Annual Meeting 2022 – Poster Session

Poster 01

Single-nucleotide resolution mapping reveals patterns of O^6-methylguanine formation and repair in a human glioblastoma cell line exposed to the anticancer drug temozolomide

Jasmina Büchel, Cécile Mingard, Patricia Reinert, Sabrina Huber, Shana J. Sturla

Laboratory of Toxicology, Department of Health Science and Technology, ETH Zurich

The widely used chemotherapeutic drug temozolomide kills cancer cells by inducing DNA alkylation such as by forming O^6 methylguanine (O^6 MeG), which causes mismatches upon replication and leads to apoptosis by mismatch-repair overload. However, O^6 MeG can be directly repaired by the enzyme O^6 methylguanine-DNA methyltransferase (MGMT), therefore causing resistance towards temozolomide. While understanding individual genomic patterns of temozolomide-induced DNA alkylation may improve patient stratification for therapy, there are no methods established for mapping O^6 MeG at single nucleotide resolution. To address this limitation, we developed a new method for precisely locating O^6 MeG in the whole genome and used it to analyze patterns of O6 MeG formation and repair. The method is based on immunoaffinity enrichment of DNA fragments containing O^6 MeG with an anti-O^6-MeG antibody and subsequent stalling of a high-fidelity polymerase at O^6 MeG. The glioblastoma-derived human cancer cell line LN229, which is deficient in MGMT, as well as LN229 cells transfected with MGMT, were exposed to cytotoxic levels of temozolomide. Genomic mapping of O^6 MeG revealed its accumulation in certain trinucleotide sequence contexts, which are comparable to the mutational signatures found in patients treated with temozolomide. These findings suggest that mapping O^6-MeG at single-nucleotide resolution improves our understanding of O^6 MeG formation in cells as well as how MGMT influences its genomic distribution pattern as a potential factor for resistance to chemotherapy with temozolomide.

Keywords: DNA methylation, whole genome sequencing, temozolomide, O^6-methylguanine.

Poster 02

Kidney cell models for drug safety profiling: Susceptibility of classical 2D cultures vs. integrated biomimetic array chips (IBAC) under flow

Benedetta Carasi, Laurence Hilfiger, Tanja Minz, Marcel Gubler

Investigative and Immuno Safety, F. Hoffmann-La Roche Ltd, Basel

Kidney is susceptible to drug treatment with renal liabilities compounds and this is due to the fact that a large number of commercially available drugs has access to proximal tubule epithelial cells (PTEC), either mediated by active transport or passive diffusion. While renal transport systems and urine flow are important intrinsic mechanisms of drug clearance, the same mechanisms can be responsible for drug accumulation and renal tubular cell toxicity.

Because of the high susceptibility of renal compartment, safety profiling on PTEC is crucial in order to ensure a predictive picture of their potential harmful effect in patients.

Although well known and reproducible, 2D models lack a sufficient degree of translatability. In this study with RPTEC/TERT1 cell line, we aimed to investigate the superiority of a novel microfluidic model, IBAC - Integrated Biomimetic Array Chip, over the classical 2D. We wondered if the introduction of bidirectional flow, provided by the IBAC system, has a critical effect on the biology and drug susceptibility of PTEC

A side by side comparison of the two systems was performed at three different levels:
1) Susceptibility to four nephrotoxic reference compounds, by the use of three injury readouts (ATP content, LDH release and Dead cell count by imaging).
2) Morphological features assessment in terms of tight junctions and cytoskeleton structure.
3) Gene expression profile of PTEC markers and apical and basolateral transporters.

We have identified distinct differences in the cytotoxicity profiles of the investigated drugs in 2D vs. IBAC models while morphological features and the gene expression profile did not change in the two model systems. Additional improvement of microphysiological culture conditions like e.g. co-culture with other renal cell types or use of extracellular matrix scaffolds are expected to further enhance the performance and translatability of the renal cell models under flow.

Keywords: Renal Proximal Tubule Epithelial cells (PTEC); Integrated Biomimetic Array Chip under flow (IBAC); Safety profiling; 2D vs microphysiological cell models

Poster 03

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

Natasha Fehrenbach (1), Stephan Krähenbühl (1,2), Alex Odermatt (1) and Jamal Bouitbir (1)

(1) Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Switzerland

(2) Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Switzerland

Sunitinib as multi-target anticancer drug is associated with severe hepatotoxicity in patients. To date, the mechanisms underlying this toxicity remain still unclear. We recently showed that treating wild-type (WT) mice with sunitinib increased the accumulation of mitochondrial reactive oxygen species (mtROS) in liver, leading to apoptosis and cell death. In this study, we investigated whether superoxide dismutase 2 (SOD2), located exclusively in the mitochondrial matrix and responsible for mtROS detoxification, represents a potential susceptibility factor in sunitinib-associated hepatotoxicity. Therefore, we treated male WT and Sod2+/- mice for two weeks by oral gavage with vehicle or sunitinib at 7.5 mg/kg/day.

Body and liver weight was similar between WT and Sod2+/- mice treated or not with sunitinib. We then measured the mitochondrial respiratory capacities and the mitochondrial membrane potential (ΔΨm) in isolated mitochondria using the Oroboros Oxygraph-2k. Sunitinib did not affect mitochondrial respiration in WT mice. Interestingly, the CIII- and CIV-linked respiration was decreased in the sunitinib-treated mutant mice compared to control mutant mice. Compared to the control WT, control Sod2+/- mice showed a higher CIII-linked respiration. ΔΨm decreased in both sunitinib-treated groups compared to the respective control groups. The mRNA expression of the antioxidative enzymes SOD1 and catalase was not affected by sunitinib. Interestingly, catalase mRNA expression was upregulated in the control Sod2+/- compared to the control WT. Since mitochondria are physically connected with endoplasmic reticulum (ER), we investigated whether sunitinib treatment causes ER stress induced-unfolded protein response (UPR) in liver. Sunitinib increased the mRNA expression of the chaperones GRP94 and BiP in both WT and Sod2+/- mice whereas the mRNA expression of ATF4, ATF6 and sXBP1 was not affected.

In conclusion, our preliminary results indicate that sunitinib seems to impair mitochondrial function in Sod2+/- mice. Further experiments such as mitochondrial respiratory complex activity, markers of mtROS production, apoptosis, and UPR are planned for the validation of our hypothesis.

