Annual Meeting 2023 – Poster Session

Poster 01

In vitro Pharmacokinetic modeling of the gut microbial metabolite urolithin A

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Hepatic xenobiotic metabolism is classically considered the most defining process of toxicokinetics and its assessment is central to predict systemic concentrations of chemicals. Additionally, the human gut microbiome has recently emerged as a significant contributor to chemical metabolism, potentially equaling the relevance of the liver. Therefore, methods to incorporate it as a metabolic compartment in toxicokinetic modeling are currently being developed and refined.

Urolithin A (UA) is a beneficial gut microbial metabolite of dietary ellagitannins, for which a pharmacokinetic model is lacking. Mimicking postbiotic supplementation of the pure chemical, we established a first physiologically-based pharmacokinetic (PBPK) model for UA, including gastrointestinal absorption, partitioning to organs of interest, glucuronidation in the liver and small intestine, urinary excretion as well as enterohepatic recirculation for both UA and its glucuronide. Predicted plasma concentrations compared favorably with plasma values measured previously in a human supplementation study.

In a second step, we used stool samples from 22 healthy volunteers to obtain *ex vivo* biotransformation rates in anaerobic batch fermentations using individual and pooled fecal slurries. The human gut was then included in the PBPK model as an additional compartment of metabolism. With this model, we could directly compare UA pharmacokinetics for postbiotic supplementation vs its natural formation scenarios and understand how concentrations for biological effects previously reported *in vitro* relate with predicted target tissue concentration. The developed strategy is now used as a blueprint for assessing the toxicological relevance of other gut microbial transformation products.

Poster 02 ***(selected for Short Presentation)***

Assessing the safety of food chemicals without animal testing: A Next-Generation Risk Assessment approach

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Chemical risk assessment is currently undergoing a paradigm shift driven by ethical considerations, regulatory action and the need to ensure the safety of chemicals using efficient, cost-effective and robust methods. Non-animal approaches represent a powerful alternative to improve safety assessments by using more human-relevant tools providing a good coverage of key biological targets. Next-generation risk assessment (NGRA) provides a framework integrating data coming from diverse so called new approach methodologies (NAMs) into the decision-making process, allowing for safety assessments to be conducted without the use of animal data. The assumption behind the use of NGRA is, that if the exposure level of a chemical in humans is below the concentration needed for exhibiting any biological effect, it is unlikely it would trigger any toxicity. Whitin this context, estimates of exposure can be obtained using physiologically based kinetic (PBK) models while potential biological effects are assessed by means of points of departure (PODs) from a range of *in vitro* assays. These assays are selected to both provide a good biological coverage and to detect very early biological perturbations before the onset of any adverse effect. Then, the PODs and exposure estimates can be combined into a single metric often referred to as margin of safety.

Here we present a NGRA framework under development at Nestlé, built to support the safety assessment of food chemicals and food contact materials without the need of animal data. The whole approach relies on the chemical mode of action (MoA). Depending on whether the MoA is known or not, a defined panel of *in silico* and *in vitro* assays will be used to estimate internal exposures and *in vitro* PoDs. The derived margins of safety will be then used to define the risk. The current framework will undergo validation by running multiple case studies to assess accuracy and reliability with the final goal of being implemented as a routine approach for safety assessment.

Poster 03

Developing fish cell-based model for neurotoxicity testing

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The extensive use of chemicals and their release into water bodies pose a major environmental problem. In aquatic ecosystems as well as for humans, fish play an integral role. Among other effects, chemicals can affect the neurophysiology of organisms, impairing their chances of survival. Chemical risk assessment largely depends on time-consuming and ethically questionable animal experiments, which provide limited understanding of the molecular mechanisms underlying the action of neurotoxicants. Here, *in vitro* approaches represent a promising alternative for hazard identification of anthropogenic substances.

Accordingly, this research aims first to characterize the current fish invitrome, which includes a set of fish cell lines derived from different organs, and second to develop the first fish cell-based model for neurotoxicity testing. This should advance our understanding of the features of fish cell lines to facilitate the development of organ-specific *in vitro* toxicity assays. By focusing on brain cells, we want to explore the mechanisms of neurotoxicity with an alternative animal-free method developed for the screening of potentially neuroactive agents. Through transcriptomics and proteomics studies, we are analyzing the gene expression and protein profile of six fish cell lines. In parallel, we are investigating the growth, molecular/structural profile, and functional capabilities of a yet uncharacterized cell line established from the rainbow trout brain (RTbrain). We work towards identifying different neurotoxic endpoints related to various neurotoxic mechanisms in the cell line that will form the basis of a rapid and convenient *in vitro* assay for the screening of neuroactive agents. Chemical testing with a set of negative and positive control chemicals will assess the robustness and predictivity of the RTbrain *in vitro* assay.

We expect to gain a thorough understanding of the potentials and limitations of the RTbrain cell line as a model for *in vitro* neurotoxicity. The assay ideally will provide an animal-free method in ecotoxicology for high-throughput screening of data-poor compounds. Furthermore, the gained insight into underlying molecular mechanisms of neurotoxicity will be informative for the further development of the adverse outcome pathway (AOP) framework.

Keywords: Ecotoxicology, neurotoxicity, *in vitro* assay.

Poster 04

Advancing risk assessment of Bisphenol A analogues with toxicokinetic modeling

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As industry exceedingly replaces the endocrine disruptor bisphenol A (BPA) by its analogs, human exposure has increased. In comparison to BPA, these substitutes are much less researched regarding their potential of affecting the human endocrine system, but initial *in vitro* data suggest similar activity. Moreover, given the relatively limited available administration, distribution, metabolism and excretion (ADME) characterization of these substances, the utilization of kinetic modeling is essential to initiate their comprehensive risk assessment.

To this end, we extended a previously published human physiologically based kinetic (PBK) model for BPA, BPS, BPF and BPAF to now address other replacement analogs that have current exposure relevance: BPB, BPE and BPM.

Hepatic glucuronidation kinetics were assessed experimentally from liver S9 incubations. Partition coefficients were predicted using quantitative *in-vitro*-to-*in-vivo* extrapolation (QIVIVE). The quantification of enterohepatic recirculation (EHR) was derived from the outcomes of a PBK model we specifically developed for rats, where toxicokinetic data was available for BPF and BPAF. We also refined the model by improving the intestinal compartmentalization, incorporating endocrine organs of interest for later evaluation of toxicity in target tissues, and developing a submodel for glucuronide metabolites. The influences of parameter uncertainty and variability on the modeled concentrations were evaluated by sensitivity analysis and Monte Carlo simulations.

