EU-ToxRisk
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ABSTRACTS BOOKLET
SPEED PRESENTATIONS

Session 3:

Wed. 24 February 2021

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TALK 1 Why are there popcorn lungs? – Advanced transcriptome analysis through reconstructed networks

Speaker: Christine Drake, Fraunhofer ITEM, Germany

Abstract:

In the past, workers involved in the preparation of microwave popcorn had an increased risk of developing bronchiolitis obliterans as a result of exposure to high concentrations of diacetyl, a compound used as butter flavorant.

In the EUTOXRISK case study 8 we studied the early transcriptional response upon exposure to diacetyl and 5 other short-chain aliphatic diketones (representing alpha, beta and gamma diketones) in primary human bronchiolar (PBEC) cell cultures using air-liquid exposure. Differential gene expression after 24h and 72h was assessed based on transcriptome data generated with Temp-O-Seq® technology. For each individual substance and for each group of diketones, genes that displayed a consistent differential expression across dose and exposure duration were identified. In order to obtain more mechanistic understanding pathway analysis was performed. Furthermore, we reconstructed networks of genes that interact with one another and are associated with fibrosis, inflammation or apoptosis using the TRANSPATH-database. This analysis revealed valuable and relevant mechanistic insights for the toxicological profiling of diketones.

TALK 2 Diversity of transcriptome responses of reference chemicals in EU-ToxRisk multi-organ test battery

Speaker: Nanette Vrijenhoek, Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research, Leiden University, The Netherlands

Abstract:

We systematically compared the transcriptomics response of a multi-organ test battery consisting of 7 in vitro test systems with in total 19 compounds in a broad concentration range. We deployed high-throughput transcriptomics TempO-Seq technology and to detect transcriptional regulation of ~2700 genes from thousands of samples. After establishing a testing pipeline, we showed that the activity across test systems by these compounds was diverse and provided quantitative and mechanistic insight in the differences in responses between test systems.

TALK 3 Functional genomics to uncover critical determinants of Adverse Outcome Pathways: uncovering the endoplasmic reticulum stress response

Speaker: Marije Niemeijer, Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research, Leiden University, The Netherlands

Abstract:

Mechanistic understanding of drug-induced liver injury (DILI) is currently still lacking and therefore hard to predict. To improve prediction, it is key to understand the critical events leading to adverse outcomes defined as adverse outcome pathways (AOPs). Some of these drugs induce endoplasmic reticulum (ER) stress and activate the unfolded protein response. However during chronic ER stress, activation of the UPR will be insufficient and will activate apoptotic pathways mediated by CHOP leading to hepatotoxicity. To improve mechanistic understanding to uncover key events leading to adversity by ER stress, we aimed to identify novel key regulators of CHOP. We applied an imaging-based RNAi screen of the druggable genome targeting 3457 genes in HepG2 CHOP-GFP cells to identify novel regulators of the tunicamycin-induced ER stress response. CHOP-GFP expression was evaluated after 16 hours of tunicamycin exposure which was altered by 201 genes upon knockdown. These potential regulators were further evaluated with other ER stress inducers or DILI compounds and their role in induction of other UPR-related genes such as ATF4, XBP1 and BIP. To evaluate the relevance of 10 selected novel regulators for the human liver during ER stress, we evaluated the transcriptome in both HepG2 and primary human hepatocytes (PHHs) after knockdown and subsequent exposure for 16 hours of tunicamycin. Three potential regulators were confirmed in PHHs which showed upon knockdown perturbation of UPR activation after tunicamycin. Pathway analysis revealed their key role in multiple ER signaling pathways, namely translation, protein degradation, UPR and protein trafficking. Overall, the combined approach of RNAi screening and transcriptome analysis allowed the identification of critical determinants of the drug-induced ER stress response and will further shape our understanding and prediction of DILI liabilities.

TALK 4 Effects of neonicotinoid pesticides on nicotinic acetylcholine receptor activity in neuronal cell models – indications of developmental neurotoxicity?

Speaker: Ylva Johansson, Stockholm University, Sweden

Abstract:

In case study 14, we have investigated if neonicotinoid pesticides may disturb neurodevelopment by modulating nicotinic acetylcholine receptor (nAChR) activity in the SH-SY5Y and LUHMES neuronal cell models.

At concentrations relevant for human exposure, nicotine, acetamiprid, imidacloprid, thiacloprid and clothianidin induced Ca^{2+} influx via the α7 homomer nAChR and non-α7 subtype nAChRs, whereas dinotefuran and thiamethoxam did not increase the $[Ca^{2+}]_i$. The agonistic response caused by nicotine, acetamiprid, imidacloprid, thiacloprid and clothianidin could in turn be blocked by nAChR pharmaceutical antagonists. In addition, it has been shown that pre-exposure with the four active neonicotinoids blocked the response of acetylcholine or nicotine. Considering the importance of α7 nAChR homomer and αβ heteromers in brain development, these findings are altering.