Keywords: Sunitinib; hepatotoxicity; SOD2, oxidative stress; endoplasmic reticulum.

Poster 04

Organ-specific biotransformation in salmonids: insight into enzyme kinetics and micro-pollutant clearance

Marco E. Franco (1), René Schönenberger (1), Juliane Hollender (2,3), Kristin Schirmer (1,3,4)

(1) Department of Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology, Eawag, 8600 Dübendorf, Switzerland
(2) Department of Environmental Chemistry, Swiss Federal Institute of Aquatic Science and Technology, Eawag, 8600 Dübendorf, Switzerland
(3) Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland
(4) School of Architecture, Civil and Environmental Engineering, EPF Lausanne, 1015 Lausanne, Switzerland

For aquatic animals, like fish, bioaccumulation assessments have largely focused on the biotransformation capacity of the liver, as the organ responsible for a large proportion of the biotransformation activity. However, there is limited knowledge regarding extrahepatic biotransformation and its role in estimating the bioaccumulation potential of chemicals.

In the present study, we explored the ability of different organs to support biotransformation processes through the evaluation of in vitro phase I and II biotransformation enzyme kinetics and the estimation of intrinsic clearance of pyrene, used as reference chemical, and two micro-pollutants: the fungicide azoxystrobin and the pharmaceutical propranolol. Comparative studies using S9 subcellular fractions from the liver, intestine, gills, and brain of brown trout (Salmo trutta) and rainbow trout (Oncorhynchus mykiss) suggested significant phase I and II enzyme activity in all organs, particularly for CYP1A and Glutathione-S-transferase, respectively. CYP2B and UDP-glucuronosyltransferase in the intestine and gills also displayed similar activity as in the liver. All chemicals tested were biotransformed by liver S9 fractions. However, extrahepatic biotransformation was only observed for pyrene and azoxystrobin, with particular interspecific differences regarding the magnitude of intrinsic clearance.

Altogether, these observations provide evidence that extrahepatic biotransformation may depend on specific organ-chemical interactions. Thus, considering the biotransformation potential of different organs could help in refining the estimation of bioaccumulation parameters during environmental hazard and risk assessments.

Keywords:

Poster 05

Biological evaluation of pharmaceuticals inhibiting 11β-hydroxylase identified by virtual screening

Marie-Christin Jäger (1), Jacek Kędzierski (3), Victoria Gell (2), Tim Wey (1), Denise V. Winter (1), Daniela Schuster (2), Martin Smieško (3), Alex Odermatt (1)

(1) Swiss Centre for Applied Human Toxicology and Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland.

(2) Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Paracelsus Medical University, Strubergasse 21, 5020 Salzburg, Austria.

(3) Swiss Centre for Applied Human Toxicology and Division of Computational Pharmacy, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 61, 4056 Basel, Switzerland

Secondary hypertension and hypokalemia are risk factors arising from disruption of steroidogenesis via inhibition of the steroidogenic enzyme 11β-hydroxylase (CYP11B1). Clinical approval of pharmaceuticals not tested for their inhibitory potential on CYP11B1 pose a certain risk for unexpected adverse effects. Its inhibition restricts the conversion of 11-deoxycortisol and 11-deoxycorticosterone to cortisol and corticosterone, respectively, thereby resulting in feedback stimulation of adrenal steroidogenesis and leading to an accumulation of the CYP11B1-substrates. At excessive levels, they activate the mineralocorticoid receptor, eventually leading to hypertension. This study applied a ligand-based pharmacophore- and an induced-fit docking based in silico approach to screen the DrugBank Database for pharmaceuticals potentially inhibiting CYP11B1. Identified hits were initially tested for their inhibitory potential in a mitochondrial-based assay. IC50 values were determined in a cell-based model with V79 cells stably expressing CYP11B1. Confirmed hits inhibiting CYP11B1 comprise the α2-adrenoceptor agonists dexmedetomidine, medetomidine and detomidine and the antagonists 1-benzylimidazole and atipamezole. Other confirmed inhibitors belong to the class of azole fungicides and include tioconazole, climbazole, butococnazole, terconazole, luliconazole, oxiconazole and econazole. Further inhibitors include the retinoic acid-metabolism blocking agents liarozole and talarozole as well as the farnesyltransferase inhibitor tipifarnib.

This project combined different computational approaches leading to chemically diverse and numerous hits, illustrating the advantage of the application of both in parallel, pharmacophore-based as well as structure based models. The project not only identified pharmaceuticals potently inhibiting CYP11B1, thus posing a risk for a patient to suffer from secondary hypertension, it also provides a strategy for identifying steroidogenesis disruptors.

Keywords:

Poster 06 (selected for oral presentation)

Ovarian Toxicity of Bisphenol A, Phthalates and their substitutes

Friedrich Joos, Alex Odermatt, and Jamal Bouitbir

Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel

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 widely used EDCs, have attracted considerable attention from 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. For this reason, we aimed to investigate the potential toxicity of these substitutes and characterize the mechanisms underlying this toxicity.

We included BPA and its alternatives BPS, BPF, BPAF and BADGE, as well as DEHP and its derivatives MEHP, BzBP, and DEHA. We treated immortalized human granulosa cell line COV434 with compounds at human relevant concentrations from 10 nM to 10 µM for 24 h; 48 h and 72 h.

XTT cell viability assay showed that none of the above-mentioned compounds did affect cell viability after 24 h of exposure. Exposure to 10 nM BzBP for 48h decreased cell viability. After 72h of exposure cell viability decreased in cells exposed to 10 nM MEHP, 10 µM BPS and 10 µM BPAF. However, mitochondrial superoxide production did not change when cells were exposed to BPA and phthalates or their substitutes for 24h, 48h and 72h. The gene expression of Sod 1 increased when cells were treated with 10 µM BADGE. The mRNA expression of SOD2, exclusively located in the mitochondrial matrix, decreased in granulosa cells exposed to 10 µM DEHP and BzBP.

In conclusion, our preliminary results show that the selected compounds did not exert relevant effects on cell viability and induced some changes in antioxidative enzymes of granulosa cells at human relevant exposures. Further analyses are still needed to confirm these observations. As a next step, we would like to investigate two possible susceptibility factors SOD2 and H6PD among which polymorphisms are associated with PCOS in humans. We would also like to lay a special focus on potential changes in steroidogenesis.

