations. Common publications, op-eds and press coverage in EU journals are also used to inform the stakeholders about the cluster work.

The ASPIS communication plan also includes unwired activities, such as participation in joint conference sessions at international conferences and the organization of the ASPIS annual open symposium. The symposium series aims to involve cluster members, stakeholders, and cluster regulatory advisory board to discuss together crucial achievements and challenges in the implementation of NAMs into chemical risk assessment in Europe and beyond. This will allow ASPIS to support EU green deal objectives such as the toxic-free environment.

Poster #39: Science Policy on NAMs – A current overview of opportunities for implementation

Author(s): Laura Holden

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Over the last decades, ecotoxicological and toxicological research has developed a wide range of alternative approaches to animal testing (New Approach Methodologies (NAMs)), some of which are already available for regulatory use and others continue to be developed. In parallel, the European regulatory landscape has created several opportunities for the uptake of NAMs, however the acceptance of NAMs and their data by regulators is still limited.

There are a series of opportunities that can provide impetus to advance the use of alternative methods, including the upcoming revisions of REACH and CLP regulations; the implementation of the EU Chemical Strategy for Sustainability; and the European Parliament's resolution calling for an action plan to accelerate the transition from using animals in research. Additionally, EU projects, clusters, and partnerships are working at the science-policy interface on the topic of NAMs, of which ASPIS is one. These each play a role in the future implementation strategy of NAMs in chemical safety assessment for regulatory purposes and are addressed by the Embedded Translation pillar of PrecisionTox. The movement of science towards the 3Rs policy and more protective chemical data involves engagement with stakeholders such as policymakers, regulators, research institutions, and industry. The science-policy interface seeks to build confidence in the use of NAMs and we aim for NAM science and technology to reach its protective potential through regulatory implementation.

Our research has identified barriers to the uptake of NAMs, enabling us to consider solutions to address them. The identification of and continued engagement with projects, activities, actors, and upcoming regulatory revisions has provided further drive for change behind the NAM transition, through our Group First Regulate Better White Paper; a variety of media about NAMs to increase awareness and understanding; and webinars, discussions, and presentations with policy and decision makers.

Poster #40: Main Drivers for Use and Regulatory Acceptance of New Approach Methodologies: a Survey of European Risk Assessors

Author(s): Nicolas Roth

Affiliation: University of Basel

Project: PrecisionTox

Abstract:

Over the last decades, ecotoxicological and toxicological research has developed a wide There is widely acknowledged scientific, social, ethical, and financial motivation to promote the integration and use of non-animal testing methods in research and chemical risk assessment to advance the 3R principles. The paradigm shift in toxicity testing towards the use of alternative methods has led to major advances in the field of predictive toxicology and systems toxicology, and to a substantial increase in available in vitro, in silico, and mechanistic data derived from New Approach Methodologies (NAMs). However, integrating these new scientific developments in risk assessment remains an important challenge. While policies have long had the goal of protecting human health and ecosystems with new and innovative methods, solid downstream implementation has been slow, often because novel scientific methods have failed to consider regulatory needs as a driver for successful translation into risk assessment and management practice. As part of Task 6.4/Activity 6.4.2 of the Horizon Europe co-funded 'Partnership for the Assessment of Risks from Chemicals' (PARC), we present a project that aims to promote innovation in risk assessment, and facilitate the use and regulatory acceptance of NAMs to support Next Generation Risk Assessment. The project combines literature review, mixed methods research (expert elicitation, survey), and gaps and needs analysis for knowledge consolidation and further prioritization in PARC. Taking a bottom-up approach, it focuses on risk assessors' needs in their daily workflow and decision-making. An online survey with European risk assessors (and possibly beyond) will be implemented to get a deepened understanding of the technical, structural and cultural barriers and drivers that may facilitate/hinder the integration and use of NAMs at desk level, in various EU risk assessment frameworks and application contexts for authorization of chemicals. Active engagement with the ASPIS cluster will be sought, given the similarities in aims and objectives, to explore synergies and potential for collaboration.

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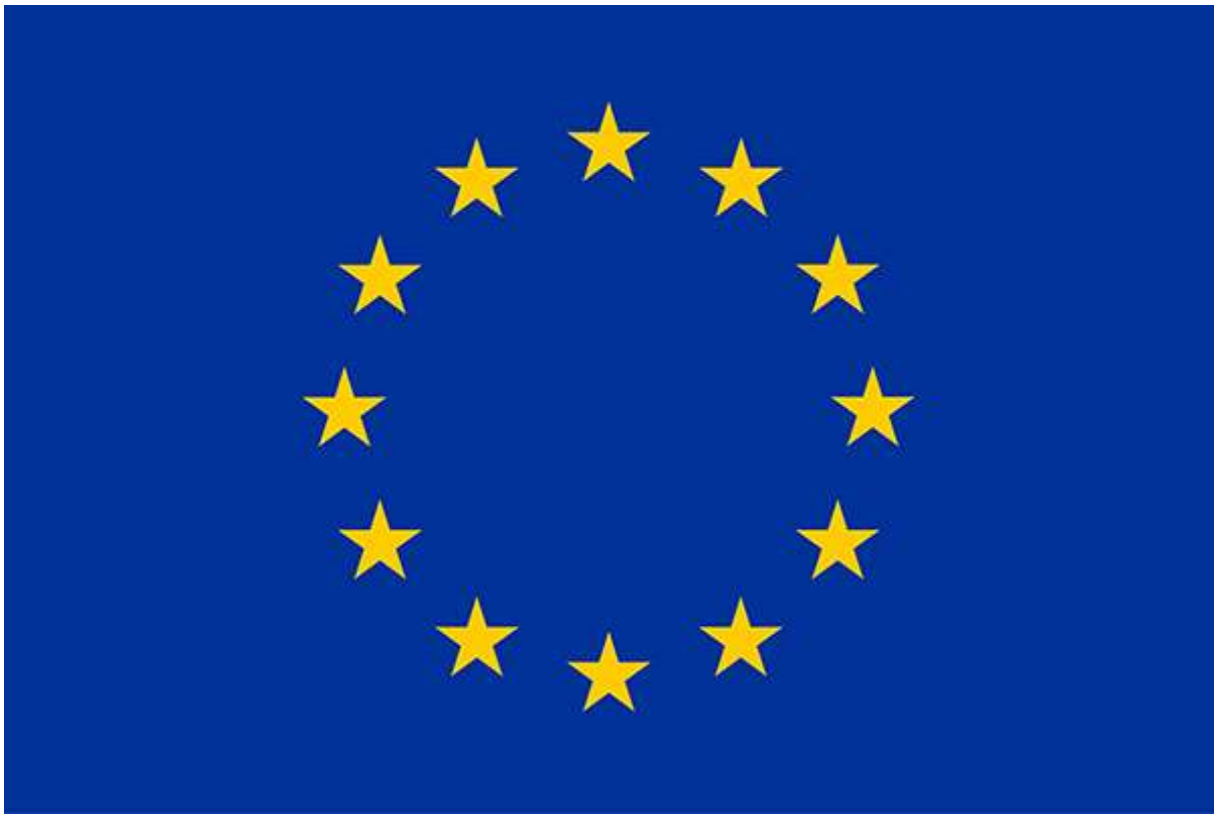

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