The resulting human PBK model enables the prediction of systemic levels of bisphenol A and six of its most prevalent analogs after oral exposure. It also supports the prediction of time-concentration profiles of these substances in the thyroid and gonads, a prerequisite for assessing their potential endocrine toxicity. The framework introduced here marks a notable advancement in conducting risk assessments for a group of substances lacking human *in vivo* data.

Keywords: BPA ; bisphenols; PBK modeling; QIVIVE; Glucuronidation.

Poster 05

Unraveling Toxicological Challenges in Medical Devices: A Case Study of 3-Iodo-2-propinylbutylcarbamate

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Medical devices are expected to be without unacceptable risks, including biological and toxicological risks in clinical practice. The toxicological risk assessment of medical device constituents within the biological evaluation process shall follow ISO 10993-17. The latest standard version was published in September 2023, showcasing a new title, "Biological evaluation of medical devices — Part 17: Toxicological risk assessment of medical device constituents". Although the standard's aim remains unchanged, it introduces several new concepts and tools into the toxicological risk assessment of medical devices, like the new toxicological screening limit (TSL) concept, which strictly correlates to the protectiveness of the threshold of toxicological concern (TTC) defined in ISO/TS 21726:2019.

This case study aims to shed some light on the complexity of toxicological risk assessments when dealing with challenging medical device constituents, for which expert judgment is indispensable, by applying the new ISO 10993-17:2023 methodology to 3-Iodo-2-propinylbutylcarbamate present on a long-term implant contacting tissue/bone.

3-Iodo-2-propinylbutylcarbamate is a carbamate ester and belongs to the same TTC group as organophosphates. An evaluation of the applicability of the TSL was performed. However, in ISO/TS 21726:2019, organophosphates are excluded since they are considered as cohort of concern compounds. Hence, the TSL method was deemed inappropriate for 3-Iodo-2-propinylbutylcarbamate.

Therefore, a compound-specific toxicological risk assessment was performed. A literature search was conducted to identify potential harms and retrieve the most suitable point of departure (POD). The POD was then adopted to derive tolerable intakes for the relevant toxicological endpoints, applying appropriate uncertainty factors. Estimated exposure doses and margin of safety (MOS) values for the applicable time periods were also calculated. The MOS values were then evaluated to determine whether the newly predefined risk acceptability criteria are met.

Poster 06

Next-generation human iPSC-derived 3D brain systems to study chemical-induced myelin disruption

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While in the recent years, the exposure of children to chemicals has become a major concern in the population, data on their toxic effects on the nervous system are still lacking. Indeed, the current developmental neurotoxicity (DNT) testing relies on *in vivo* experiments that are time and animals consuming, expensive, and which results are difficult to apply to humans. Due to these difficulties, the DNT is therefore not systematically assessed in new marketed chemicals. Experts in the field of toxicology as well as regulatory agencies such as the Organization for Economic Co-operation and Development (OECD) and the European Food Safety Authority (EFSA) have proposed a battery of *in vitro* tests covering the key steps of the brain development (DNT-IVB). While methods have already been endorsed for several of the processes, myelination is still lacking an assay.

This project aims to determine the efficacy of the 3D brain microphysiological system called BrainSpheres as a model of myelination to be integrated into the DNT-IVB. BrainSpheres are a new *in vitro* model composed of neurons, oligodendrocytes and astrocytes that are capable of synaptic activity as well as myelin production. To test its potential as a model for neurotoxicity, Haloperidol and Bisphenol A (BPA), two compounds toxic to the myelin, have been used at different sub-cytotoxic concentration to treat the BrainSpheres.

The effects of the treatments on myelin-associated proteins and genes have been quantified using qPCR and immunohistochemistry.

Poster 07

Superoxide dismutase 2 (SOD2) as a potential susceptibility factor for sunitinib-associated hepatotoxicity

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Sunitinib is a multi-targeted tyrosine kinase inhibitor used to treat metastatic renal-cell carcinoma and gastrointestinal stromal tumors. Despite its clinical benefits, it carries a black-box warning for hepatotoxicity, which can be severe and fatal in few cases. To date, the mechanisms underlying this toxicity remain still unclear. Here, we investigated the role of superoxide dismutase 2 (SOD2), located exclusively in the mitochondrial matrix and responsible for ROS detoxification. Therefore, we treated male WT and $Sod2^{+/-}$ mice daily for two weeks by oral gavage with sunitinib at 7.5 mg/kg.

We first performed a pharmacokinetic study in WT and $Sod2^{+/-}$ mice after a single dose of sunitinib. Blood analyses by LC-MS/MS revealed that compared to WT, $Sod2^{+/-}$mice showed lower exposure and lower maximal sunitinib concentration. As expected, SOD2 protein expression decreased in $Sod2^{+/-}$ mice compared to WT mice and was similar between both $Sod2^{+/-}$ groups. Sunitinib decreased SOD2 and catalase, but increased thioredoxin 2 (TRX-2) protein expression in WT mice. Additionally, protein expression of catalase and TRX-2 was higher in $Sod2^{+/-}$ mice.

Sunitinib decreased the mitochondrial membrane potential in both groups. Interestingly, the CIII-linked respiration was higher in $Sod2^{+/-}$ than in WT mice. The CIII- and CIV-linked respiration was decreased in sunitinib-treated $Sod2^{+/-}$ compared to the vehicle-treated $Sod2^{+/-}$ mice. The assessment of individual complex activity showed that the activity of complex II and IV was higher in $Sod2^{+/-}$ than in WT mice. Complex II activity in sunitinib-treated $Sod2^{+/-}$ mice was significantly lower than in vehicle-treated $Sod2^{+/-}$ mice.

The mRNA expression of the mitophagy marker Park2, and of the regulator of mitochondrial biogenesis Pgc1-α, was higher in the $Sod2^{+/-}$ mice than in WT mice.

In conclusion, we show that $Sod2^{+/-}$ mice presented a decreased exposure to sunitinib and presented compensatory upregulation of antioxidative capacities and mitochondrial homeostasis. Furthermore, we show a potential role for SOD2 in sunitinib-associated hepatotoxicity, which is mainly related to mitochondrial dysfunction. Taking into account the clinical context, polymorphisms in SOD2 reported in humans could represent a risk factor for hepatotoxicity observed with sunitinib.