Keywords: endocrine disrupting chemicals; granulosa cells; cell viability; oxidative stress.

Poster 07

Design and optimization of a serum-free culture medium for the rainbow trout cell line RTgill-W1

Barbara Jozef (1), Zhao Rui Zhang (1,2), Hans-Michael Kaltenbach (3), Melanie Fischer (1) and Kristin Schirmer (1,2,4)

(1) Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
(2) ETH Zürich: Department of Environmental Systems Science, Zurich, Switzerland
(3) ETH Zürich: Department of Biosystems Science and Engineering, Basel, Switzerland
(4) EPF Lausanne, School of Architecture, Civil and Environmental Engineering, Lausanne, Switzerland

Efforts to replace fish for regulatory testing have resulted in the development of strategies and assay procedures based on permanent fish cell lines, especially of rainbow trout (Oncorhynchus mykiss). At the core of one such assay, capable to predict fish acute toxicity on chemical exposure is the rainbow trout gill cell line (RTgill-W1). This assay has recently been approved by international standardization organizations, leading to the ISO standard 21115 and the OECD test guideline 249. However, while the assay as such is performed in a simple exposure medium without the addition of animal components, RTgill-W1 cells are grown routinely in Leibovitz' L-15 medium (L-15) supplemented with 5-10% fetal bovine serum (FBS). FBS is a problematic supplement, both scientifically and ethically. We, therefore, have set out to develop a serum-free formulation, capable of supporting RTgill-W1 cell proliferation, by a systematic, bottom-up medium design.

A high-throughput 96-well plate RTgill-W1 cell proliferation assay was developed and optimized to enable systematic screening of serum-free media components, individually and in a mixture. Numerous supplements were tested. Some showed improved metabolic activity compared to cells in L-15, while additional led to significant cell proliferation, comparable to cells cultured with 5% FBS.

Based on these developments, a formulation was identified that enables RTgill-W1 cells to proliferate in the absence of fetal calf serum for repeated passaging. Adaptation of RTgill-W1 clones to this new serum-free medium has been successfully performed: the RTgill-W1 serum-free clones were able to be continuously passaged up to 31 times without the need for serum. Furthermore, the suitability of the newly developed serum-free media was tested for the cryopreservation of RTgill-W1. Fine-tuning of supplement concentrations, relying on the Design of Experiments and Response Surface Methodology, will allow for further improvements toward a fully optimized medium design.

While the presented methodology to achieve a novel serum-free medium formulation is focused on the RTgill-W1 cell line, transfer to other fish cell lines or even cell lines of other vertebrates should be possible. What is more is that we envision easy adaptation of cell culture media to address

specific questions beyond routine culturing, such as the change of the cells' fatty acid composition in response to specific medium formulations.

Keywords: serum-free medium, fish cells, cell culture medium, toxicity testing

Poster 08

Assessment of anabolic steroids impact on 5β-reductase by virtual screening and *in vitro* approaches

Jacek Kedzierski (1), J. A. Allard (2), A. Odermatt (2), M. Smieško (1)

(1) University of Basel, Division of Computational Pharmacy, Department of Pharmaceutical Sciences, University of Basel, Basel, Basel-Stadt, Switzerland

(2) University of Basel, Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Basel-Stadt, Switzerland

Metabolism of xenobiotics often occurs in the liver which is also responsible for producing bile and its derivatives. Bile acids and their conjugates are critical for dissolving lipophilic compounds in a strictly hydrophilic environment of the human gastrointestinal tract. However not all bile possesses equal surfactant properties. The critical micellization concentration of bile acids depends on the type of the Δ4 reduction stereochemistry. Bile acids (5β reduced) exhibit on average 2mM lower critical micellization concentration, when compared to their 5α reduced counterparts (allo-bile acids). The cis steroid confirmation is introduced by 3-oxo-5β-steroid-Δ4-dehydrogenase (AKR1D1). The mutation within the AKR1D1 gene may lead to liver cholestasis which untreated can progress to steatosis. Interestingly, AKR1D1 is not only involved in bile acid pathways but also androgens and progestogens clearance. Thereby synthetic testosterone derivatives like anabolic–androgenic steroids (AAS) may pose a real threat of altering the physiological function of the AKR1D1. AAS are often taken off-label in high dosage regiments and can therefore pose a target and pathway-specific homeostasis thread.

We investigated commonly used AAS and their impact on the AKR1D1. We have found that nandrolone, clostebol, methasterone, drostanolone, and methenolone alter the AKR1D1 activity of endogenous testosterone when assessed by the in vitro assay at 5uM concentration in a statistically significant manner. Whereas structures of nandrolone suggest that it might be metabolized by AKR1D1, molecular scaffolds of clostebol, methasterone, drostanolone, and methenolone indicate that competitive inhibition may occur. This disturbance of the AKR1D1 activity may lead to a shift in bile acid profiles which can then relate to bile acid deficiency syndrome.

In this contribution we shed light on the structure-activity relationship of the AAS treatment in the scope of the AKR1D1 enzyme.

Keywords:

Poster 09

Assessing critical windows of insecticide exposure during zebrafish development

Sarah Könemann (1,2), Melissa von Wyl (1), Colette vom Berg (1)

(1) Department of Environmental Toxicology, Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland

(2) École Polytechnique Fédéral de Lausanne, EPFL, Route Cantonale, 1015 Lausanne, Switzerland