TALK 5 Structural modelling of MIE interactions: docking neonicotinoids into the human nAChR

Speaker: Karin Grillberger, Department of Pharmaceutical Chemistry, University of Vienna, Austria

Abstract:

The purpose of this project is to investigate new approach methods (NAM) for toxicity risk assessment, for which this case study (CS 14) about a prominent group of pesticides known as neonicotinoids and their potential developmental neurotoxicity (DNT) hazard was implemented. The goal here is, to gather data and information concerning the molecular initiating event of these compounds with the human nicotinic acetylcholine receptor (nAChR) with a focus on the subtypes α7 and α4β2. In order to achieve that induced fit docking and subsequent hierarchical clustering methods were applied and illustrated that there is a common binding mode of neonicotinoids in the nAChR, which has also been reported in co-crystalised structures and relevant literature. Further analysis revealed that there is a difference concerning the interactions of neonicotinoids and the more potent nicotinoids, which is mainly based on a flip of the imidazole ring thus mimicking the agonistic binding mode of nicotine. Depending on the electronegative pharmacophore being a nitrogen- or a cyano-functionality, different interactions in the binding pocket can be formed. Furthermore, another inverted binding orientation of the neonicotinoids was observed which has also been postulated in literature.

TALK 6 NAM-based strategies to uncover bioactivation of trichloroethylene: Species differences and implications for human risk assessment

Speaker: Liliana Capinha, Division of Molecular and Computational Toxicology, Vrije Universiteit Amsterdam, The Netherlands

Abstract:

Trichloroethylene (TCE) is a volatile organic solvent that has been commonly used in industry as a precursor of other industrial halogenated alkenes. Due to its widespread use and very low water solubility TCE is a persistent environmental pollutant. This halogenated alkene has been extensively studied in metabolism and toxicological contexts and has been demonstrated to exhibit renal toxicity. The nephrotoxic effects of TCE have been attributed to TCE conjugation by glutathione-S-transferases (GSTs) followed by renal metabolism involving ϒ-glutamyl-transferase (GGT), cysteinylglycinase and cysteine conjugate β-lyase bioactivation. Despite the extensive animal and in vitro studies on TCE data gaps remain.

Here, we developed a liquid chromatography mass spectrometry (LC-MS) method that allows the identification of three regioisomeric glutathione (S-(1,2-trans-dichlorovinyl)-glutathione (1,2-trans-DCVG), S-(1,2-cis-dichlorovinyl)-glutathione (1,2-cis-DCVG) and S-2,2-dichlorovinyl-glutathione (2,2-DCVG) and their corresponding cysteine conjugates and mercapturic acids of TCE. Additionally, we investigated the role of human recombinant GSTs and liver fractions (rat, mouse and human) in TCE metabolism.

With these results we aim to provide the tools and essential knowledge for the improvement of established PBPK models for TCE metabolism while considering species variability and its effects on metabolite formation crucial for risk assessment of TCE for human health.

TALK 7 Towards an ADME competent four-organ-chip

Speaker: Cormac Murphy, Division of Molecular and Computational Toxicology, Vrije Universiteit, Amsterdam, The Netherlands

Abstract:

A PBPK modelled ADME Four-Organ-Chip (Chip4) was developed to with two separate microfluidic circuits acting as “blood” and “kidney” circuits. Proximal tubular-like cells (PTL) and podocyte-like cells, derived from human induced-Pluripotent stem cells (iPSC), were introduced to form a barrier along a filter in the kidney circuit. In the blood circuit human primary liver spheroids were included for main metabolism, human primary intestinal barrier for absorption and human IPSC derived neuronal spheroids as a potential target organ.

The initial experiments were dedicated to optimizing co-culture conditions and the formulation of common media for each circuit.

In addition, a one-week repeated exposure of 1□M of the antipsychotic medication haloperidol (Cmax estimated at approximately 15nM), that is known to have a neurotoxic metabolite, was conducted as a preliminary experiment from how this chip can be used for testing xenobiotics.

TALK 8 Quantitative AOPs: computational modelling of the DNA damage response

Speaker: Muriel Heldring, Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research, Leiden University, The Netherlands

Abstract:

In silico and *in vitro* methods are essential tools to reduce the number of *in vivo* assays performed to test for safety of chemicals and drugs. However, the use of *in vitro* testing is potentially compromised by a lack of knowledge on the predictability of *in vitro* data for the behavior of cells in healthy human tissue. Extrapolation of DNA damage-induced protein dynamics is of special interest, since the central regulator of this pathway, transcription factor p53, is involved in the regulation of important cell outcomes such as cell cycle arrest, senescence and apoptosis. We questioned whether the dynamics of proteins involved in p53 signaling measured in the frequently used HepG2 cell line are representative for the dynamics of these proteins in primary human hepatocytes (PHHs). To study the correspondence between HepG2 cells and PHHs from approximately 50 donors in their response to the DNA damaging compound cisplatin, we collected gene and protein expression data in both cell types with high-throughput transcriptomics and imaging of reporter cells. Specifically, we examined the predictive capacity of p53 expression levels for expression of downstream targets Mdm2, p21 and Btg2 in PHHs. To this purpose, we constructed an ODE model that describes the dynamics of p53 and its downstream targets Mdm2, p21 and Btg2 upon DNA damage in HepG2 cells. This model was used to generate simulations of p53 signaling dynamics in virtual PHH samples. Subsequently, we investigated whether virtual samples behaved similarly to PHH samples by studying the correlations between expression levels of p53 and its downstream target genes in the absence and presence of cisplatin. The virtual samples generated based on the HepG2 dynamics could well explain the correlations observed in PHHs, yet for explaining the p53-Mdm2 correlation a reduced inhibitory effect of Mdm2 on p53 was required. Thus, such reduced inhibition needs to be considered when extrapolating data from human cell lines to primary cells for p53 signaling. In general, our study highlights the importance of studying base-line differences in signaling dynamics between *in vitro* and *in vivo* settings for extrapolation purposes.

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