P05-03
Study on the effects of 19 perfluoroalkyl substances on gene expression and biokinetics of PFOS and PFOA in human HepaRG liver cells

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Poly- and perfluoralkyl substances (PFASs), like perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have been reported to cause liver toxicity in experimental animals and to disturb lipid homeostasis in experimental animals and humans. To obtain more insight into the cellular effects of PFASs on the human liver, we assessed the effects on gene expression of 19 PFASs in HepaRG cells by performing microarray studies for PFOS and RT-PCR analyses of selected genes for all PFASs. We also assessed the biokinetics of PFOS and PFOA in the *in vitro* model, determining time- and concentration-dependent cell-associated PFAS levels. BMDExpress analysis of the PFOS microarray data point to various affected processes, with cholesterol biosynthesis (downregulated) and ATF4-related signaling (upregulated) being among the processes with the lowest BMC values. Results from RT-PCR analyses for genes related to ATF4 signaling, cholesterol biosynthesis, PPAR signaling and other sensitive genes point to differences in potencies for the tested PFASs. The shorter chain PFASs (PFPeA, PFHxA, PFBS, HPFO-DA) only impacted on the expression of PPAR-regulated genes. Interestingly, BMC values for different PFASs related to ATF4 activation were correlated with those for decreased expression of the cholesterogenic genes, suggesting a possible relation between these processes. Of the tested PFASs, HFPO-TA was shown to be the most potent modulator of gene expression. The *in vitro* biokinetic data indicate that at the applied culture conditions maximum cell-associated PFOS and PFOA levels are obtained around 1 hour after exposure, remaining stable up to the end of exposure (24 hours), being up to 10-fold higher for PFOS compared to PFOA, depending on the nominal concentrations applied. Altogether, the study provides mechanistic insights into the effects and relative potencies of PFASs on the human liver, pointing to a possible association between ATF4 signaling and the PFAS-induced decrease in cholesterogenic gene expression. In combination with *in vitro* biokinetic data, as obtained for PFOS and PFOA in the present study, these results will be used as a basis for quantitative *in vitro* to *in vivo* extrapolations (QIVIVE), with the application of physiologically-based kinetic (PBK) modelling, to assess whether effects found *in vitro* can be expected at relevant *in vivo* exposure scenarios.

P05-04
Including the intestinal microbiome incubations in physiologically based kinetic modeling of pyrrolizidine alkaloid N-oxides

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The intestinal microbiome is able to affect the susceptibility to a wide range of pharmaceutical and foodborne chemicals through a broad range of reactions. An example is the reduction of food borne pyrrolizidine alkaloid N-oxides to the parent pyrrolizidine alkaloids (PAs) enabling their further bioactivation in the liver to reactive toxic pyrrole metabolites. To include the reactions by the intestinal microbiome in physiologically based kinetic (PBK) models requires not only definition of a conceptual model that includes an intestinal compartment containing the intestinal microbiota, but also a way to determine the related kinetic constants in an *in vitro* model and to scale the resulting kinetic constants to the whole organism. Using pyrrolizidine N-oxides as the model compounds it was shown that anaerobic fecal incubations provide a way to define kinetic constants for pyrrolizidine alkaloid N-oxidation by the intestinal microbiota of both rats and human. The Vmax values thus obtained require subsequent scaling to the whole organism. This can be done is various ways including i) fitting of PBK model predicted data to available *in vivo* data, ii) based on the fecal fraction of body weight and iii) using the bacterial counts and volumes of the various intestinal compartments. Using the PBK models thus obtained the role of the intestinal microbiota in the bioactivation of pyrrolizidine N-oxides to the parent PAs can be taken into account. The PBK models enable definition of relative potency factors for the N-oxides relative to their parent PAs by comparison of the area under the concentration time curves for the parent PA upon dosing either the N-oxide or an equimolar amount of the parent PA. This reveals that the relative potency of PAs is predicted to be lower than that of the corresponding PA.

P05-05
Identification of Monotonic Trends in the Dose-response Relationship of Nanomaterial Toxicity Data using the R package NMTox

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Nanomaterials, present in many products nowadays, have different physico-chemical, biological and toxicological properties compared to the same material in the bulk form. Therefore, their potential toxicity needs to be analyzed to ensure their safety for human, animals and the environment. *In vivo* studies are commonly used to assess the risk of chemicals. However, due to the large amounts of nanomaterials that can be produced and for reducing animal testing and financial and time costs, there is a growing interest in incorporating *in vitro* and *in silico* models in the risk assessment process and increasing the focus on *in vitro-in vivo* extrapolation (IVIVE) methods.