During its development, the nervous system is most vulnerable to chemical exposure. Particularly, neuroactive chemicals, designed to target the nervous system, can interfere with tightly orchestrated developmental processes. Any disturbance of these processes can lead to impaired neuronal functioning, which severely reduces the fitness of the organism. Among others, insecticides represent a large group of neuroactive chemicals, which are inevitably released into the environment. Insecticides are applied primarily during spring and early summer such that insecticide application coincides with spawning season of many aquatic species, including fish. It is therefore unavoidable that fish and other species are exposed during early development. This study aimed to investigate which stages of the early development of zebrafish were most susceptible to sub-lethal insecticide exposure. To cover different periods of neuronal development, zebrafish were exposed to two organophosphate (OP), two carbamate (CBM), and two neonicotinoid (NIC) insecticides in eight exposure scenarios. The latter included exposure from 0-1 day post fertilization (dpf), 1-2 dpf, 2-3 dpf, 3-4 dpf, 4-5 dpf, 5-6 dpf, 0-6 dpf, and unexposed control. After each exposure, except for the continuous, larvae were transferred to chemical-free medium and were raised until 6 dpf. Effects on the nervous system were assessed based on three behavioural assays. At 1 dpf, spontaneous movements of embryos within the chorion were measured with an automated tracking device. At 3 dpf, the touch-evoked response was tested by touching the tail of hatched larvae with a needle and measuring the distance moved. From 4 to 6 dpf, the locomotion in response to changing light conditions was assessed using the light-dark transition assay. Briefly, the OP dimethoate and the CBM pirimicarb significantly increased the number of spontaneous movements, while for thiacloprid (NIC) a significant reduction was observed. In the touch-evoked response assay, almost all insecticides, except imidacloprid (NIC), induced a reduction in distance moved. However, this effect predominantly occurred immediately after exposure, while larvae seem to have recovered from earlier exposure. Similarly, the locomotion at 4 to 6 dpf only was reduced when larvae were exposed shortly before. Although none of the exposure windows seemed to be critical when looking at locomotion, there is a tendency that earlier exposure leads to longer lasting effects.

Keywords:

Poster 10

Save Time & Money by Smart Planning – Efficient product life cycle management by tailored chemical characterization

Charlotte E. Laupheimer (1), Jenny Keicher (2) and Arne Jaksch (2)

(1) Head of Biocompatibility & Toxicology at Jaksch Life Science Consulting GmbH, An der Aare 2, 4663 Aarburg Switzerland.

(2) Senior Partner at Jaksch Life Science Consulting GmbH, An der Aare 2, 4663 Aarburg Switzerland

Medical device manufacturers regularly face challenges such as raw material scarcity and changes in suppliers, material composition, and manufacturing processes including auxiliary materials that bring their product portfolio at risk. Changes to the products' design not only pose threats from a business perspective but may also alter the biological safety of a medical device.

Herein, we describe a case study showing how a tiered approach based on a detailed analysis of the existing biocompatibility data, followed by a tailored chemical characterization testing set-up led to an efficient procedure to evaluate a raw material change and to establish the biological safety of several medical devices by selecting a suited worst-case test article. A chemical characterization including a deep dive mass spectra analysis, followed by a toxicological risk assessment allowed us to determine chemical equivalence between the new and the clinically established raw materials. In addition, biological equivalence according to ISO 10993-18 Annex C could be shown between the new and the clinically established medical devices.

Tailor-made testing strategies significantly reduce the number of biological tests and associated costs while shortening the required testing time and reducing animal use. Such fast-track change management provides manufacturers with a powerful tool at hand to secure a stable product portfolio and respect 3R principles (replacement, reduction, and refinement of animal experimentation).

Keywords: smart planning, medical devices, chemical equivalence, biological equivalence, ISO 10993.

Poster 11

Identification of marker genes to monitor residual iPSCs in iPSC-derived products

M Lemmens (1,2), J Perner (1), L Potgeter (1), M Zogg (1), S Thiruchelvam (1), M Müller (3), T Doll (3), A Werner (3), Y Gilbart (1), P Couttet (1), HJ Martus (1), S Libertini (1)

(1) Preclinical Safety Department, Translational Medicine, Novartis Institutes for BioMedical Research; Basel, CH

(2) Division Molecular and Systems Toxicology, Department Pharmaceutical Sciences, University of Basel, CH

(3) Chemical Biology & Therapeutics Department, Novartis Institutes for BioMedical Research; Basel, CH

Engineered tissues and cell therapies based on human induced pluripotent stem cells (iPSCs) represent a promising approach for novel medicines. However, iPSC-derived cells and tissues may contain residual undifferentiated iPSCs that could lead to teratoma formation after implantation into patients. As consequence, highly sensitive and specific methods to detect residual undifferentiated iPSCs are indispensable for safety evaluation of iPSC-based therapies.

Here, we used RNA-seq data to identify potential marker genes for iPSC contaminations in iPSC-derived cells. Identifying such iPSC marker genes for each cell type individually provided a larger and more specific set of potential marker genes than considering all cell types in the analysis. Thus, we focused on one cell type for validation experiments. By spiking different amount of iPSCs into iPSCs-derived cardiomyocytes (iCMs), we evaluated the sensitivity of the selected candidate genes by RT-qPCR, and compared their performance to the previously suggested marker LIN28A. ESRG, LINC00678, CAMKV, IDO1, CNMD, L1TD1, LIN28A, LCK, VRTN and ZSCAN10 detected contaminant iPSCs amongst iCMs with a limit of detection ranging from 0.001 to 0.1%, depending on the gene and the iCM batch used.

In conclusion, using the example of iCMs, we provide an approach to identify a set of highly specific and sensitive markers that can be used for quality assessment of iPSC-derived products.

Keywords:

Poster 12

Comparative transcriptomics in developing rat hippocampus after perinatal exposure to 9 endocrine active chemicals reveals effects on different interneuron groups with chemical-specific gene expression patterns

W. Lichtensteiger (1), C. Bassetti-Gaille (1), H. Rehrauer (2), J. Félix (4), B. Linillos-Pradillo (3), H. Idrissi (3), L. Miguélez-Salas (3), L. Rancan (3), S.D. Paredes (3), M. De la Fuente (4), C.-G. Bornehag (5), P. Leonards (6), J. Rüegg (7), J. A.F. Tresguerres (3), M. Schlumpf (1)

(1) GREEN Tox & Institute of Veterinary Pharmacology & Toxicology, University of Zurich, Zurich.

(2) Functional Genomics Center Zurich, ETH & University of Zurich, Zurich.

(3) Faculty of Medicine, Complutense University, Madrid.

(4) Faculty of Biology, Complutense University, Madrid.