Keywords: Sunitinib, Hepatotoxicity, SOD2, Oxidative stress, Mitochondria.

Poster 08

Unraveling the origins of carboxymethylated DNA adducts in the human gut

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Carboxymethylated DNA adducts are found in elevated levels in people who eat meat, and give rise to mutational profiles similar to what is found in colorectal cancer patients. These adducts are hypothesized to arise from metabolites in the human gut. For example, commensal bacteria in the gut can reduce nitrogen containing groups, forming reactive metabolites that carboxymethylate DNA. On the other hand, they may biosynthesize diazo-containing compounds, such as azaserine, which carboxymethylates DNA. Recently, a biosynthetic gene cluster for the production of diazo-compounds has been discovered, and these genes have been aligned with databases of human-relevant gut bacteria, resulting in bacterial candidates that are present in human gut microbiota and may form azaserine and carboxymethylate DNA.

To determine whether such bacteria biosynthesize azaserine and what carboxymethylation profiles result, the extracellular medium is collected and analyzed after culturing the bacteria with naked DNA. Thus, a mass spectrometric method was developed to identify carboxymethylated structures and to identify potentially DNA damaging compounds. The method was optimized using an authentic synthetic standard of the adduct O6-carboxymethyl-2'-deoxyguanosine (O6-CMdG). We characterized the chemical structure and stability of O6-CMdG, and found that it is susceptible to hydrolytic cleavage of the glycosidic bond with a half-life of approximately 6 months.

The reagents and bioanalytical methodology resulting from this work will enable more accurate and high throughput research to understand, and possibly avoid, the formation of these DNA adducts.

Keywords: gut microbiota, DNA damage, DNA carboxymethylation.

Poster 09

Tracking transformations of exogenous metabolites through gut microbial metabolism

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Transformation of exogenous chemicals by the gut microbiota profoundly impacts human health.

We present here an approach to quantify the rate of gut microbial metabolism for pharmaceutical, environmental exposure, and diet related chemicals. Calculated reaction rates can then be applied in physiological based kinetic models to predict human exposure levels. Additionally, our approach enables identification of yet unknown modifications performed by gut microbes. Using *ex vivo* fermentations of human and rat gut microbial communities coupled with merged targeted/untargeted LC-MS/MS metabolomics analyses, supported by *in silico* tools, we calculated rates of chemical reactions and linked experimentally measured metabolites to predicted reaction products.

We detected metabolic alterations in microbiota communities after oral antibiotic exposure and consumption of plant natural products. We further correlated microbial taxa to reaction rates providing a link between specific bacteria and chemical transformations. Further applications of this metabolic capacity quantification platform include addressing how exposome chemicals are transformed in the gut and predicting chemical-microbiota relationships that impact human health.

Poster 10

Targeting transcription-coupled nucleotide excision repair to overcome resistance to DNA alkylating drugs

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Cellular DNA repair pathways such as transcription-coupled nucleotide excision repair (TC-NER) are responsible for chemotherapeutic drug resistance by removing DNA adducts formed by DNA alkylating drugs.

We hypothesize that drug sensitivity of cancer cells proficient in TC-NER may be increased by blocking this pathway from effectively repairing drug-induced DNA damage. A potential approach to impede the excision of damaged nucleotides may be to covalently inhibit TC-NER endonucleases ERCC1-XPF or XPG by chemical cross-linking to DNA modifications.

To explore such a modality, as a first step, we aimed to synthesize and characterize chemical probes consisting of a covalent inhibitor attached to an oligonucleotide that can be used for optimizing the chemistry required for activating TC-NER and subsequently blocking its repair function.

Thus, we designed a library of compounds with varying reactive groups, i.e. vinyl sulfonamides, acrylamides and sulfonylfluorides, and two linkers of different sizes.

These probes were chemically characterized and have the potential for further addition by copper catalyzed azide alkyne cycloaddition to a modified oligonucleotide bearing an alkyne group, and as a substrate for further biological characterization with regards to capacity to cross-link DNA repair proteins.

Keywords: DNA repair, covalent inhibitors

Poster 11

In silico and _in vitro_ workflow to identify pharmaceuticals inhibiting CYP17A1 and CYP11B1

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Inhibition of cytochrome P450 enzymes CYP17A1 and CYP11B1 can cause secondary hypertension and hypokalemia. Adrenal steroidogenesis is not covered in routine pre-clinical investigations in drug development, which may lead to unexpected side-effects. CYP17A1 is a bifunctional enzyme exhibiting 17α-hydroxylase and 17,20-lyase activity. The 17α-hydroxylase is essential for the production of cortisol. Its inhibition results in a feedback induction of steroidogenesis, with an excessive formation of mineralocorticoids, ultimately causing sodium and water retention, hypokalemia, and hypertension. Similarly, CYP11B1 inhibition blocks cortisol biosynthesis and results in mineralocorticoid accumulation and MR-induced hypertension.

To identify drugs potentially causing secondary hypertension via CYP17A1 or CYP11B1 inhibition, the DrugBank database was screened for potential inhibitors in a combined workflow using *in silico* and *in vitro* methods. Compounds predicted to inhibit were evaluated in enzyme activity assays based on mitochondrial fractions from V79-4 cells stably expressing CYP11B1 or microsomes from COS-1 cells transiently expressing CYP17A1 and POR in the absence or presence of cytochrome b5, depending on the CYP17A1 reaction analyzed.

This workflow led to the identification of several so far unknown pharmaceuticals potently inhibiting CYP17A1 and/or CYP11B1. Several of these compounds inhibited both enzymes, including retinoic acid metabolism blocking agents and azole antifungals, indicating additive or synergistic effects and a risk for secondary hypertension. As a common moiety, the identified inhibitors share a nitrogen containing aromatic heterocycle. The nitrogen`s pKa value was found to be an indicator for the interaction potency with the iron atom present in the active center.

In conclusion, the presented workflow successfully identified numerous CYP17A1 and CYP11B1 inhibitors potentially posing a risk for secondary hypertension and hypokalemia.

Keywords: Hypertension, Off-Target Binding, Pseudohyperaldosteronism, Virtual Screening, Enzyme Activity Assays.

Poster 12

Evaluating Ovarian Toxicity associated with Bisphenol A, Phthalates and their substitutes

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Endocrine-disrupting chemicals (EDCs) are exogenous substances or chemical mixtures affecting the hormonal system and causing adverse health effects. Given the rising rates of infertility in developed countries, a particular concern is the potential influence of EDCs on the female reproductive system. Moreover, female disorders such as polycystic ovary syndrome (PCOS) and endometriosis are endocrine-metabolic diseases likely affected by EDCs. Phthalates and bisphenol A (BPA), belonging to the most used EDCs, have attracted attention of the regulatory authorities and are under extensive investigation. In contrast, the newer BPA and phthalate substitutes are less well characterized, emphasizing the need to assess their potential toxicities. We aimed to investigate the potential toxicity of these substitutes and characterize the underlying mechanisms.

We selected several derivatives of BPA (BPAF, BPB, BPF, BPS, BPZ, BADGE, cyclo-di-BADGE) and DEHP (MEHP, DBP, MBP, BzBP, MBzP, DEP, DINP, DiBP, DEHA, and ATBC). We established a cell-free assay for human hydroxysteroid dehydrogenase HSD3B2 activity, measuring the conversion of pregnenolone to progesterone. We screened the compounds for their ability to inhibit HSD3B2 at a concentration of 10 µM.

As expected, BPA inhibited human HSD3B2 with an IC50 of ~8 µM (Ye et al. 2011). BPAF and BPB showed HSD3B2 inhibition in the screening. Subsequent determination of IC50 values showed 2.0 µM and 1.9 µM for BPAF and BPB, respectively. Interestingly several phthalates (MEHP, BzBP, DiBP, DEHA) seem to increase progesterone levels, contrary to previously published inhibitory properties of phthalates for HSD3B2 (Yuan et al. 2012).

In conclusion, our results show that in addition to BPA, two replacement compounds, BPAF and BPB, inhibited human HSD3B2 with a higher potency than BPA itself. Next, we want to further examine steroidogenesis with activity assays for enzymes downstream of HSD3B2 in steroid biosynthesis, namely HSD17B1 and CYP19A1, as well as treating human granulosa cell line COV434 to observe potential changes in ovarian steroidogenesis. Since cells with a knock-out of ER-resident, NADPH producing enzyme H6PD, had decreased HSD3B2 and HSD17B1 mRNA levels, we plan to investigate H6PD as a potential susceptibility factor in the context of exposure to EDCs.

Keywords: endocrine disrupting chemicals; HSD3B2; steroidogenesis; granulosa cells.

Poster 13

Anti-androgenic effects of parabens and benzophenone-type UV-filters inhibiting 17β-hydroxysteroid dehydrogenase 6

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The short-chain dehydrogenase/reductase 17β-hydroxysteroid dehydrogenase 6 (17β-HSD6) is highly expressed in prostate, liver and lung. It catalyzes the oxidation of the 3α-hydroxy group on 5α-androstane-3α,17β-diol (3α-Adiol) to the 3-oxo group to form 5α-dihydrotestosterone (DHT). This backdoor pathway generates DHT in several steps from the intermediate androsterone, thereby circumventing the classical pathway of DHT formation by 5α-reduction of testosterone (T).

The production of DHT and T plays an important role in male physiology and development where they drive gene expression by activating androgen receptors (AR). Pathological mutations in genes involved in the backdoor pathway result in a phenotype with under-masculinized external genitalia in genetically male patients. Accordingly, xenobiotics inhibiting 17β-HSD6 may exert anti-androgenic effects that potentially lead to a similar phenotype.

Parabens and UV-filters, used as additives in cosmetics and body care products, have been detected in human serum, urine and breast milk, and more importantly in umbilical cord blood, indicating fetal exposure. While anti-androgenic effects of parabens and benzophenone-type UV-filters by inhibition of AR transcriptional activity have frequently been reported, effects on androgen formation have received less attention.

This study assessed parabens and benzophenone-type UV-filters that potentially interfere with DHT generation by inhibiting 17β-HSD6 using an *in vitro* enzyme activity assay. This led to the identification of the so far first known 17β-HSD6 inhibitors with nanomolar IC50 values and revealed initial insights into the structure-activity relationships of 17β-HSD6 inhibitors.

The results suggests that commercially used parabens and UV-filters may exhibit anti-androgenic effects by inhibiting the backdoor pathway of DHT formation, an issue that should be further considered in human health risk assessments.

Poster 14

A battery of *in silico* models application for pesticides exerting reproductive health effects: Assessment of performance and prioritization of mechanistic studies

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Given the high attention to endocrine disrupting chemicals (EDC), there is an urgent need for the development of rapid and reliable approaches for the screening of large numbers of chemicals with respect to their endocrine disruption potential.

This study aimed at the assessment of the correlation between the predicted results of a battery of *in silico* tools and the reported observed adverse effects from *in vivo* reproductive toxicity studies. We used VirtualToxLab (VTL) software and the EndocrineDisruptome (ED) online tool to evaluate the binding affinities to nuclear receptors of 17 pesticides, 7 of which were classified as reprotoxic substances under Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP). Then, we aligned the results of the *in silico* modelling with data from ToxCast assays and *in vivo* reproductive toxicity studies. We combined results from different *in silico* tools in two different ways to improve the characteristics of their predictive performance.

Reproductive toxicity can be caused by various mechanisms; however, in this study, we demonstrated that the use of a battery of *in silico* tools for assessing the binding to nuclear receptors can be useful for identifying hazardous compounds and for prioritizing further studies.

Poster 15

Methodology approach in evaluation reproductive and developmental toxicity endpoints *in vivo* studies to reduce numbers of animal use

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Nowadays, *in vivo* reproductive toxicity studies cannot be entirely replaced by *in vitro* studies. For covering bioethics and 3R principles focused on decreasing the experimental usage of animals, the present work provides an approach to evaluate reproductive toxicity and developmental toxicity (teratogenic activity) with maximal targeted endpoint assessments.

There are two main OECD test guidelines for chemical reproductive and developmental toxicity testing: OECD 443 "Extended one-generation reproductive toxicity study" and OECD 414 "Prenatal Developmental Toxicity Study". When conducted independently, the overall number of animals used in these two studies includes around 440 adults and an average of 1600 foetuses.

In contrast, combining these studies in a single approach allows to reduce the amount of animals. The main difference between these OECD tests is the duration of the exposure period, which is much longer in a reproductive toxicity study than in a developmental study. Consequently, the dosages used are also usually much lower in reproductive research. To harmonise them, existing information about test substance toxicity and implemented doses (repeated-dose studies or screening reproductive toxicity studies, etc.) are essential. Furthermore, the combined study enables defining NOAEL and LOAEL levels for reproductive and developmental toxicity endpoints in a single approach. The next important thing that should be considered is the test substance´s influence on implantation during gestation. This is a critical part of the development study because it could bias the number of foetuses for further evaluation. Interestingly, in the combined experimental approach, animals mated with untreated intact animals fulfil the developmental study requirements according to the OECD guideline and provide additional valuable information regarding sex sensitivity.

While far from a complete replacement of *in vivo* studies by *in vitro* approaches, the crucial benefit of a combined study is animal use reduction and refinement, which are essential as two of the 3R principles and can be considered a step toward. As a result, one combined study reduced the number of animals used in the experiment by 50 % adults and 35 % progeny.

Poster 16 ***(selected for Short Presentation)***

Discerning cellular targets to Adeno-Associated Virus-induced immune-associated hepatotoxicity

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Adeno-associated virus (AAV) vectors have emerged as promising *in vivo* gene delivery tools for gene therapy. They are mostly non-integrative, can transduce various tissues and have a low immunogenic profile. Despite these advantages, there are reports of severe immune-associated hepatotoxicity after AAV administration in clinical trials. The mechanism behind the reported liver immune responses is still not clearly understood. Here, we investigated the immune-related hepatotoxic effect of AAVs on individual liver cells. Several human liver *in vitro* models were used: HepaRG (hepatocyte cell line), hTERT-HSC (hepatic stellate cell line) and THP-1 (macrophage cell line, used as a Kupffer cell surrogate) that were exposed to different AAV-GFP serotypes (AAV2,3,8 & 9) and titers (MOI 10^3 – 10^6). Transduction efficiency was investigated by PCR, fluorescence microscopy and image quantification. Cellular responses were studied using cell viability and gene expression assays, immunofluorescent staining, and multiplex cytokine panel screens. Titer- and serotype-dependent decreases in cell viability were observed in HepaRG and hTERT-HSC but not in THP-1. The vector genome (GFP gene) could be detected in all three cell lines transduced with all four tested serotypes. However, GFP protein expression was cell and serotype dependent. AAV2 was able to transduce all three tested cell lines, whereas AAV3, 8 and 9 were able to transduce only HepaRG and THP-1. Furthermore, AAV2 increased αSMA gene and protein expression and decreased TGF-β gene expression in hTERT-HSC suggesting an activation of HSC. THP-1 were also activated by AAV2 and AAV8 as indicated by increased gene expression of CD80, CD206, HMOX and CXCL10. AAV2 and AAV8 induced increased secretion of various pro-inflammatory cytokines in all three cell lines, with IL-6 levels strongly increased in HepaRG and THP-1. In conclusion, an immune response to AAV-mediated liver gene therapy may be driven by the early activation of hepatic stellate cells and Kupffer cells, and increased secretion of pro-inflammatory cytokines by parenchymal and non-parenchymal cells. Further studies will focus on the use of advanced 3D human *in vitro* models to gain further insight into AAV-induced immune-associated hepatotoxicity and further improve the safety of therapeutic AAV vectors.

Poster 17

Combining *in vitro* and *in silico* experiments to assess the neurotoxicity of organic solvents such as glycol ethers

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Environmental and occupational exposure to chemicals may contribute to the development of several neurological diseases, however chemicals are not required to be tested systematically for their neurotoxic potency. The absence of systematic testing may partially be explained by the current OECD test guidelines relying on animal experiments that are expensive, laborious, as well as scientifically and ethically debatable. The goal of this study is to provide a strategy to rank solvents according to their neurotoxicity, using the much-used glycol ethers as a case study. The proposed strategy focuses on a complex 3D *in vitro* brain model (BrainSpheres) derived from human induced pluripotent stem cells (hiPSCs), as well as *in vivo*, *in vitro* and in silico models for blood-brain barrier (BBB) and *in vitro* models for liver metabolism. Data are integrated in a toxicokinetic (TK) model. Internal concentrations predicted with this TK model are validated with results from *in vivo* human controlled exposure experiments. Results show that the cytotoxicity of propylene glycol ethers (PGEs) in BrainSpheres, liver and BBB models, as well as in Zebrafish larvae is correlated to the carbon chain length of the compounds. One-week repeated exposure of BrainSpheres to PGEs show a decreased in synaptic and astrocytes markers at subcytotoxic concentrations. In Zebrafish larvae acute PGEs exposure decrease the integrity of the blood-brain barrier, revealed by a significant increase in extracellular fluorescent tracer permeability into brain parenchyma, and impaired behavioral patterns. Finally, the concentration of PGEs predicted in human brain for workers exposed 8h/day and 5 days per week at the occupational exposure limit is in the order of magnitude of the concentration of PGs found neurotoxic *in vitro*. Altogether, these results suggest that PGEs are potentially neurotoxic for the human brain. The TK model will be used in reverse dosimetry to predict air concentrations deemed safe for the human brain. These predictions will contribute to the protection of workers and the general population exposed to PGEs.

Keywords: Organic solvent exposure, neurotoxicity, blood-brain-barrier, liver toxicity, microphysiological system.

Poster 18

Navigating international PFAS regulatory updates and restrictions: A case study of the impact analysis in the medical device industry

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Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic organic compounds that are used in numerous applications such as textiles, (food) packaging, lubricants, refrigerants, electronics, medical devices, etc. PFASs or their metabolites are highly persistent substances that if not controlled, their concentrations will continue to increase in the environment. Due to their persistence, PFAS remediation is extremely difficult and costly. Furthermore, certain PFASs are known toxicants and they bioaccumulate, thus posing a threat to human health.

Currently, numerous legislative updates and regulatory initiatives are being undertaken to tackle PFAS. In the USA, the Environmental Protection Agency proposed a new PFAS drinking water regulation for six PFAS. In the EU, a universal restriction proposal (REACH Annex XV) was recently published by ECHA affecting more than 10 000 PFASs, including PVDF and PTFE fluoropolymers. Two restriction options (ROs) have been accessed in the restriction proposal. The first (RO1) consists of a full ban with an 18-month transition period, while the second (RO2), includes a few use-specific derogations of up to 12 years. This would be the first time that such a broad restriction would be applied. Unless manufacturers take immediate action, these initiatives will tremendously impact multiple industries and affect market access to vital products.

Here, we present the impact analysis of the upcoming restrictions in the medical device industry. Manufacturers will have to assess alternatives and understand their supply chain to detect and amend potential repercussions. These regulations may require the re-evaluation, restructuring, or revalidation of manufacturing processes, technologies, and facilities. Moreover, analytical/reporting regulatory requirements and waste management responsibilities will further increase legal obligations for medical device manufacturers.

In summary, upcoming PFAS regulations will reshape the medical device industry. Manufacturers must proactively assess, strategize, and comply to provide safe and effective medical devices.

Poster 19

Assessment of human toxicokinetics and metabolism of propylene glycol ethyl ether (PGEE)

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Glycol ethers are organic solvents mainly present in cleaning or paint products for consumer and professional uses. Propylene glycol ethers are progressively replacing ethylene glycol ethers, known to induce reproductive and neurological toxicities. However, propylene glycol ethers have unknown toxicities and are rarely assessed for neurotoxicity. Humans are exposed to these chemical substances by ingestion, inhalation, and skin absorption. One of the most present on the Swiss market is the propylene glycol ethyl ethers (PGEE) and toxicokinetic studies are absent for it. We conducted human inhalation studies by exposing human volunteers (N=4) to PGEE vapours under controlled conditions for four hours at different air concentrations (N=3). We assessed Michaelis-Menten-Kinetic for the formation of metabolites using human S9 liver fractions. We quantified the parent compound (GC-MSMS) and the respective metabolites (LC-MSMS) in blood, urine, exhaled air, and cell lysate samples. PGEE concentration increased in blood, urine, and exhaled air until they reached a maximal concentration at the end of exposure (Cmax = 0.8-1.4 μg/mL). The elimination curves of PGEE showed a fast exponential decrease with a particularly short half-life in exhaled air (t1/2 = 1.8-2.8 min). Internal doses in blood increased with increasing air con-centrations. Metabolites were quantified in cell lysate of S9 fraction and kinetic parameters de-termined (Km = 1133 μM ; Vmax 1.37 nmol/min/mg). Given the reported neurotoxic effects of some glycol ethers in humans, we decided also to investigate their potential effect on blood-brain-barrier (BBB) using zebrafish larvae model. We evaluated the BBB integrity following exposure to PGEE. The results showed that PGEE exposure have an impact on BBB decreasing its integrity. A significant increase in extracellular fluorescent tracer permeability from blood vessels into the surrounding brain parenchyma in zebrafish larvae was observed following solvent exposure. Thus, further tests should be performed for the neurotoxicity assessment.

Keywords: Toxicokinetics, organic solvents, human inhalation uptake, biomonitoring, blood-brain barrier

Poster 20

Using a mathematical model for human lupus nephritis to increase understanding of the effect of anti-inflammatory agents on parameters mediating renal damage

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Like other autoimmune diseases, systemic lupus erythematosus (SLE) induced lupus nephritis (LN) manifests itself with a complex pathogenesis. In recent years, a number of novel diagnostic, prognostic and treatment response biomarkers were evaluated in LN patients. In order to better understand the interplay between pathological and therapeutic processes, a previously established mathematical model (Budu-Grajdeanu et al., 2010) was reconstructed and a sensitivity analysis performed. For the sensitivity analysis, each parameter value was increased and decreased by 10 percent at a time and percentage changes of the Cmax and AUC for each of four components (i.e. immune complexes [I], pro-inflammatory mediators [P], damaged tissue [D], anti-inflammatory mediators [A]) that mediate renal damage were computed. The parameter which has the most impact on all four components when changed slightly is μ_a, the rate of decay of anti-inflammatory agents. Other parameters which showed considerable impact on immune complexes, pro-inflammatory mediators and damaged tissue include A_{inf}, the anti-inflammatory concentration that provides half of the maximum inhibitory effect, and s_a, the concentration of anti-inflammatory therapy.

Overall, the compartments most sensitive to minor parameter changes are P and D. Budu-Grajdeanu et al. found that simulations of P and D for four patients qualitatively and (to some degree) quantitatively reproduced measured clinical concentrations of urinary monocyte chemotactic protein-1 (uMCP-1) and urine protein to urine creatinine ratio (uP:C), respectively.

Ongoing research efforts investigate the use of this model with alternative individual-level biomarker data and a precise setting of parameters which during the sensitivity analysis were identified to have the most impact on parameters mediating renal damage in LN.

Poster 21

In silico and *in vitro* established physiologically based kinetic model for diterpene glycosides

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Next-generation risk assessment (NGRA) is a hypothesis-driven approach based on exposure, *in vitro*, and *in silico* methodologies, that can potentially support animal-free safety decision-making for regulatory purposes. Internal chemical exposure can be estimated using physiologically based kinetic (PBK) models. These models are based on mathematical equations that simulate the absorption, distribution, metabolism, and excretion (ADME) of a chemical within a living organism. The organism's organs and blood are represented by a set of interconnected compartments. Physiological (tissue volumes, blood flows, etc.), biochemical, and physicochemical data are needed to parameterize PBK models. Although currently several PBK platforms exist, integrating physiological and biochemical parameters for multiple animal species and human populations; chemical-specific input parameters are often unavailable or difficult to find in the literature. Thus, there is a need to generate such data using *in silico* or *in vitro* methodologies.

We report a stepwise methodological approach to investigate the suitability and limitations of applying PK-Sim® and its parameterization for data-poor substances. We used physiological data provided by PK-Sim®, and biochemical and physicochemical values obtained solely by applying **in silico** predictors (e.g. SwissADME, OPERA, and ADMET). The selection of the parameters to be further verified *in vitro* was informed by a sensitivity analysis. Both the rodent and human PBK models were evaluated using available published *in vivo* data.

This methodological approach will be illustrated using chemicals from the diterpene glycoside family, following the OECD PBK model guidance document.

In conclusion, we present here a methodological approach for PBK model parameterization using solely *in silico* and *in vitro* data and leveraging open science, since PK-Sim® is part of the open-source software Open Systems Pharmacology Suite (OSPS, www.open.systems-pharmacology.org), specifically tailored towards open-user-friendly modelling and simulation.

Poster 22

Toxicological risk assessment of niobium and zirconium-containing implants

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Individuals who suffer from accidents or pathological conditions, such as osteoarthritis, rheumatoid arthritis, and post-traumatic arthritis, may require surgical intervention, such as orthopaedic prosthesis implantation. Orthopaedic implants are medical devices that serve to sustain or substitute the function of injured tissue, totally introduced into the human body by clinical intervention and are intended to remain in place after the procedure.

Nowadays, millions of people live with implants such as joint replacements. More recently, zirconium and niobium-containing implants have also been used due to their unique properties, biomechanical behaviour and history of safe use. Nevertheless, manufacturers are obliged for implantable medical devices to demonstrate biological safety according to ISO 10993-1 and to conduct a toxicological risk assessment in compliance with ISO 10993-17. However, the toxicological database, including suited health-based guidelines values, is very limited for niobium and zirconium.

Therefore, the present work aimed to derivate parenteral tolerable exposure levels for niobium and zirconium for implantable medical devices, including the toxicological risk assessment according to ISO 10993-17.

In this context, a systematic literature search was planned and conducted to collect chemical, physical, and toxicological data on niobium and zirconium as required by ISO 10993-1 and 10993-17. Primary and secondary literature were searched in suited databases using defined inclusion and exclusion criteria. Studies reports were systematically identified, screened and appraised for eligibility, followed by data extraction and evaluation of included studies in order to synthesise evidence. Afterwards, suited studies for setting the point of departure were selected to derive tolerable intake and tolerable exposure values. The toxicological risk assessment was completed by deriving worst-case exposure doses and calculating the corresponding margin of safety. Besides this, the remaining uncertainties due to identified data gaps were elaborated.

Poster 23

Development of a "plug & play" microphysiological system to mimic liver fibrosis *in vitro*

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Non-alcoholic fatty liver disease (NAFLD) parallels the increase in obesity rates with a cur-rent estimated global prevalence of 30%. It is recognized as one of the most common causes of chronic liver diseases among adults. Untreated NAFLD can progress to liver cirrhosis or even hepatocellular carcinoma. We developed a flexible microphysiological system (MPS) that recapitulates progression of liver fibrosis by incorporating the cell types driving the key events of the liver fibrosis adverse outcome path-way (AOP). This novel device offers the possibility to perfuse the cells individually or sequentially, allowing a comprehensive study of the complex processes of liver fibrosis.

The MPS prototype features polymethylmethacrylate well plates and interchangeable lids. The microchannels were formed using pressure-sensitive adhesive tape. Two different lids were de-signed for "Culture" and "AOP" mode. The Culture lid serves to maintain the cells in separated compartments, while the AOP lid facilitates cell-to-cell communication through medium flow (generated by a peristaltic pump). HepaRG and hTERT-HSC (representing hepatocytes and hepatic stellate cells, respectively) were cultured in 3D, while THP-1 cells (surrogate of Kupffer cells) were maintained in monolayer cultures within the MPS. Cell viability (ATP content) and morphology were monitored for 3 days to evaluate possible toxicity of the MPS. In addition, functional markers such as albumin expression for hepatocellular health and alpha smooth muscle actin staining for stellate cell activation were assessed.

The cell lines maintained their morphology during 3 days in both setups (Culture and AOP) within the MPS. The albumin expression in HepaRG and the ATP content of HepaRG, hTERT-HSC and THP-1 cultured in the MPS were similar to that in standard culture dishes. Additionally, spontaneous activation of the hTERT-HSC cultured in the MPS was not observed. These data confirmed biocompatible properties of the MPS.

Our preliminary data demonstrate the potential use of this innovative in vitro MPS. Exposure of this novel AOP-MPS to fibrogenic drugs will be the next step towards elucidating the mechanisms underlying liver fibrosis. The online monitoring of glucose, lactate, pH and ROS using specially de-signed sensors will further enhance the applicability of the MPS.

Poster 24

Machine learning for predictive ecotoxicology in fish

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New approach methods, such as machine learning, have promising potential as alternative to animal testing for ecotoxicology. However, comparing the performance of models across studies to predict toxicity values is difficult because it is highly dependent on the data set and the applied train-test-split.

To motivate machine learning experts without a strong biological background to work on ecotoxicological challenges, we provide a clean and well-characterized data set as a potential benchmark.

The data set is comprised of effect values (EC50/LC50) for acute mortality (≤ 96 h) in fish, crustaceans, and algae. We describe feature curation and data set assembly and give insights into our modeling approach, based on the combination of a variety of models, including Linear-regression-based least Absolute Shrinkage and Selection Operator (LASSO), tree-based models (RandomForest, XGBoost), and a Gaussian Process (GP) regression model. These models are paired with five molecular representations (four common fingerprints and the mol2vec molecular embedding). The performance of all possible model and representation pairings were evaluated against a dataset on fish acute mortality with 140 different species.

Results showed that the combination of the tree-based XGBoost model and the MACCS fingerprint performed most accurately and that the prediction usually was within an order of magnitude of the log-transformed true value. To make the model performance more transparent and understandable, and to gain insights into what kind of additional data could improve model performance, we performed feature importance analyses. Additional analyses served to identify local models of chemicals where predictions were particularly good or particularly poor.

Keywords: Predictive toxicology, ecotoxicology, acute mortality, machine learning.

Poster 25

Elucidating the transcriptional effects of oxidative DNA damage at a genome-wide level

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Chemically induced DNA damage has been linked to premature aging, cancer, and neurodegeneration. While the major DNA oxidation product 8-hydroxyguanine, (8-oxoG) can induce mutations, recent data suggests it may also regulate gene expression.

To better understand such epigenetic and toxicological properties of 8-oxoG, we determined the distribution of 8-oxoG genome-wide and related the locations of oxidation with transcriptional activity in cells. Click-code-seq is a single-nucleotide resolution DNA damage sequencing method that can be used to detect genomic locations of 8-oxoG by labeling damage positions with an oligonucleotide barcode. We applied this method to examine the endogenous genome-wide distribution of 8-oxoG in a fibroblast-like human cell line (HAP-1). By examining the local sequence context of 8-oxoG, we found evidence for a mutually exclusive relationship between DNA oxidation and methylation. Interestingly, we also observed a guanine oxidation strand bias, which increases with gene expression level, suggesting an association between transcription and 8-oxoG. Finally, we analyzed oxidation levels in gene promotor regions in relation to the expression of downstream genes and identified a non-monotonic relationship between promoter DNA oxidation and gene expression.

These data advance our understanding of cellular consequences of oxidative DNA damage and its impact on disease etiology.

Keywords: DNA damage, gene expression, DNA damage sequencing, 8-hydroxyguanine.

Poster 26 ***(selected for Short Presentation)***

Kinetic modeling and *in vitro* fermentation reveal how gut microbiota impacts bioactivation and bioavailability of phytoestrogens

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Prenylated polyphenols, such as isoxanthohumol (iXN), are natural components of hops and are found in beer. The gut microbiome can convert iXN to 8-prenylnaringenin (8-PN), the most potent known phytoestrogen. It strongly interacts with estrogen receptors, potentially disrupting endocrine signaling. Apart from beer, dietary supplements used to alleviate postmenopausal symptoms also contain 8-PN and its precursor iXN. It is difficult to assess the potential adverse effects of 8-PN exposure from consuming beer or using dietary supplements because there is a lack of methods to estimate systemic concentrations of gut microbial metabolites.

Therefore, we developed a human-specific physiologically based kinetic (PBK) model to predict, based on realistic exposure scenarios, the biologically available levels of hop polyphenols activated microbial metabolites in human blood and target tissues. To include metabolism rates in the model, glucuronidation was measured by incubating hepatic and intestinal S9 fractions with iXN and 8-PN. Kinetics of microbial metabolism were determined by using an *in vitro* anaerobic fecal fermentation using pooled and individual fecal slurries, and the conversion of iXN to 8-PN was analyzed by liquid chromatography-mass spectrometry. The resulting *in vitro* kinetic constants were scaled to *in vivo* human physiology. The gut microbiome was included in the PBK model as a dedicated compartment of metabolism, and the interindividual variation in gut microbiome composition was included in the assessment. The predicted concentrations were then used to test *in vitro* estrogen receptor activation to assess the safety of hop polyphenols. The PBK model serves as a framework for a quantitative assessment of gut microbial metabolites.

We successfully developed a PBK model that predicts the levels of hop phytoestrogens in the body and demonstrated its applicability for evaluating the influence of microbial metabolites.

Keywords: Microbial metabolism, pharmacokinetics, PBPK modeling, biotransformation, hop polyphenols, estrogenicity.

Poster 27

Study of Chemical-Induced Myelin Disruption Using Human iPSC-Derived 3D Brain Spheroids

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Growing evidence suggests that environmental factors contribute to the development of neurodevelopmental disorders. However, the impact of exposure to xenobiotics during crucial developmental stages remains unclear. The study of developmental neurotoxicity (DNT) has been limited by high costs, extensive animal use and low translational power. To address these challenges, there is an increasing need for a battery of New Approach Methodologies (NAM) assays covering key neurodevelopmental processes (KNDP), such as proliferation, migration, and myelination. Recently, an *in vitro* battery (IVB) was assembled to investigate chemical effects on KNDP. However, gaps exist due to the absence of assays targeting specific KNDP.

Myelination is a critical event in brain development and a sensitive endpoint for DNT. However, a myelin assay in a human context has not been integrated into the IVB due to difficulties in generating myelin *in vitro*. Here, we utilized human induced pluripotent stem cells (hiPSCs) to create a 3D brain spheroid model, which contains neurons and glial cells. This model presents compact myelin wrapped around axons, offering an ideal platform for myelin research. The evaluation of myelin involves quantification through immunostaining and confocal microscopy analysis, with a specific focus on assessing proteolipid protein 1 (PLP1) fluorescence intensity.

In our study, we investigated the effects of seven compounds: Cuprizone, Methamidophos, Diazonine, Vanadium, Diamethoate, BDE-99 and a negative control Acetominophen. Myelination disruption was observed in spheroids treated with Cuprizone, Diazonine and BDE-99 at non-cytotoxic concentrations, but not in those treated with Ibuprofen and others, demonstrating the assay's ability to specifically detect demyelination.

However, for high-throughput screening of more compounds, we encountered challenges due to the labor-intensive and time-consuming nature of the quantification process. To overcome this, we are currently developing a semi-automated myelin quantitative assay for toxicological assessment and screening, with the aim to significantly improve efficiency in terms of time and resources. Ideally, this innovative assay will allow the analysis of a substantial volume of chemicals using this streamlined approach.

Poster 28

In vitro characterization of the alcohol- and aldehyde dehydrogenase enzymes in the liver, the blood-brain barrier, and the brain

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Several neurological disorders have been linked to occupational exposure to chemicals. Propylene glycol ethers (PGEs) are commonly used as mixtures of a non-toxic α-isomer and a β-isomer that is oxidized to a potential noxious acid metabolite via the alcohol dehydrogenase (ADH) and the aldehyde dehydrogenase (ALDH). However, studies about the neurotoxicity of these solvents are rare. Knowing the rate of solvent metabolism is important to estimate the brain exposure to metabolite(s). Although the liver is the main organ for ADH- and ALDH-mediated metabolism, the activity of mitochondrial ALDH2 in the brain and the blood-brain barrier (BBB) has been described. However, the presence and activity of ADH1 in both organs remain unknown. Here, we demonstrated the presence of the two enzymes ADH1 and ALDH2 in *in vitro* models for the liver, BBB and brain. Gene expression of ADH1 isoforms and ALDH2 was assessed using RT-qPCR. Enzymes were detected on the protein level using western blot and immunostaining. The activity of the enzymes was determined by measuring the production of metabolites using LC-MS/MS. Subcellular fraction (S9) was also used to determine the Michaelis-Menten kinetic parameters. ADH1 and ALDH2 were expressed in all tested *in vitro* tissues (gene and protein expression). The 3D HepaRG cells were able to generate PGE-metabolites to a similar extent to primary human hepatocytes. Moreover, liver S9 incubations served as a system to estimate the enzyme kinetic of metabolite formation, whereby lower Km values were observed with increasing carbon length of the solvents. In conclusion, the data show expression of ADH1 and ALDH2 in all three tested *in vitro* systems, indicating that metabolite formation can take place in the liver and in the central nervous system (brain and BBB). Future steps will quantify *in vitro* metabolite generation by the liver, the BBB, and the brain. The data will help further optimize the generated in vitro physiological based toxicokinetic (PBTK) model to predict human systemic and brain exposures, thereby supporting the risk assessment of occupationally relevant chemicals.

Keywords: Propylene glycol ethers, alcohol dehydrogenase, aldehyde dehydrogenase, *in vitro* metabolism, neurotoxicity.