To support research on nanomaterial toxicity, including IVIVE, several project initiatives aim to enhance the usability of nanomaterial data by developing databases containing information from various available experimental nanomaterial datasets. The H2020 NanoInformaTIX project is one of these initiatives. Based on data collected over the last decades, this project aims to build a user-friendly platform for risk management of engineered nanomaterials. Our aim is to utilize the data gathered within the NanoinformaTIX project to develop an approach to perform *in vitro-in vivo* extrapolation analyses for nanomaterial risk assessment. In order to select nanomaterials within the vast amounts of data within the NanoInformaTIX database, which have to be analyzed for dose-response trends, we developed a software tool (the R package NMTox) to explore the database and to identify monotonic trends in the dose-response relationship of nanomaterials for toxicity endpoints. In the second stage, dose-response models are fitted (using a software tool that is currently being developed) on the nanomaterials for which a monotonic trend is identified. These nanomaterials/toxicity endpoint combinations will be the focus for *in vitro* to *in vivo* extrapolation.

As a case study, we focus on the cell viability data available within the NanoinfomaTIX database. Inference was performed by testing the significance of monotonic dose-response relationships using Likelihood ratio tests. Since a high number of data subsets were test-

ed, a method to adjust for the multiplicity was implemented. The analysis was performed on 14 nanomaterials, across different study providers, cell types, methods and exposure time. A significant monotonic dose-response relationship was found for 20 subsets of the data. Dose-response curves for the cell viability endpoints are fitted and we identify toxic concentrations that will be implemented in the risk assessment process.

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P06 – New approach methodologies: 3D models, stem cells, organ-on-a-chip, microfluidics

P06-01
Development of multiparametric kinetic measurements and analytics workflows for the generation of an IPSC derived cardiomyocyte based cardiovascular model

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Purpose: Unexpected cardiotoxicity underlies high levels of late-stage attrition and post-market withdrawals, accounting for up to 45% of the liability. Typically, cardiotoxic effects of new drugs are predicted using *in vitro* expression of single cardiac ion channels, *in vivo* and *ex vivo* animal studies. However, these models fail to reflect the complexity of the human cardiac microenvironment and are contrary to the principles of the 3Rs (Replacement, Reduction, and Refinement) in animal research. Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have now become an attractive platform for capturing the effects of chronic modulators or toxicants and could complement existing assays to improve cardiac safety assessments. We profiled a panel of 42 compounds with known cardiotoxic effects on monolayer cardiomyocytes however, despite good predictively a few compounds were missed in this assay. Our research aims to improve this model by taking into consideration the role of non-cardiomyocytes to better recapitulate the *in vivo* myocardial microenvironment. This would lead to a more physiologically relevant model and therefore a more predictive functional and structural cardiovascular toxicity assay.

Methods: Cardiomyocytes, cardiac endothelial cells, and fibroblasts cells were generated from human pluripotent stem cells using established differentiation protocols. The baseline function and structure of hiPSC-CMs were assessed using calcium flux, kinetic live-cell imaging, and fixed cell imaging for sarcomere organisation and cellular morphology. These assays were conducted for both in-house differentiated cardiomyocytes and commercially available iCell cardiomyocytes provided by GSK. 2D co-cultures of cardiomyocytes with fibroblast and endothelial cells were assessed against monoculture cardiomyocytes with regards to cardiac electrophysiology, ion channel expression & function, cellular metabolism, and sarcomere organisation.

Results: Monolayer cardiac differentiation of hiPSC produced high-purity beating cardiomyocytes (>85% alpha-actinin positive). We used CellOPTIQ-based optical imaging to assess contractility and intracellular Ca^{2+} transients. For cardiac nonmyocytes, differential expression of pluripotency, endothelial and fibroblast markers were seen throughout endothelial and fibroblast lineage specialisation. We further tested and characterised these three cell types individually (e.g. endothelial tubulogenesis assay and cellular metabolism) and examined different conditions for co-cultures. The presence of each cell type within the co-culture models was confirmed at the protein and gene expression level. These co-culture models differed in viability, morphology and pharmacological responses when compared to monolayer cardiomyocytes. These results indicate that co-culture hiPSC-CM models may increase the scope, maturity, and predicitivty of this assay.

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P06-02
This abstract has been withdrawn.

P06-03
Kidney-on-a-Chip – Integrating glomerular filtration and tubular reabsorption models

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The kidney's excretory function is crucial in drug development, as it dictates drug clearance and reabsorption. Furthermore, nephrotoxic-