(5) Department of Health, Karlstad University, Karlstad

(6) Faculty of Science, Vrije Universiteit, Amsterdam,

(7) Environmental Toxicology, Uppsala University, Uppsala

Transcriptomics analyses in developing rat hippocampus were performed in two projects after perinatal exposure to 9 chemicals with confirmed or suspected endocrine activity. In the first study, choice of chemicals and dosage was based on literature data documenting effects on development of learning and memory: Arochlor 1254 (Aro1254, 5.0 and 0.5 mg/kg), Bisphenol A (BPA, 5.0 and 0.5 mg/kg), and Chlorpyrifos (CPF, 3.0 and 1,0 mg/kg) (Lichtensteiger et al., 2021). In the second project, chemicals were chosen from recent epidemiological data in the Swedish SELMA study, where prenatal exposure to a mixture of these compounds was associated with cognitive deficits in children. This investigation is part of a project of novel approaches to developmental neurotoxicity (DNT) testing for endocrine disrupters (ENDpoiNTs). Effects of chemicals on developing rat hippocampus are investigated as a link between in vitro and in vivo data with focus on OMICs. Bisphenol F (BPF, 3.6 and 0.036 mg/kg), butylbenzyl phthalate (BBzP, 200 and 20 mg/kg), 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH, 300 and 30 mg/kg), permethrin (PMT, 3.6 and 0.36 mg/kg), Perfluorooctanesulfonic acid (PFOS, 0.75 and 0.3 mg/kg), and Triphenylphosphate (TPHP, 20 and 2 mg/kg) were studied. All 9 chemicals were administered in chow to parent rats from pre-mating until lactation. The higher dose was chosen from DNT data in rodents or, in the absence of such data, from reproductive toxicity data. Hippocampus was taken on postnatal day 6 (PND6). In the second investigation, one pup/sex/litter of the higher dose was raised to adulthood. Behavioral tests of adult offspring revealed sex-dependent effects on learning and memory. In males, memory impairment was observed after BPF, BBzP, PFOS, PMT, and DINCH, while TPHP was effective only in female offspring (Felix et al., Abstract No. 1085, ICT 2022, Maastricht).

Transcriptomics in hippocampus of male PND6 offspring (RNAseq) revealed significant effects of the higher dose group on genes expressed by interneurons, with two chemical-specific expression patterns: Aro1254, CPF, BPA, and BPF affected gene expression in medial ganglionic eminence (MGE)-derived interneurons, with upregulation of estrogen receptor-alpha (Esr1), Sox6, and Zeb2, and downregulation of parvalbumin (Pvalb), whereas genes expressed by caudal ganglionic eminence (CGE)-derived interneurons were unaffected. In contrast, BBzP, PFOS, and PMT affected genes expressed by CGE-derived interneurons, with upregulation of Gata2, Tcf7l2, COUP-TFII (Nr2f2), and calretinin (Calb2), and downregulation of VIP, but left genes expressed by MGE-derived interneurons unchanged. DINCH influenced DNT-related GO processes, but did not significantly affect expression of interneuron related genes. Target genes of DINCH include Lhx5 regulating neural precursor cell proliferation, and Otx2 involved in MGE-neurogenesis and oligodendrogenesis. TPHP, which did not affect memory in male offspring, influenced very few genes in male PND6 hippocampus. RNAseq of female hippocampus and of lower dose groups will follow.

Downregulation of Pvalb in MGE-derived interneurons is probably caused by upregulation of Sox6, which suppresses Pvalb expression. Data obtained in the first study with Aro1254, BPA and CPF suggest that upregulation of Sox6 may have resulted from estrogen-induced suppression of miR-24 by these compounds (Lichtensteiger et al., 2021). Gene network analyses of genes expressed by CGE-derived interneurons indicate involvement of Wnt signalling and possible links with RAR-alpha and PPAR-gamma.

Our results illustrate the use of transcriptomics for the characterization of specific gene expression patterns of groups of chemicals that are linked with impaired behavioral development in animal models and humans. The analysis will be extended by epigenetic and metabolomic investigations in the same hippocampus samples. Together, these data should help in the design of new in vitro models for DNT testing with a better link to developmental processes in humans, and increased predictive value.

Supported by Horizon2020 Grant 825759 (ENDpoiNTs) and by the Swiss Office of Public Health (first investigation).

Keywords: endocrine disrupters, hippocampus, development, transcriptomics, memory function

Poster 13

Fluorescence-based quantification and single nucleotide resolution mapping of oxidative DNA damage in the human genome

Nikolai J.L. Püllen, Vakil Takhaveev, Navnit K. Singh, Hailey Gahlon, Shana J. Sturla

ETH Zurich, Schmelzbergstrase 9, 8092 Zurich, Switzerland

Chemically induced DNA damage can accumulate in the genome or transform into mutations causing cancer or accelerated ageing. Major DNA damage types are single strand breaks, apurinic sites and oxidatively modified nucleobases. New DNA damage sequencing methods are rapidly emerging, but accurate damage sequencing is impaired by naturally abundant generic damage sites and artifacts produced during sample processing and determining overall damage levels is technically difficult.

Therefore, we developed a fluorescence-based method for rapid and cost-effective quantification of DNA lesions in the human genome. We accurately detected UV- and chemically induced oxidative lesions and apurinic sites in human cells and verified the results using mass spectrometry. Furthermore, we applied our method to design a low-background sequencing protocol to map oxidative lesions and apurinic sites in the human genome. Single nucleotide resolution and the application of molecular barcodes allowed us to characterize the sequence context of damaged bases and damage distribution in genomic regions of functional importance.

We found a depletion of damage in defined nucleobase sequences and transcriptionally active regions suggesting epigenetic functions and an active mechanism safeguarding these areas. The new fluorescence-based method was used to improve damage sequencing protocols and furthermore enables rapid quantification of a variety of common DNA damage types.

Accurate damage sequencing and quantification paves the way for novel diagnostic tools and an in-depth understanding of the link between DNA damage and disease.

Keyword: DNA damage

Poster 14

Combinatorial Nanoparticle Design for Efficient Delivery of Therapeutic Enzymes through the Blood Brain Barrier

Natascha Santacroce (1), Congyu Wu (1), Floriana Burgio (1), Patrick Shahgaldian (1), Laura Suter-Dick (1,2)

(1) University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Muttenz, Switzerland

(2) Swiss Center for Applied Human Toxicology (SCAHT), Basel, Switzerland

The blood-brain barrier (BBB) is a highly selective semipermeable barrier, which controls the influx and efflux of substances, and maintains the metabolic activity and neuronal function in the brain. As a consequence, benefits of biological drugs, achieved in a large num-ber of diseases (e.g., cancer, autoimmune diseases), do not significantly impact pathologies where the therapeutic target is in the brain. To overcome this limitation, a variety of nanopar-ticle (NP) drug transporters have been designed to favor their transport through the BBB. Here we show how different surface modifications of NPs influence uptake and transcytosis through the brain endothelium using an in vitro cell model made of hCMEC/D3 (D3).

To assess cytotoxicity, D3 cells were treated with several NP constructs at concentra-tions ranging from 0 to 400 µg/ml for 24 h. Viability was determined by the quantification of intracellular ATP. For uptake studies, D3 cells were incubated with 100 µg/ml NPs for 1 h and then lysed to quantify the amount of internalized NPs by measuring the fluorescence intensity. For permeability assay, D3 cells were grown on the apical side of transwell inserts till tight bar-rier formation and exposed to 100 µg/ml NPs. After 3 h, the medium in the basolateral cham-ber was collected to quantify the amount of transcytosed NPs. Mechanistic evaluations of NP endocytosis was carried out via confocal microscopy and image analysis (Cellprofiler).

No severe cytotoxicity was observed even at the highest concentration regardless of the surface modification. However, some surface modifications increase cellular uptake and enhance transcytosis. Mechanistic investigation revealed that NPs can enter cells via specific receptor-mediated endocytosis. We could demonstrate that the functionalization of NPs can influence transcytosis through a brain endothelial barrier in vitro. Nevertheless, endo-thelial function is highly dependent on intercellular interactions and flow-related shear stress, which needs to be considered for future assessments.

Keywords:

Poster 15 (selected for oral presentation)

Methotrexate-induced liver fibrosis is associated with oxidative stress, impaired mitochondrial respiration and endoplasmic reticulum stress *in vitro*

Saskia Schmidt (1,2), Catherine Messner (1,2,3), Carine Gaiser (1), Carina Hämmerli (1) and Laura Suter-Dick (1,3)

(1) University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Muttenz, Switzerland

(2) University of Basel, Department of Pharmaceutical Sciences, Basel, Switzerland

(3) Swiss Centre for Applied Human Toxicology (SCAHT), Basel, Switzerland

Low-dose methotrexate (MTX) is a standard therapy for rheumatoid arthritis given its low cost and efficacy. Despite these benefits, MTX has been reported to cause chronic drug-induced liver injury, namely liver fibrosis. The hallmark of liver fibrosis is the excessive scarring of liver tissue, triggered by hepatocellular injury and subsequent activation of hepatic stellate cells (HSCs). However, little is known about the precise mechanisms through which MTX causes hepatocellular damage and activates HSCs. Here, we set off to investigate the mechanisms leading to hepatocyte injury in the human surrogate cell line HepaRG and used the immortalized stellate cells hTERT-HSCs to elucidate the mechanisms leading to HSC activation.

HepaRG and hTERT-HSC were cultured in mono- or co-cultures and subjected to varying concentrations of MTX with or without N-acetylcysteine (NAC) for 3 to 7 days. HepaRG monocultures were used to evaluate cell viability, gene expression of SLC19A1, protein expression of dihydrofolate reductase (DHFR), superoxide production in mitochondria, and mitochondrial respiration. Similarly, hTERT-HSC mono- or co-cultures with HepaRG, were used to study HSC activation by immunofluorescence, and endoplasmic reticulum (ER) stress by assessing gene expression levels of C/EBP homologous protein (CHOP).

MTX at human-relevant concentrations (7 – 30’000 nM) led to a non-dose-dependent de-crease of HepaRG viability, which plateaued at roughly 80%. The MTX-uptake transporter SLC19A1 and the drug target DHFR are expressed in HepaRG, and their expression was induced by MTX treatment. Superoxide formation was significantly increased, and spare respiratory capacity (SRC) significantly decreased when HepaRG were exposed to MTX at concentrations higher than 117 nM. Pre-treatment with NAC markedly reduced the formation of superoxide but failed to rescue the SRC. Activation of hTERT-HSC, represented by an increased expression of α-SMA stress fibers in the hTERT-HSC, was observed in mono- and co-cultures starting from 117 nM MTX. The ER-stress marker CHOP was highly elevated in both mono- and co-cultures above 117 nM, consistent with the activated hTERT-HSCs detected by immunofluorescence staining.

In conclusion, we were able to link hepatocellular damage caused by MTX to GSH-dependent and GSH-independent pathways. We further conclude that oxidative stress in HepaRG and ER stress in hTERT-HSC may play a role in MTX-induced activation of HSC. In addition, we demonstrated that HepaRG are a pharmacologically relevant *in vitro* model to evaluate MTX-induced hepatotoxicity.

Keywords

Poster 16 (selected for oral presentation)

Preparation and characterization of a benchmark data set for machine learning in ecotoxicology

Christoph Schür (1,2), Lilian Gasser (3), Jimeng Wu (2), Fernando Perez-Cruz (3), Kristin Schirmer (1,4,5), Marco Baity-Jesi (2)

(1) Department of Environmental Toxicology, Eawag, Swiss Federal Institute of Aquatic Science and Technology
(2) Department of Systems Analysis, Integrated Assessment and Modelling, Eawag, Swiss Federal Institute of Aquatic Science and Technology
(3) Swiss Data Science Center
(4) ETH Zürich: Department of Environmental Systems Science, Zurich, Switzerland
(5) EPF Lausanne, School of Architecture, Civil and Environmental Engineering, Lausanne, Switzerland

The global landscape of registered chemicals on the market is growing at a fast pace. Meanwhile, the regulation of compounds requires extensive animal testing to ensure their safety for both the public and the environment. Beyond its ethical implications, animal testing is labor-, cost-, and time-intensive. Here, new approach methods can be a remedy by reducing, if not ultimately replacing, conventional animal tests. An increase in computational power and accessibility of machine learning methods enable the use of in silico methods for ecotoxicological questions such as the hazard assessment of chemicals. However, the currently available literature on applying machine learning methods to aquatic ecotoxicology is usually based on data sets that are not clearly characterized, limited in species and chemical space. Likewise, the reproducibility of results is hindered through intransparent reporting on model performance metrics and the availability of data and code. Selecting informative features and interpreting modeling results in a meaningful way require ecotoxicological expertise. We see this as a hindrance for out-of-domain machine learning experts to apply their knowledge to ecotoxicological challenges. As a team of ecotoxicologists and machine learning experts, we, therefore, set out to develop an expertly curated, defined, and well-described ecotoxicological benchmark data set as a crucial component to furthering the use of in silico methods in our field. Our data set contains information on mortality-related endpoints in three distinct taxonomic groups (fish, crustaceans, algae) representative of the main trophic levels in the aquatic food web. We describe which components we deem suitable to enhance the predictive power of models trained on that data. We present insights and patterns that can be deduced from the raw data alone, such as species sensitivity distributions (SSD). Additionally, we discuss the challenges associated with adequate train-test-splitting of such data and propose concrete tasks to the community that could help answer pressing questions from our field. These include predictions across taxonomic groups and the identification of potential surrogate species.

Keywords:

Poster 17

Utilizing urolithin A as a representative compound to develop physiologically based pharmacokinetic models for gut microbial metabolites

Georg Aichinger, **Maja Stevanoska**, Shana J Sturla

Laboratory of Toxicology, D-HEST, ETH Zurich

The gut microbiome is emerging as a key contributor to xenobiotic metabolism, potentially impacting circulating levels of chemicals in the host. To quantitatively investigate the influence of the gut microbiome on chemical toxicity or bioactivity, the spotlight is on well-researched gut bacterial products that may serve as representatives for method development. This includes urolithin A (UA), a gut microbial metabolite of ellagitannins that is well-described for its beneficial effects on human health.

With the goal to develop and evaluate physiologically based pharmacokinetic (PBPK) models as an alternative to animal testing, we assessed the glucuronidation of UA in liver and small intestine S9. We included dissolution kinetics, built a PBPK model, and evaluated it against available data from human intervention studies. The model was used to predict tissue concentrations after realistic dosing scenarios to perform quantitative in vitro to in vivo extrapolation focusing on the beneficial effects of UA on the brain and muscle tissue, but also to determine potential toxicity in gut and gonad tissue.

As next steps, we are determining gut microbial metabolism kinetics using anaerobic fecal fermentation techniques coupled with liquid chromatography. This includes the assessment of interindividual differences in formation rates as well as in predominantly produced metabolites. The gut microbiome will be added to the PBPK model as a dedicated compartment of metabolism, and the interindividual variation in gut microbiome compositions will be included in the assessment. The generated methodology will be used as a blueprint for research on the quantitative assessment of the impact of gut metabolism on the pharmaco/toxicokinetics of different chemicals, particularly for endocrine active substances.

Keywords: microbial metabolism, pharmacokinetics, PBPK modeling, biotransformation

Poster 18

Fluorescence-based Quantification of Chemically Damaged DNA

Prosper N. Takam, NJL. Püllen, ES. Sandell, K. Schreier, NK. Singh, S. Diedrich, SJ. Sturla

Laboratory of Toxicology, Department of Health Sciences and Technology, ETH Zürich, Switzerland

Exposure to chemicals from food, drugs and the environment can increase the risk for diseases such as cancer or Alzheimer's. At a cellular level, these chemicals generate reactive intermediates that can covalently modify DNA, resulting in DNA damage. Measuring DNA damage arising from chemical exposures is central to characterizing its mutagenic potential and elucidating genotoxicity mechanisms. To detect and quantify DNA damage, there are several methods available such as 32P-postlabelling, immunoassay/immunohistochemistry and gas or liquid chromatography coupled with mass spectrometry. Mass spectrometry-based approaches are the gold standard but require expensive instrumentation and specific expertise. Therefore, there is a need for more accessible alternative methods.

To this end, we developed a rapid DNA damage quantification approach that works on the basis of ligating a fluorescent chemical reporter probe to sites of DNA modification. Initially tested in oligonucleotide DNA, the method could be used to accurately detect enzyme-induced single-strand breaks with concentrations of oligonucleotide DNA as low as 200 pM. We similarly processed genomic DNA and could detect native levels of common DNA lesions such as oxidative modifications, apurinic sites and single-strand breaks. Furthermore, we quantified abasic sites and oxidative modifications in chemically exposed human cells.

For the oxidizing agent potassium bromate, we detected a two-fold increase in oxidative damage for cells exposed to 50 mM potassium bromate compared to unexposed cells. For the anti-cancer drug irofulven, we could detect a five-fold increase in apurinic sites arising from alkylation-promoted depurination following exposure of a human cancer cell line to 15 μM drug. Fluorescence data were validated by parallel measurements with HPLC/MS.

As a result of this study, we have established a convenient and effective general method for the rapid quantification of common DNA damage products in the human genome using a fluorescence-based ligation strategy.

The method is anticipated to make DNA damage quantification more readily accessible for use in mechanistic toxicity studies, as a dosimeter for chemical exposure and risk assessment, and in precision medicine.

Keyword chemicals, oxidative stress, DNA damage, fluorescence, quantification

Poster 19

The natural-product anticancer drug ET-743 induces strand-specific DNA breaks in the transcribed regions and promoters of active genes

Vakil Takhaveev (1), Emma Dillier (1), Kook Son (2), Hobin Yu (2,3), Visesato Mor (2), Orlando D. Schärer (2,3), and Shana J. Sturla (1)

(1) Department of Health Sciences and Technology, ETH Zürich, Zürich 8092, Switzerland,

(2) Center for Genomic Integrity, Institute for Basic Science, Ulsan 44919, Republic of Korea,

(3) Department of Biological Sciences, Ulsan National Institute of Science and Technology, Ulsan 44919, Republic of Korea.

ET-743 (Trabectedin, Yondelis) is a clinically approved anticancer drug isolated from the sea squirt Ecteinascidia turbinata. ET-743 alkylates DNA, yet, paradoxically, it causes cytotoxicity more efficiently in cancer cells highly proficient in transcription-coupled nucleotide excision repair (TC-NER). In our recent work employing COMET Chip assays and knockouts of DNA repair genes, we discovered that ET-743 induces cytotoxic DNA breaks in a fashion dependent on the catalytic activity of ERCC1-XPF but independent from that of XPG. Therefore, we proposed a mechanistic model where ET-743 inhibits 3' incision by XPG and leads to the accumulation of breaks caused by 5' incisions by ERCC1-XPF. To further dissect this aspect of the mechanism of ET-743-induced cytotoxicity, we mapped with single-nucleotide resolution these persistent ERCC1-XPF-dependent ssDNA breaks induced by the drug via the method of genome-wide ligation of severed 3'-OH ends followed by sequencing (GLOE-Seq). We compared profiles of strand breaks in a TC-NER-proficient bone osteosarcoma cell line (U2OS), and corresponding TC-NER-deficient CSB knockout, global-genomic-NER-deficient XPC knockout and NER-deficient XPA knockout cells. We found that in the TC-NER-proficient backgrounds, ET-743-induced DNA breaks occur on transcribed strands of active genes, and that the number of breaks strongly correlates with the gene transcription level. Intriguingly, ET-743 was also found to induce DNA breaks in the promoter regions of active genes, however, in this case, on the non-transcribed strand. With these data, we provide genome-wide evidence for the transcription dependency of ET-743-induced DNA break formation and uncover novel elements in the puzzle of ET-743 cytotoxicity, which could advance precision medicine applications of this drug.

Keywords: ET-743, Trabectedin, DNA damage, sequencing, DNA repair

Poster 20

Selected endocrine disrupting chemicals exacerbate free fatty acid induced lipid accumulation in human liver cells

Manuel Tschan (1), Fabrice Müller (1,2), Laura Suter-Dick (1,2)

(1) University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Muttenz, Switzerland

(2) Swiss Centre for Applied Human Toxicology (SCAHT), Basel, Switzerland

Exposure to EDCs represents a risk factor for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD encompasses a spectrum of diseases ranging from liver steatosis to steatohepatitis which can progress to cirrhosis and hepatocellular carcinoma. Nuclear receptor (NR) activation by EDCs has been proposed as a molecular initiating event for liver steatosis. However, detailed mechanistic understanding of how EDCs lead to fat accumulation is lacking. In this study, we investigated the effects of DEHP, BPA and PCB-153 on viability and lipid metabolism using an *in vitro* human hepatocyte model (HepaRG). Liver toxicity of the EDCs was evaluated after 48 h and 7 d by measuring ATP-content and albumin release. Lipid accumulation in HepaRG cells was quantified using the lipophilic stain nile-red and image analysis. Lipid accumulation was tested at non-lethal EDC concentrations with or without a co-exposure to free fatty acids (FFAs). FFA co-treatment was used to simulate a diseased obese phenotype, to achieve this we added a 2:1 ratio of oleic acid and palmitic acid to the culture medium. To assess the activation of AHR and PXR as well as the expression of several genes involved in lipid metabolism, we conducted gene expression studies using RT-qPCR. Exposure to PCB-153 and BPA led to a decrease in ATP content and albumin release in HepaRG in a concentration- and time-dependent fashion. At the highest non-lethal concentration, PCB-153 increased lipid accumulation after 48 h. After 7 d all the tested EDCs significantly increased lipid accumulation at the highest concentration. Co-exposure of EDCs with FFA led to a synergistic effect on lipid accumulation. Gene expression analysis showed upregulation of DGAT2 and PCK1 in DEHP-treated cells after 7 d. The data shows that EDCs alter lipid metabolism in HepaRG. Further, we demonstrated that FFA and EDCs act synergistically on hepatic lipid accumulation. Gene expression analysis indicated that DEHP might exacerbate free fatty acid induced lipid accumulation by upregulation of the genes DGAT2 and PCK1. Future studies include testing EDCs in more complex 3D liver spheroid models to further investigate EDCs as potential risk factor for NAFLD.

Keywords: endocrine disrupting chemicals, liver, lipid accumulation, NAFLD

Poster 21

Consumers' views of natural cosmetic products and ingredients in light of marketing claims, toxicological properties and regulatory principles

Lisa Wiesner (1) and Angela Bearth (2)

(1) University of Geneva

(2) ETH Zurich, Consumer Behavior - Department Health Sciences and Technology

A paradigm shift in the usage of conventional consumer care products towards more natural cosmetic products is supported by the public promotion of ethical marketing claims and the increasing market revenue of the latter.

Together with health benefits, natural cosmetic products often advertise as "not being tested on animals" or "being good for humans and the environment." Consumer frequently equate "natural" with better, safer or healthier, which is not always the case, as natural ingredients express the same toxicity as chemically synthesized ingredients. Similarly, "not tested on animals" might also entail misconceptions or oversimplified notions of toxicological safety testing. Ingredients used in natural cosmetic products may have a history in a tiered animal testing strategy or new chemical entities – depending on their worldwide market usage – may undergo animal testing.

Our work investigated consumer views of natural cosmetic product claims, and the consumers' knowledge about toxicology, animal testing strategies and their willingness to accept safety testing alternatives via an online questionnaire.

Our results show an association between the participants' knowledge about toxicological principles and regulatory principles for animal testing, and their consumer choices. Trust in regulatory bodies and support for testing alternatives was related to consumer choice and knowledge in this specific field.

Overall, our results can (i) provide insights for unified labelling and advertisement claims on natural cosmetic products, (ii) make toxicology knowledge available to a broader audience, and (iii) support the efforts aimed to reduce conventional animal testing methods and raise awareness towards animal testing alternatives among the public.

Keywords: risk perception, natural cosmetics , animal testing, 3R safety

Poster 22 (Late submission)

Pharmacokinetic modeling of Bisphenol A analogs BPF and PBAF and their gastrointestinal metabolites behavior

Inga Potapova, Shana J Sturla, Georg Aichinger

Laboratory of Toxicology, D-HEST, ETH Zürich, LFO Schmelzbergstrasse 9

Physiologically-based pharmacokinetic (PBPK) modeling is a methodology to predict absorption, distribution, metabolism and excretion (ADME) of chemicals in an organism based on data obtained from computational tools and in vitro experimentation. Thus, it is a vital tool of worldwide effort to decrease the need for animal experiments. As a novel approach, the implementation of microbial metabolism in gut-competent PBPK models aims to link microbiology with quantitative pharmacokinetics.

Bisphenol A is a contaminant that is controversially discussed for their endocrine-disruptive potential. It is increasingly replaced by other bisphenols of which the toxicity, but also toxicokinetics are much less evaluated. Additionally, little is known of the gut microbiome's impact on bisphenol stability in the gut, and thus on ADME processes in general.

Thus, we refine an existing PBPK model for bisphenols A, F, AF and S. This human model was translated to the rat species by adjusting the physiology and remeasuring bisphenol glucuronidation kinetics in rat S9 fraction. It will be matched with in vivo data to accurately predict enterohepatic recirculation and gastrointestinal concentrations. After re-translating it to the human species, the PBPK model will be linked with microbial metabolism kinetics which are measured in anaerobic fermentation experiments, the influence of microbiome-catalyzed biotransformation reactions and possibly arising interindividual differences will be elucidated.

Keywords: