21ST INTERNATIONAL CONGRESS

ESTIV

ABSTRACT BOOK

BARCELONA 2022

21 - 25 NOVEMBER

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WELCOME ADDRESS

Dear delegates,

The long-awaited 21st International Congress of the European Society of Toxicology In Vitro (ESTIV 2022) is happening in Barcelona-Sitges, Spain, from 21st to 25th November 2022. The 21st ESTIV congress had to be postponed from 2020 to 2022 due to public health concerns and limitations in international travels. The ESTIV Board also took into consideration the postponement of the World Congress on Alternatives from 2020 to 2021 when deciding to move the ESTIV congress to 2022.

The Organising and the Scientific Committees of ESTIV 2022 took very seriously all the organisational and programme commitments made before the postponement of the congress. To make the congress even more attractive and to reflect on the developing science, the Scientific Committee decided to implement for the first time parallel sessions, allowing for the inclusion of several emerging topics, such as the role of in vitro and in silico approaches in COVID-19 research, Organ-on-a-chip & Microphysiology systems and Developmental Neurotoxicity (DNT) research.

The ESTIV Board and the Scientific Committee are grateful to all Session chairs and abstract reviewers for their excellent evaluations of the submitted abstracts and for ensuring a very high quality and high standards of the scientific programme.

Last, but certainly not least, the ESTIV Board cordially thanks all authors, who submitted their scientific works to the ESTIV 2022 Congress.

The Congress Committees of the ESTIV 2022 warmly welcome you at the 21st ESTIV Congress in Barcelona-Sitges and look forward to see so many colleagues working in the area of in vitro and in silico toxicology finally reuniting!

Helena Kandarova and João Barroso

Acknowledgements

The ESTIV 2022 congress would not have been possible without the logistic assistance of the conference organiser Klinkhamer Group | conferences & events as well as the financial support of the ESTIV supporters and sponsors.

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KEYNOTE AND INVITED SPEAKERS

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Monday, November 21st 2022
14:20 - 15:10

Session: 4a. Computational toxicology – in silico modelling, read-across, artificial intelligence and machine learning

KEYNOTE:
Use of Artificial Intelligence and Machine Learning in Chemical Risk Assessment
ABSTRACT #459

Timothy Allen, PhD

Tim Allen is a Research Associate at the MRC Toxicology Unit, University of Cambridge. He completed his PhD in 2016 on Molecular Initiating Events (MIEs) and how computational methods can be used to predict them in the group of Professor Jonathan Goodman at the Department of Chemistry in Cambridge. Since then he has undertaken post-doctoral work in the Department of Chemistry in Cambridge and at the United States Environmental Protection Agency in North Carolina. This has included work using quantum chemistry density functional theory calculations to predict the mutagenicity of alpha-beta unsaturated carbonyls and understand their reactions with DNA, using 3D Quantitative Structure Activity Relationships to quantitatively predict MIEs and explore the chemical-biological interactions in several cases, and developing in-house computational tools for use by his industrial partners at Unilever's Safety and Environmental Assurance Centre for use in safety decision making. Tim has also served as a member of ILSI Europe's expert group on the application of Adverse Outcome Pathways (AOPs) in food ingredient risk assessment and has presented his research at over 20 national and international conferences. In 2019 Tim moved to the MRC Toxicology Unit to continue his work in predictive toxicology, including new investigations into how we can use and understand state-of-the-art machine learning approaches such as deep learning neural networks.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Monday, November 21st 2022
16:30 - 18:30

Session: 1. Bio-engineering, stem cells and disease models

INVITED LECTURE:
Recapitulating Complex Human Tissues using Organ-on-chip and Organoid Technologies
ABSTRACT #499

Prof. Peter Loskill, PhD

Peter Loskill is W3-Professor for Organ-on-Chip Research at the Eberhard Karls University Tübingen and the Natural and Medical Sciences Institute (NMI) as well as Chair of the European-Organ-on-Chip-Society (EUROoCS). He graduated in 2012 from Saarland University with a PhD in Physics and thereafter worked as a postdoctoral fellow at UC Berkeley. In 2015, he was named as one of Technology Review's "Innovators under 35 Germany" and awarded a Fraunhofer ATTRACT starting grant. He now heads the µOrgano-Lab and the 3R Center Tübingen for In vitro Models and Alternatives to Animal Testing. The interdisciplinary µOrgano-Lab (https://www.organ-on-chip.uni-tuebingen.de) combines approaches from engineering, biology, physics and medicine to generate and apply novel microphysiological tissue models recapitulating complex human biology in vitro.

The 3R Center Tübingen (https://www.the3rs.uni-tuebingen.de) aims to provide all scientists in Baden-Württemberg with low-threshold access to novel alternative methods to animal testing.
Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Tuesday, November 22nd 2022 08:30 - 10:30

Session: 2a. Models, biomarkers and assays for endocrine disruption and developmental toxicity

INVITED LECTURE: Computational systems models for human-predictive developmental toxicity ABSTRACT #160

Dr. Thomas Knudsen

Thomas Knudsen is a Developmental Systems Biologist at the US EPA National Center for Computational Toxicology and Exposure, where he is a lead in the Virtual Tissue Models project. His research on prenatal developmental toxicity and systems biology has led to over 150 scientific papers. Current research is focused on building and testing a 'virtual embryo' framework for predictive modeling of developmental toxicity. This entails integration of in-vitro data from HTS (high-throughput screening) profiling in ToxCast/Tox21 with biological knowledge of in-vivo embryology and in-silico cell agent-based models for synthetic reconstructing morphogenesis, leading to a quantitative prediction of chemical dysmorphogenesis. Dr. Knudsen is a Past-President of the Teratology Society, Former Editor-in-Chief of Reproductive Toxicology, and currently serves as Editor-in-Chief of Current Research in Toxicology.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Tuesday, November 22nd 2022 11:00 - 13:00

Session: 3a. Models, biomarkers and assays for systemic and immune toxicity

INVITED LECTURE: New approach methodologies in immunotoxicology with a focus on immunosuppression ABSTRACT #465

Prof. Emanuela Corsini, PhD

Emanuela Corsini is a tenured full professor in toxicology at the School of Pharmacy at the Università degli Studi di Milano, Milan, Italy. Her research focuses on the refinement of alternative in vitro tests for immunotoxicity, promoting the regulatory acceptance of alternative methods, and understanding the mechanism of action of immunotoxic/immunomodulatory compounds at the molecular level. Dr. Corsini has served on multiple ECVAM and ICCVAM Panels and Working groups to establish scientific confidence in alternative methods in immunotoxicology testing, performance standards for these novel assays, and the development of integrated testing strategies for their use as part of comprehensive and predictive assessments. She has authored over 190 research publications in toxicology and related disciplines. She is active in numerous scientific and professional organizations and serves on several editorial boards of toxicology journals.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Tuesday, November 22nd 2022
14:00 - 16:00

Session: 4a. Computational toxicology – in silico modelling, read-across, artificial intelligence and machine learning

INVITED LECTURE:
Bioinformatics and Network science applied in toxicology
ABSTRACT #463

Prof. Olivier Taboureau

Olivier Taboureau is a Professor at Université Paris Cité, Faculty of Science, SDV department, Sciences du Vivant conducting research at the BFA laboratory, Unité de Biologie Fonctionnelle et Adaptative, CNRS UMR 8251. The BFA unit conducts research in Integrative Biology. Research topics in BFA try to understand the biological mechanisms underlying human adaptation to environmental and/or internal perturbations (endocrine, metabolic or genetic inputs) in physiological or pathophysiological

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Tuesday, November 22nd 2022
14:00 - 16:00

Session: 4a. Computational toxicology – in silico modelling, read-across, artificial intelligence and machine learning

INVITED LECTURE:
The key role of computational toxicology for Toxicology for the 21st Century 2.0
ABSTRACT #460

Prof. Thomas Hartung

Thomas Hartung, MD PhD, is the Doerenkamp-Zbinden-Chair for Evidence-based Toxicology with a joint appointment for Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health, Baltimore. He holds a joint appointment as Professor for Pharmacology and Toxicology at University of Konstanz, Germany; he also is Director of Centers for Alternatives to Animal Testing (CAAT, http://caat.jhsph.edu) of both universities with the portal AltWeb (http://altweb.jhsph.edu). CAAT hosts the secretariat of the Evidence-based Toxicology Collaboration (http://www.ebtox.com), the Good Read-Across Practice Collaboration, the Good Cell Culture Practice Collaboration, the Green Toxicology Collaboration, and the Industry Refinement Working Group. As PI, he heads the Human Toxome project (http://humantoxome.com), funded as an NIH Transformative Research Grant. He is the former Head of the European Commission's Center for the Validation of Alternative Methods (ECVAM), Ispra, Italy, and has authored more than 490 scientific publications.
Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Wednesday, November 23rd 2022 08:30 - 10:30

Session: 5a. Toxicokinetics and in vitro – in vivo extrapolation

INVITED LECTURE:
An Open, Accessible Interface for Contextualizing Chemical Effects
ABSTRACT #464

Nicole C. Kleinstreuer, PhD

Nicole Kleinstreuer is the acting director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), leading domestic and international efforts to develop novel testing and analysis strategies that provide more rapid, mechanistic, and human-relevant predictions of potential environmental chemical hazards. She has a secondary appointment in the NIEHS Division of Intramural Research Biostatistics and Computational Biology Branch, and adjunct faculty positions in the Yale School of Public Health and the Eshelman School of Pharmacy at UNC-CH. Kleinstreuer's research focuses on mathematical and computational modeling of biological systems and their susceptibility to perturbations that result in adverse health outcomes. She is the recipient of numerous prestigious awards including the 2019 Society of Toxicology Achievement Award.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Wednesday, November 23rd 2022 11:00 - 13:00

Session: 6a. In vitro methods for safety testing of biopharmaceuticals/biotherapies/vaccines

INVITED LECTURE:
In vitro assays to predict retroviral vector-induced genotoxicity
ABSTRACT #461

Dr. Michael Rothe

Michael Rothe is a lead scientist at the Hannover Medical School's Institute of Experimental Hematology in Hannover, Germany. He is a biologist by training and focuses on the safety assessment of retroviral vectors that are used in gene therapy. Many inherited diseases can be treated with gene modification of hematopoietic stem cells, but in early clinical trials, several patients suffered from retroviral vector-induced leukemia. The risk for malignant mutagenesis due to gene insertion can be addressed by viral integration site analysis in animal models, but the predictive value of these studies is limited and the burden to the animal is not justified when an alternative exists. The In Vitro IMmortalization assay (IVIM), developed at the Hannover Medical School (MHH) in 2006, is a powerful in vitro test and has become a de facto gold standard assay for retroviral vector safety analysis and is considered a crucial element of an investigational new drug or clinical trial application by regulatory agencies (EMA, FDA, TGA, Health Canada). IVIM still has limitations associated with the use of myeloid cytokines and sensitivity. To overcome these limitations, Dr. Rothe and his team has developed the Surrogate Assay for Genotoxicity Assessment (SAGA). SAGA determines the risk of vector mutagenicity based on machine learning techniques applied to gene expression data. Currently, Dr. Rothe is working on an all-in-one safety test that detects insertional mutants, also

from the lymphoid lineage. This work is conducted within the framework of the R2N (https://r2n.eu/ – Replace and Reduce in Lower Saxony, Germany), a consortium devoted to developing alternative methods to replace and reduce animal models in biomedical research.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

Thursday, November 24th 2022
11:00 - 12:40

Session: 8a. In vitro methods for safety assessment of medical devices

INVITED LECTURE:
ISO 10993 SERIES: ADDED VALUES OF 3D MODELS FOR BIOCOMPATIBILITY MEDICAL DEVICES
ABSTRACT #451

Christian Pellevoisin, PhD, ERT

Christian Pellevoisin is a toxicologist and scientific director at Mattek. During his career, he has been involved in numerous projects that have led to the introduction of new reconstructed human tissue models and in vitro methods for evaluating the safety and efficacy of products in different industries, including cosmetics, medical devices and drugs. As chairman of the French AFNOR commission for biocompatibility of medical devices and convenor of working group 8 of the ISO technical committee 194, he participated in and conducted projects that led to the publication of ISO standards 10993-23 for irritation and 10993-10 for skin sensitization. Strongly committed to the recognition of alternative methods to animal experimentation, he is involved in several university courses and organizes practical training for the implementation of validated methods based on the use of reconstructed human tissues.

Please find the abstract in the specific session in ALL ORAL PRESENTATIONS.

ORAL PRESENTATIONS

ALL ORAL PRESENTATIONS

Monday, November 21st 2022
16:30 - 18:30

Session: 1. Bio-engineering, stem cells and disease models

Chairs: *Peter Loskill (University of Tübingen, Germany) & João Barroso (JRC, Ispra, Italy - ESTIV Vice-president)*

O-1-1 (INVITED TALK)

Recapitulating Complex Human Tissues using Organ-on-chip and Organoid Technologies
ABSTRACT #499

Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem cells (hiPSC) has led to the emergence of a new paradigm of drug screening using human patient- and disease-specific organ/tissue-models. One promising approach to generate these models is by combining the hiPSC technology with microfluidic devices tailored to create physiological microenvironments and recapitulate 3D tissue structure and function. Such Organ-on-Chip (OoC) platforms or microphysiological systems (MPS) combine human genetic background, physiological tissue structure and functionality, and "vasculature-like" perfusion. Using microfabrication techniques, we have developed a variety of OoCs that incorporate complex human 3D tissues and keep them viable and functional over multiple weeks, including "Tumor-on-chip", "Retina-on-chip", "Choroid-on-chip", "Heart-on-chip", "Pancreas-on-chip and a "White adipose tissue(WAT)-on-chip". The OoCs generally consist of three functional components: organ-specific tissue chambers mimicking in vivo structure and microenvironment of the respective tissues; "vasculature-like" media channels enabling a precise and computationally predictable delivery of soluble compounds (nutrients, drugs, hormones); "endothelial-like" barriers protecting the tissues from shear forces while allowing diffusive and active transport processes. The small scale and accessibility for in situ analysis make our OoCs amenable for both massive parallelization and integration into a high-content-screening approach. The adoption of OoCs in industrial and non-specialized laboratories, moreover, requires enabling technologies that are user-friendly and compatible with automated workflows. We have developed tailored platforms for automation of tissue generation and culture as well as for the flexible plug&play connection of individual OoCs into multi-organ-chips. These complex human-relevant models and enabling technologies put the research community now in a position to provide answers to questions that animal models have not been able to address and to explore new paradigms in drug development, personalized medicine, toxicity screening, and mechanistic research.

References

J. Rogal, K. Schlünder, & P. Loskill, "Developer's Guide to an Organ-on-Chip Model", ACS Biomater. Sci. Eng., 2022, DOI: 10.1021/acsbiomaterials.1c01536 P. Loskill, R. Hardwick, & A. Roth, "Challenging the pipeline", Stem Cell Rep., 2021, 16, 2033-2037, DOI:10.1016/j.stemcr.2021.08.004

O-1-2

iPSC-derived Brain Organoids as versatile research tool for Developmental Neurotoxicity and Neurological disorders
ABSTRACT #48

Lena Smirnova[1], Xiali Zhong[1], Sergio Modafferi[1], Thomas Hartung[1], Carolina Romero[1], Jesse Plotkin[12], Caroline Krall[1], Alan Kim[1]

[1]*Johns Hopkins University*
[2]*CCDC Chemical Biological Center, Edgewood, USA*

Exposure to environmental chemicals during early life is suspected to contribute to the increasing incidence of neurodevelopmental disorders, especially autism. Currently, 1 in 49 children in US is diagnosed with autism spectrum disorders (ASD), and this cannot be explained only by the genetics, suggesting that environmental exposures contribute. Demanding animal tests for Developmental Neurotoxicity (DNT) have been devised, but because of complex underlying mechanisms, limitations of those current approaches are enormous. Several tests covering different key events of neural development have been developed over the last decade. The main goal of our research is to develop a testing strategy for DNT, based on our human 3D iPSC-derived brain model, for a DNT IATA (Integrated Approaches to Testing and Assessment). Our iPSC-derived brain organoid model covers many key events of neural development, which allows multiplexing of several endpoints in one assay. We

have been developing an assay using high-content imaging and CRISPR/Cas9 knock-in fluorescent tags for neural markers (6-in-1 BrainSphere assay or mini brainbow). In addition, we introduced the microglia, immune cells of the brain, to make our brain organoids immunocompetent. iPSC used for organoids allow to study molecular mechanisms of neurological disorders by using patient-derived or genetically modified cells. To study gene environmental interactions (GxE) in autism we used CRISPR/Cas9 modified iPSC with mutation in autism risk gene CHD8. We found a synergy in miRNA and metabolic biomarkers between CHD8 mutation and the organophosphate pesticide chlorpyrifos. Taking together, brain organoids represent a versatile tool for mechanistic understanding of diseases, studying GxE and screening chemicals.

References

Wang, P., Lin, M., Pedrosa, E., Hrabovsky, A., Zhang, Z., Guo, W., et al. (2015). CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. Molecular Autism, 6(1), 1. Pamies D, Barreras P, Block K, Makri G, Kumar A, Wiersma D, Smirnova L, Zang C, Bressler J, Christian KM, Harris G, Ming GL, Berlinicke CJ, Kyro K, Song H, Pardo CA, Hartung T, Hogberg HT. A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. ALTEX. 2017;34(3):362-376. Modafferi S, Zhong X, Kleensang A, Murata Y, Fagiani F, Pamies D, Hogberg HT, Calabrese V, Lachman H, Hartung T and Smirnova L. Gene environment interactions in developmental neurotoxicity - a case study of synergy between chlorpyrifos and CHD8 knockout in human BrainSpheres. EHP, 2021, 129:77001. Doi: 10.1289/EHP8580

O-1-3

Evaluation of 3D-Bioprinted Human Skin Equivalents for In Vitro Permeation Testing ABSTRACT #312

Alec T. Salminen[1], Kristy Derr[2], Kelly J Davis[3], Linda S VonTungeln[1], Marc Ferrer[2], Suzanne C Fitzpatrick[4], Linda M Katz[4], Prashiela Manga[4], Nakissa Sadrieh[5], Paul C Brown[5], Menghang Xia[2], Nicole Kleinstreuer[6], Luísa Camacho[1]

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[6]*NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA*

Background and Objectives Predicting dermal absorption of topical drugs and cosmetic ingredients constitutes a necessary step in safety assessment. In vitro permeation testing (IVPT) with excised human and animal skin has been employed extensively for basic science and regulatory research. While excised human skin remains the 'gold standard' for IVPT studies, unreliable supply and high cost continue to motivate the push for alternative skin models for such applications. Materials and Methods Full thickness skin equivalents (FTS) were constructed in a layer-by-layer fashion using a volumetric plunger dispensing-based bioprinter. Gel-embedded human dermal fibroblasts support human epidermal keratinocyte stratification in an air-liquid culture. Matured FTS were directly mounted on static Franz diffusion cells (0.2 cm2 application area) for IVPT. The permeation of prototypic cosmetic ingredients and topical drugs, including the OECD-reference caffeine, was quantified. A commercially available reconstructed human epidermis (RHE) model, an artificial barrier membrane, and dermatomed excised human skin were tested in an identical fashion in parallel. Transepithelial water loss and histology of the models were also compared. Results Bioprinted FTS exhibit native skin-like morphology, including a characteristic stratified epidermis containing a robust stratum corneum. Cumulative permeation of a finite dose of caffeine, 6 h after application, was quantified and resulted in the following rank of barriers: Artificial membrane (least caffeine permeation observed) > Excised human skin >> RHE > Bioprinted FTS. Discussion and Conclusion As observed herein and elsewhere, excised human skin is a substantial barrier to caffeine. Reconstructed biological skin models remain limited in their potential to replicate directly this physiology. Despite such results, alternative

skin models benefit from low cost, sustained viability, and the potential for customization, giving them select advantages over excised skin tissue. Continued improvement, coupled with systematic evaluation, of alternative skin models could lead to their adoption for IVPT in regulatory science.

O-1-4

iPSC-derived hepatic stellate cells for in vitro fibrosis modelling
ABSTRACT #450

Raquel A. Martinez Garcia de la Torre[1], Mikel Azkargorta[2], Félix Elortza[2], Juan José Lozano[3], Pau Sancho-Bru[13]

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[2]*Proteomics Platform, CIC bioGUNE, Basque Research and Technology Alliance (BRTA), CIBERehd, ProteoRed-ISCII, 48160 Derio, Bizkaia, Spain*
[3]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain*

Introduction: Upon liver injury and toxicity, activated Hepatic Stellate cells secrete proinflammatory and profibrogenic cytokines and increase the production of ECM components, becoming the main fibrogenitc cell type. By sequential addition of factors, we have generated functional human HSCs from Induced Pluripotent Stem Cells (iPSC-HSC). Here we performed a proteomic analysis to understand how the differentiation occurs and to uncover the pathways involved in liver fibrosis. Methods: Human iPSC-HSC were obtained. Samples were processed every two days for MS-proteome analysis and human primary HSCs were used as control. iPSC-HSCs were tested in vitro in 2D and in 3D spheroids with HepG2. qPCR analysis was used to evaluate the response. Results: The sequential proteomic profiling of the differentiation showed a reduction of pluripotent markers (RIF1, POU5F1) and an increase in HSC markers (LUM, PTN, COL1A1 and MMP2). Comparison of the proteomic profile of iPSC-HSC with primary HSCs revealed that signalling pathways involved in HSC activation (TGFb, PDGFR) were expressed at middle stage of the differentiation.This analysis revealed RORA as an important transcription factor involved in HSC differentiation and activation. Experimentally, we found that the addition of RORA agonist SR1078 along the differentiation increased the expression of HSC markers such as RELN, LRAT and LHX2. While the stimulation of iPSC-HSCs with the agonist decreased the expression of ACTA2 and Col1A1 and increased the expression of quiescent markers LRAT and LHX2, the stimulation with the RORA antagonist promoted HSCs expression of fibrogenic markers. Moreover, the treatment of 3D iPSC-HSC/HepG2 spheroids with a hepatotoxic agent as thioacetamide (TAA) increased the activation state of iPSC-HSCs that was reversed with RORA agonist treatment. Conclusion: The present study demonstrates that the differentiation protocol is a unique tool to explore the mechanisms of human fibrosis, and to test in vitro the effect of antifibrogenic drugs.

O-1-5

TRANSPORT OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) ACROSS HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC) DERIVED INTESTINAL EPITHELIAL CELLS IN COMPARISON WITH HUMAN PRIMARY INTESTINAL EPITHELIAL CELLS AND CACO-2 CELLS
ABSTRACT #409

Aafke Janssen[1], Loes Duivenvoorde[1], Nicole Pinckaers[1], Bart van der Hee[2], Annelies Noorlander[1], Liz Leenders[1], Jochem Louisse[1], Meike van der Zande[1]

[1]*Wageningen Food Safety Research*
[2]*Department of Animal Sciences, Wageningen University*

Background and Objectives: Humans can be exposed to PFAS via many exposure routes, including diet, which may lead to several adverse health effects. So far, little is known about PFAS transport across the human intestinal barrier. The Caco-2 model is the most widely applied and accepted in vitro model to study intestinal transport. This model only consists of enterocytes, has high transepithelial electrical resistance values, and lacks a mucus layer, and may therefore not adequately represent human physiology. Human induced pluripotent stem cell (hiPSC)-derived intestinal epithelial cell (IEC) layers are monolayers with tight junctions and a mucus layer and they consist of enterocytes, goblet cells, enteroendocrine cells and Paneth cells that are also present in native tissue. They may represent a more physiologically-relevant model for transport studies, but so far limited data are available on transport of chemicals across these cells. Material and Methods: hiPSCs were differentiated into

hindgut endoderm, intestinal stem cells and subsequently into IEC monolayers. In the resulting hiPSC-derived IEC monolayers, transport of 5 PFAS (PFOS, PFOA, PFNA, PFHxS, HFPO-DA) was studied and compared with transport across Caco-2 cells, and a human primary IEC-based model. Results: The Papp values of PFAS transport in the hiPSC-derived IEC model were in the following order: PFHxS>PFOA>PFNA>HFPO-DA>PFOS. Although the order of PFAS transport was highly similar between the models, Papp values were highest for hiPSC-derived IECs and lowest for Caco-2 cells. Interestingly, HFPO-DA transport varied between the three models with highest transport in the hiPSC-derived IEC model and little to no transport in the other models. Overall, PFHxS transport was highest in all models and HFPO-DA transport was rather limited. Discussion and Conclusion: The hiPSC-derived IEC model is a promising novel in vitro model to study transport of chemicals across the intestinal barrier, showing slight differences with the conventional Caco-2 model.

Tuesday, November 22nd 2022
08:30 - 10:30

Session: 2a. Models, biomarkers and assays for endocrine disruption and developmental toxicity

Chairs: *Thomas Knudsen (US EPA, Durman, USA) & Arno Gutleb (LIST, Luxembourg, Luxembourg)*

O-2A-1 (INVITED TALK)

Computational systems models for human-predictive developmental toxicity
ABSTRACT #160
Thomas Knudsen[1]

[1]*USEPA*

Assessing developmental toxicity has a critical role in environmental health policy. New approach methodologies (NAMs) that enable in vitro profiling aim to quickly evaluate the human toxicity potential of thousands of chemicals with less reliance on animal testing. This comes with the need for computational models to translate data into toxicological prediction. A ToxCast platform using a metabolic biomarker-based pluripotent human (H9) stem cell assay (ToxCast_STM) identified a signal for developmental toxicity in 183 of 1062 chemicals [1]. Performance-based models classified the potential for developmental toxicity with balanced accuracies (BAC) of up to 84% for 42 well-characterized reference compounds. Although highly specific, the dataset showed weaker sensitivity where developmental toxicity was less concordant between rat-rabbit fetal outcomes, coincided with adverse effects on the pregnant mother, or were missed due to limited biological coverage of the assay. Here, we used recursive partitioning against the broader ToxCast assay portfolio evaluated 432 chemicals having developmental toxicity data in pregnant rats and/or rabbits. We built a training model with 183 well-qualified developmental toxicants and non-toxicants (BAC 78%), then tested it against an out-of-sample set of 249 chemicals where developmental toxicity was concurrent with maternal toxicity and less concordant in rat-rabbit studies. ToxCast_STM emerged as the strongest predictor in the training set and, together with several additional ToxCast assay features classified, with high confidence, 143 of the 249 out-of-sample test chemicals. While these findings support the use of in vitro data-driven models to confidently predict developmental toxicity across a structurally diverse landscape of chemicals in ToxCast, cell-oriented computational systems models with sufficient intelligence of embryology to quantitatively simulate morphodynamics are being evaluated to augment the in vitro bioactivity profiles [2,3]. Case examples will be demonstrated with disruption of the retinoid signaling pathway. This abstract does not reflect EPA policy.

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Sky Workshop. Reg Tox Pharm 114: 104668.

O-2A-2

Upcoming validated mechanistic in vitro methods for the identification of thyroid disruptors.

ABSTRACT #120

David Asturiol, Ingrid Langezaal, Joanna Bartnicka, Camilla Bernasconi, Ann-Charlotte Bostroem, Gerard Bowe, Sandra Coecke, Elise Grignard, Marlies Halder, Aude Kienzler, Roman Liska, Anne Milcamps, Sharon Munn, Amalia Muñoz Pineiro, Francesca Pistollato, Anna Price, Jukka Sund

The currently available tests for regulatory assessment of endocrine properties of chemicals available in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals are mainly in vivo, and do not offer much information on the chemical's mode-of-action. The European Commission published scientific criteria for the identification of endocrine disrupters under the Biocidal Products and Plant Protection Products Regulations, respectively in November 2017 and April 2018, which include the use of mechanistic information. Thus, the development of mechanistic methods as well as Adverse Outcome Pathways is needed in order to identify whether a chemical produces an adverse effect via an endocrine mode of action. This requirement for mechanistic assays is facilitating the introduction and use of in vitro and in silico mechanistic-based New Approach Methodologies (NAMs) into regulatory chemical safety assessments. The validation by EURL ECVAM of in vitro methods to study the various thyroid disrupting modes-of-action contributes to the availability of such mechanistic methods. The thyroid validation study was performed together with the European Union Network of Validation Laboratories (EU-NETVAL). It contains methods relevant for: - Thyrotropin Releasing Hormone receptor (in)activation - Thyrotropin Stimulating Hormone receptor (in)activation - Thyroid hormone receptor (in)activation - Thyroid Perioxidase inhibition - Thyrosine iodination inhibition - Sodium Iodide Symporter inhibition - Human serum protein binding - Deiodination inhibition - Glucuronididation inhibition - Sulfation inhibition - Monocarboxylate Transporter 8 inhibition The achievements during 2-3 years of experimental assessment to demonstrate reproducibility and reliability of the methods will be presented, including overall results obtained from the implementation, the description of the thyroid methods in SOPs and the testing of 30 blind-coded chemicals. Challenges encountered and lessons learnt during the different steps of the validation study, from selection of the methods from scientific literature, to reporting of experimental results and next steps towards regulatory acceptance will be shared with the audience.

O-2A-3

Interlaboratory OECD Validation of the Rapid Androgen Disruption Adverse outcome Reporter (RADAR) assay

ABSTRACT #129

Andrew Tindall[1], Sarah Bancel[1], Mathe Chabanon[1], Ioanna Katsiadaki[2], Marion Sebire[2], Azora König[3], Elke Eilebrecht[3], Lena Kosak[3], Jessica Rieder[4], Helmut Segner[4], Tetsuro Okamura[5], Maki Sakurai[5], Gregory Lemkine[1]

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In the past ten years, increasing interest has been paid to the identfication of chemicals interefering with the normal functioning of the androgen axis. Two key in vitro studies identified 66/200 and 37/134 pesticides tested as anti-androgenic. However, the effects of many of these pesticides have yet to be confirmed in vivo due to the absence of medium throughput in vivo assays. With this in mind, we developed a transgenic medaka line capable of revealing the level of activity of the androgen axis by emmision of green fluorescence. This line harbours a portion of the spiggin1 gene promoter upstream of GFP coding sequence. The spiggin1-GFP line has been demonstrated to respond specifically to androgens and to correctly identify pro-androgenic and anti-androgenic chemicals acting via a variety of modes of action. The sensitivity of the line is similar to that of the 21-day androgenised female stickleback screen. Using eleuthero-embryonic life stages, which are non-compliant with the EU definition of a laboratory animal, we developed the 72 h Rapid Androgen

Disruption Adverse outcome Reporter (RADAR) assay which is carried out in six-well plates. This assay is currently under OECD validation as a potential test guideline. In an OECD interlaboratory validation study, the RADAR assay was performed in five different laboratories in France, the UK, Switzerland, Germany and Japan. The results were coherent between each of the partner laboratories and showed a similar sensitivity. The transferability of the RADAR assay has been confirmed as well as its ability to be read using a variety of different fluorescence imaging systems. The reproducibility of the assay has also been demonstrated with a high level of coherence between the results from the different partners. Evaluation of the results by the OECD will indicate the next steps towards the possible publication as an OECD test guideline.

O-2A-4

Validation of a New Approach Method (NAM) testing DIO1 inhibition using a human microsome based, colorimetric assay

ABSTRACT #289

Andreas Weber[1], Barbara Birk[1], Chantal Herrmann[1], Hans-Albrecht Huener[1], Nina Hambruch[1], Kostja Renko[2], Sandra Coecke[3], Steffen Schneider[1], Dorothee Funk-Weyer[1], Robert Landsiedel[1]

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Impairment of thyroid hormone signaling has been associated with several adverse effects. Regulatory requirements are increasing to identify different mode of actions (MoA) impacting thyroid hormone (TH) signaling pathways. The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) is coordinating the validation of multiple in vitro methods focusing on different thyroid MoA by cooperating with a network of EU laboratories (EU-NETVAL). Deiodinases (DIO) are local regulators of TH action by activating or inactivating TH via deiodination. DIO1, one of the three human isoforms, is mainly expressed in thyroid, liver, and kidney tissue. It serves as one main source for circulating T3 via deiodination of T4 and plays a role in recycling of iodide via deiodination of inactive TH metabolites. A non-radioactive approach to determine DIO1 inhibition based on enzymatic activity in human liver microsomes was transferred to our laboratory [1] and further developed. The released iodide was quantified via the Sandell-Kolthoff (SK) reaction. 40 blinded items were assessed to test the predictivity of the method. 22 test items were de-blinded and compared to available in vitro data. Additional testing strategies were implemented to show specificity of the observed DIO1 inhibition. Reproducibility is given for tested controls with IC50 values in range of literature (e.g., IC50: 6-Propyl-2-thiouracil: 3.8 µM in this study, compared to [2]: 5.4 µM). High concordance with in vitro data obtained in recombinant enzyme [2] is given for the unblinded substances. The DIO1 inhibition assay using human liver microsomes is a robust and reproducible in vitro assay to assess potential DIO1 inhibition of chemicals with high concordance with known in vitro data. Additional tests on specificity improve the data assessment Finally, integrative testing strategies considering other thyroid related MoA are needed to assess the biological relevance of the assay.

References
[1] https://doi.org/10.1210/en.2011-1863 [2] https://doi.org/10.1093/toxsci/kfy302

O-2A-5

Immunological implications of Endocrine Disrupting Chemicals (EDCs): RACK1 as a bridge between the endocrine and the immune systems

ABSTRACT #337

Erica Buoso[12], Mirco Masi[13], Valentina Galbiati[4], Ambra Maddalon[4], Martina Iulini[4], Marina Marinovich[5], Marco Racchi[1], Emanuela Corsini[4]

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[4]*Laboratory of Toxicology, Dept. of Environmental and Political Sciences, University of Milan, Italy*
[5]*Dept. of Pharmacological and Biomolecular Sciences (DISFeB), Section of Toxicology and Risk Assessment, University of Milan, Italy*

Background and Objectives: RACK1 plays a central role in the immune system after the strong correlation demonstrated between its expression and immune cells activation, resulting in the modulation of pro-inflammatory cytokines [1,2].

Because of the presence of an androgens and glucocorticoids responsive element in RACK1 gene promoter, we hypothesized that EDCs can affect the immune response via RACK1 modulation. Since EDCs represent a public health issue, research efforts want to identify molecular biomarkers and methods to predict their immunotoxicity. The aim of our study is to assess how EDCs interfere with the immune response by modulating RACK1 expression and to elucidate the mechanisms behind their immunological implications. Material and Methods: To investigate EDCs effects on RACK1 expression, THP-1 cells and PMBCs were treated with increasing concentrations of anti-androgen p,p'DDT, p,p'DDE, vinclozolin, atrazine and cypermethrin, estrogen-active compounds 17β-estradiol, 17β-estradiol-BSA, diethylstilbestrol, zearalenone and ethynyl-estradiol and, finally, perfluorooctanesulfonic acid, diethyl-phthalate, bisphenols A, AF and S, mifepristone, flutamide, BAY 11-7082 (NF-κB inhibitor), GPER agonist (G1) and antagonist (G15). Luciferase reporter assay, qPCR, western blot analysis, specific sandwich ELISA, flow cytometric analysis and in silico molecular docking simulation were performed. Results: All the EDCs acting as AR antagonists, perfluorooctanesulfonic acid and diethyl-phthalate as GR agonists induced a significant decrease in RACK1 expression, LPS-induced IL-8 and TNF-α production and CD86 expression whereas the estrogen-active compounds showed the opposite effect through GPER activation. Finally, while BPS displayed upregulating effects on RACK1 production and consequent cytokine release, BPA and BPAF initially downregulated RACK1 but mifepristone, flutamide and BAY 11-7082 unmasked upregulating effects and shed new light on their mechanism of action. Discussion and Conclusion: RACK1 represents a bridge between the immune and the endocrine systems, indicating its relevance as target of steroid-active substances and EDCs thus offering the possibility to be a screening tool for their immunotoxic potential.

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O-2A-6

Beyond AOPs: A mechanistic evaluation of OF NAMs in dart testing
ABSTRACT #397

Predrag Kukic[1], Ramya Rajagopal[1], Maria T Baltazar[1], Paul L Carmichael[1], Matthew P Dent[1], Julia Head[1], Hequn Li[1], Iris Muller[1], Joe Reynolds[1], Kritika Sadh[1], Wendy Simpson[1], Sandrine Spriggs[1], Andrew White[1]

[1]*Unilever*

New Approach Methodologies (NAMs) offer a unique opportunity to enable human-relevant safety decisions to be made without the need for animal testing. Protecting human health against the potential effects a chemical may have on embryo-foetal development and/or aspects of reproductive biology using NAMs is particularly challenging. There are numerous Adverse Outcome Pathways (AOPs) that can lead to Developmental and Reproductive Toxicity (DART), which means defining and developing strict testing strategies for every AOP, to predict apical outcomes, is neither a tenable goal nor a necessity to ensure NAM-based safety assessments are fit-for-purpose. Instead, a pragmatic approach is needed that uses the available knowledge and data to ensure NAM-based exposure-led safety assessments are sufficiently protective. To this end, the mechanistic and biological coverage of existing NAMs for DART were assessed and gaps to be addressed were identified, allowing the development of an approach that relies on generating data relevant to the overall mechanisms involved in human reproduction and embryo-foetal development. Using the knowledge of cellular processes and signalling pathways underlying the key stages in reproduction and development, we have developed a broad outline of endpoints informative of DART. A total of 103,607 articles served as the comprehensive pool from which biological marker terms relevant to reproductive and developmental mechanisms were extracted. These included 3,551 genes, 474 biological processes and 338 miRNAs. When the existing NAMs integrated in a framework were compared against this outline, we found them to generally cover the reproductive and developmental processes underlying the traditionally evaluated apical endpoint studies. The application of this safety assessment framework was illustrated using an exposure-led case study. Work is ongoing to generate NAMs data for a

substantial number of known human teratogens and non-teratogens with known human exposure to further build confidence that NAM-based exposure-led safety assessments can be protective of DART.

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Session: 2b. Challenges in cosmetics safety

Chairs: *Nathalie Alépée (L'Oréal, Paris, France) & Erwin van Vliet (Houten, Utrecht, Netherlands)*

O-2B-1

Global Regulatory Landscape for Cosmetics - Challenges and Opportunities in Utilising NAMs
ABSTRACT #396

Jay Ingram[1,2]

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[2]*Humane Society International Animal Free Safety Assessment Collaboration*

Governments around the world are increasingly implementing legislation that prohibits the testing of cosmetic products and ingredients on animals. However, banning animal testing does not necessarily equate to the acceptance of animal free safety assessment methodologies. Significant progress has been made in advancing scientific practices to enable animal free safety assessment. Despite the scientific advancements that have been made and the increasing prevalence of animal test ban legislation, due to testing requirements in other sectors or geographies, animal testing of cosmetics continues. This presentation will aim to provide an overview of the global regulatory landscape as it pertains to animal test ban legislation relating to cosmetic products and ingredients. It will also go into further detail on some key regional and national regulatory frameworks. The complications and challenges faced in implementing impactful regulatory frameworks as well as leveraging New Approach Methodologies (NAMs) will be discussed. Finally, the presentation will provide opportunities and solutions in driving the acceptance and application of NAMs in cosmetic regulatory safety assessment in a way that will leverage robust scientific practices to protect human safety.

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O-2B-2

Overview of New Approach Methodologies for eye hazard identification according to UN GHS
ABSTRACT #437

Els Adriaens[1], Nathalie Alépée[2]

[1]*Adriaens Concuslting, Belgium*
[2]*L'Oréal R&I, France*

Background: Over the last decades, many efforts were made to develop New Approach Methodologies (NAMs) that follow recommendations and combinations of modules as specified in the Guidance Document No. 263 on Integrated Approaches to Testing and Assessment (IATA) for serious eye damage and eye irritation, originally adopted by the OECD in 2017. Discussion: One of the standalone methods which is capable to distinguish between the three UN GHS categories for eye hazard identification of chemicals is the SkinEthic™ Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT) test method that uses reconstructed human cornea-like epithelium (RhCE) (Alépée et al., 2020). The EyeIRR-IS standalone method uses a toxicogenomic approach on the SkinEthic™ HCE model to categorize liquid chemicals into the three UN GHS categories (Cotrez et al, 2021). Cosmetics Europe has developed two defined

approaches (DAs) for non-surfactant liquid chemicals (Alépée et al., 2021). DAL-1, applicable to neat liquids only, combines 4 physicochemical properties with a RhCE test method (EpiOcular™ EIT or SkinEthic™ HCE EIT; OECD TG 492), and the Bovine Corneal Opacity and Permeability laser light-based opacitometer (BCOP LLBO) test method (OECD TG 437). DAL-2, applicable to neat liquids and to liquids and solids dissolved in water, is based on the combination of the BCOP LLBO and the Short Time Exposure test method (STE, OECD TG 491). A new approach for eye hazard assessment of surfactants has been developed recently. This DA is based on the combination of RhCE (EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the STE test method. Conclusion: The standalone methods and defined approaches have shown to successfully distinguish between the three UN GHS categories for eye hazard identification. Also recently, OECD finalized the review of the SkinEthic™ HCE TTT method, DAL-1, and DAL-2 which now become integrated in new guidelines for eye hazard assessment.

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O-2B-3

Application of an updated Next Generation Risk Assessment (NGRA) framework for skin allergy: a case study for Diethanolamine

ABSTRACT #405

Nathalie Alépée[1], Dagmar Bury[1], Sebastian Hoffmann[2], Petra Kern[3], Masaaki Miyazawa[4], Hayato Nishida[5], Erwin van Vliet[6], Nicola Gilmour[7]

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[4]*Kao Corporation, 2606 Akabane, Ichikai, Haga, Tochigi, 321-3497, Japan*
[5]*Shiseido Global Innovation Centre, 1-2-11, Takashima, Nishi-ku, Yokohama-shi, Kanagawa, 220-0011, Japan*
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Risk assessment of cosmetics and their ingredients has shifted towards the use of new approach methodologies (NAM) and Defined Approaches (DA), which can provide information on skin sensitisation hazard and potency. Progress has been made in acceptance of DA for regulatory purposes, with three DA now published within an OECD Guideline 497. However, the challenge remains as to how DA can be incorporated into a next generation risk assessment (NGRA) to ensure consumer safety. Here we provide an update to our previously published NGRA framework (Gilmour et al. 2020) and applied it to two hypothetical consumer safety risk assessment scenarios; 0.75% Diethanolamine (DEA) used in a shampoo (rinse off product) and 0.5% DEA used in a deodorant (leave on product). DEA was selected based upon the existing NAM information demonstrating inconsistencies with respect to the outcomes from the in silico predictions and the in chemico / in vitro data. The following six DA were applied to obtain hazard potential and potency information; Integrated Testing Strategy (ITS version 1 and 2), Artificial Neural Network, Sequential Testing Strategy, Bayesian Network ITS and the Skin Allergy Risk Assessment model. The inconsistent NAM information reduced confidence in the ability to conclude that DEA was a non-sensitiser. These inconsistencies also introduced differences with respect to the hazard potential and potency information obtained from the applied DA. Risk assessment for 0.75% DEA in a shampoo resulted to an overall decision of safe for all DA applied. Risk assessment for the higher consumer exposure resulting from use of 0.5% DEA in a deodorant was safe for some DA, but un-safe for others. The uncertainty arising from use of each DA in risk assessment was evaluated, as well

if consideration of additional information in the NGRA was able to increase confidence.

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Gilmour, et al. (2020). Development of a next generation risk assessment framework for the evaluation of skin sensitisation of cosmetic ingredients. Regulatory Toxicology and Pharmacology 116, 104721.

O-2B-4

New challenges for environmental safety of cosmetics
ABSTRACT #431

Véronique Poulsen[1]

[1]*L'Oréal*

Environmental concerns are raising worldwide. This leads either to local regulations such as the ban of some UV filters in Hawaii, or regional regulations such as the European Green Deal. In this framework, the European Commission launched the Chemicals Strategy for Sustainability (CSS) in 2020. The objective is to move towards a Toxic-Free Environment by substituting substances having a chronic effect for human health or the environment. New environmental hazard categories will therefore be added to the existing ones, e.g. endocrine disruptors or PMT (Persistent, Mobile and Toxic) substances. The industry will need to fulfil new requirements linked to these new categories, keeping in mind that the cosmetic industry should ensure that its ingredients are not harmful for living organisms without the help of tests on vertebrates. L'Oréal is actively involved in the method development to better assess and reduce the environmental impact of our ingredients and formulas. For example, L'Oréal developed a test protocol to assess the effects of UV filters on corals, and is involved in the development of alternatives to fish tests to assess the potential endocrine activity of cosmetic ingredients. The Cosmetic trade associations are also involved in method development to address different concerns. Two years ago, Cosmetics Europe initiated a project for the development of exposure models to estimate exposure profiles of UV filters in marine and freshwater environments. In addition, joined research projects will be covered within the ICCS (International Collaboration for Cosmetics Safety) to develop fish test alternatives, address persistence and mobility of ingredients, and better assess the fate of UV filters and polymers.

Tuesday, November 22nd 2022
11:00 - 13:00

Session: 3a. Models, biomarkers and assays for systemic and immune toxicity

Chairs: *Emanuela Corsini (Universita degli Studi di Milano, Milan, Italy) & Jochem Louisse (Wageningen University, Wageningen, The Netherlands)*

O-3A-1 (INVITED TALK)

New approach methodologies in immunotoxicology with a focus on immunosuppression
ABSTRACT #465

Emanuela Corsini[123]

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[3]*Università degli Studi di Milano, Milan, Italy*

Background and Objectives: A well-functioning immune system is essential for maintaining the integrity of an organism. Exposure to immunotoxic compounds can have serious adverse health consequences affecting responses to both communicable and non-communicable diseases. It is therefore important to understand the immunotoxic potential of xenobiotics and the risk(s) they pose to humans. Materials and Methods: The term NAMs refer to any non-animal-based approaches that can be used to provide information in the context of chemical hazard identification and characterization, including integrated approaches to testing and assessment (IATAs) and defined approaches for data interpretation (DAs). Results: While animal testing has been the gold standard for hazard identification of immunotoxic compounds, a lot of efforts and successes have been made to develop non-animal-based test systems. When using in vitro assays for screening purposes, it is clear that one assay alone will not be able to cover all of the potential adverse effects of chemicals on the immune system and that several assays will be required to identify immunotoxicants. A tiered testing strategy currently represents a valuable approach to assess immunotoxicity in vitro. Pre-screening for direct immunotoxicity in vitro should begin by evaluating myelotoxicity. If not myelotoxic,

chemicals should be tested for leukotoxicity, compounds should then be tested for immunotoxicity at non-cytotoxic concentrations using various approaches, e.g. cytokine production, lymphocyte proliferation assay, and natural killer cell assay, etc. Discussion and Conclusion: The state of the art of available NAMs to assess immunotoxicity, with a focus on immunosuppression, will be presented together with an in deep discuss of the recent detailed review paper on this topic approved at OECD.

O-3A-2

In vitro evaluation of both skin irritation and sensitization hazard of mixtures and finished products using the SENS-IS assay.
ABSTRACT #156

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Background and objectives Nowadays, for cosmetics finished products confirmatory compatibility test in humans maybe needed since test on animals and most alternative methods are of limited value with respect to human exposure. We had previously shown that using a in vitro alternative method combining a 3D human reconstituted epidermis (Episkin) and the quantitative measurement of 62 specific gene expression biomarkers, the SENS-IS assay, one could evaluate the sensitization hazard of mixtures and finished cosmetics products. Here we shown the ability to measure, on finished product, the impact of mixing irritant and sensitizers at different doses in different cosmetic bases. Material and Methods, 3 different commercially available cosmetics bases (2 creams and one cleansing gel) were used mixed with 2 different irritants (SLS and lactic acid) and 2 different sensitizers (cinnamaldehyde and isoeugenol) at different doses. The different mixtures were tested onto the 3D epidermis using the SENS-IS assay. Results, We confirmed the ability of the SENS-IS assay to measure the enhanced sensitization potency induced by irritant. Moreover, a slight but significant enhancement of potency was also observed with the cleansing gel as expected. Discussion and Conclusion These results indicate that the SENS-IS assay is a valuable source of information when analyzing mixture component effects and finished products.

O-3A-3

Multicellular, liver microtissues reliably depict key events of the liver fibrosis AOP
ABSTRACT #212

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An adverse outcome pathway (AOP) is a risk assessment tool that identifies the sequence of biochemical events that lead to an adverse outcome in an organism exposed to a substance. In the liver fibrosis AOP (#38), the sequence of key events that ultimately lead to fibrosis involve the interaction of three cellular players: hepatocytes, Kupffer cells and hepatic stellate cells. We have generated an in vitro, 3D-co-culture model with human cell lines that mimics the key events and the ensuing liver fibrosis phenotype. Here, we show the response of the 3D-co-culture liver model to several substances including known pro-fibrotic stimuli (TGF-β, thioacetamide and methotrex-ate), cholestatic actuators (Bile Salts), non-profibrotic hepatotoxic compounds (acetaminophen) and several environmental toxicants with unclear pro-fibrotic potential. The results demonstrate that the 3D-hepatic co-cultures mimic the key events characteristic of the liver fibrosis AOP (hepatocellular damage, activation of Kupffer cells, activation of stellate cells and deposition of extracellular matrix) when exposed to pro-fibrotic and pro-cholestatic stimuli. This was determined using parameters such as cytotoxicity, albumin and cytokine secre-tion, deposition of collagen and gene expression analysis (qPCR and RNA-sequencing). Moreo-ver, the model was able to discriminate toxicants leading to fibrosis from those (like acetamino-phen) that cause clinically relevant hepatotoxicity not linked to hepatic fibrosis. Thus, this system is a robust, specific, and reliable model to assess fibrotic potential of substances.

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O-3A-4

Immunotoxicity of crystalline silica particles: in vitro screening models and use of high-dimensional immune cell profiling
ABSTRACT #325

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Exposure to respirable crystalline silica particles (CS) in occupational settings is associated with lung diseases and systemic autoimmunity. Failure of particle clearance by alveolar macrophages can lead to sustained inflammatory responses. However, the understanding of the cellular mechanisms linking local pulmonary toxicity and autoimmune diseases is not well understood. The aim of the present study is to characterize early effects of CS exposure on the phenotype and function of innate and adaptive immune cells. The study will provide mechanistic information to support data generated in the occupational and autoimmune disease cohorts of the EXIMIOUS project (Mapping exposure-induced immune effects: connecting the exposome and the immunome) . Changes in immune cell function were measured following direct and indirect CS exposure of whole blood from healthy donors. In the indirect exposure model, blood cells were exposed to conditioned media from CS treated THP-1 macrophages followed by analysis of immune cell responses by single cell mass cytometry (CyTOF). The CyTOF antibody panel has been optimised and includes 42 markers for identifying innate and adaptive immune-cell subsets as well as their functional states. Unsupervised algorithms and statistical analyses were used for the data analyses. In the direct exposure model, whole blood was exposed to CS followed by cytokine profile assessment. Multiplex analysis of blood plasma revealed induction of specific patterns of secreted cytokines and chemokines following direct (IL-1β, IL-18, TNFα, CCL3, CCL4, CXCL2, CXCL8) and indirect (IL-1β, IL-18, IL-4, IL-23, TNFα, CCL2, CXCL8, CXCL10) exposure models, respectively. CyTOF analysis is ongoing and preliminary results suggest changes in phenotype and cytokine expression in specific immune cell subpopulations.

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O-3A-5

A 3D Alveolar In Vitro Test System for the Prediction of Chemical Respiratory Sensitizers
ABSTRACT #363

Sabina Burla[1], Aline Chary[1], Pamina Weber[1], Melissa Saibene[1], Tommaso Serchi[1], Jessica Ponder[2], Kristie Sullivan[2], Arno Gutleb[1]

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The prevalence of respiratory allergic diseases caused by chemicals increased in the last decades, resulting in high morbidity. Consequently, chemical respiratory sensitizers should be correctly classified and labelled to ensure the hazard is communicated and the compounds are safely handled and used. The gaps of knowledge in the immunological mechanisms underlying respiratory sensitization slowed down the development of test methods. As the validated methods for the identification of skin sensitizers fail to distinguish skin from respiratory sensitizers, test systems specific to the respiratory tract are required to predict the sensitizing potential of a chemical. We evaluated the efficiency of a 3D alveolar in vitro test system designed for respiratory sensitization, built on microporous membrane of cell culture inserts, using human cell lines: alveolar type II pneumocytes - A549, endothelial - EA.hy926 and monocytic leukaemia-THP-1. The test system was exposed at the air-liquid-interface (ALI) to known low-molecular weight chemical respiratory

sensitizers and non-sensitizers, to verify its performance to identify the respiratory allergens. Cell viability in response to increasing exposure doses was assessed in the resazurin assay following 24h exposure at ALI, and the dose-response curves were generated for the apical, basolateral compartments, and the complete test system. The test system was subsequently exposed to a dose inducing a maximum of 25% cytotoxicity and the expression of CD54, CD86 and TSLPr cell surface markers on THP-1 cells was measured by flow cytometry. The obtained data show that the test system can discriminate respiratory sensitizers from non-sensitizers. Additionally, the tested pro-haptens were correctly identified as respiratory sensitizers, chemicals classified as false negatives by other new approach methodologies for respiratory sensitization evaluation. This work contributes to the understanding of respiratory sensitization and supports the need for further testing of chemicals able to elicit an allergic response.

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O-3A-6

In vitro assessment of skin sensitizing potential of process-related impurities in polymeric materials during product development.

ABSTRACT #407

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Skin sensitization testing represents a key toxicological endpoint during safety evaluation of ingredients intended for consumer products with topical exposure, both in a regulatory context and during product development, to early understand the toxicological profile of the end-product and potential impurities. The inclusion of New Approach Methods (NAMs) for skin sensitization testing into OECD TGs have resulted in a broader acceptance of such methods as replacements to animal models. However, these methods are not universally applicable, and compounds with certain properties, such as lipophilicity, or of complex composition, are frequently considered outside the applicability domain. GARDskin is a genomic-based next-generation in vitro assay for assessment of skin sensitizers progressing towards regulatory acceptance. The assay is highly sensitive, is compatible with a variety of solvents and has a demonstrated applicability for testing of lipophilic materials. The aim of this study was to evaluate the skin sensitization potential of a lipophilic polymeric material (Mw > 2000g/mol) containing approximately 2% impurities, during product development. The polymeric material was initially flagged as a skin sensitizer. A preparative procedure was applied to purify the polymer from impurities, while generating enriched by-product fractions (oligomeric fraction, Mw: 500-1000 g/mol and small molecule fraction, Mw: <500 g/mol). The fractions were evaluated in GARDskin, using acetone or DMSO as solvents, and classified as skin sensitizers (by-products) and non-sensitizers (purified polymers, n=2), respectively, indicating that the impurities were responsible for the positive classification of the initial non-purified polymeric material. In conclusion, the GARDskin protocol enabled for testing of the lipophilic materials, using a selection of solvents to increase solubility. Results from this study informed that actions to reduce concentration of impurities may be a useful strategy to prevent skin sensitization properties of the final end-product, highlighting the importance of skin sensitization testing during the production development.

Session: 3b. Organ-on-a-chip & Microphysiological Systems

Chairs: *Sofia Batista Leite (JRC, Ispra, Italy) & Pau Sancho-Bru (Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain)*

O-3B-1

A new immunocompetent OOC platform for culturing 3D human tissues with capillary flow-driven immune cells and investigating their cross-talk

ABSTRACT #204

silvia scaglione[12], Monica Marzagalli[1], Maurizio Aiello[1], Arianna Fedi[2]

[1]*React4life*
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Background and Objectives: One of the most unmet challenges of microphysiological systems is related to more predictive and fully humanized in vitro models for investigating cross-talk among immune cells and other healthy/pathological tissues. The preclinical assessment of immunotherapies is currently carried out through 2D cell culture in static conditions, and in vivo xenografts or genetically engineered animal models, generated by the engraftment of PDXs into immunodeficient mice bearing human immune cells, but cost, time, and complete immune-compatibility remain important challenges. Material and Methods: A novel Multi-In Vitro Organ (MIVO) organ on a chip (OOC) platform has been recently developed to culture immunocompetent tumor models, with circulating immune cells under proper physiological culture conditions. Biologically-relevant cancer samples (up to 5 mm) or patient biopsies are cultured within the MIVO chamber, while human immune cells (e.g. Natural Killer cells, NK) are able to (i) circulate in the OOC mimicking the blood capillary flow, (ii) extravasate through a permeable barrier resembling the vascular barrier, (iii) infiltrate the cancer tissue. Results: A human 3D neuroblastoma model with proper immunophenotype was optimized to develop a complex tumor/immune cell coculture as a paradigm of an immune-oncology screening platform [1]. NK cells have been introduced within the capillary fluid flow circulation of the OOC and their migration and infiltration towards the 3D tumor model was analyzed and quantified. Importantly, a tumor-specific NK cell extravasation was observed, with DNAM1+ NK cells infiltrated within 3D tumor tissue. Flow cytometry analysis also highlighted an effective anti-tumor NK cell-mediated activity and an initial tumor cells apoptosis. Discussion and Conclusion: We generated a functional and relevant human model, through the adoption of OOC device, that can be efficiently employed as an immune-oncology screening platform, both for pharmacological treatments and for cell-based therapies.

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O-3B-2

INTEGRATION OF HUMAN-STEM-CELL-BASED EMBRYOID BODIES INTO A MICROFLUIDIC MULTI-TISSUE PLATFORM FOR SYSTEMIC EMBRYOTOXICITY TESTING ABSTRACT #303

Julia Boos[1], Isabel Wegner[1], Andreas Hierlemann[1]

[1]*ETH Zürich*

Assessing compound embryotoxicity constitutes a central part of every drug development process. However, current in vitro assays do not include complex embryo-maternal interactions during pregnancy and are mostly based on the use of murine-derived cell models, which are of limited predictive power due to considerable inter-species differences. Here, we present a multi-organ platform, which combines a microphysiological model of the placental barrier with 3D embryoid bodies (EBs), derived from human induced pluripotent stem cells (hiPSC). The platform consists of two independent fluidic networks, representing maternal and embryonic blood circulation. Both fluidic networks are separated by a semipermeable membrane, which serves as a scaffold to form and culture a human placental-trophoblast barrier in the maternal culture compartment. The hiPSC-derived EBs are cultured in immediate vicinity to the placental barrier in a hanging drop on the embryonic side, which enables direct interaction and molecule exchange between the tissue models through the liquid phase. In a first step, we successfully established the formation and cultivation of hiPSC-derived EBs in our microfluidic device and compared their growth behavior and morphology to those achieved with standard well plates. To evaluate toxicity effects on embryonic development, we established an optical clearing method to visualize the spatial distribution and differentiation of hiPSCs into derivatives of the three germ layers. We further developed a qPCR-based panel of genes, expressed during early embryonic development, to evaluate altered gene expression patterns in differentiating EBs. In a next step, we integrated the placental barrier into the system and confirmed hEB growth and differentiation under co-culture conditions. These results show the potential of the platform to mimic physiologically relevant conditions on chip and lay a promising foundation to study the effects of compounds at the embryo-maternal interface in an entirely human-based system.

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O-3B-3

Multi-Organ-Chip Technologies: The effect of application route on the metabolism
ABSTRACT #402

Ilka Maschmeyer[1]

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Microfluidic microphysiological system (MPS) are considered an enabling technology to reliably emulate complex human or animal biology in vitro. Despite this hype in academia, MPS approaches have not yet been widely adopted by the pharmaceutical and cosmetics industries due to lack of qualified assays to predict the safety of novel drug candidates and cosmetics. Here, we present a 6 day co-culture of skin and liver in the HUMIMIC Chip2 to investigate the metabolic fate of the hair dye 4-amino-2-hydroxytoluene (AHT) focussing on the first pass metabolism in the skin model. We evaluate the system's robustness as well as its capacity to provide in vivo relevant information about exposure scenario-dependent bioavailability by comparing the results with in vivo findings. Additionally, selected single- and multi-organ models bearing advanced therapy test potential, such as human bone marrow equivalents, chip based co-culture of tumour with a healthy organ equivalent and immune tissues on a chip will be introduced. The presentation concludes on the status quo of pharmaceutical and cosmetic assay adoption for the presented Multi-Organ-Chip platform in particular, and the entire MPS field in general. It emphasis challenges towards MPS-based organismal homeostasis, which bear the potential of a paradigm shift in advanced therapy development.

O-3B-4

A BREATHING LUNG-ON-CHIP MODEL: AN ADVANCED IN-VITRO TOOL FOR DRUG TESTING AND TOXICOLOGY
ABSTRACT #420

Aude Rapet[1], Léa Todeschini[1], Nuria Roldan[1], Giulia Raggi[1], Lea de Maddalena[1], Laurène Froment[1], Nina Hobi[1], Janick D. Stucki[1]

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Background and Objectives Complex in vitro lung models are becoming key assets in drug development, disease modeling and toxicology. In addition, interest in these more predictive models has increased because of the pressure from regulatory agencies to reduce animal studies in order to achieve more ethical and translatable testing. Material and Methods The AXLung-on-Chip System allows to recreate the human lung environment. By culturing primary human alveolar epithelial cells and endothelial cells on the ultra-thin membrane inside the AX12, the alveolar barrier can be recreated. The cells were cultured until stable barrier formation and then treated with e.g. FolR1-TCB and ProLeukin® at different concentrations. Results This technology allows various readouts to obtain the maximum amount of data. Non-invasive, real-time barrier function is routinely assessed by TER measurements and permeability assays are performed to examine molecule transport across the barrier. On-chip transcriptomic analysis, (sc) RNAseq, enables in-depth studies of cell populations, when proteomic approaches (multiplex ELISA, mass-spectrometry), help quantify secreted biomarkers or immune-mediated cytokine storm. Further, immunofluorescence, electron microscopy and immunohistochemistry-on-chip are used to observe detailed cell structure and markers. This wide asset of readouts enables predictive pulmonary toxicity studies such as safety assessment of immunotherapeutic drugs like FolR1-TCB, where animal models are irrelevant due to species differences in the immune system. The AXLung-on-Chip System has also shown clinically relevant patient-to-patient variability in the response to therapeutics (ProLeukin®) and its application as an efficacy tool in modelling chronic diseases with limited treatment options (ARDS, pulmonary fibrosis, COVID-19). Discussion and Conclusion Overall, our data confirm the predictive ability of this advanced in-vitro tool, which facilitates better toxicology assessments and decision-making during the drug development process. Thus, this lung-on-chip model is a promising tool for robust in vitro testing, including safety, efficacy, and toxicology, while providing an alternative to animal testing.

O-3B-5

A microfluidic bone marrow chip for the safety profiling of complex large molecules in pre-clinical drug development
ABSTRACT #423

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Background and Objectives Hematologic toxicities are common dose-limiting adverse events during drug development. Highly species-specific therapeutic antibodies have limited the usability of classical animal models for preclinical safety assessment due to insufficient cross-reactivity to non-human homologous proteins. Hence, a human bone marrow microphysiological system (MPS) on the basis of TissUse's HUMIMIC Chip2 technology was developed and assessed for its capability to predict hematopoietic liabilities. Material and Methods A zirconium oxide scaffold mimicking the structure and surface of trabecular bone was seeded with bone marrow mesenchymal stromal cells. Human CD34+ hematopoietic stem and progenitor cells (HSPCs) were added into the stromal cell-covered scaffold and subsequently placed into the HUMIMIC Chip2, where the complete model was cultured for up to 31 days. The differentiation and maturation of HSPCs into myeloid, erythroid and lymphoid blood cells was induced by the addition of different hematopoietic growth factors. Treatment effects were analyzed by weekly flow cytometry staining, erythroid enucleation and cytokine levels in the cell culture medium. Results In line with literature, adding pro-inflammatory factors and cytokines such as IL1b impacted lineage balance and output. Furthermore, inhibition of erythroblast differentiation by bispecific TfR-engaging antibodies previously overserved in cynomolgus monkey studies was recapitulated on chip. Data from the chip indicate a potential role of antibody-dependent cell-mediated cytotoxicity in the reduction of erythropoiesis. Application of T-cell bispecifics (TCBs) resulted in T-cell activation and killing of target cells. However, the T cell response level was significantly stronger in an allogeneic setup compared to an autologous setup, indicating that the therapeutic index for TCBs could be underestimated when working with allogeneic cells. Discussion and Conclusion In summary, this study provides proof-of-concept that this MPS can be used as an alternative to classical animal models for in vitro hematopoiesis safety profiling of complex large molecules.

O-3B-6

A human beating heart on chip for on-line screening of drug cardiotoxicity
ABSTRACT #442

Roberta Visone[12], Ferran Lozano[1], Simona Marzorati[3], Caterina Pernici[1], Alessandro Cordiale[2], Claudio Bernardi[3], Enrico Pesenti[3], Marco Rasponi[12], Paola Occhetta[12]

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Background and Objectives Cardiotoxicity is one among the most prominent causes of drug attrition. The development of relevant in vitro models able to effectively predict clinical outcomes in early stages of drug development is thus of paramount importance. Here we present a human functional 3D cardiac model developed in a beating Organs-on-chip (OoC) integrating fit-to-purpose assays for detecting drug cardiotoxicity in pre-clinical stages. Material and Methods The OoC integrates two key technologies: uBeat®, providing a 10% uniaxial cyclic strain to cardiac microtissues [1] and µECG, to record on-line cardiac electrophysiology [2]. Human induced-pluripotent-stem-cell derived cardiomyocytes and fibroblasts (3:1 ratio) were embedded in fibrin and mechanically trained at 1Hz for 7 days. The model was exploited to assess functional cardiotoxicity and inotropic effects of drugs (e.g. Dofetilide, Ranolazine, Nifedipine, Isoprenaline) administered at increasing doses. Drug-induced changes in electrophysiological parameters (i.e. beating period-BP, spike amplitude-AMP, field potential duration-FPD) were assessed through µECG. Contraction parameters of spontaneously beating models (e.g. contraction amplitude-CA) were evaluated through Musclemotion software[3]. Results The human cardiac microtissues showed a synchronous beating already after 5 days, with a BP of 1.9±0.7s and an FPD of 0.69±0.25s. Drug screening results evidenced that DMSO (vehicle) and Aspirin (negative control) did not alter cardiac electrophysiology. Dofetilide and Quinidine prolonged the FPD at concentration near the Cmax, while Verapamil and Nifedipine shorten it at 5-50nM. Mexiletine (10µM) statistically decreased the AMP and Terfenadine elicited arrhythmic events. Isoprenaline shorted the BP and improved the CA in a dose-dependent manner (1-1000nM). Discussion and Conclusion The pharmacological campaign confirmed the suitability of the model to effectively predict compounds cardiotoxic effects at concentrations near the Cmax and matching indications reported on drug FDA labels. The

platform resulted highly versatile, providing cardiac microtissues with stimulations and multiple read-outs, enabling the generation of relevant pre-clinical data for cardiotoxicity screening.

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Tuesday, November 22nd 2022 14:00 - 16:00

Session: 4a. Computational toxicology – in silico modelling, read-across, artificial intelligence and machine learning

Chairs: *Olivier Taboureau (University Paris Diderot, Paris, France) & Tim Allen (Ladder Therapeutics, Toronto, Canada)*

O-4A-1 (INVITED TALK)

Bioinformatics and Network science applied in toxicology
ABSTRACT #463
Olivier Taboureau[1]

[1]*Université Paris Cité*

For over a decade, computational chemical biology has contributed to a wide array of scientific tasks from analytical chemistry and biochemistry to pharmacology and toxicology. With the increasing availability of data from the"omics" technologies, we start to be able to profile chemical effect, not only at the molecular level, but also at more complex layers (cells, tissues, organs) allowing a better understanding of the mechanism of action underlying complex diseases. Under the RiskHunt3R consortium, one of our challenges is to develop computational method that can assess chemical safety and adverse outcomes for humans. Taking advantage of chemical-biology, toxicology, genomics and epidemiologic data available, proteins, genes and pathways associated to specific toxicity endpoints or diseases can be identified. Human protein/gene modulation (genetic variability) that potentially affect chemical exposure and might influence human disease states can be extrapolate. Finally, common biological mechanisms of diseases associated with chemical exposure can be explored through human environmental disease network, helping us to gain insight into disease etiology and comorbidity. Through different examples, I will describe how bioinformatics and network science might help in the study of chemical action across multiple scales of complexity from molecular and cellular to tissues and organ levels and how it may provide a deeper understanding of the chemical risk assessment in human health.

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O-4A-2 (INVITED TALK)

The key role of computational toxicology for Toxicology for the 21st Century 2.0
ABSTRACT #460
Thomas Hartung[1]

[1]*Johns Hopkins University*

The 2007 NAS report on Toxicity Testing for the 21st Century was a watershed moment for toxicology. Since then, the discussion is no longer whether to change but how and how fast? With knowledge in the life sciences doubling every seven years, we now have four times more understanding and actually a number of disruptive technologies have evolved, which were not anticipated in the report, such as Microphysiological Systems (MPS) and Machine Learning, aka Artificial Intelligence (AI). In order to embrace these developments and move toxicology to a more wholistic and integrated paradigm, the Basic Research Office of the Office of the Under Secretary of Defense for Research and Engineering, OUSD(R&E), hosted a Future Directions workshop Advancing the Next Scientific Revolution in Toxicology on April 28-29, 2022, at the Basic Research Innovation Collaboration Center (BRICC), in Arlington, VA. A vanguard of scientific and technical experts and agency observers developed a report, laying out how recent developments can be embraced and set the direction of "Toxicology for the 21st Century 2.0" in the next decades. Computational approaches, especially AI, play a key role here: 1. A central role of Exposomics to change to more exposure-driven toxicology, with AI enabling us to make sense of ~omics (big) data 2. Predictive toxicology through automated read-across such as read-across-based structure-activity relationships (RASAR) 3. The computational modeling of in vitro tests and MPS 4. Digital pathology through

image analysis 5. Information extraction by Natural Language Processing of scientific literature and the grey information of the internet as well as curated databases of legacy data 6. Evidence integration of different evidence streams allows probabilistic risk assessment The EU project ONTOX is working toward the implementation of some of these goals.

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O-4A-3

Turning AOPs into testable hypotheses with natural language processing and tissue-specific knowledge graphs
ABSTRACT #376

Ignacio Tripodi[1], Lena Schmidt[1], Brian Howard[1], Ruchir Shah[1]

[1]*Sciome, LLC*

Objectives: The Adverse Outcome Pathway (AOP) framework is crucial to success of new approach methodologies for toxicity testing. To maximize the potential of AOPs by allowing scientists to easily turn them into testable hypotheses, we have developed a method to translate the English descriptions of each AOP component to unique ontology descriptors. This generates a fully semantic representation of an AOP, by easily mapping all its events to the most appropriate ontology concepts, also denoted by nodes in a massive knowledge graph (KG). Methods: Using a specialized artificial neural network model with pre-trained word embeddings and carefully curated pairs of similar/dissimilar biological event descriptions, we produce high-accuracy candidate ontology mappings for every molecular initiating event, key event and adverse outcome to a controlled vocabulary. For example, AOP events “protein alkylation” and “liver fibrosis”, are mapped to Gene Ontology concept GO:0008213 and Human Phenotype Ontology concept HP:0001395, respectively. Using in vitro transcriptomics data, and gene relations from a tissue-specific KG, we then calculate statistical significance of each AOP event and provide a transparent justification of which genes are involved in each event. Results/Discussion: After curating three OECD-endorsed AOPs quickly and painlessly using these mappings, we demonstrate a use case that takes advantage of our novel semantic AOP definition, showcasing how manifestation of an AOP can be queried using post-exposure transcriptomics data for known stressors. The experimental evidence becomes increasingly compelling when, if measured as a time series and the time points match the molecular dynamics of the toxicant, we can observe enrichment of AOP events shifting towards the adverse outcome over time. Furthermore, the KG used for AOP scoring is tissue-specific and expandable; and our AOP-mining approach is applicable to all types of ‘omics (gene, protein, metabolite), in-vitro assays, and other platforms for NAMs.

O-4A-4

Integrating in vitro and in silico data for screening and characterization of tattoo pigments for human health risk assessment
ABSTRACT #429

Prachi Pradeep[1], Ajay Vikram Singh[1], Ralph Pirow[1], Peter Laux[1], Andreas Luch[1]

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Tattoo inks contain several substances (organic and inorganic pigments, solvents etc.) which may be associated with adverse health effects. Consequently, tattoos may pose a significant health risk to not only the skin but also other parts of the human body/health due to intradermal exposure. Substances in tattoo inks are regulated by entry 75 in Annex XVII of REACH Regulation (EC) No 1907/2006. However, despite these legal requirements, a well-defined criterion for the safety assessment of tattoo inks remains lacking. In this context, a recent BfR proceeding titled “Tattoo inks: minimum requirements and test methods” proposed a comprehensive risk assessment of pigments using in-vitro/in-chemico data in accordance with the OECD Guidelines and CLP Regulation. However, in the absence of experimental data, in-silico data are often used for data-gap filling. Here, a categorization framework was developed to evaluate the genotoxicity potential of 97 tattoo pigments obtained from the ECHA registered substances database. The registered data for these pigments was compared with in-vitro genotoxicity data assembled from several publically available data sources (NTP,

eChemPortal etc.). Next, in-silico genotoxicity predictions for these pigments were generated from publically available QSAR tools (EPA TEST and Vega) and OECD Toolbox structural alerts. The in-vitro and in-silico genotoxicity data were utilized in this framework to flag potential genotoxicants for further screening. Finally, chemical clustering based on structural similarity was used to derive genotoxicity predictions for pigments with little/no data. 46 of the 97 pigments had reliable data available from REACH dossier, of which, 7 had contradicting in-vitro data from other sources, and 11 had contradicting in-silico predictions. Of the remaining 51 pigments, 41 were categorized as genotoxic and 5 as non-genotoxic based on this categorization framework. The framework developed herein will be used for human health risk assessments for tattoo pigments in the absence of experimental data.

O-4A-5

Modelling in vitro time-concentration response of gene expression to chemical exposure
ABSTRACT #453

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Background and Objectives The use of omics, as for example transcriptomics, in non-animal chemical risk assessment, is particularly promising. Allowing for the high level of correlation between genes, this study proposes an innovative multivariate approach, based on One Factor modelling, to model transcriptomics time-concentration response. The approach aims at deriving a system-level benchmark concentration (BMC), i.e. concentration of a substance that produces a predefined response. Material and Methods A metabolic competent model of primary human hepatocytes (HepaRG) was treated with five well known substances. For each chemical, the whole transcriptomics profile was obtained by targeted RNA-Seq at 8 concentrations and 5 times of exposure in 9 replicates (3 technical * 3 biological). Data pre-processing and gene expression differential analysis was performed before applying One Factor modelling to model the response of all genes jointly, across concentrations and exposure times. Result 10270 genes passed the quality filters applied in the pre-processing steps. The One Factor model accounts for the main part of gene expression variation and allows to express the pair of exposure values (time and concentration) needed by each chemical to elicit the predefined response by a curve, which represents the BMC in a dynamic way.. Discussion and Conclusion A multivariate approach, as One Factor Modelling, is able to handle the interdependency of genes. This approach is particularly robust and suitable to analyse outputs from complex experimental design, even including different time points. Although in this study the BMC is calculated at a system-level, it is possible to retrieve information on individual genes and gene-sets, useful in comparing the responses of different chemicals. Thanks to gene expression modelling, we obtained new insights in in vitro transcriptomics BMC calculation and explored how and to what extent it varied as a function of exposure time.

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Session: 4b. Local toxicity testing (safety and efficacy)

Chairs: *Montserrat Mitjans (Universitat de Barcelona, Barcelona, Spain) & Helena Kandarova (CEM SAS, Bratislava, Slovakia)*

O-4B-1

A new OECD guideline based on Defined Approaches for Eye Hazard Assessment of non-surfactant liquids combining Physicochemical Properties and OECD-approved Test Methods
ABSTRACT #335

Nathalie Alépée[1], Els Adriaens[2], Takayuki Abo[3], Dan Bagley[4], Jason Magby[4], Arianna Giusti[5], Karsten Mewes[6]

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Background and Objectives: The validation and regulatory acceptance of in vitro test methods for assessing the eye irritation/damage potential of chemicals remains an active topic for regulatory toxicology. Cosmetics Europe (CE) has developed

two defined approaches (DAs) for non-surfactant liquid chemicals. DAL-1, applicable to neat liquids only, combines 4 physicochemical properties with a Reconstructed human Cornea-like Epithelium test method (Validated Reference Method 1, (VRM1): EpiOcular™ EIT and VRM2: SkinEthic™ HCE EIT; OECD TG 492), and the Bovine Corneal Opacity and Permeability laser light-based opacitometer (BCOP LLBO) test method (OECD TG 437). DAL-2, applicable to neat liquids and to liquids and solids dissolved in water, is based on the combination of the BCOP LLBO and the Short Time Exposure test method (STE, OECD TG 491). Materials and methods: The performance of the DAs was evaluated in accordance with the United Nations Global Harmonized System and compared against the proposed minimum performance of 75% for Cat. 1, 50% for Cat. 2 and 70% for No Cat. agreed by the OECD experts. Results: The balanced accuracy (BA) of DAL-1 was 68.7% (with VRM1) and 75.2% (with VRM2). DAL-1 with VRM1 identified 75.0% of Cat. 1 (N=17), 59.1% of Cat. 2 (N=22) and 70.5% of No Cat. (N=55) correctly. DAL-1 with VRM2 identified 76.5% of Cat. 1 (N=17), 68.7% of Cat. 2 (N=23) and 79.7% of No Cat. (N=46). DAL-2 had a BA of 74.3% and identified 81.2% of Cat. 1 (N=17), 56.3% of Cat. 2 (N=24) and 85.3% of No Cat. (N=123) correctly. Conclusion: The performance reached the proposed minimum values, therefore, DAL-1 and DAL-2 have shown to successfully distinguish between the three UN GHS categories for eye hazard identification. OECD finalized the review of the DAs which now become integrated in a new guideline for eye hazard assessment.

O-4B-2

MODELING SKIN INFLAMMATION USING 2D AND 3D IN VITRO MODELS
ABSTRACT #344

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Background and Objectives Skin inflammation can be due to a variety of factors, including immune system dysfunction, infections, and injury or wound. Atopic dermatitis is a typical chronic inflammatory skin disease that affects children and adults. Topical corticosteroids are first-line topical therapies for eczema. However, side effects can sometimes occur after chronic use. Natural compounds with anti-inflammatory properties are now commonly being incorporated into skin care products to combats skin inflammation. The objective of the study was to validate an in vitro assay using 2D and 3D skin models to evaluate anti-inflammatory properties of new topical products. Materials and Methods Normal human epidermal keratinocytes were irradiated with UVB, or treated with lipopolysaccharide (LPS), or with cytokine cocktails IL-4 and IL-13. Dexamethasone (DEX) and Pyridone 6 (JAK kinase inhibitor 1) were used as anti-inflammatory positive controls. 3D reconstructed human epidermis (RHE) tissues were treated with LPS alone, or in combination with systemic treatment of DEX or topical treatment of Betamethasone cream.Treatment duration was 24 hours. mRNA expression of TNF-alpha and CXCL8 (IL-8) was measured by quantitative RT-PCR and secreted IL-8 was measured by ELISA. In addition, expression of filaggrin (FLG) and involucrin (IVL) (markers of skin barrier) was also measured. Results In 2D keratinocytes, UVB irradiation, LPS and cytokine cocktails induced significant increase of secreted IL-8, while dexamethasone and JAK inhibitor significantly decreased IL-8 production. In addition, cytokine cocktails down-regulated the expression of both filaggrin and involucrin, while JAK inhibitor reversed this effect. In 3D RHE tissues, LPS and cytokine cocktails induced significant increase of IL-8 and TNF-alpha mRNA expression and secretion. Betamethasone cream and JAK inhibitor significantly decreased TNF-alpha and IL-8 mRNA expression. Discussion and Conclusion The human in vitro skin models mimicking skin inflammation developed in the study will be valuable in assessing the efficacy of new anti-inflammatory drugs.

O-4B-3

In vitro investigation of aluminum bioavailability after exposure of oral care products
ABSTRACT #357

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Background and Objectives Aluminum is an ubiquitous element found in nature and in human-made products. It may trigger several adverse health effects in humans. In fact, aluminum may interfere with metabolism of other cations and induce gastrointestinal disorders and possibly neurotoxicity. In cosmetics, aluminum is used in antiperspirants, lipsticks, and toothpastes. In the light of recent data, the Scientific Committee on Consumer Safety (SCCS) considers that the use of aluminum compounds is safe at the percentage of 2.65% in toothpastes. No data are available on aluminum leach, eventually ingested with toothpaste. In this study, the bioavailability of aluminum included in cosmetic products used for oral care was assessed. Material and methods To assess the effect of the aluminum in the case of accidental ingestion, toothpastes were subjected to in vitro digestion according to INFOGEST model [1]. The bioavailability of accidentally ingested Al3+ was evaluated by incubating Al3+ solutions on EpiIntestinal 3D model (MatTek). At the end of the experiment, the EpiIntestinal tissue barrier integrity was analyzed by measuring transepithelial electric resistance (TEER) [2]. Moreover, tissue architecture was analyzed under light microscope. Margin of safety (MoS) was also calculated. Results The bioavailability of Al3+ was below the detection limit after the EpiIntestinal model's incubation. TEER measures didn't underline any differences for all the treatments with respect to negative control, thus showing absence of toxicity for all the Al3+ concentrations tested. These results were confirmed by histological evaluation of tissues. Moreover, MoS assessed for this experiment was more than 100. Discussion and Conclusion The data presented here suggest that only a very small amount of Al3+ is able to trespass intestinal mucosa. This is in line with SCCS opinion stating about safety of aluminum daily applications through cosmetic products.

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O-4B-4

Integrative evaluation strategy for skin sensitization : the mixtures challenge
ABSTRACT #406

Eric Andres[1]

[1]*Oroxcell*

Sensitization evaluation is an essential part of the safety assessment of substances in the dermo-cosmetic industry. In the past, sensitization was evaluated on rodents using the LLNA or GPMT, however, the in vivo assays are now banned in Europe for the evaluation of cosmetics raw materials. In response, some in vitro assays for the detection of sensitization were developed to permit the toxicological evaluation of these substances without the use of animals, with the main purpose of distinguishing sensitizing substances from non-sensitizing substances. Considerable research into the mechanism of skin sensitization has permitted the sensitization process to be characterised as an adverse outcome pathway identifying four key events (KE). Among them, the first 3 KE can be evaluated in vitro by the evaluation of covalent binding to proteins (DPRA, ADRA, and kDPRA), the ARE-Nrf2 Luciferase Test (Keratinosens™ and LuSens), and the activation of dendritic cells (h-CLAT, U-SENS™, and IL-8 Luc assay). However, these tests were not optimized for the evaluation of substances difficult to test such as lipophilic substances requiring the use of organic solvents, or complex mixtures such as botanical extracts. Although possible, the testing was shown to be difficult mainly due to solubilization issues, to interference related to cytotoxic effects from the organic solvent or from a component of the mixture, or to the unavailability of the composition of the mixture. We discuss here the limits of the tests described in the standard guidelines, together with the options developed at Oroxcell to address these issues, both by the development of new assays and by the adaptation of existing tests to substances difficult to test. Our integrative approach considerably extends the field of

application of the assays towards complex substances and mixtures, both for toxicological assessment and efficacy against sensitization effect.

O-4B-5

Application of a next generation risk assessment framework for skin sensitisation using new approach methodologies (NAMs): geraniol case study

ABSTRACT #421

Renato Ivan de Ávila[1], Nicola Gilmour[1], Nora Aptula[1], Katarzyna Przybylak[1], Georgia Reynolds[1], Joe Reynolds[1], Maja Aleksic[1], Ramya Rajagopal[1], Gavin Maxwell[1]

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Background and Objectives: Skin Allergy Risk Assessment (SARA) model is a Bayesian statistical approach, developed to infer a human-relevant metric of sensitiser potency and a measure of consumer risk to chemicals. SARA can take different combinations of historical in vivo (animal and clinical human) information and in chemico and in vitro NAM data. The SARA model was applied within a next generation risk assessment (NGRA) framework for skin allergy to a hypothetical case study based on 0.02% geraniol in a face cream. Materials and Methods: Consumer exposure information and in silico predictions were used to define the risk assessment and experimental data need. Publicly available data on geraniol (DPRA, KeratinoSensTM, h-CLAT and U-SENSTM) were used within the SARA model to predict human skin sensitisation potency. A risk measure for exposure was then calculated by comparison to benchmark exposures which, based upon documented history of use and clinical evidence have been assigned high/low risk for skin sensitisation induction. Results: In silico tools predicted geraniol to be a skin sensitiser due to abiotic activation. This was confirmed by peptide reactivity profiling. As SARA can take partial datasets, an analysis of the impact of using different data combinations on the risk metric was performed, e.g. excluding DPRA data. The SARA model benchmarked geraniol with moderate sensitisers and predicted that use of 0.02% geraniol in face cream is low risk with 95% probability. Discussion and Conclusion: This case study provides an example of how NGRA approaches can be applied to skin allergy risk assessment to sufficiently protect consumers. The SARA model is a versatile approach that allows different combinations of NAM inputs. Moreover, it was shown that historical exposures can provide a means to benchmark risk assessment outcomes using clinical experience. However, additional clinical benchmarks still need to be identified.

Wednesday, November 23rd 2022
08:30 - 10:30

Session: 5a. Toxicokinetics and in vitro – in vivo extrapolation

Chairs: *Nicole Kleinstreuer (NICEATM, Durham, USA) & Jochem Louisse (Wageningen University, Wageningen, The Netherlands)*

O-5A-1 (INVITED TALK)

An Open, Accessible Interface for Contextualizing Chemical Effects

ABSTRACT #464

Nicole Kleinstreuer[1]

[1]*NICEATM*

Regulatory toxicology testing has traditionally relied on in vivo methods to inform decision-making. However, scientific, practical, and ethical considerations have led to an increased interest in the use of in vitro and in silico methods to fill data gaps. While in vitro experiments have the advantage of rapid application across large chemical sets, interpretation of such mechanistic data can be challenging without appropriate biological context. Assay endpoints that are mapped to mechanistic targets and toxicological modes of action can be combined into bioactivity profiles for chemical assessments. Physiologically based pharmacokinetic (PBPK) models can be applied to estimate tissue-level chemical concentrations based on various dosing parameters, and paired with in vitro to in vivo extrapolation (IVIVE) to estimate the administered dose needed to achieve in vitro bioactivity concentrations within the body. IVIVE results can be useful to inform on metrics such as margin of exposure or to prioritize potential chemicals of concern, but the PBPK models used in this approach have extensive data requirements. Thus, access to input parameters, as well as the technical requirements of applying and interpreting models, has limited the use of IVIVE as a routine part of in vitro testing. Access to open computational support tools and reliable parameter predictions for PBPK modeling and IVIVE are essential for facilitating

broader application and acceptance of these techniques, as well as for encouraging the most scientifically sound interpretation of in vitro results. This talk will highlight recent developments in open-access computational support tools for bioactivity profiling, PBPK modeling, and IVIVE accessible via the Integrated Chemical Environment (https://ice.ntp.niehs.nih.gov/), demonstrate the types of insights these tools can provide, and discuss how these analyses may inform in vitro-based decision making.

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O-5A-2

Crucial role of Toxicokinetics and ADME methods in application of Next generation Risk Assessment

ABSTRACT #391

Andreas Schepky[1]

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Besides ethical considerations, scientific reasons have led to a worldwide shift towards human health safety assessments of chemicals without using animal testing. The Next Generation Risk Assessment (NGRA) approach is exposure-led and hypothesis-driven, based on the combination of non-animal data derived from in silico, in chemico and in vitro methodologies named “New Approach Methodologies” or “NAMs”. To come to a reliable assessment first key step are toxicokinetics. A variety of ADME tools are developed and used to support, e.g. PBPK modeling, one of the most crucial parts of NGRA. Performing this modeling on a high level is mandatory for the outcome of whole NGRA. It will be demonstrated how application of modern ADME tools impacts highly on successful NGRA of chemicals, e.g. UV filters, preservatives. Additionally, the roles of ADME, esp. metabolism, and PBPK modeling on internal Threshold of Toxicological Concern (iTTC) will be discussed.

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O-5A-3

Generic PBK models based on in vitro and in silico input data: How to gain trust in the results?

ABSTRACT #426

Ans Punt[1], Jochem Louisse[1], Eric Fabian[2], Bennard van Ravenzwaay[2], Dawei Tang[3], Hequn Li[3]

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PBK modelling forms an essential component of the risk assessment process based on animal free methodologies. In this context, PBK models are used to calculate human equivalent doses of in vitro effect concentrations, also referred to as quantitative in vitro to in vivo extrapolations (QIVIVE). Taking into account the numbers of chemicals for which a risk assessment needs to be performed, simple generic PBK modelling approaches parameterised solely with in vitro and in silico input data are essential. However, for regulatory application, insight into the predictive performance and applicability domain of such generic PBK models is required. Therefore, we recently evaluated the performance of a generic human PBK model to predict peak plasma concentrations (Cmax) for 44 chemicals based on a minimal set of in vitro- or in silico-derived input parameters for passive intestinal absorption, liver clearance, and plasma and tissue binding (1). For

the majority of chemicals in the dataset adequate Cmax predictions could be made within 5-fold of in vivo observed Cmax values. For the chemicals that could not be adequately predicted with the generic human PBK model (>5-fold different from the observed in vivo Cmax), we explored whether in silico and in vitro screening tools can be used as indicators of whether additional kinetic processes like extrahepatic metabolism and/or transporter-mediated kinetics, need to be included in the PBK model to improve the PBK model predictions. Overall, the findings from these studies are critical to gain confidence in the predictive performance of these generic PBK models based on in vitro and in silico input data, and to facilitate the application of such PBK models in next generation risk assessment.

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O-5A-4

USE OF A 3D-IN VITRO MODEL FOR THE ASSESSMENT OF LIVER METABOLISM RELATED TO NEUROTOXICITY OF OCCUPATIONALLY RELEVANT CHEMICALS ABSTRACT #318

Sophie Werner[123], Fabrice Müller[13], Laura Suter-Dick[13]

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Several neurological disorders have been linked to occupational exposure to chemicals. Propylene glycol ethers are commonly used as mixtures of a non-toxic α-isomer and a β-isomer that is oxidized to a potential noxious acid metabolite. However, studies about the neurotoxicity of these solvents are rare. Knowing the rate of solvent metabolism is important to estimate the metabolite exposure for the brain. Here, we studied the toxicity of selected solvents and their corresponding acid metabolites on 2D and 3D liver cells and proved the 3D in vitro HepaRG liver model to predict hepatic metabolism. We evaluated cell viability 48 h and 7 days after exposing the cells to the solvents and their metabolites by measuring ATP content. The synthesis and release of albumin as a functional hepatocyte marker were detected by immunostaining and ELISA albumin assay. Effects on cell proliferation were shown by immunostaining and the activities of metabolic enzymes were confirmed with known substrates. After exposing the 3D HepaRG model to the solvents, we measured metabolite concentration by LC-MS. The 3D HepaRG cells were able to perform phase 1 metabolism. Cytotoxic studies showed decreased cell viability accompanied by decreased cellular albumin levels after exposure to the metabolites but not to the solvents, whereby the 3D cultures were more sensitive than the 2D cultures. Moreover, exposure to the solvents led to increased cell proliferation. The formed metabolites were measured in the 3D HepaRG model and kinetic parameters calculated. In conclusion, we assessed the toxic effects of solvents and their acid metabolites on liver cells and established a metabolically competent 3D HepaRG model suitable to measure metabolite formation. Future steps include the integration of our in vitro toxicity data into a physiological based toxicokinetic (PBTK) model to predict human systemic and brain exposures and thus support risk assessment of occupationally relevant chemicals.

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O-5A-6

High-Throughput Assessment of the Abiotic Stability of Test Chemicals in In Vitro Bioassays ABSTRACT #253

Julia Huchthausen[1], Luise Henneberger[1], Sophia Mälzer[1], Beate Nicol[2], Chris Sparham[2], Beate Escher[13]

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Abiotic stability of chemicals is not routinely tested prior to in vitro bioassays, although abiotic degradation can reduce the concentration of test chemicals leading to misinterpretation of bioassay results. If nominal effect concentrations of unstable chemicals in in vitro assays are used as input parameters for quantitative in vitro-to-in vivo extrapolation, the effect in vivo may be underestimated. A high-throughput workflow was developed to measure the abiotic stability of 22 test chemicals in protein-rich aqueous media under typical bioassay conditions at 37 °C for 48 h. Four representative degradation processes were evaluated: hydrolysis, reactivity toward proteins, photodegradation, and oxidation/autoxidation. The chemicals were extracted from the exposure media at different time points using a novel 96-pin solid-phase microextraction. For most hydrolyzable chemicals, pH-dependent degradation in phosphate-buffered saline indicated that acid-catalyzed hydrolysis was less important than reactions with hydroxide ions. Reactions with proteins were mainly responsible for the depletion of the test chemicals in the media, which was simulated by bovine serum albumin (BSA) and glutathione (GSH). GSH is a good proxy for reactivity with electrophilic amino acids but may overestimate the actual reaction with three-dimensional proteins. Hydroquinones or polyunsaturated chemicals are prone to autoxidation, but this reaction is difficult to differentiate from hydrolysis and could not be simulated by the oxidant N-bromosuccinimide. Photodegradation played a minor role because cells were incubated in the dark and simulations with high light intensities did not yield realistic degradation. Stability predictions from in silico models for environmental conditions can give initial indications of the stability but were not always consistent with the experimental stability in bioassays. Experimental stability measurements are indispensable for a reliable evaluation of the abiotic stability of chemicals and should be routinely integrated in future in vitro bioassay workflows if the data are to be used for human health risk assessment.

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Session: 5b. In vitro systems to assess respiratory toxicity

Chairs: *Erin Hill (IIVS, Gaithersburg, MD, USA) & Clive Roper (Roper Toxcilogy Consulting Limited, UK)*

O-5B-1

Evaluating human cell-based in vitro systems to assess respiratory toxicity – is more complex better?
ABSTRACT #425

Andreas Stucki[1], Monita Sharma[1], Sandra Verstraelen[2], An Jacobs[2], David Poelmans[2], Sylvie Remy[2], Frederick Maes[2], Evelien Frijns[2], Karen Hollanders[2], Lieve Geerts[2], Stefaan Voorspoels[2], Jo Van Laer[2], Amy J Clippinger[1]

[1]*PETA Science Consortium International e.V.*
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Background and Objectives: Many tools for in vitro respiratory toxicity testing have become available in recent years including new human cell-based systems and exposure systems. In this study, we compare the ability of a two- and three-dimensional (2D and 3D) cell systems to predict portal-of-entry effects of four different chemicals in the human respiratory tract. At least three concentrations per chemical, different exposure scenarios (liquid, aerosol and vapor), and biological endpoints were assessed. Materials and Methods: human bronchial epithelial cell line (BEAS-2B) and reconstituted human airway tissues (MucilAir™) were exposed to silanes (triethoxysilane [TES] or trimethoxysilane [TMS] as vapor) or surfactants (Triton X-100 or Oleoyl Sarcosine as aerosol) at the air-liquid interface using a VITROCELL® 6/4 exposure system for 30 minutes. In addition, exposure by pipetting was done for surfactants. Endpoints assessed include cell viability, cytotoxicity, and secretion of inflammatory markers and, additionally for the 3D tissues, histology, barrier integrity, and cilia beating frequency. Results: Concentration-dependent decrease in cell viability and an increase in cytotoxicity was

observed in both, 2D and 3D tissues as compared to the sham exposure. An increase in secretion of inflammatory markers (i.e. Interleukins 6 and 8) was also observed for the silanes. In the 3D system, the active cilia beating areas and barrier integrity were also reduced in a concentration-dependent manner at 19-24 h post-exposure. Interestingly, after 7 days of recovery, barrier integrity returned to normal pre-exposure values. Discussion and Conclusion: More human-relevant in vitro models have become available recently but it is difficult to judge when the costs and complexity of these models overweigh the added physiological relevance. The results of this project may help better understand the usefulness of different test systems to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and to inform regulatory decision-making.

O-5B-2

Development and characterization of a decellularized bronchial scaffold for application in physiologically relevant 3D airway models reconstruction
ABSTRACT #311

Artur Christian Garcia da Silva[1], Sérgio de Morais Carvalho Filho[1], Tatyane Gonçalves Hayasaki[1], Marize Campos Valadares[1]

[1]*Laboratory of Research and Education in In vitro Toxicology (Tox In) – Faculty of Pharmacy – Federal University of Goiás (UFG)*

Background and Objectives: The search for in vitro physiologically relevant 3D airway models has advanced considerably in the last decades, aiming to replace animal-based methods with assays that resemble human response to toxicants exposure through inhalation. However, mimicking the whole composition of the extracellular matrix (ECM) microenvironment is a difficult task that has been overcome by some strategies, such as 3D bioprinting and commercial hydrogels and biomatrixes. On the other hand, it is still challenging to resemble the tissue topography and location of anchorage proteins, as well as to reproduce the exact proteic and non-proteic composition of ECM. So, this work aimed to produce and characterize a decellularized bronchial porcine scaffold obtained from food industry waste for further reconstruction of 3D bronchial models using human cells for long-term cultivation. Material and Methods: Porcine lungs were donated from a local slaughterhouse, and fragments from the right and left main bronchus were cut using a circular scalpel. The tissues were submitted to different methods of matrix decellularization (surfactant solutions, osmotic gradient and nucleases treatment) and then characterized regarding tissue morphology, DNA staining, collagen fibers arrangement, collagen Type IV immunolocalization and glycosaminoglycan composition. Results: The results demonstrated that all the evaluated decellularization methods were efficient for tissue cell removal. However, the SDS 1% treatment and osmotic gradient methods best preserved the ECM architecture, preserving the collagen Type IV location and glycosaminoglycan composition. Furthermore, SDS 1% guaranteed the best removal of genetic material content from the tissues. Discussion and Conclusion: The findings showed that surfactant-based decellularization methods can be efficiently employed to generate high-quality and low-cost extracellular matrix scaffolds for employment in tissue engineering. The following steps include biocompatibility studies and 3D airway model reconstruction using the obtained scaffolds.

O-5B-3

The application of alveolar macrophage responses for respiratory safety assessment
ABSTRACT #382

Victoria Hutter[1], Abigail Martin[1], Amjad Saeed[1], Noelia Perez-Diaz[1], Ewelina Hoffman[1]

[1]*ImmuONE Ltd*

Whilst human in vitro models of the airway epithelium provide valuable information regarding the interaction of inhaled substances within the airways, it is becoming increasingly evident that tissue-resident immune cell responses are pivotal in the tissue responses of many organs including the alveolar region of the lungs [1,2]. To date, there are limited immunocompetent human alveolar models available with the ability to appropriately mimic the epithelial and immune responses in the respiratory airways and many assessment strategies for the immune cell component fail to capture relevant endpoints to support in vivo-in vitro correlations [3]. The aim of this study was to demonstrate how ImmuLUNGTM, an in vitro human 3D epithelial-macrophage co-culture model, can be utilised for safety assessment with a combination approach of morphological, functional and biochemical endpoints to support a mechanism-based toxicity assessment approach. Alveolar-like macrophages in mono-culture

(ImmuPHAGETM) and co-cultured with alveolar epithelial cells (ImmuLUNGTM) and were exposed for 24 h to a panel of compounds representing different mechanisms of action: amiodarone (phospholipidosis), staurosporine (apoptosis induction), DQ particles (frustrated phagocytosis), cigarette smoke (autophagy), LPS (inflammation) and marketed inhaled medicines (salbutamol, fluticasone). After 24 h incubation a range of cell health, biochemical and functional responses were assessed together with detailed quantified morphological features and high content analysis. By combining key cell features and responses, a phenotype profiling approach was adopted to identify a 'fingerprint' of immune cell responses within both models to different compounds. This approach was highly reproducible and enabled clear differentiation of the cellular response based on the established mechanism of toxicity. This combination approach of the tissue resident immune responses supports the evaluation of the mechanism substance toxicity and tissue pathology providing new insights to support better and earlier decision making to support safety assessment.

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O-5B-4

Prediction of acute respiratory toxicity of volatile liquids using physiologically relevant in vitro inhalation model
ABSTRACT #447

Jan Markus[1], Yulia Kaluzhny[2], George R. Jackson[2], Silvia Letasiova[1], Olivia Gabriel[2], Paul Kearney[2], Mitchell Klausner[2], Alex Armento[2]

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To assess the health effects of inhaled substances the acute respiratory toxicity (ART) testing is required. OECD accepted methods utilize GHS categorization that depends on animal based tests. There is no validated in vitro ART assay, even though animal tests have been discredited as predictors of human responses and on ethical grounds. The goals of this work were to develop physiologically relevant tests using the EpiAirway™ tissue model, demonstrate interlaboratory transferability, and correlate the results to an established categorization system relevant to human respiratory irritation. Test articles (TA, n=53) were applied to EpiAirway tissues in 2 separate laboratories with ART protocols developed for exposure to mists/sprays (Direct Application Protocol, DAP) and vapors/volatile liquids (Vapor Cap Protocol, VCP). In both protocols, tissues were exposed for 4 hours to 4 fixed doses of the TA diluted in corn oil or water. In the DAP, TAs were applied to the apical tissue surface and in the VCP, to an absorbent material in a specially designed cap that forms a tight seal above the tissue allowing exposure to TA vapor. The effects on tissue viability (MTT assay) and barrier properties (Transepithelial Electrical Resistance, TEER) were determined. The effective doses (ED) which reduced tissue viability by 25% (ED-25) or by 75% (ED-75) were mathematically interpolated for the DAP and VCP methods, respectively. The ED-25 and ED-75 were correlated to the acute irritation Health Effects (HE) Codes (HE14/15/16) listed by OSHA, which are relevant to human exposure. Both protocols demonstrated high predictivity of human HE Codes, which are more relevant to human respiratory toxicity than the GHS categories. Good inter-laboratory reproducibility was observed for the VCP and DAP methods. Both protocols provide physiologically relevant, organ-specific in vitro tests that can improve the predictivity of human responses and significantly reduce the number of animals being used.

O-5B-5

IMPACT OF AIRBORNE PARTICULATE MATTER FROM PORT, INDUSTRIAL AND URBAN AREAS ON OXIDATIVE AND INFLAMMATORY RESPONSES ON LUNG CELLS.
ABSTRACT #298

Margaux Cochard[1], Frédéric Ledoux[1], Dominique Courcot[1], Yann Landkocz[1]

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Interactions sur le Vivant, UCEiV UR4492, SFR Condorcet FR CNRS-3417, Université du Littoral Côte d'Opale (ULCO), Dunkerque, France

Exposure to fine particulate matter (PM2.5) has been known for years for its adverse effects on human health [1]. Moreover, the nature of emission sources governs the particle chemical composition, leading to various toxicity responses [2]. Mechanisms like oxidative stress and inflammation can be induced by PM2.5 exposure [3]. The aim of this work is to chemically characterize PM2.5 from distinct origins and to study the toxicological potency of PM2.5 on pulmonary cells in order to establish the link between specific particle constituents with specific cell responses. PM2.5 were collected at four sites influenced by urban, industrial, road traffic, and port and maritime traffic emissions. PM2.5 from all four sites were extensively chemically characterized, notably by quantification of metals, water-soluble ions, total carbon, and organic components. Oxidative potential was also evaluated. Results highlight a clear difference in some metals concentration, notably for PM2.5 collected under industrial and road traffic influences. A549 lung cells were then exposed to organic and water-soluble fractions of PM2.5 from the four influences. In vitro cytotoxicity and viability were studied by exposing cells up to 100 µg/cm² for 24, 48 and 72 hours. No significant cytotoxic effect was observed but cells viability was altered. Oxidative stress, antioxidant response, as well as inflammation by ELISA of pro-inflammatory cytokines IL-6, IL-1β, TNF-α and IFN-γ, were studied. Depending on PM2.5 origin, oxidative stress and inflammation responses vary. These preliminary results need to be consolidated in order to identify biological markers specifically activated depending on the PM2.5 origin and chemical characteristics. This work was supported by ADEME (Convention no. 1962C0005) and Région Hauts-de-France.

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Wednesday, November 23rd 2022
11:00 - 13:00

Session: 6a. In vitro methods for safety testing of biopharmaceuticals/biotherapies/vaccines

Chairs: *Michael Rothe (Hannover Medical School, Hannover, Germany) & Anne Marie Vinggaard (Technical University of Denmark, Denmark)*

O-6A-1 (INVITED TALK)

In vitro assays to predict retroviral vector-induced genotoxicity
ABSTRACT #461

Michael Rothe[1], Antonella Bastone[1], Axel Schambach[1]

[1]*Hannover Medical School, Hannover, Germany*

Insertional mutagenesis is a bottleneck in retroviral vector-mediated gene therapy. Instead of laborious in vivo models with limited predictive value, scientists try to employ in vitro assays to screen for insertional mutagenesis. A decade ago, our lab developed the in vitro immortalization (IVIM) assay to quantify the genotoxic potential of vectors (1). Many academic and industrial partners have used IVIM to complete their preclinical safety documentation. Despite general acceptance in the field of haematopoietic gene therapy, bias for insertional mutants of the myeloid lineage and a low sensitivity are clear limitations. We recently developed the molecular surrogate assay for genotoxicity assessment (SAGA). The new test is more robust, sensitive and biologically informative. Instead of replating cells for later analysis of proliferating mutants, we isolate total mRNA of the bulk cultures and performed microarray analyses. With the help of machine learning algorithms, we can identify mutagenic vectors by the deregulation of an oncogenic gene expression signature (2). However, both IVIM and SAGA have an intrinsic myeloid bias due to culture conditions. To detect lymphoid insertional mutants, we differentiated mHSPC to mature T cells using the OP9-DL1 co-culture system. Transduction with the LTR-driven RSF91 mutagenic vector induced a block in T cell differentiation at the early double-negative 2 (DN2) progenitor stage, in contrast to fully differentiated untransduced mock cultures. Arrested samples upregulated the leukemogenic transcription factor Lmo2 and harbored vector insertions in its vicinity, as frequently observed in the patients from the clinical trials with severe adverse events. The new lymphoid branch of IVIM/SAGA complements the already accepted myeloid approach and contributes to a safer clinical translation of gene

therapy strategies.

References
(1) Modlich et al., Blood. 2006 Oct 15;108(8):2545-53. (2) Schwarzer et al.,Mol Ther. 2021 Dec 1;29(12):3383-3397.

O-6A-2

State-of-the-Art in Secondary Pharmacology and its Impact on the Safety of New Medicines
ABSTRACT #49

Jean-Pierre Valentin[1], Steve Jenkinson[2], Lyn Rosenbrier-Ribeiro[3], Friedemann Schmidt[4], Vicencia Toledo Sales[5], Yoav Timsit[6], Mohan Rao[7], Annie Delaunois[8], Richard Brennan[4], IQ DruSafe Secondary Pharmacology Working Group[9]

[1]*UCB Biopharma, Development Science, Braine l'Alleud, Belgium*
[2]*Pfizer*
[3]*Grunenthal*
[4]*Sanofi*
[5]*Takeda*
[6]*blue print medicines*
[7]*Johnson & Johnson*
[8]*UCB Biopharma*
[9]*IQ DruSafe Consortium*

Adverse effects often occur as a result of off-target or secondary pharmacology (2P). Consequently, 2P profiling of new chemical entities across a range of protein targets with known association to safety adverse events (e.g., GPCRs, ion channels, transporters, kinases and other enzymes) is routinely used in pharmaceutical R&D. However, there is variability across industry in the target panels screened, technologies applied, and when and how screening is conducted. As part of the IQ DruSafe consortium, a 2P working group was established, comprising representatives from 18-member pharmaceutical companies, to conduct a review of 2P strategies and share experiences, and to propose recommendations for best practice and future application for the industry. Member companies contributed proprietary data across parameters such as target panel composition, position in the R&D process, sourcing of the panels, the assay types and technology used. Performance and outcome, including total number of compounds run, concentrations tested, and hit rates, were also captured. A detailed database capturing an overview of the strategic, scientific, and operational aspects of 2P screening was created. The database currently holds 3729 records describing assays on 747 different biological targets or anti-targets. Analysis of the data will be presented highlighting some key trends that should be considered for future evolution of 2P practices across the industry. For example, there is strong influence of previous published work on 2P panel composition by Bowes et al 2012 and Lynch et al, 2017 with high prevalence of the targets they propose applied across the industry, but also a set of bespoke or uncommon targets identified that should be considered from a safety perspective. Furthermore, given the recent trends in pharma project focus, extending the diversity of target classes screened to include a set of safety-related kinases, as well as potentially enzyme and epigenetic classes should be considered.

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Bowes et al 2012, Bowes, J., Brown, A. J., Hamon, J., Jarolimek, W., Sridhar, A., Waldron, G., & Whitebread, S. (2012). Reducing safety-related drug attrition: The use of in vitro pharmacological profiling. Nature Reviews: Drug Discovery, 11(12), 909–922. Lynch, J. J., Vleet, T. R. V., Mittelstadt, S. W., & Blomme, A. G. (2017). Potential functional and pathological side effects related to off-target pharmacological activity. Journal of Pharmacological and Toxicolgical Methods, 87, 108–126.

O-6A-3

Exploiting Real-Time Assays to Multiplex Orthogonal Methods and Determine Mechanism of Action During In Vitro Safety Testing
ABSTRACT #107

Terry Riss[1]

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New assays continue to be developed to enable more efficient in vitro safety testing of biopharmaceuticals. Recent advances include the ability to use a multi-mode plate reader to record real-time cell health data continuously for days from the same cells. In contrast to endpoint assays that destroy cells and limit downstream sample processing, real-time orthogonal assay methods can be multiplexed to confirm results and eliminate variability caused by using parallel samples. One example is the use of orthogonal assays to monitor a decrease in a metabolic marker indicating loss of cell viability while simultaneously monitoring an increase in loss of membrane integrity as a confirmatory marker of dead cells. A second

example is to use the real-time detection of dead cells as a trigger to initiate assays to detect which signal transduction pathways are activated in response to a specific drug treatment. When a population of cells is treated with a toxin, not all cells die at the same time. During the early phase of toxin treatment, some cells may be dead whereas others are activating stress response signal transduction events to support survival. Real-time monitoring of each well to identify a detectable level of cell death can trigger removal of that individual sample for analysis without destroying the rest of the assay plate. Cherry-picking individual samples from a multi-well plate for RNA extraction enables identification of activated signal transduction pathways resulting from exposure to test compounds. These new real-time cell health assay methods that enable multiplexing orthogonal assays can contribute to rapid and efficient development of in vitro alternatives to using animals for safety testing of biopharmaceuticals and new chemical entities.

O-6A-4

Botulinum neurotoxin potency testing in vitro – Motor neurons differentiated from human induced pluripotent stem cells serve as highly suitable target cells

ABSTRACT #123

Maren Schenke[1], Hélène-Christine Prause[12], Wiebke Bergforth[1], Alice Dick[1], Andreas Rummel[3], Brigitte Gertrud Dorner[4], Frank Klawonn[56], Bettina Seeger[1]

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[2]*Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria*
[3]*Institut für Toxikologie, Medizinische Hochschule Hannover, Hannover, Germany*
[4]*Biological Toxins (ZBS 3), Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany*
[5]*Biostatistics Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany*
[6]*Department of Computer Science, Ostfalia University, Wolfenbüttel, Germany*

Background and Objectives: Approximately 400,000 mice are used annually in Europe for potency testing of pharmaceutical preparations of Botulinum neurotoxins (BoNTs), although some in vitro methods are already in use. BoNTs inhibit the release of neurotransmitter containing neuronal vesicles, by cleavage of proteins involved in their exocytosis. Alternatives for the mouse bioassay are product-specific, as they are mostly aimed at identifying serotype-specific protein cleavage. Since we are developing a cell-based and serotype-independent method for BoNT potency assessment, this study focused on sensitivity assessment of induced pluripotent stem cell (iPSCs)-derived motor neurons (MNs) to pharmaceutically relevant BoNT serotypes /A1, /B1, /E1 and /F1. Material and Methods: MNs were differentiated from iPSCs according to three different protocols and their sensitivity to BoNT/A1, /B1, /E1 and /F1 was measured by assessing substrate cleavage via Western Blot. Results were compared to Mouse LD50 values obtained for previously tested BoNT charges. Results: MNs are highly sensitive to the human-relevant BoNT serotypes (Schenke et al. 2020; Schenke et al. 2021). One of the protocols (Maury) gave highly human representative results and was 20-fold more sensitive to BoNT/A1 than mice with 0.05 Mouse LD50/mL, and slightly less sensitive than BoNT/B1 with 4.12 Mouse LD50/mL, BoNT/E with 4.54 Mouse LD50/mL and BoNT/F with 2.46 Mouse LD50/mL. Discussion and Conclusion: In vitro differentiated human MNs appear to be well suited for serotype independent BoNT potency testing. Human-relevant sensitivity to the pharmaceutically relevant BoNT serotypes could be demonstrated by Western blot analysis. Optimally, an alternative method should be at least as sensitive as the replaced animal test, but the human cells being slightly less sensitive than mice, could even help to boost predictability, since humans are 30 to 100 times less sensitive to BoNT/B than mice. Acknowledgement: Funded by the German Federal Ministry of Education and Research (FKZ 031L0132B).

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O-6A-5

Supported Gel Slabs: Decreasing the barrier of entry for use of 3D cell cultures in the drug discovery workflow
ABSTRACT #287

Zachary Sitte[1], Matthew Lockett[1]

[1]*University of North Carolina*

Bringing a drug to market is a costly and time-intensive process, partially due to the lack of physiologically relevant in vitro models, in the drug discovery workflow, that can predict clinical outcomes. Three-dimensional (3D) culture platforms provide tissue-like microenvironments that result in more representative cellular responses and phenotypes than traditional monolayer culture formats. The number of 3D cell culture techniques available continues to increase with advances in microfabrication techniques and cell-compatible 3D printing techniques, however, their wide adoption is limited due to a high barrier of entry in terms of cost, expertise, or equipment. The paper-based cell cultures (PBCCs), developed by our laboratory and others, uses accessible materials to rapidly generate tissue- and tumor-like structures. Despite their ease of use, the paper scaffolds are incompatible with some experimental techniques, such as confocal microscopy, due to the cellulose fibers being optically opaque and exhibiting high autofluorescence. Here we describe an alternative to the paper-based scaffolds, a supported gel slab (SGS) 3D culture platform. This platform incorporates the functionality of PBCCs (e.g., rapid culture generation and simple segmentation of tissue- and tumor models) but overcomes its imaging limitations. The SGSs, much like the PBCCs, are constructed from low-cost and accessible materials. To characterize the SGS platform, we compare the analytical figures of merit with the PBCCs in terms of reproducibility, molecular biology-based readouts of cellular responses, the ability to quantify invasion, and responses to chemotherapies. Our preliminary results show the SGS platform generates comparable or improved figures of merit, providing a method that can be more easily incorporated into the drug discovery workflow, which is compatible with optical, molecular biology, and other commonly used cell-based readouts.

O-6A-6

Mechanistic investigations and early de-risking of QRS complex widening to improve cardiac safety profile of novel anti-malarials
ABSTRACT #332

Jean-Pierre Valentin[1], Alvaro Cardenas[1], Vitalina Gryshkova[1], Chloé Korlowski[1], Pierre Bonnaillie[1], Nabila Chabbi[1], Rosana Chirico[1], Teresa de Haro Garcia[1], Benoit Laleu[2], Martin Lowe[3], Annie Delaunois[1]

[1]*UCB Biopharma*
[2]*MMV*
[3]*Exscientia*

Quinoline-related antimalarial drugs such as chloroquine have been associated with cardiotoxicity risk, in particular QT prolongation and QRS complex widening. In the frame of the Medicines for Malaria Venture (MMV) partnership, UCB developed novel plasmepsin X (PMX) inhibitors for malaria treatment. The first lead compounds tested in anesthetized guinea pigs (GP) induced profound QRS widening, although exhibiting weak if no inhibition of NaV1.5-mediated currents in patch clamp assays. To understand the mechanism underlying QRS widening and rapidly identify further compounds devoid of such risk, we established a set of in vitro models including NaV1.5 rate-dependence and NaV1.8 patch clamp assays, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), and Langendorff-perfused isolated GP hearts. Six compounds were tested in all models including anesthetized GP, and 9 additional compounds were tested in hiPSC-CM and isolated GP hearts only. All compounds showed roughly similar cardiovascular profile, consisting of QRS widening, bradycardia, negative inotropy, hypotension, and for some, QT prolongation. There was a good consistency between data obtained in the two in vitro models (decrease in sodium spike amplitude in hiPSC-CM, QRS widening in isolated GP hearts) and in the anesthetized GP, although a right shift of the concentration-response curves was noted from in vivo to in vitro. Patch clamp assay results showed that the QRS widening observed with PMX inhibitors is likely multifactorial, with involvement of sodium and/or calcium channel-mediated mechanisms. In conclusion, early in vitro de-risking of QRS widening allowed to improve the cardiac safety profile of novel PMX inhibitors.

Session: 6b. Knowledge sharing and education

Chairs: *Pilar Vinardell (Universitat Barcelona, Barcelona, Spain) & Erin Hill (IIVS, Gaithersburg, MD, USA)*

O-6B-1

The science of sharing knowledge about models and methods
ABSTRACT #110

Sofia Batista Leite[1], Clemens Wittwehr[1], Ivana Campia[1], Marcelle Holloway[1], Pierre Deceuninck[1], Maurice Whelan[1]

[1]*European Commission, Joint Research Centre (JRC)*

'It takes a village to raise' an idea, to make change, to disseminate knowledge. Scientific advancement is fuelled by increasingly sophisticated methods of investigation that emerge, evolve and ultimately thrive. The ultimate value of a method is often demonstrated by how it serves multiple purposes beyond its originally intended use. The recently published JRC report(1) suggests that methods should be the vehicle to bridge across different scientific communities to achieve greater levels of crossdisciplinarity. For that, the strategy and tactics we adopt to share knowledge about our methods and models (M&Ms) become key. Sharing strategies should not simply focus on the descriptive information about methods, but on the knowledge and practices that surround them, including the inspiration that led to their creation, understanding the context of their development, their relevance and reliability. This comprises more than the simple explicit 'content' share – through journal publications, platforms or data catalogues, but also the social – the people that use and promote them. With this talk, the authors plan to share with the ESTIV community the EURL ECVAM perspective on how the knowledge-sharing paradigm needs to shift to better exploit M&Ms, put them at the centre of a change in scientific approach. The strategy has to be people and community centric, using unstructured social environments to create the right ecosystem for optimal sharing. Additionally, knowledge of M&Ms needs to be explicit and adapted to communities receiving it, targeting their motivation and values, and using a translation system that can bridge different community-specific languages and concepts. "Knowledge Sharing is about making the right knowledge or the right knowledge sources (including people) available to the right people at the right time!"(2) As a community, we need to work on the key aspects: the right knowledge on M&Ms, right sources, right people and right time.

References

(1) https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/bridging-across-methods-biosciences (2) http://www.knowledge-management-tools.net/knowledge-sharing.html

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O-6B-3

Scientific Validity of Non-Animal-Derived Antibodies
ABSTRACT #153

João Barroso[1]

[1]*European Commission, Joint Research Centre*

Antibodies are binding molecules that have a high specificity for their unique target (antigen). They are crucial tools for research, diagnostics, therapeutic and regulatory applications. Based on their recognition properties and binding specificity, protein-based antibodies are currently still the most important tools for the specific detection of proteins or other molecules. Animals are still widely used for the development and production of monoclonal and polyclonal antibodies despite the availability of alternative non-animal technologies for more than 20 years. In line with the legal requirements of EU Directive 2010/63/EU on the protection of animals used for scientific purposes, animals should not be used in procedures where a non-animal alternative that provides the same or higher level of information exists. For this reason, the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) mandated its Scientific Advisory Committee (ESAC) to review the available evidence and deliver an opinion on the scientific validity of antibodies produced using animal-free technologies, such as phage-display. Taking into consideration the available evidence, the ESAC concluded that non-animal-derived antibodies are mature reagents generated by a proven technology, being able to replace animal-derived antibodies in the vast majority of applications. They have no general or systematic disadvantages with respect to affinity, stability, shelf life and specificity, and offer significant scientific and economic benefits. Based on the ESAC Opinion, EURL ECVAM issued its own recommendation that the provisions of Directive 2010/63/EU should be respected and the use of animals for the development and production of antibodies should no longer be authorised in the absence of robust and legitimate scientific justification [1]. An overview of the ESAC findings

and of the EURL ECVAM Recommendation on non-animal-derived antibodies will be presented.

References
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O-6B-4

Animals in the (Petri) Dish: Towards a Truly Animal-free Laboratory
ABSTRACT #299

Tilo Weber[1]

[1]*Animal Welfare Academy of the German Animal Welfare Federation, Neubiberg, Germany*

Directive 2010/63/EU calls for a full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so. One approach for non animal methods (NAM) is the use of cell and tissue cultures. To facilitate an optimal environment for cells to thrive in vitro, they have to be held in homeostasis by maintaining media supplement, attachment factors, coating materials and even preparation of the used plastic ware [1]. Unfortunately, many commonly used laboratory reagents and materials are still of animal origin. This does not only cause immense ethical and animal welfare issues and dilemmata. Moreover, their use can result in reduced reproducibility, reliability, transferability and biosafety of any scientific data derived from these methods. Therefore, animal-derived materials and reagents have to be replaced by human-derived or (chemically) defined materials to fully end the exploitation of animals in science. In this presentation, different sources of animal-derived laboratory products will be described; alongside with production procedures and the implication for involved animals. Main focus will be on production of fetal bovine/calf serum (FBS/FCS) [2], but also murine sarcoma cells, animal-derived antibodies and pyrogen detection tests. Furthermore, an ethical discussion of the use of non-human-derived cells will be encompassed [1]. Finally, cruelty-free replacements will be highlighted, including strategies to accelerate transition to non-animal-derived laboratory reagents and materials [3]. Thence, setting the path to achieve a truly animal-free laboratory working environment to the benefit of animals, scientists and patients.

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[1] Weber, T., Wiest J., Oredsson, S. and Bieback K.: Case studies exemplifying the transition to animal-component-free cell cultures, ATLA, in press, (2022) [2] Weber, T., Wirths, F., Brakebusch, N. and Van der Valk, J.: Reply to comment “Animal welfare and ethics in the collection of fetal blood for the production of fetal bovine serum”. ALTEX, 38.2 (2021), 324-326, doi:10.14573/altex.2103191 [3] Manuela Cassotta, M., Pistollato, Bartnicka, J.J., Saravanan, S., Weber, T., Bastos, L.F., Vito D’Alessandro, V. and Coecke, S.: A worldwide survey on the use of animal derived materials and reagents in scientific experimentation, Eng Life Sci, in press, (2022)

O-6B-5

OUTCOMES OF A WORKSHOP TO ADDRESS ANIMAL METHODS BIAS IN SCIENTIFIC PUBLISHING
ABSTRACT #320

Catharine Krebs[1], Helder Constantino[2], Lilas Courtot[3], Kathrin Herrmann[4], Ann Lam[1], Sofia Batista Leite[5], Janine McCarthy[1], Brinda Poojary[6], Kristie Sullivan[1]

[1]*Physicians Committee for Responsible Medicine*
[2]*Humane Society International Europe*
[3]*Animal Free Research UK*
[4]*Johns Hopkins University, Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT)*
[5]*European Commission Joint Research Centre*
[6]*Humane Society International, India*

Background and Objectives: Publishing plays a crucial role in the advancement of science, the implementation of interventions, and the progress of researchers’ careers. Thus, there is a need to identify and address biases that occur within the publishing process. We recently identified a bias called “animal methods bias in publishing:” a preference for animal-based methods where they may not be necessary or where nonanimal-based methods may be suitable, which affects the likelihood of a manuscript being accepted for publication. Material and Methods: On April 20 and 21, 2022, stakeholders gathered virtually for a workshop to address animal methods bias in scientific publishing. The charge for the workshop was: (1) Explore a range of stakeholder

perspectives, including from academic and industry researchers, journal editors, and government representatives; (2) Describe the current state of animal- and nonanimal-based experimental systems; (3) Describe animal methods bias in publishing and related biases in publishing and peer review; (4) Identify potential causes, consequences, and mitigation strategies for animal methods bias in publishing . Results: Common and salient themes of the workshop were: (1) Different research domains have different needs for model systems and have made progress in nonanimal models at different rates; (2) Animal methods bias extends beyond publishing; (3) There is a need for more evidence of bias. Additional recommendations to address animal methods bias in scientific publishing that emerged from the workshop included: Build awareness; Educate editors and reviewers; Implement different incentive models; Require preregistration and retrospective review; Implement open science; Prioritize funding for nonanimal methods; Advance validation, standardization, and reporting of nonanimal methods; Change regulatory requirements. Discussion and Conclusion: This workshop was successful in its charge and created an ongoing taskforce committed to addressing animal methods bias in publishing, which will build more evidence and further explore contributing factors, impacts, and mitigation strategies.

O-6B-6

A Course on Animal-Free Risk Assessment of Cosmetics and Cosmetic Ingredients
ABSTRACT #462

Renato de-Avila[1], Catherine Willett[2]

[1]*Unilever*
[2]*Humane Society International*

There has been significant progress globally over recent years in advancing the science to underpin non-animal cosmetic safety assessment. In addition, restrictions on animal testing of cosmetics and cosmetic ingredients are expanding geographically every year. These advances are leading to the need for building capacity for completely animal-free safety assessment of consumer products amongst the regulated and regulatory communities globallly. Achieving a confident risk assessment of a consumer product or ingredient without data from new animal testing requires a novel approach to the assessment as well as integration of several types of in silico and in vitro data. To enable confident decision-making regarding the safety of cosmetics and personal care products, it is important to build confidence is the requisite methodologies based on experience. Toward this aim, an in-depth educational program has been developed by the AFSA (Animal-Free Safety Assessment) Collaboration, a partnership of NGOs and industry. The course covers the risk assessment process from beginning to end in 8 modules: Problem formulation, Consumer Exposure, Exposure-based Waiving, History of Safe Use, In silico Tools & Read-Across, Internal Exposure: Dosimetry, In Vitro Data Synthesis, and the Overall Risk Assessment. A ninth module covers the global regualtory landscape for cosmetics and chemicals. We have presented overviews of several of the modules in webinars, and are planning to begin rolling out modules in their entirety this fall via webinar series. In 2023 we plan to create a free, online self-paced course.

Thursday, November 24th 2022
08:30 - 10:30

Session: 7a. Developmental Neurotoxicity (DNT)

Chairs: *Ellen Fritsche (IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany) & Clive Roper (Roper Toxcilogy Consulting Limited, UK)*

O-7A-1

Developmental neurotoxicity (DNT) as a case study on how to assemble a an in vitro testing battery for a complex regulatory in vivo endpoint
ABSTRACT #398

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Testing for developmental neurotoxicity (DNT) is currently performed in rats according to OECD/US-EPA guidelines. These methods are resources demanding, have unknown sensitivity and uncertainties in their interpretation. To overcome these issues and allow a large number of

chemicals to be tested for DNT, a DNT in vitro testing battery (DNT IVB) has been assembled under the guidance of the European Food Safety Authority (EFSA) in collaboration with the Danish- and US-Environmental Protection Agency and under the umbrella of the Organisation for Economic Cooperation (OECD). The DNT IVB consists of test methods based on primary human neural progenitor cells (hNPC), human induced pluripotent stem cell (hiPSC)-derived neural crest cells and neurons, as well as LUHMES cells and model the key neurodevelopmental processes hNPC proliferation, migration and differentiation into neurons and oligodendrocytes, neurite morphology, neural crest cell migration and neurite outgrowth. This IVB was challenged with 120 chemicals. In addition, a hiPSC-based test method for human neuronal network formation was set up and challenged with 28 pesticides. Concentration-response curves reveal benchmark concentrations (BMCs) for the 120 compounds in the individual test methods. Classification models for data interpretation were applied. For interpretation of compound results across the whole battery, respective most sensitive endpoints (MSEs) were determined. Battery results were used in two case studies, i.e. hazard characterization of deltamethrin and flufenacet in an Adverse Outcome Pathway-informed Integrated Approach for Testing and Assessment (by EFSA) and flame retardant prioritization (by the US-National Toxicology Program). These data demonstrate the successful set-up of a DNT-IVB for fit-for-purpose regulatory problem formulations. An OECD guidance document has been prepared (Crofton & Mundy 2021) that informs on use and interpretation of the DNT-IVB for regulatory application. Increasing trust in battery performance by testing more chemicals and lab-to-lab transfer will aid its implementation into regulation.

O-7A-2

FIRST STEPS FOR CREATING AN ONTOLOGY FOR COGNITIVE FUNCTION DEFECTS FOR REGULATORY APPLICATION
ABSTRACT #416

Eliska Kuchovska[1], Alessio Gamba[2], Luiz Carlos Maia Ladeira[2], Bernard Staumont[2], Raphaëlle Lesage[3], Inger-Lise Steffensen[4], Graciela Lopez Soop[5], Tim Hofer[5], Oddvar Myhre[5], Hubert Dirven[5], Liesbet Geris[236], Ellen Fritsche[17]

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Cognitive functions such as learning, thinking, reasoning, remembering, problem-solving, and attention are critical for day-to-day life. They are developed in a series of complex and sensitive brain developmental processes (differentiation, synaptogenesis, etc.). These processes can be disturbed by various environmental cues and lead to neurodevelopmental disorders such as cognitive function defects (CFD). This study aims to review the known links between prenatal exposure to chemicals, disturbed neurodevelopmental processes, and CFDs. The final goal of this initial approach is to create an ontology – a framework quantitatively and qualitatively integrating biological, toxicological, kinetic, and chemical data – as a new innovative strategy to predict repeated-dose developmental neurotoxic (DNT) effects of chemicals within the ONTOX project [1]. First, a literature study on CFDs was carried out. Second, a physiological map (PM) of the developing brain was created and CFD-relevant adverse outcome pathways (AOP) were identified. Finally, a tailored in vitro battery with human primary and iPSC-derived cell lines (2D and 3D) was proposed based on [2] and [3]. The created PM shows the neurodevelopmental processes and physiological mechanisms underlying neurodevelopmental disorders and enables the derivation of new CFD-relevant AOPs. Collected existing AOPs were mapped into an AOP network containing information about available in vitro assays for assessing compound effects on relevant molecular initiating events (MIE) and key events (KE) in the network. The described results are the first steps for the generation of a DNT ontology related to CFDs. The proposed ONTOX in vitro battery or an in silico approach will be used for filling artificial intelligence-identified data gaps in the ontology. Ultimately, the combination of these methods serves to create a new approach methodology that can, in combination with exposure assessment, advance human risk assessment in line with Next Generation Risk Assessment principles and

without the use of animals.

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O-7A-3

Evaluation of a human iPSC-derived BBB model for repeated dose toxicity testing
ABSTRACT #286

Maxime Culot[1], Sara Wellens[1], Lucie Dehouck[1], Vidya Chandrasekaran[2], Pranika Singh[3], Rodrigo Azevedo Loiola[1], Thomas Exner[3], Paul Jennings[2], Fabien Gosselet[1]

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Background and Objectives : The blood-brain barrier (BBB) is a highly restrictive barrier that preserves central nervous system homeostasis and ensures optimal brain functioning. Using BBB cell assays makes it possible to investigate whether a compound is likely to compromise BBBs functionality, thereby probably resulting in neurotoxicity. Recently, several protocols to obtain human brain-like endothelial cells (BLECs) from induced pluripotent stem cells (iPSCs) have been reported. Within the framework of the European MSCA-ITN in3 project, we explored the possibility to use an iPSC-derived BBB model to assess the effects of repeated dose treatment with chemicals. Methods: Our first objective was to evaluate different published protocols to differentiate iPSCs into BBB like endothelial cells regarding their expression of endothelial markers, formation of tight barrier and the presence of functional efflux pumps (e.g. ABCB1, ABCG2, ABCC1). After some protocol optimizations, the iPSCs derived BBB cells were found to exhibit important BBB characteristics up to 15 days after the end of the differentiation and could be used to assess the effects of repeated dose treatment, using Cyclosporine A (CsA) as a model compound. Results: Although iPSCs derived BBB cells were still undergoing transcriptional changes over time, a targeted transcriptome analysis (TempO-Seq) indicated a time and concentration dependent activation of ATF4, XBP1, Nrf2 and p53 stress response pathways under CsA treatment. Conclusion: Taken together, these results demonstrate that this iPSC-derived BBB model and iPSC-derived models in general hold great potential to study the effects of repeated dose exposure with chemicals, allowing personalized and patient-specific studies in the future.

O-7A-4

Comparative developmental neurotoxicity of NSAIDs in the zebrafish
ABSTRACT #353

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Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed medications worldwide. NSAID have been shown to alter the development of the central nervous system and behaviour alterations have been observed in zebrafish embryos (1). Although their pharmacological mechanism of action is well-known, information about their adverse effects on neurological development is still unclear. The aim of this study was to compare the ability of NSAID to induce behavioral alterations in zebrafish embryos. Methods: Zebrafish embryos were exposed to increasing concentrations of various NSAIDs non-selective and selective cyclooxygenase, COX-1 and COX-2, inhibitors

(SC-560, NS-398, indomethacin, oxaprozin and celecoxib). Firstly, developmental effects of NSAIDs were evaluated using the FishInspector software (2) to select the appropriate concentrations for behaviour analysis. Two behavioral parameters, spontaneous tail coling (STC) and thigmotactic behaviour, were analysed. Results were also compared to their acute thigmotactic effect at 3 days post-fertilization. Results: The selective COX-1 inhibitor (SC-560) showed fewer developmental defects compared to the COX-2 selective inhibitors (NS-398 and celecoxib). Only zebrafish embryos exposed to COX-1 inhibitors showed an increased frequency of STCs. Therefore, thigmotaxic behaviour was analysed for COX-1 inhibitors. Oxaprozin exposure increased significantly thigmotaxis while indomethacin showed a trend towards increased thigmotaxis. Discussion: COX-2 selective inhibitors produced a widely variety of morphological defects compared to COX-1 inhibitors, that contrasts with the morpholino knockdown experiments in which COX-2 knockdown fail to produce any phenotype (3). STC analysis revealed that inhibition of COX-1 induced an hyperactive phenotype in zebrafish. From the thigmotaxic assays, results suggest that inhibition of COX-1 appears to induce acute and developmental anxiety-related alterations in zebrafish larvae. It can be concluded that inhibiting one isoenzyme may have a greater neurotoxic effect than the other, which can be further explored in future studies. As such, this study reinforces the need to investigate neurotoxic effects of NSAIDs.

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O-7A-5

AN ADVERSE OUTCOME PATHWAY (AOP) BASED APPROACH TO IDENTIFY CHANGES IN PRENATAL BRAIN PROGRAMMING

ABSTRACT #378

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In previous studies we identified that EGCG (epigallocatechin-gallate) interferes with neural progenitor cells (NPCs) migration by its interaction with the Integrin-β1 cell adhesion molecule. However, since the dose makes the poison, we investigated in the Neurosphere Assay if 5, 50 or 500 times lower concentrations of EGCG could be safely used as a neuroprotective prenatal therapy against the myelination defects induced by intrauterine growth restriction (IUGR). IUGR was surgically induced in one horn in pregnant rabbits on gestation day (GD) 25. Neural progenitor cells (NPCs) growing as neurospheres were obtained from whole brains of rabbit pups at GD30. Neurospheres were in vitro exposed to increasing concentrations of EGCG and the neurodevelopmental key events migration distance, migration corona, oligodendrocyte differentiation and cell viability were assessed. As expected, IUGR neurospheres presented a significant reduction in oligodendrocyte differentiation, and control neurospheres presented a significant reduction in migration distance and corona formation after exposure to EGCG 1µM. Surprisingly, EGCG did not disturb the migration pattern of IUGR neurospheres at any concentration. Investigating the mechanism behind this difference in response we discovered that IUGR neurospheres have a significant 10-times higher expression of β1-integrin than controls. Since this subunit is also involved in dendrite formation, we further explored the consequences of EGCG exposure on dendrite formation and continued building the previously outlined AOP complementing it with a combination of key events related with both pathways and leading to the adverse outcome learning deficits. We have discovered for the first time that IUGR neurospheres respond differently than control to

the exposure of a compound triggering migration alterations and we have unravelled the mechanism behind this difference. The Neurosphere Assay and the AOP frame are useful tools to assist on the discovery of molecular initiating events by reducing the number of animals needed to characterize neurodevelopmental alterations.

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O-7A-6

EFFECTS OF SPIKE PROTEIN AND TOXIN-LIKE PEPTIDES FOUND IN COVID-19 PATIENTS ON HUMAN 3D NEURONAL/GLIAL MODEL UNDERGOING DIFFERENTIATION: POSSIBLE SARS-COV-2 IMPLICATIONS FOR BRAIN DEVELOPMENT.

ABSTRACT #456

Laure-Alix Clerbaux[1], Francesca Pistollato[1], Mauro Petrillo[2], Emilio Mendoza-de Gyves[3], Helena Soares[4], Maurice Whelan[3]

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Background and objectives. It is known that infections during pregnancy may increase the risk for offspring to develop neurodevelopmental disorders. However, the possible neurodevelopmental consequences of SARS-CoV-2 are presently still unknown. In utero exposure to SARS-CoV-2 has been hypothesized to affect the developing brain. Notably, Spike (S) protein interactors, such as ACE2, have been found expressed in the fetal brain, and could play a role in potential SARS-CoV-2 fetal brain pathogenesis. Apart from the possible direct involvement of SARS-CoV-2 (as a whole or due to specific viral components) in neurodevelopmental manifestations, in a previous study (1), the presence of toxin-like peptides in plasma, urine and fecal samples specifically from symptomatic COVID-19 patients was reported. The sequences of these (oligo)peptides map with the those of known neurotoxic proteins. Method. We investigated the possible neurotoxic effects elicited by acute exposure and by repeated exposure to Spike (S) protein (recombinant S1+S2), toxin-like peptides found in COVID-19 patients, and a combination of both on 3D human iPSC-derived neural stem cells (NSCs) differentiated towards neurons/glia (2). Results. Our data show that S protein at non cytotoxic concentrations caused a decrease of spontaneous electrical activity 2 days after exposure, and when combined with toxin-like peptides, such decrease was observed earlier. Whole transcriptome analysis revealed the dysregulation of five specific genes, i.e., SPHK1, ELN, GASK1B, HEY1 and UTS2, upon acute exposure, involved in the regulation of inflammation, hypoxia, and neuronal/glial differentiation, as well as some genes critical during brain development (3). Discussion. The perturbations of these neurodevelopmental endpoints are discussed in relation with concentrations found in COVID-19 positive pregnant women and in the context of recent knowledge about key events described in Adverse Outcome Pathways relevant to COVID-19-associated brain dysfunctions, gathered wihtin the CIAO project (Modelling the pathogenesis of COVID-19 using the Adverse Outcome Pathways, www.ciao-covid.net).

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Session: 7b. Implementation of NAMs into regulatory frameworks – establishing scientific confidence, development of standards and good practices

Chairs: *Marketa Dvořáková (NIPH Prague, Prague, Czech republic) & Jochem Louisse (Wageningen University, Wageningen, The Netherlands)*

O-7B-1

Method developers are key players ensuring in-house reproducibility and relevance of in vitro methods paving the way for regulatory acceptance

ABSTRACT #242

Sandra Coecke[1], Gerard Bowe[1], Anna Beronius[2], Thomas Cole[1], Elise Grignard[1], Marcelle Holloway[1], Ingrid Langezaal[1], Sofia Leite[1], Roman Liska[1], Susanna Louhimies[3], Sharon Munn[1], Katrin Schutte[3], Patience Browne[4], Maurice Whelan[1]

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Applying Good In Vitro Method Practices (GIVIMP) by the global life science community is leading to more harmonisation of in vitro method related processes and procedures. Method developers are key players to ensure use of current best scientific and quality practices across all 36 OECD member countries and beyond. Therefore, the best ambassadors in stimulating use of in vitro methods as key building blocks in modern life science practices (research and regulatory decision-making) are the method developers. The European Commission Joint Research Centre's European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is working on developing and validating innovative, mechanistic methods and approaches based on current harmonised scientific and quality standards complying with the GIVIMP guidance document (OECD, 2018) and is aligning with multi-disciplinary research clusters that are complementing these efforts. The instruments used to disseminate the harmonised GIVIMP practices are conferences, face-to-face meetings and trainings, webinars, templates (Krebs et al., 2019), reporting and evaluation tools (SciRAP, 2020) and e-learning training materials. During these activities, various critical aspects of the GIVIMP guidance document are highlighted since they impact the reliability and robustness of test systems, choice of relevant reference and control items, the in vitro experimental designs, practices for in-house validation and their transfer to other laboratories. An important driver of these knowledge sharing and exchange activities is to strive for the production of harmonised, concise and clear, globally understood and well-performing in vitro method procedures detailing e.g. acceptance criteria, data analysis and interpretation of the results. These life science community alignment efforts are of critical importance to make available the necessary fully animal-free in vitro tools to ease, prevent and manage the disease burden (e.g. thyroid disruption, neurodevelopmental effects, cancer) caused by toxicants in people and the environment.

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O-7B-2

Good In Vitro Method Practices Certification: A Roadmap for Implementation and Harmonization

ABSTRACT #284

Amanda Ulrey[1], Susanne Kolle[2], Robert Landsiedel[2], Erin Hill[1]

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The Organization for Economic Cooperation and Development (OECD) guidance document on Good In Vitro Method Practices (GIVIMP) details a set of quality standards to improve both the quality of and confidence in newly developed, and routinely executed in vitro methods. Currently a practical guide to implement GIVMP standards is missing; leaving organizations to define the best approach for themselves. Given the broad range of scientific and quality topics in GIVIMP, there is the potential for varying interpretations of the guidance and thus significant differences in implementation. The Institute for In Vitro Sciences, Inc. (IIVS) has created a business to business certification program to help commercial and academic institutions achieve the best value from this guidance document. The certification program harmonizes GIVIMP interpretation and standardizes "claims" of compliance with the document among participating laboratories. GIVIMP certification can be useful for academic and commercial facilities already functioning under other quality standards such as Good Laboratory Practices (GLPs) and ISO since GIVIMP provides unique recommendations for in vitro work not covered under those standards. A pilot certification between IIVS and BASF SE toxicology laboratories (Ludwigshafen, DE) has been launched to provide proof-of-concept for the program. This poster discusses the need for the GIVIMP certification program and provides details on its structure and administration.

O-7B-3

TOWARDS STANDARDIZATION OF TESTING METHODS FOR COMPARATIVE IN VITRO METABOLISM (CIVM) STUDIES
ABSTRACT #389

Annelies Noorlander[1]

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Performance of comparative in vitro metabolism (CIVM) studies for pesticide active substances related to human health is set by Commission Regulation (EU) No 283/2013. However, harmonized and validated approaches to perform such CIVM studies are currently lacking, hampering the evaluation of CIVM study results. For adequate interpretation of CIVM data, it is of importance that the in vitro test systems produce consistent data that are reliable (reproducible), requiring a standardized in vitro testing methods. EFSA's Scientific Panel on Plant Protection Products and their Residues (PPR Panel) recently published an Opinion with a proposed study set-up for CIVM studies in order to detect possible unique human metabolites (UHM) or disproportionate human metabolites (DHM; metabolite that is formed ≥ four-fold in human hepatocytes than in animal hepatocytes). Currently, studies using this proposed approach are not yet available. Therefore, the aim of this study was to perform a CIVM study for the herbicide chlorpropham according to the proposed approach in the PPR Panel Opinion using primary human hepatocytes (PHH), HepaRG cells, and primary rat hepatocytes (PRH). Before the main study, solubility, non-specific binding and cytotoxicity of chlorpropham are determined. The test systems are characterised for their metabolic activity by reference compounds for a series of specific CYPs and for UGT and SULT activity. For the main study, cell concentration, test item concentration and incubation time are optimized. Under optimized conditions CIVM studies are performed to obtain data for at least the minimal requirements mentioned in the PPR Panel Opinion (3 time points, 3 test item concentrations, all with 3 replicates). Example datasets as that produced in the present study are important for evaluation of the proposed study set-up in the PPR Panel Opinion.

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O-7B-4

IMPACT OF CURRENT REGULATORY CHANGES ON TOXICOLOGY TESTING REQUIREMENTS FOR CHEMICALS IN EUROPE
ABSTRACT #511

Manon Beekhuijzen[1]

[1]*Charles River Laboratories*

On 14 October 2020, the European Commission published its chemicals strategy for sustainability

towards a toxic-free environment as part of the European Green Deal. It presents several actions to bring about a toxic-free environment and to protect people and the environment from hazardous chemicals. One of the main drivers behind this new strategy is the expected increase in chemical production and the unknown territory of chemicals risks. Therefore, authorities are aiming to acquire more information on the intrinsic properties of many chemicals. CARACAL (expert group) is working on these extended REACH information requirements. Currently, they have proposed five options, of which all include merging of Annex VII and VIII. This will result in an substantial increase of animal studies needed for chemicals manufactured in low amounts. Depending on the option that will be selected, it could also increase the requests of NAM-based testing for ADME and critical hazards (e.g. DNT, ED). The preferred option would include these NAMS but also revisions to Annex IX, which would result in a significant decrease of animal studies. It will take several years before this will be implemented (expected in 2026). In addition, the European Commission has published a draft delegated regulation for the introduction of hazard classes for ED (endocrine disruption), PBT (persistent, bioaccumulative, toxic), vPvB (very persistent, very bioaccumulative), PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile) compounds. The criteria mention that classification shall be largely based on evidence from human or animal data, and it also mentions that the adverse effect should be determined in an intact organism or its offspring and future generations. It is therefore expected that ECHA will request additional animal studies for these type of compounds. Implementation of these hazard categories is expected in Q1 2023.

Thursday, November 24th 2022
11:00 - 12:40

Session: 8a. In vitro methods for safety assessment of medical devices

Chairs: *Christian Pellevoisin (MatTek Life Sciences, France) & Helena Kandarova (CEM SAS, Bratislava, Slovakia)*

O-8A-1 (INVITED TALK)

ISO 10993 SERIES: ADDED VALUES OF 3D MODELS FOR BIOCOMPATIBILITY MEDICAL DEVICES

ABSTRACT #451

Christian Pellevoisin[1], Silvia Letasiova[1], Seyoum Ayehunie[2], Mitchell Klausner[2]

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The ISO 10993 series of standards for the biocompatibility assessment of a medical device incorporates the 3Rs concept but the lack of qualification for medical devices (MDs) of several in-vitro methods already validated for chemicals and cosmetics leads to a high number of animal tests each year. This situation evolved considerably last year with the publication of the ISO 10993-231 standard for irritation of MDs. The multi-centric study, conducted under the auspices of Working Group 8 of Technical Committee 194, validated two in-vitro methods with reconstructed human epidermis (RhE) to replace topical and intracutaneous tests in rabbits (1). The ISO 10993-23 standard requires that MD extracts must be performed in-vitro before in-vivo testing is considered. Additional studies with mild irritants tested at low concentrations in MD extracts reinforced the added value of RhE models (2). For medical devices intended to be applied to a specific area, i.e., mucosal or eye epithelium, the standard still states that RhE models are not recommended. The results of studies using the EpiOcular, EpiVaginal or EpiOral models show that these in-vitro methods are promising to replace special irritation tests on hamsters and rabbits. For skin sensitization, also mandatory to evaluate any MD, the publication of the TS 11796 gives the framework to verify the applicability of the OECD validated methods for medical device biocompatibility. Preliminary results with some methods using RhE models showed very promising results. The SENS-IS assay, a transcriptomic method, was evaluated with success to classify medical devices polymers extracts spiked with skin sensitizers (3). In addition, the test alerted to an allergenicity risk of a glucose sensor already on the market with several cases of allergic contact dermatitis reported in humans. Ongoing and future studies should accelerate integration of these in-vitro methods into ISO standards and their resulting regulatory acceptance.

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Vitro. 2018 Aug;50:439-449. doi: 10.1016/j.tiv.2018.01.001. Epub 2018 Jan 8. PMID: 29326048. 2) Pellevoisin C, Coleman KP, Hoffmann S. ISO 10993-23 In vitro irritation testing for medical devices: Substantiating applicability to mild irritants and non-extractables. Toxicol In Vitro. 2022 Apr 26;82:105371. doi: 10.1016/j.tiv.2022.105371. Epub ahead of print. PMID: 35487444. 3) Pellevoisin C, Cottrez F, Johansson J, Pedersen E, Coleman K, Groux H. Pre-validation of SENS-IS assay for in vitro skin sensitization of medical devices. Toxicol In Vitro. 2021 Mar;71:105068. doi: 10.1016/j.tiv.2020.105068. Epub 2020 Dec 8. PMID: 33301901.

O-8A-2

Why in-vivo methods for the assessment of sensitization might be outdated in the near future

ABSTRACT #272

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Within the scope of ISO 10993 for some medical devices it is necessary to assess their sensitizing potential. In the EU the in-vivo LLNA is still state of the art. Critical political and social debates and the implementation of animal welfare requirements led to several in-vitro methods covering different key events in order to support or substitute animal testing. The combination of different in-vitro methods can be used supportively to evaluate the sensitizing potential of medical devices. Two methods, suitable for polar and non-polar extracts, have been adopted and validated for this application. The Direct Peptide Reactivity Assay (DPRA) mimics the process of haptenation, the covalent binding of low-molecular weight substances to proteins (Key event 1). The depletion of cysteine and lysine detected via HPLC-MS can be used for the discrimination between sensitizers and non-sensitizers. Secondly, a bioassay equivalent to the Nrf2 reporter gene assay KeratinoSens™ was adopted and used to evaluate the skin sensitization potential of samples. The activation of the Nrf2-pathway in cultured cells is a valuable endpoint for in-vitro detection of skin sensitizers (Key event 2). Results have shown that both methods can detect certain skin sensitizers at far lower concentrations than the LLNA. Following the IATA strategy, combining the results of these methods lead to a conclusive prediction if materials are sensitizing. As a future perspective the OFI is currently developing a 3D skin-based model for quantifying chemical sensitization potency of medical devices. Recent literature and first results of the comparative qPCR method show that specific gene regulations, known to be crucial in dermal sensitization processes are likely to be another serious alternative to in-vivo sensitization tests.

O-8A-3

HEMOCOMPATIBILITY OF BACTERIOPHOBIC COATED CATHETERS

ABSTRACT #314

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Background and objectives: Central venous catheters (CVCs) present a higher infection risk and to avoid mortality and morbidity associated with CVCs infection a promising approach consist in applying a bacteriophobic coating on the CVC surface. For its use in clinical practice, these devices must present low cytotoxicity and high hemocompatibility. The objective of this study is to compare the hemocompatibility of coated and un-coated bacteriophobic CVC using human blood to validate the safety of the device. Material and methods: This study was approved by the Bioethics Committee at University of Barcelona, Spain, and informed consent was given to all participants. Six healthy adult volunteers (3 males and 3 females) were included in each experiment. 2 cm of each catheter (coated and uncoated), equivalent to about 3.14 cm2 were exposed to 1 mL of blood. 3 tubes with only 1 mL of blood were used as controls. The tubes were incubated at 37°C for 1 hour with rotation. Blood was centrifugated and plasma was used to determine hemolysis, prothrombin time and complement activation. Results and Discussion: Although the existence of individual and sex differences, the increase of

hemolysis observed after blood exposition to catheters can be considered negligible (< 2%); thus, coated and uncoated catheters can be considered as no hemolytic. There are no differences in the prothrombin time. The exposition of blood to foreign devices can activate the complement system as an immune response with an increase in the C3a. Concerning the innate immune system activation, evaluated by the complement system, did not resulted in an increase on the C3a fragment. Therefore, our results do not seem to indicate innate immune response generation when blood was exposed to the coated tubes. Conclusion: The coated catheters showed a similar hemocompatibility to the uncoated catheters validating the blood compatibility of the coating.

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O-8A-4

BIOCOMPATIBILITY OF MEDICAL DEVICES: EYE IRRITATION OF PRESERVED AND UNPRESERVED EYE DROPS ON HCE MODEL ABSTRACT #448

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Background and Objectives: Transient (acute), short term (daily repeated) and long term (daily repeated for months) application conditions are known as relevant for eye drops assessment. However, the current ISO 10993-10 neither provides protocols to assess this endpoint nor proposes alternative to animal testing as requested by the MD Regulation. To overcome these limitations, a multiple endpoint analysis (MEA) approach on human reconstructed corneal epithelium (HCE) was used to assess the biocompatibility (cytotoxicity and irritation potential) of commercially available eye drops without using animals. Material and Methods: Benzalkonium chloride (BAK) was used as positive control. The products were tested with two different protocols: an acute exposure (24h application followed by 16h post-incubation), and repeated exposure (two exposure a day for 72h). The MEA approach included viability evaluation, fence properties measurements, LDH release and gene expression (qRT-PCR) of occludin known for its role in recovering tight junctions structure when damaged thus allowing damage reversibility investigation. Results: The results have confirmed the toxicity of BAK 0.01% on HCE for both exposures protocols by a reduction of cell viability (<50%). The other endpoints support this toxicity (TEER reduction, LDH release increase, up-regulation of occludin gene expression). After acute exposure none of the products reveal signs of toxicity as compared to the negative control (saline solution treatment). On the contrary, after 72h exposure, the results have demonstrated that repeated treatments induce different degree of corneal damages according to the presence/absence of preservatives. Discussion and Conclusion: While preservative free products were well tolerated and did not affect corneal epithelium, 'soft' preserved eye drops caused signs of toxicity and damage to the ocular surface. These data support the use of reconstructed models associated with the multiparametric analysis in detecting delayed mechanisms of toxicity responsible for the adverse reactions associated with long-term use of ophthalmological products.

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O-8A-5

Measurement of metal sensitizer potency with the SENS-IS assay. Application to Medical devices
ABSTRACT #142

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Background and objectives Skin sensitization, one of the three biocompatibility tests recommended for all medical devices, is still based on in vivo approaches (ISO 10993-10). Yet, the recent validation of in vitro skin irritation of medical device extracts demonstrated the added value of reconstructed human models (RhE), in the context of medical devices (ISO DIS 10993-23). Metal alloy are frequently used for medical devices in orthopedic surgery, cardiology or dentistry. The goal of this study was to evaluate the capacity of SENS-IS assay, a quantitative analysis of specific genes expressed in RHE models, to predict in vitro the skin sensitization potential of different metal salts. Material and Methods Sixteen (9 sensitizers and 7 non sensitizer) metal salts were tested in the SENS-IS assay. Nickel and titanium were tested in 3 different salts form, Chromium and palladium in 2 different salts form. The lowest positive concentration and the highest negative concentration (NOEL) were measured using the SENS-IS assay. Results In contrast to other in vitro tests, all 9 sensitizers metal salts and all 7 non sensitizer were correctly predicted using the SENS-IS assay. Discussion conclusion. The SENS-IS assay which has already been shown to be able to detect the sensitizing potential of medical devices extracts, is also able to measure metal salts sensitization potency. Further studies are engaged with a more comprehensive set of molecules, but this work paves the way for a broader multicenter study to validate the integration of SENS-IS in future in vitro testing strategies to address the sensitizing potential of medical devices.

Session: 8b. In vitro COVID-19 research / Lung and cardiovascular models

Chairs: *Arno Gutleb (LIST, Luxembourg, Luxembourg) & Yasunari Kanda (National Institute of Health Sciences, Japan)*

O-8B-1

Knowledge from Human Relevant Cell, Tissue and Mathematics-based Methods as Key Tools for Understanding COVID-19 Dynamics, Kinetics, Symptoms, Risk Factors and Non-conventional Treatments

ABSTRACT #243

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Although knowledge from previous SARS infection has been instrumental, not all specific SARS-CoV2 features have been clarified nor are the underlying molecular and cellular mechanisms fully understood. It is therefore necessary to understand in detail the dynamics and kinetics of the SARS-CoV-2 virus, which includes the means of entry into the organism via the external body barriers (e.g., nose, lung, eye, intestine), its distribution and passage through internal body barriers (e.g., placenta barrier, blood-brain barrier) into the various human body organs, and its subsequent viral dynamics. It is important to understand the effects the virus triggers in the whole organism, mapping its cellular entrance via the external body barriers by binding to cellular receptors of these barriers, its replication and the subsequent mechanism of action SARS-CoV-2 has on the cells and tissues of its human target organ systems. The cellular immune response and specific cellular responses to the pathogen can contribute to multi-organ dysfunction. The mechanistic understanding on modulators of the immune response and cell homeostasis balance and specific risk factors is critical to understand the systems biological processes underlying the multi-organ systems effect and will improve diagnosis, prevention and therapeutic strategies. Knowledge from human relevant cell, tissue and mathematics-based methods are key tools for understanding COVID-19 dynamics, kinetics, symptoms, risk factors and treatments. Cross-community research on SARS-CoV-2 is essential to understand its detailed pathophysiology. Greater investment and innovative methodological approaches are needed to accelerate and continue knowledge gathering on SARS-CoV-2 in all the aspects of the disease. Due to the seriousness of the global health situation with this extraordinary crisis of the human race caused by COVID-19 and other pathogens, medical researchers, cell biologists, life science experts, mathematical modellers and bioengineers across the world are actively collaborating to accelerate detailed mechanistic knowledge of new diseases.

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O-8B-2

3D Human airway epithelial models to study SARS-CoV-2 pathogenesis and to discover antivirals

ABSTRACT #274

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[1]*Epithelix*

The recent outbreak of SARS-CoV-2 (COVID-19) is a major threat to human beings. The respiratory system is the main entry portal of SARS-CoV-2 which infects initially and principally the airway epithelia; then it gradually propagate to other human organs, causing symptoms such as fever, dry cough, fatigue, diarrhoea, conjunctivitis, Pneumonia, respiratory failure, loss of taste, etc... To fight against SARS-CoV-2, confinement is necessary but not sufficient. Vaccination is certainly a priority, but new anti-viral drugs are also indispensable. Since the first step of SARS-CoV-2 infection is taking place in airway epithelial cells, it is logic to use 3D airway epithelial model as drug testing platform. Epithelix has developed and is offering standardized air-liquid interface 3D human airway epithelial cultures from nasal or bronchial (MucilAir™) and small-airway (SmallAir™) origins. These epithelial models closely mimic the morphology and function of the native tissues: such as cilia formation and beating, mucus production and secretion, mucociliary clearance, and secretion of antiviral molecules. These models have been successfully used for the development of antivirals against influenza, rhinoviruses, respiratory syncytial virus, amongst others. This talk will highlight how these reconstituted human airway epithelial models can be used to characterize viral infection kinetics, tissue-level tropism and transcriptional immune signatures induced by SARS-CoV-2. Relevance of these models for the preclinical evaluation of antiviral candidates will also be addressed in the context of repositioning of marketed drugs or evaluation of novel therapies and combinations delivered systematically or through aerosol therapy.

O-8B-3

Effects of drugs formerly repurposed for COVID-19 treatment on connexin43 hemichannels and pannexin1 channels

ABSTRACT #278

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Background: One of the clinical features of Coronavirus disease 2019 (COVID-19) is the occurrence of systemic inflammation with concomitant cytokine storms. In this respect, many drugs with anti-inflammatory characteristics were proposed for the treatment of COVID-19, including azithromycin, chloroquine, hydroxychloroquine, dexamethasone, favipiravir, remdesivir, ribavirin and the combination of lopinavir and ritonavir. Connexin43 (Cx43) hemichannels and pannexin1 (Panx1) channels play a pivotal role in inflammatory processes and thus innate immunity. Aim: To study the impact of potentially repurposed COVID-19 drugs on Cx43 hemichannels and pannexin1 channels. Material and Methods: Cells overexpressing human Cx43 and Panx1 were exposed for 24 hours to the drug panel and levels of mRNA and proteins were evaluated by means of real-time reverse transcriptase quantitative polymerase chain reaction analysis and immunoblot analysis, respectively. The effects of the drugs on channel activity were assessed via bioluminescent measurement of Cx43-mediated and Panx1-mediated adenosine triphosphate release in the extracellular environment. Results: Combined exposure to lopinavir and ritonavir as well as to remdesivir reduced Cx43 mRNA amounts. None of the tested drugs affected Cx43

protein expression. No drugs were found to block Cx43 hemichannel activity. Panx1 channel activity, on the other hand, was inhibited by hydroxychloroquine, favipiravir, lopinavir and the combination of the latter with ritonavir, yet Panx1 mRNA and protein levels remained unchanged. Discussion and Conclusion: The combination of lopinavir and ritonavir was able to reduce both Cx43 protein expression levels and Panx1 activity. Thus, this combination seems to offer a promising treatment strategy and should be investigated further. In addition, remdesivir or hydroxychloroquine and favipiravir can be of interest to be used as specific blockers for Cx43 expression or Panx1 activity, respectively.

O-8B-4

Use of a human bronchial epithelium model to assess the impact of PM2.5 exposure on the severity of viral infections

ABSTRACT #300

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Epidemiological studies have shown an association between exposure to fine particles (PM2.5) and the severity of respiratory infections such as influenza and COVID-19 [1,2,4,5]. PM2.5 and viruses target the bronchial epithelium suggesting that chronic exposure to PM2.5 could make it more susceptible to viral infections. The objective of this project is to study the impact of repeated exposure to PM2.5 on the severity of influenza virus and understand the underlying mechanisms. An in vitro model of human bronchial epithelium developed from the Calu-3 cell line grown at the air-liquid interface (ALI) is used. This original differentiated model mimics the human bronchial epithelium in a realistic way as the secretome is similar to that of primary bronchial cells [3] and conditions have been optimized to do repeated exposures since we are chronically exposed to pollutants. PM2.5 were collected in winter close to Paris and their chemical composition characterization is in progress. As our hypothesis is that exposures to PM2.5 would favour viral severity, we seek to decipher how it can interfere with antiviral defence (viral receptors, pro-inflammatory cytokines, interferon (IFN) pathway, antimicrobial peptides…). We characterised the responses to a single 24h exposure to PM2.5 or diesel particles (DEP1650b) at ALI at 5, 10 or 20 µg/cm². Particles induced the expression of the pro-inflammatory cytokines IL-6 and IL-8 and seem to impact the antiviral signalling pathway involving IFN-β and the antimicrobial peptide β-defensin-1. We also characterised responses during a viral infection, confirming the functionality of the anti-viral pathways. We are performing single PM2.5 exposure followed by viral infection, before moving to PM2.5/virus co-exposures and then repeated pre-exposures to PM2.5, to study the different possible scenarios and then understand the mechanisms involved. This work is support by the Foundation for Medical Research (ENV202003011541), the French Environment and Energy Management Agency and DIM Qi².

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O-8B-5

Metabolic Regulation of SARS-CoV-2 Infection

ABSTRACT #395

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Viruses are efficient metabolic engineers that actively rewire host metabolic pathways to support their lifecycle, presenting attractive metabolic targets for intervention. Here we chart the metabolic response of lung epithelial cells to SARS-CoV-2 infection in primary cultures and COVID-19 patient samples. Virus infection produced transcriptional changes associated with increased glycolysis and lipid accumulation. Metabolism-focused drug screen showed that fenofibrate reversed lipid accumulation and blocked SARS-CoV-2 replication through a PPAR□-dependent mechanism in both alpha and delta variants. Analysis of 3,233 Israeli patients hospitalized due to COVID-19 supported in vitro findings. Patients taking fibrates showed significantly lower markers of immunoinflammation and faster recovery. Additional corroboration was received by comparative epidemiological analysis from cohorts in Europe and the United States. A subsequent prospective non-randomized interventional open-label study was carried out in 15 patients hospitalized with severe COVID-19. The patients were treated with 145 mg/day of nanocrystallized fenofibrate in addition to standard-of-care. Patients receiving fenofibrate demonstrated a rapid reduction in inflammation and a significantly faster recovery compared to patients admitted during the same period. Taken together, our data suggests that pharmacological modulation of PPAR□ should be strongly considered as a potential therapeutic approach for SARS-CoV-2 infection and emphasizes the need to complete the study of fenofibrate in large randomized controlled clinical trials.

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Tuesday, November 22nd 2022
16:30 - 17:30

Session: Early Stage Researcher Session I

Chairs: *Erwin van Vliet (Houten, Utrecht, Netherlands) & Pau Sancho-Bru (Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain)*

O-ESCS1-1

A single procedure to generate functional hiPSCs-derived liver organoids - Towards an innovative tools suitable for drug screening
ABSTRACT #165

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We previously showed that human pluripotent stem cells (hiPSCs) provide a suitable model to study metabolic diseases upon hepatocyte-like cell (HLC) differentiation. With a non-invasive approach, hiPSCs can be generated from urine samples of patients and HLCs have been used to model cholesterol metabolism regulation, by the study of LDLR- and PCSK9-mediated autosomal dominant hypercholesterolemia (ADH) as well as PCSK9-mediated familial hypobetalipoproteinemia (FHBL). This model provides promising advantages with a direct link to the patient and with an unlimited source of HLCs. But like all models, there are limitations, mainly by the neonatal characteristic of HLCs lead to difficulties for pharmacological investigations. Therefore, to overcome these burdens, we chose to 1. Differentiate hiPSCs into HLCs in an innovative 3D modified Hyaluronic Acid hydroscaffold, BIOMIMESYS® produces by HCS Pharma to enhance their maturation. 2. Adapt our 3D

differentiation process to a 96-well format to make it compatible for drug screening. 3. Characterization of the 3D HLCs model by metabolism tests and compare to primary human hepatocyte (PHH). We gathered 3' SRP data all along the differentiation process and RNAseq has been performed by comparing 2D and 3D differentiation conditions to characterize hiPSCs differentiation into liver organoïds. We observed an enhanced expression of most hepatic genes and genes expressed by non-parenchymal cells such as stellate cells. Immunofluorescence data confirmed the co-localization of albumin-positive hepatocytes, desmin-positive stellate cells and LYVE1-positive endothelial cells in liver organoïds. Finally, at a functional level, several CYP activities including CYP3A4 were detected at the basal level and successfully induced. Liver organoïds responded to pharmacological treatments as shown by their ability to accumulate lipids upon amiodarone treatment or uptake LDL-bodipy upon statin treatment. Altogether, our development gave rise to functional liver organoïds generated with a unique and common procedure, in a process of automating for future high throughput screening.

O-ESCS1-2

In vitro screening for developmental neurotoxicity of flame retardants using a 3D human primary neural progenitor cell assay: a case study
ABSTRACT #133

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Brominated flame retardants (BFRs), like BDE-99, have been identified as threats to human health. They have been eliminated and less persistent alternative flame retardants (aFRs), such as organophosphorus FRs (OPFRs), have been released on the market. However, kinetics and toxicities of these compounds have not been investigated. Considering the fact that the developmental nervous system is a sensitive target organ for flame retardants (FRs), it is essential to assess the developmental neurotoxicity (DNT) potential of these aFRs. We have developed 3D neurosphere in vitro test methods (NPC1-6) based on human primary neural stem/progenitor cells (NPCs), which represent distinct neurodevelopmental key events (KEs), including NPC proliferation, migration, differentiation into neural effector cells (astrocytes, neurons and oligodendrocytes) and thyroid hormone (TH)-dependent oligodendrocyte maturation. We used FRs as a case study to investigate the usefulness of the NPC test methods for DNT evaluation. Using the neurosphere methods, we have evaluated the adverse effects of several FRs including phased-out polybrominated FRs, in use polybrominated and organophosphorus FRs: 2,2',4,4'-tetrabromodiphenylether (BDE-47), 2,2',4,4',5-pentabromodiphenylether (BDE-99), Tetrabromobisphenol A, Triphenyl phosphate, Tris(2-butoxyethyl) phosphate and its metabolite Bis-(2-butoxyethyl) phosphate, Isodecyl diphenyl phosphate, Triphenyl isopropylated phosphate, Tricresyl phosphate, Tris(1,3-dichloro-2-propyl) phosphate, Tert-butylphenyl diphenyl phosphate, 2-Ethylhexyl diphenyl phosphate, Tris(1-chloroisopropyl) phosphate and Tris(2-chloroethyl) phosphate). The studied aFRs altered distinct neurodevelopmental KEs in the range of 0.03-20 µM. Especially for the disturbance of differentiation into oligodendrocytes, most of them had comparable activities to BDE-47 and TBBPA. Here, hierarchical clustering of DNT ToxPies of FRs differ from ToxPies using the ToxCast assays. In addition, microarray analyses were performed from TBBPA-exposed neurospheres to get further insights into its mode-of-action. The neurosphere assay assess an array of neurodevelopmental endpoints and can be used for the DNT assessment of compound classes, like FR. Therefore it is suitable as a part of a DNT in vitro testing battery.

O-ESCS1-3

Predicting developmental toxicity of pyrethroid insecticides in vitro using human induced pluripotent stem cells
ABSTRACT #276

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Background and Objectives: Metabolites of pyrethroid insecticides are detected in most urine samples from the general population. Pyrethroids act primarily by interfering with voltage-gated sodium channels, which are essential for nerve cell function and cardiac muscle contraction. Therefore, pyrethroids are suspected to be cardiotoxic. Furthermore, pyrethroids are suggested to interfere with thyroid hormones that are essential for foetal brain development(1). Thus, exposure to pyrethroids during vulnerable windows in pregnancy may adversely affect child neurodevelopment and cardiovascular health. Material and Methods: The adverse effects of three pyrethroid insecticides (deltamethrin, α-cypermethrin and etofenprox) and the common metabolite 3-phenoxybenzoic acid (3-PBA) on cardiomyocyte differentiation were assessed in an in vitro 3D model of human induced pluripotent stem cell-derived embryoid bodies (EBs) that mimic the very early stage of the human embryo – the blastocyst. A cardiac-specific reporter gene assay - the PluriLum(2), was used as a cardio-developmental toxicity endpoint. The binding ability of pyrethroids to transthyretin (TTR) was assessed using the cell-free ANSA-TTR binding assay. Results: The EBs were differentiated into cardiomyocytes and the effects of the pyrethroids on this process was measured. Deltamethrin, α-cypermethrin and etofenprox had a significant negative impact on differentiation (LOECs: 13, 6.3 and 1.6 µM, respectively). 3-PBA showed no effect at any tested concentrations (up to 100 µM). However, it significantly displaced ANSA from TTR (LOEC = 1.6 µM), whereas the parent pyrethroids showed no TTR-binding potential at the range of tested concentrations (up to 200 µM). Discussion and Conclusion: Our results indicate that pyrethroid parent compounds have the potential to adversely affect cardiac differentiation. On the other hand, only the metabolite, 3-PBA, could bind to TTR and potentially disturb thyroid hormone transportation. These findings highlight the risks posed by insecticides on human foetal development, supporting the need to restrict their use in order to reduce human exposure.

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O-ESCS1-4

CHARACTERIZATION OF EXTRACELLULAR VESICLES RELEASED BY PERIPHERAL BLOOD MONONUCLEAR CELLS EXPOSED TO POLYCYCLIC AROMATIC HYDROCARBONS AND STUDY OF THEIR INTERACTION WITH ENDOTHELIAL CELLS
ABSTRACT #351

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Background and Objectives: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Several studies have highlighted their toxic properties for human health, such as carcinogenic and immunosuppressive effects for the prototypical PAH benzo[a]pyrene (B[a]P). Our previous data reported that circulating cells such as Peripheral Blood Mononuclear Cells (PBMC) are targeted by PAHs exposure. Extracellular vesicles (EVs), present in the body fluids, have a major role in cell-to-cell communication. They are released by cells into the extracellular compartment, and interact by transferring their content in targeted cells. In this context, we were interested in determining the impact of PAHs on the release of EVs by these PBMCs and exploring their role on adjacent cells to potentially understand the toxicity mediated by these contaminants. Materials and Methods PBMCs were isolated from different blood donors and then exposed to PAHs for 48 hours. EVs were isolated by ultracentrifugation and characterized using Nano Tracking Analysis and western blotting.

Then, EVs were added on cultured Hmec-1 endothelial cells and the ability of EVs to be internalized was validated by PKH67 labeling. The impact of miRNA transfer on the processes of inflammation, proliferation or remodeling of endothelial cells was then analyzed by qPCR and by functional tests. Results Our data highlight the ability of PBMCs to secrete EVs has been highlighted. Interestingly, exposure of PBMCs to 10nM and 2µM B[a]P for 48h increases this secretion, whereas Pyrene, a non-carcinogenic PAH, does not modify it. We also show the internalization of EVs secreted by PBMCs in Hmec-1 endothelial cells and we endeavor to show the impact of this on the main functions of endothelial cells. Discussion and Conclusion Studying the dysregulation of EVs and their miRNAs upon PAH exposure and their transfer to adjacent cells will be decisive in understanding complex biological processes altered by these environmental contaminants.

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O-ESCS1-5

Towards a personalized kidney-on-a-chip derived from induced pluripotent stem cells

ABSTRACT #390

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Our understanding of the mechanisms involved in the development and progression of renal disease is still limited due to the lack of functional in vitro models that can accurately emulate human physiological processes. Microphysiological systems enable the long-term co-cultivation of multiple cell types under nearly physiological conditions and can thereby bridge the gap between conventional in vitro models and human patients. These microphysiological systems are, however, typically equipped with heterogenous cell populations sourced from multiple donors with diverse genetic backgrounds, which limits their applications in personalized medicine and patient-specific disease modeling. In this study, we generate a personalized and autologous kidney-on-a-chip that encompasses human induced pluripotent stem (iPS) cell-derived podocytes and proximal tubule epithelial cells from a single donor. The renal cells are seeded into the HUMIMIC Chip4, which enables the long-term co-cultivation of the renal model with up to three additional organ equivalents with a defined fluid flow and shear stress. The final maturation of the iPS cell-derived podocytes and tubular cells occurs within the multi-organ-chip, thereby allowing the cells to form a barrier that enables glomerular filtration and tubular reabsorption. After the renal cells' final maturation, the co-culture with autologous equivalents of the small intestine, liver and brain can be maintained for at least 14 days. The kidney-on-a-chip exhibits a stable metabolism, a cellular barrier that prevents albumin from entering the excretory circuit, and the cells demonstrate a steady expression of key podocyte and tubular markers. When the employed organ models are differentiated from iPS cells derived from a single patient, the HUMIMIC Chip4 represents a personalized multi-organ-chip. This patient-on-a-chip can be used for elaborate efficacy studies and the development of personalized therapies. Therefore, the developed autologous kidney-on-a-chip could not only advance future studies of renal disease mechanisms, but it could also pave the way towards personalized medicine.

Tuesday, November 22nd 2022
17:30 - 18:30

Session: Early Stage Researcher Session 2

Chairs: *Erin Hill (IIVS, Gaithersburg, MD, USA) & Clive Roper (Roper Toxcilogy Consulting Limited, UK)*

O-ESCS2-1

The Electro-Mitochondrial Coupling of a Microphysiological Human Heart ABSTRACT #394

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Cardiovascular diseases are the leading cause of death worldwide. Efforts to study cardiac dysfunction are frustrated by physiological differences between humans and animal models. Here we present a sensor-embedded, hiPSC-derived model of vascularized cardiac tissue that develops into complex multi-chambered self-paced heart organoid under anisotropic stress. Sensor integration permits the simultaneous real-time measurements of oxygen uptake, extracellular field-potential, and cardiac contraction with >10-Hz resolution. Using this platform, we discovered 1-Hz cardiac respiratory cycles, whose frequency is coupled to the electrical, rather than the previously theorized mechanical, activity of the tissue. Microscopic analysis revealed that calcium oscillations drive a mitochondrial respiration cycle. Pharmaceutical or genetic inhibition of this electro-mitochondrial coupling leads to arrhythmogenic behavior. We show that the chemotherapeutic mitoxantrone induces arrhythmia by disturbing the electro-mitochondrial coupling and that this effect is partly reversed by co-administration of metformin, suggesting a mitochondrial basis for chemotherapy-induced arrhythmia. Our work describes the mitochondrial dynamics of cardiac rhythms, underscoring the utility of microphysiological systems for advancing our understanding of cardiac physiology and pharmaceutical interventions.

O-ESCS2-2

Affinity prediction between cosmetic ingredients and CD54/CD86 receptors by molecular docking: A novel approach to predict skin sensitization potential ABSTRACT #366

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The human Cell Line Activation Test (h-CLAT) is OECD adopted cell-based assay that contributes to the assessment of the skin sensitization potential of skincare ingredients. The method addresses KE3 of skin sensitization Adverse Outcome Pathway (AOP) by quantifying the expressional alteration of cell surface receptors (CD54 and CD86) associated with activation of monocytes and dendritic cells (DC). To achieve a significant reduction in time and cost for large-scale cosmetic screening, an in-silico skin sensitization prediction model assesses the interaction b/w cosmetics compounds and CD54 & CD86 receptors. As per h-CLAT OECD guidelines 442E, six positive (++), and three negative (-) chemicals with known skin sensitization effects were selected to test the concept. Nine compounds were screened against CD54 and CD86 using molecular docking. Consequently, docking results revealed that positive cosmetic ingredients i.e., Diphenyl-cyclopropenone (-11.20 kcal/mol), Phthalic anhydride (-11.00 kcal/mol), Tetramethylthiuram disulfide (-9.42 kcal/mol), Isoeugenol (-9.30 kcal/mol) predicted strong affinities, and Benzyl benzoate (-7.54 kcal/mol), Oxalic acid (-7.80 kcal/mol) predicted moderate affinities, and negative skin sensitizers, Lauric acid (-3.45 kcal/mol), and Heptyl butyrate (- 3.67 kcal/mol) showed lesser-affinities against CD54 receptor. Diphenyl-cyclopropenone (-12.82 kcal/mol), Phthalic anhydride (-11.55 kcal/mol), Tetramethylthiuram disulfide (-10.78 kcal/mol),

Isoeugenol (-11.05 kcal/mol) predicted strong affinities, and Benzyl benzoate (-8.66 kcal/mol), Oxalic acid (-9.23 kcal/mol) predicted moderate affinities, and negative skin sensitizers, Lauric acid (-4.08 kcal/mol), and Heptyl butyrate (- 5.69 kcal/mol) showed lesser-affinities against CD86 receptor. Our computational results present the proof of the concept of method in agreement with OECD guidelines 442E. This concludes that the proposed in-silico method offers a great alternative or complement to experimental pre-screening & skin sensitization potency evaluation of cosmetic compounds.

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O-ESCS2-3

ELECTROSPUN SCAFFOLD-BASED APPROACHES FOR TOXICOLOGICAL SCREENING IN SKIN, PULMONARY, AND INTESTINAL EPITHELIAL TISSUE ABSTRACT #309

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There is an increasing demand for reliable in vitro drug toxicity models that can be implemented in early-stage discovery, due to the lack of predictability of current preclinical models. As primary sites of drug absorption, epithelial barrier models are essential to predict toxicity in vitro. Accordingly, the objective of this work was to develop biomimetic intestinal, lung and skin in vitro models for pharma- and toxicological screening. In vitro epithelial tissue models based on membranes of poly(ε-caprolactone) (PCL) and cellulose acetate (CA), cellulose acetate phthalate (CAP), ethylcellulose (EC) or methylcellulose (MC) were generated via needleless electrospinning. Scaffold morphology, chemical composition and wettability were assessed using scanning electron microscopy, Fourier-transform infrared spectroscopy and contact angle measurements, respectively. To evaluate the scaffold biological response, biocompatibility studies assessing cell metabolic activity, proliferation and adhesion were performed using Caco-2 colorectal adenocarcinoma cells (intestine), 16HBE14o- bronchial and A549 alveolar epithelial cells (lung), and immortalized XB2 keratinocytes and 3T3 fibroblasts (skin). PCL, PCL:CA and PCL:EC were composed of beadless nanofibres, whereas PCL:CAP and PCL:MC scaffolds showed defect-free micro- and nanofibres. PCL, PCL:CA, PCL:CAP and PCL:EC samples were hydrophobic, unlike PCL:MC, which was hydrophilic. In in vitro studies of intestinal models, cells adhered, proliferated and formed tight monolayers similarly on all scaffolds. Studies with skin cell models revealed that PCL:CA and PCL:CAP blends outperformed all other substrates in terms of cell proliferation and distribution. Lung cell models showed that, while 16HBE cells were able to adhere and proliferate in PCL, PCL:CA, PCL:EC and PCL:MC scaffolds, A549 cells were only able to have the same biological response on PCL, PCL:CA and PCL:MC. All fibrous meshes prepared demonstrated biocompatibility towards most cell types tested, thus highlighting the potential of PCL-cellulose derivative blends as substrates suitable for in vitro epithelial tissue models for pharmacological and toxicological screening.

O-ESCS2-4

Developing a method to incorporate laminar flow into scaffold-based 3D cell cultures for more physiologically relevant nutrient and drug delivery.

ABSTRACT #317

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Three-dimensional (3D) culture models incorporate tissue-like architectures and microenvironments that are more physiologically relevant than maintaining cells as monolayers on plasticware. Despite many examples of culturing cells in a 3D environment, most models rely on static culture conditions in which diffusion-dominated environments occur. These environments result in pericellular hypoxia as the rate of oxygen consumption outpaces its rate of diffusion. Here, we describe a low-cost and modular continuous fluid delivery system (CFDS) that is compatible with cell-containing scaffolds. The CFDS continuously supplies cultures with oxygen and nutrients from a single reservoir. Using laminar flow conditions, we eliminate the oxygen and nutrient depletion zones prominent in static cell culture conditions. This system was designed to support 0.01 – 2 million cells per scaffold (1.13 – 21.24 mm3 per scaffold) for at least 72 hours. We demonstrate the utility of this modular delivery system by comparing the responses of mono- and co-cultures to chemotherapies under static and flow conditions. We also use the CFDS to determine the role stromal signaling factors play in the response and resistance to chemotherapies. In particular, we probed the effect of ratios and distances between cell types in co-cultures containing tumor and stromal cells. These examples demonstrate a first-generation device aimed at coupling multiple organ models together to generate a milli-human model to help better understand the role of multi-organ signaling in drug toxicity.

O-ESCS2-5

Kidney organoids for toxicology: application to the development of Adverse Outcome Pathway (AOP) study of nephrotoxicity induced by uranium

ABSTRACT #268

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Kidney organoid models open new perspectives in the field of nephrotoxicology to study various type of kidney impairment by proposing a human based-model that is representative of renal morphology and function. In order to characterize kidney organoid for the evaluation of nephrotoxic substances, we propose to use this organ-like model to develop the Adverse Outcome Pathway (AOP) of renal failure induced by uranium [1], a reference nephrotoxic substance. The kidney organoid model developed in collaboration with INSERM IRMETIST laboratory is generated from human induced Pluripotent Stem Cells (IPSCs) previously characterized [2] and based on the work of Przepiorski et al [3]. The characterization of the model is performed by immunolabeling of cell population (proximal and distal tubules, collecting duct, podocytes and endothelial cells), the follow-up of the development of the nephron structure by histology, the identification of subcellular structures by TEM and the study of the expression of genes of interest. Exposure to uranium of kidney organoids shows the heterogeneous distribution of uranium at cellular and subcellular level thanks to Secondary Ion Mass Spectrometry (SIMS) technology at low and high concentration (respectively 100µM and 1000µM). Apoptotic and necrotic cell death are investigated depending on exposure conditions (concentration, time) thanks to phenotypical analysis (apoptosis and necrosis biomarkers, morphological study by histology). Uranium at 100µM induced a 2.5 to 3.5 fold increase in Caspase3/7 and LDH levels compared to basal levels in kidney organoids. These results on the distribution and adverse effects of uranium such as apoptosis and necrosis have contributed to the consolidation of the AOP in progress on AOP-Wiki (447). This AOP highlighted the relationships between key event and remaining gaps to finalize the understanding of toxicity mechanism. For this purpose, the key events will be studied using the kidney organoid, in particular the apoptosis, inflammation and oxidative stress pathways.

POSTER PRESENTATIONS

ALL POSTER PRESENTATIONS

Session: 1. Bio-engineering, stem cells and disease models

P-1-1

The effect of methylmercury chloride on three germ layer formation during hiPSC-derived Embryoid Bodies development
ABSTRACT #245

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Background and Objectives: Human induced pluripotent stem cells (hiPSC) provide unique non-controversial model of early development to study the effect of drugs and toxins on three germ layer formation, thus we have investigated the effect of MeHgCl, well-known developmental neurotoxin. Material and Methods: hiPSC were cultured in 2D conditions or stimulated in matrigel-based 3D culture for spontaneous differentiation to form Embryoid Bodies (EB) with 3 germ layers, typical for early development. 2D or 3D cultures were exposed to various doses of MeHgCl. Expression of typical markers of germ layers was investigated on gene and protein level Results: the strong cytotoxic effect of the MeHgCl for EB formation was observed after 24h of exposition to dose of 1µM and 0,5µM, in contrary hiPSCs in undifferentiated stage in 2D culture were not sensitive. In such experimental doses, the ROS level was significantly up-regulated (DCF-HDA) while mitochondrial membrane potential (MitoTracker Red CMXRos) was down-regulated. Low, no cytotoxic dose of MeHgCl (0,001µM) for 14 days allowed for spontaneous differentiation of hiPSC into EB, however germ layer formation was disturbed: the gene expression of NES (ectoderm), SOX17 (endoderm), and TBXT (mesoderm) were strongly down-regulated. Lowering of gene expression by 0,001µM MeHgCl was confirmed immunocytochemically at the protein level. DNA damage analysis and apoptosis were measured in EB respectively by immunodetection of gammaH2AX and Casp3 and did not reveal significant difference between EB derived from control or experimental cultures. Discussion and Conclusion: Biomimetic cell culture in 3D conditions changes susceptibility of iPSC to developmental toxins. Data indicate that during early human development mimike by EB growth from hiPSC, process of three germ layer formation is sensitive to very low, not cytotoxic doses of developmental neurotoxins This work has been supported by National Science Centre (NSC), PRELUDIUM 9 grant no UMO-2015/17/N/NZ7/04096 and statutory funds to MMRC.

P-1-2

Preliminary evaluation of extracted collagen from food fish-processing side streams as an active ingredient for cosmetic application.
ABSTRACT #277

Lorenzo Dondero[1], Giulia De Negri Atanasio[1], Federica Robino[2], Anastasiia Kharina[2], Micaela Tiso[3], Francesca Rispo[1], Giorgia Allaria[1], Francesca Tardanico[1], Federica Turrini[4], Federica Grasso[4], Valentina Orlandi[4], Matteo Zanotti-Russo[2], Raffaella Boggia[4], Elena Grasselli[1]

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Background and objectives The generation of fish-processing side streams by the fish supply chain, has increased in the recent time which has a significant impact on the environment and moreover it represents a big economic problem. The aim of this study is to identify a pool of active ingredients from food fish-processing side streams with some advantages for human skin. In particular, the H2020 EcoeFISHent project (101036428) is based on the collagen's analysis from marine sources for a human cosmetic application. Material and Methods Collagen was extracted from mixed fish-

processing products using a green technology to respect the environment and to increase the extraction efficiency process using Pulsed Ultrasound-Assisted Extraction (PUAE). Then Sircol Collagen assay (SCA) was performed to quantify the amount of collagen extracted. HECV (human endothelial cells, isolated from the umbilical cord vein) was used as a cellular model to evaluate the collagen safety by using the MTT assay. T-Scratch test was also carried out to evaluate collagen wound healing effect on HECV. Results SCA analysis showed an extractive yield ranging from 22.63 µg/ml to 14.409 µg/ml. MTT assay permitted to determine a no toxicity activity for all the samples at all the concentration investigated for the cellular model tested (HECV). Thanks to T-Scratch test it was possible to determine that all the extracts, tested at different concentrations, promoted the healing of a wound using HECV as a cellular model. The extracted collagens demonstrated an increased wound healing effect compared to negative controls. Discussion and Conclusion This path will be aimed at the validation of collagen as an active ingredient from animal processing side streams by establishing a sustainable project, based on a circular economy idea, to not create waste.

P-1-3

An innovative and robust strategy to generate hepatocyte-like cells from individual patients to investigate idiosyncratic hepatotoxicity of drugs ABSTRACT #358

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Idiosyncratic DILI (iDILI) constitutes one of the most challenging scenarios to investigate drug hepatotoxicity. The toxic events seem to be more closely related to the host rather than to the drug itself and occur only in certain individuals at drug doses normally well tolerated by the rest of treated patients. Apart from the immune-based idiosyncratic DILI, the toxic phenomenon seems to be due to phenotypic characteristics of the individuals that make their hepatocytes more “sensitive” to the drug. Thus, the use of “normal” human hepatocytes to investigate these “abnormal” responses is not conclusive and results are difficult to interpret. Ideally hepatocytes isolated from the patient would be the most appropriate cellular model to investigate an iDILI event. However this is not feasible for medical and bioethical reasons. Models based on the generation of iPSC are long-lasting, costly, and difficult to implement in the clinical world. Alternatively, we have explored the feasibility of generating hepatocyte-like cells (HLCs), by direct reprogramming of patient’s somatic cells, based on the combination of a single doxycycline-inducible polycistronic expression vector containing key hepatic factors (HNF4, HNF1 and FOXA3), introduced in hTERT immortalized fibroblasts. The cells easily grow under standard culture conditions and can be expanded without signs of transformation or senescence. Upon addition of doxycycline, cells acquire the hepatic phenotype in 10 days, expressing hepatic functions and biotransformation activities. Cells retain the phenotypic characteristics of the donor, as demonstrated with patients with liver metabolic deficiencies. A clinical study is ongoing where the toxicometabolomic responses of primary hepatocytes of a given patient to drugs are compared to those of HLCs derived from the same patient. Results indicate that our strategy can generate an unlimited source of clonal, homogeneous and non-transformed HLCs, capable of performing typical hepatic functions and suitable for pharmaco-toxicological testing for iDILI studies.

P-1-4

Human peripheral neurons with enhanced nociceptor features for the study of pain-related dysfunctions ABSTRACT #215

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Background and Objectives: In vitro models of the peripheral nervous system would benefit from further refinements to better support studies on neuropathies. In particular, the assessment of pain-related signals is still difficult in human cell cultures. We harnessed induced pluripotent stem cells to generate peripheral neurons enriched in nociceptors. The objective was to establish a culture system with signaling endpoints suitable for pharmacological and toxicological studies. Methods: The well-established PeriTox test was used to screen compounds regarding their effect on neurites of early-stage peripheral neurons. For follow up-testing, a more mature, nociceptor-enriched sensory neuronal culture was established by ectopic expression of the transcription factor NGN1 during a defined time window of differentiation. For functional studies, single cell recordings of Ca2+-indicator fluorescence from >9,000 cells were used to establish the "fraction of reactive cells" in a stimulated population as experimental endpoint that appeared robust, transparent and quantifiable. Results: To provide an example of application to biomedical studies, functional consequences of exposure to chemotherapeutic drugs were examined at non-cytotoxic concentrations. Oxaliplatin was found to induce (i) neuronal (allodynia-like) hypersensitivity to otherwise non-activating mechanical stimulation that could be blocked by modulators of voltage-gated sodium channels; (ii) hyper-responsiveness to TRPV1 receptor stimulation. Moreover, proteasome inhibitors, such as bortezomib and carfilzomib, exhibited a distinct pattern of toxicant-induced alterations in the neurons. Attenuation of P2X3 signaling, increased levels of resting intracellular [Ca2+], and a reorganization of tubulin to dense structures around the cell somata were characteristic of proteasome inhibitor-induced cell stress. Discussion and Conclusion: Since peripheral neurotoxicants can act through various modes of action, it is not sufficient to investigate one single endpoint as demonstrated by the PeriTox screening approach. Use of further test endpoints might give a better insight on the cause of peripheral neuropathies and increase the sensitivity of the assay for (peripheral) neurotoxicants.

P-1-5

Development of in vitro cardiotoxicity assessment using human iPS cell technology
ABSTRACT #163

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Drug-induced cardiotoxicity is critical in the non-clinical testing. Applications of iPSC-derived cardiomyocytes (iPSC-CMs) hold great promise as a human cell-based platform. To date, multielectrode array (MEA) system has been widely used as a standardized assay to detect proarrhythmia risk with iPSC-CMs. In addition, evaluation of inotropic effects in vivo is recognized as a safety pharmacology in drug development. Given its impact on drug development, it should be useful to detect the drug-induced effects on contractility in vitro. In the present study, we developed motion imaging system for the contractility assessment using motion vector analysis. The effects of isoprenaline and verapamil were detected using the system. In addition, we found that several anti-cancer agents impaired contractility, suggesting the usefulness of the system for cardiac safety evaluation. Our results suggest that motion imaging system can monitor the contraction movement of iPSC-CMs. We are planning to perform multi-site validation study using the image-based contractility assessment.

References

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P-1-8

Generation of Human Induced Pluripotent Stem Cell-derived Cardiomyocytes using the Aurora Kinase Inhibitor ZM447439
ABSTRACT #360

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In drug development, many drug candidates that have passed non-clinical studies cause human cardiotoxicity in clinical trials or markets,

causing the result of the withdrawal from the stages. It suggests that there are problems in non-clinical cardiotoxicity assessment with the hERG assay and animal-based studies. Stem cell technology has developed rapidly over the past decade, and in particular, human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can provide a source of human cardiomyocytes for cell therapy, drug testing, and disease modeling. However, they have immature characteristics compared to them isolated from adult hearts and exhibit high batch-to-batch variation. It is known that the differentiation of hiPSC-CMs is related to the cell cycle and the Wnt/Gsk3 mechanism in hiPSC-CMs varies depending on the confluence of hiPSCs at the beginning of differentiation. Here, we study to produce the hiPSC-CMs using ZM447439, an aurora kinase inhibitor that regulates the cell cycle. In the results, the cell cycle ratio was changed according to the confluency of hiPSCs and by the treatment of ZM447439. We determined the optimal concentration and treatment timing of ZM447439 for cardiac differentiation. The differentiation efficiency of hiPSC-CMs was analyzed by microscopy, FACS, and qRT-PCR, and their electrophysiological function was confirmed using patch-clamp and multi-electrode array (MEA) techniques. The hiPSC-CMs induced by ZM447439 enhanced the expression of mesoderm and cardiac ion channel markers and promoted their electrophysiological functionality. In this study, we developed a novel efficient, and reproducible protocol for producing hiPSC-CMs. These results suggest that hiPSC-CMs produced using ZM447439 can provide useful in vitro models in non-clinical research in keeping with the 3Rs (refine, reduce, and replace). [Acknowledgements] This work was supported by the Technology Innovation Program (#20009774) funded by the Ministry of Trade, Industry, and Energy (MOTIE, Korea), and by a grant from the National Research Foundation of Korea (NRF-2022M3A9H1015784).

P-1-9

Mechanism of the Cyclic Stretch Induced Maturation of the Human Induced Pluripotent Stem Cell-derived Cardiomyocytes
ABSTRACT #379

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The human-induced pluripotency stem cells (hiPSCs) can differentiate into most cell types that consist of organs including the heart. The cardiomyocytes derived from hiPSCs provide the source of human cardiomyocytes for cell therapy, drug testing, and disease modeling. However, these cells do not fully recapitulate some features of the mature cardiomyocytes such as the organized sarcomere and T-tubules structure, high maximum upstroke velocity, resting membrane potential, etc. It is well known that the cardiomyocytes evolve in mechanically active environments generated by spontaneous contraction, the exploring mechanical cue for the maturation of hiPSC-CMs may thus suggest a strong biomimicking rationale for producing matured cardiac cell/tissue. Here we study the effect of mechanical stimulation on the maturation of hiPSC-CMs and its underlying mechanism using optical imaging, quantitative RT-PCR, and patch-clamp analysis. In this study, we found cyclic stretch-induced morphological maturation of hiPSC-CMs by the increased cell size and sarcomere length and the decreased circularity. Cyclic stretch also induced electrophysiological maturity by increasing the expression of ion channels related to cardiac action potentials and also induced action potential parameters to mature. These results provide new insight into the relationship between ion channels and maturation-related molecules during the physical stimulation of hiPSC-CMs. [Acknowledgements] This work was supported by the Technology Innovation Program (3D-TissueChip Based Drug Discovery Platform Technology Development Program, #20009774) funded by the Ministry of Trade, Industry, and Energy (MOTIE, Korea). This work was also supported by grants from the National Research Foundation of Korea (NRF-2022M3A9H1015784) and the Korea Institute of Toxicology (1711133839).

P-1-10

Cellular Model of Parkinson`s Disease for Safety Testing of Selenium-Based Nanodelivery System
ABSTRACT #288

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Parkinson's disease (PD) is the second most common neurodegenerative disorder [1]. The current "gold standard" therapy is levodopa (L-DOPA) due to its effectiveness and ability to permeate the blood-brain barrier. However, long-term treatment with L-DOPA can lead to serious side effects and increase oxidative stress, thereby worsening the disease [2]. The oxidative stress caused by either L-dopa could be lessened by the combination of antioxidants – one that promises is selenium (Se) – an essential trace element that can protect from oxidative damage through selenoproteins [3]. Direct use of Se supplement is toxic due to a narrow therapeutic index, so Se should be applied in other therapeutical forms. The use of Se in the form of nanoparticles (SeNPs) may elicit beneficial effects with reduced toxicity [4]. This study tested the safety of SeNPs as L-DOPA carrier using differentiated neuroblastoma (SH-SY5Y) cells as in vitro model of Parkinson`s disease [5]. SeNPs were prepared via reduction of sodium selenite by L-ascorbic acid and functionalized by polysorbate 20 (SeTWEEN) and poly(vinylpyrrolidone) (SePVP). The shape, size, and surface charge of SeNPs were determined with transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential measurements, respectively. Cell viability and apoptosis induction test were performed using flow cytometry, while oxidative stress was determined by measuring the concentration of reactive oxygen species and glutathione (GSH). In all experiments L-DOPA loaded SeNPs were compared with SeNPs and L-DOPA alone. L- DOPA loaded SeNPs (1ppm) were non-toxic to differentiated SH-SY5Y cells after 24-hour treatment, while L-DOPA reduced cell viability at the 100 μM, but not at 50 μM concentration. L-DOPA induced oxidative stress at the concentration of 100 μM which was effectively reduced after combining with SeNPs (1 ppm). Results suggest that SeNPs could be a promising approach for the reduction of toxicity caused by L-DOPA.

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P-1-12

Discovery of a novel function, immune-modulatory factor CSF-2, improves the therapeutic effect of stem cells from cell damage
ABSTRACT #273

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Background and Objectives CSF-2 (Colony Stimulating Factor 2), one of the cytokines, is known to actively stimulate the growth and maturation of immune cells. However, recently, it has been reported that not only activates the function of immune cells but also that CSF-2 is actively secreted from the damaged tissue associated with various diseases. Based on these studies, we confirmed that CSF-2 was actively secreted when stem cells are damaged by various methods including cytotoxic agent. Therefore, our findings showed that CSF-2 might have a novel function as a signaling molecule for sensitive to cytotoxicity. Additionally, we identified that stem cells treated with CSF-2 improves the various functions of stem cells, thereby enhancing the efficacy of cytotoxic treatment. Material and Methods We confirmed the secretion of CSF-2 by exposure to H2O2, serum deprivation and radiation, which induce cytotoxicity in human adipose tissue-derived mesenchymal stem cells. We also investigated whether CSF-2 enhances various functions of stem cells. We evaluated differentiation potential, migration ability, MMP-2/9 expression, stemness levels and EMT markers levels after CSF-2 treatment through Oil red O staining, alizarin red S staining, transwell assay, western blot, Real-

time PCR. Results It was evaluated that CSF-2 was actively secreted by inducing cytotoxicity. In addition, we found that various functions of stem cells were improved in CSF2-treated stem cells. Discussion and Conclusion These results indicate that CSF-2 is actively secreted in response to cytotoxic agent and injury condition and improves various functions of stem cells.

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P-1-13

Studying melanoma progression on a commensal 3D-skin model
ABSTRACT #386

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The human skin is one of the largest and most versatile organ of the human body. It harbours millions of microorganisms, namely bacteria, fungi and viruses. Together these are referred to as the skin microbiome. Particularly the bacteria are involved in many cellular processes like pathogen protection, wound healing and immune modulation. The respective symbiotic relationships contribute significantly to human health (1). Dysbiosis refers to a lack of balance among bacterial communities and the host that may lead to skin diseases. Recent studies showed that such dysbiosis are also found in melanoma, the black skin cancer (2). However, the underlying mechanism of melanoma progression and skin microbiome is not yet sufficiently understood. In preliminary experiments, the melanoma cell line A375 was treated with bacterial lysate and cell-free bacterial supernatant obtained from isolated skin bacteria. First results indicate bacterial related changes in gene expression level of ATAD2, a newly characterised epigenetic regulator found overexpressed in melanoma (3). To verify those results in a more realistic context we are now establishing a complex co-culture system based on a colonizable 3D-skin model. The system is based on the commercially available MelanomaFTTM and MelanoDermFTTM which are colonized with bacteria obtained from a skin swab from one donor for 12 days. Besides epidermal keratinocytes and dermal fibroblasts which are found in both models, MelanomaFTTM and MelanoDermFTTM consist of malignant A375 cells and melanocytes, respectively. Those melanocytes undergo spontaneous melanogenesis, which also might serve as a biomarker for melanoma. The colonized models show higher melanin production compared to untreated control. So far the experiments provided first promising results on the impact of the skin microbiome on melanoma progression. Further work will now hopefully allow a more detailed analysis of the respective underlying crosstalk between microbes and host.

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P-1-14

A novel in vitro 3D model of chronic kidney disease (CKD) in the proximal tubule for drug development and safety.
ABSTRACT #354

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Chronic kidney disease (CKD) is an irreversible disease that has a global prevalence of 13.4%. Renal fibrosis is the core process that leads to CKD, and its underlying causes are metabolic conditions and nephrotoxic injury (1). Renal fibrosis is characterized by increased deposition of extracellular matrix (ECM) in the renal tubule-interstitium by renal fibroblasts combined with epithelial-to-mesenchymal transition (EMT) of renal epithelial cells which lead to loss of renal function (2). We have developed a fully human, donor-derived renal fibrosis in vitro assay. We have isolated pure populations of proximal tubule cells (aProximate™ HPTECs) (3) and renal fibroblasts (HRFs) to assemble the aProximate™ Fibrosis model and induce fibrosis via treatment with cytokines and nephrotoxic compounds. HPTECs and HRFs were isolated from human kidney cortex via Percoll® density gradient and MACS® isolation. Cell phenotypes were confirmed via immunofluorescence staining and flow cytometry. HPTECs and HRFs were cultured onto Corning® HTS 96-Transwell® plates. The model sensitivity to pro-fibrotic compounds was investigated via high content imaging, high throughput flow cytometry, and cell viability assays. We were able to induce fibrotic injury in HPTECs and HRFs within 24 hours of treatment with combinations of TNFα, TGFβ1, Angiotensin II, Polymyxin B and Cyclosporin A. Under pro-fibrotic conditions, both cell types displayed a dose-dependent increase in cell proliferation. HPTEC displayed loss of tight-junction markers ZO-1, E-Cadherin, de novo acquisition of EMT marker Vimentin, and nodule formation. Nodule count and area also increased in a dose-dependent fashion. HRFs displayed myofibroblastic phenotype via αSMA expression and dose-dependent Fibronectin and Collagen I deposition. The aProximate™ Fibrosis model can respond to pro-fibrotic hormonal, cytokine, and nephrotoxic stimuli. The changing state of the system can be monitored via high content imaging and high throughput flow cytometry, making the model amenable to high screening of anti-fibrotic compounds and nephrotoxicants.

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P-1-15

ACCURATE EVALUATION OF DRUG METABOLISM BY ENHANCED HEPATOCYTE FUNCTIONS IN A NEW OXYGEN-PERMEABLE PLATE WITH LOW DRUG ADSORPTION

ABSTRACT #472

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[Background and Objectives]: In static culture of hepatocytes using standard tissue-culture-treated polystyrene (TCPS) plates, the very low oxygen supply flux via culture medium layer was pointed out over 50 years ago, but it is not well recognized nor solved even now. Polydimethylsiloxane (PDMS) has been reported to enable aerobic respiration of cultured hepatocytes and thus enhance the metabolic functions by its high oxygen permeability. However, its high chemical sorption property is a serious problem in evaluation of drug metabolism profiles. As an alternative material to PDMS, we developed and evaluated a new material for plate cultures, 4-polymethyl-1-pentene polymer (PMP).

[Material and Methods] PMP is an olefinic polymer that express high oxygen permeability comparable to that of PDMS when fabricated as thin films. [Results]: First, we confirmed high oxygen consumption of rat hepatocytes in PMP as can be observed in the aerobic respiration observed on PDMS and low drug sorption property of PMP comparable to those of TCPS. We also confirmed that high levels of liver function including various drug metabolisms were maintained at least for a week. Interestingly, productions of some of the drug metabolites were remarkably enhanced in PMP when compared in PDMS or TCPS. [Discussion and Conclusion]: The results suggest the observation of more accurate drug metabolism profiles due to the high metabolic functions and the low adsorption of drug and its metabolites. As such, a new material, PMP, is expected to replace PDMS in variety of drug testing systems ranging from static plates to various types of microphysiological systems.

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P-1-16

3D multi cell-type liver organoids as an alternative NAFLD model for drug safety assessment

ABSTRACT #479

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Development of in vitro hepatic models that recapitulate most of liver function are essential for successful drug toxicity assessment. Liver organoids are used for research and drug discovery but is facing some limitations due to a lack of expression and activity of xenobiotic metabolism enzyme and the difficulties to reproduce fibrotic and steatosis liver. Here we aimed to generate a 3D multi cell type liver organoids using HepaRG, primary macrophages and LX-2 (HML). We established a protocol to produce HML organoids and to induce steatosis under 14 days of culture. The HML organoids had a multicellular composition and exhibited most of the liver markers. They fully respond to steatosis induction and present marker associated with fibrosis. Furthermore, they were successfully used to assess drugs toxicity involved in hepatotoxicity. The combination of HML organoids and Benchmark doses modeling improve the detection of enhance drugs toxicity in fatty liver in vitro. In conclusion, HML organoids exhibit most of liver function and is a good model for drug testing, metabolism and studying adverse effect in patient suffering NAFLD.

P-1-17

A novel in vitro iPSC-based cardiac organoid model for personalized medicine.

ABSTRACT #497

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Induced pluripotent stem cells (iPSCs), carrying the patient genetic background, open the path to advanced in vitro modelling. Feasibility to recapitulate complex physio-pathological scenarios depends on iPSCs differentiation ability to a plurality of organ specific cells, and on their maturation and networking capacity. Strong interest has arisen for cardiac organoids (CO), 3D structures, obteined thanks to iPSC natural capability to self-assembly rebuilding organ parts. Here we describe our novel protocol for the generation of CO (400µm diameter; culture: 21 days) and their characterization by multi-omic approach, immunofluorescence (IF) and electrophysiology. CO recapitulate relevant cardiac features: i) spontaneous contraction, ii) multicellularity, demonstrated by single-cell RNAseq and validated by IF, showing clusters expressing genes specific for cardiac cellular sub-sets, such as myofibroblasts (e.g. Col1A1, αSMA), atrial/ventricular cardiomyocytes (CM), iii) geometrical organization (e.g. cardiac chambers, epicardium layer). Electron microscopy analysis showed improved

sarcomeric structures, developed junctions (desmosomes, tight junctions), according to transcriptome-based prediction. Whole CO indicated a beating frequency of 1Hz, with polarization and depolarization peaks of 60 and -60 mV. Patch-clamp showed regular spontaneous action potentials, sodium and pacemaker currents, demonstrating improved CM maturity. The transcriptome analysis indicated the presence of c-AMP and NFkB intracellular machinery, supporting the feasibility to implement our CO, respectively, to simulate treatment with betablockers and anti-inflammatory drugs, two widely used cardiac drugs. With the designed protocol, CO production is high-throughput and the scaled production of thousands of COs was highly reproducible across different CO batches, as demonstrated by consensus analysis of transcriptomic and proteomic. In conclusion, results support the feasibility of our CO-based method translation as in vitro drug screening platform for personalized medicine. Such a platform could serve a wide range of applications, such as evaluation of drug cardiotoxicity, or screening of the efficacy of drugs for the treatment of genetic origin cardiomyopathies.

P-1-18

Advanced in vitro model for drug induced kidney injury assessment - generation of kidney organoid for safety assessment purposes ABSTRACT #506

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

P-1-19

NEXT GENERATION TARGET ORGAN TOXICITY RISK ASSESSMENT: ENDOGENOUSLY TAGGED HUMAN STEM CELL REPORTERS FOR HIGH-CONTENT SCREENING OF OXIDATIVE STRESS RESPONSE. ABSTRACT #508

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Development of in vitro assays for early detection of liabilities to chemical adversity is crucial for the prediction of liver toxicity (1). Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) are an attractive in vitro model to study mechanism-based xenobiotic toxicity (2). We set out to build a panel of fluorescent hiPSC reporters, suitable for high-content-screening of cellular stress response activation, upon compound exposure. We established a pipeline for efficient CRISPR/Cas9-mediated reporter generation and reporter's functional characterization upon differentiation to relevant lineages, including HLCs. Here, we present the generation and application of fluorescent hiPSCs reporter lines for sulfiredoxin-1 (SRXN1) and pirin (PIR), which are shown to be inducible and sensitive biomarkers for the oxidative stress response. Oxidative stress induced by diethyl maleate (DEM), sulforaphane and nitrofurantoin was monitored using live-cell confocal imaging of iPSC reporter lines in HLCs differentiation state. Endogenous levels of eGFP-tagged biomarkers accumulated in the cytoplasm of HLCs over 24

hours window. Newly established isogenic fluorescent reporter lines will be used i) as a tool in understanding and quantifying target organ specific oxidative stress response, ii) point of departure modelling to further capture specific lineage sensitivities towards oxidative stress and iii) ultimately, used for the hazard characterization and IATAs for target organ toxicity in next generation risk assessment.

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Session: 2a. Models, biomarkers and assays for endocrine disruption and developmental toxicity

P-2a-1

Chlorpyrifos impairs immortalized hypothalamic murine GnRH neurons at human relevant exposure levels ABSTRACT #76

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Background: Chlorpyrifos (CPF) is a widely used pesticide exerting several neurodevelopmental effects with severe consequences on children cognitive, behavioral and motor development. Human exposure occurs mainly through diet; pregnant women and children are subpopulations at higher risk. In brain, hypothalamus is one of the main target organ affected by pre- and postnatal CPF exposure. We previously demonstrated that developmental exposure to CPF alters oxytocin, vasopressin and Estrogen receptor (ER) beta expression in mice. Objectives: To better elucidate the mode of action of CPF in hypothalamus, at molecular level, we used the fully differentiated GT1-7 mouse hypothalamic cell line, analyzing a battery of endpoints at human relevant concentrations. Material and Methods: GT1-7 cells were treated for 72h with CPF in a range of six 10-fold diluted concentrations (1 nM-100 μM) assessing cell proliferation, metabolic activity, apoptosis and necrosis. Cells treated at the three lowest CPF doses (1-10-100 nM) were used to assess GnRH secretion by ELISA assay, gene expression of GnRH, ERα, ERβ, aromatase and oxytocin by qPCR and protein expression profiles by Mass Spectrometry. Electronic microscopy (TEM) was performed on GT1-7 treated with 100 nM CPF. Results: CPF dose-dependently reduced metabolic activity and decreased cell proliferation only at the highest dose. At same dose, apoptosis was observed after 48h and 72h treatment. The selected genes were all up-regulated by CPF, with different patterns according to the concentration. TEM analysis evidenced severe mitochondrial damage, mitophagy, increased mielino-like figures and reduced cell-cell contact. ELISA and proteomics analyses are in progress. Discussion and Conclusion: Exposure of hypothalamic GT1-7 cells to CPF, at human relevant concentrations, demonstrated to impair mitochondria integrity also inducing neuroendocrine markers' expression, thus supporting the concern for neurodevelopmental effects exerted by this pesticide. Bioinformatics analysis of differentially expressed proteins will further elucidate the mode of action of CPF in hypothalamus.

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P-2a-2

Evaluation of the local tolerance of flagellin aerosol therapy (FLAMOD) on primary human cell-based 3D in vitro nasal, bronchial, small-airway and alveolar models
ABSTRACT #117

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Bacterial pneumonia is a major cause of morbidity and mortality in humans. To counter this, the European consortium FAIR aims to develop more efficient therapies, based on recombinant flagellin FliCΔ174-400, to treat pneumonia with or without a concomitant uptake of antibiotics. Recombinant flagellin works as an immune-modulator which boosts the innate immunity of airway epithelia via the activation of TLR-5. Delivery of recombinant flagellin to the lung via nebulisation has the advantage of directly targeting the airway epithelial cells while conferring minimal systemic immune activation. We herein describe the local tolerance evaluation of a flagellin-based formulation (FLAMOD) on primary human airway and lung epithelial models. Regional effects on fully differentiated nasal, bronchial (MucilAir™), small airways (SmallAir™) and alveolar (AlveolAir™) epithelial function were evaluated using a multi-parametric approach and a dynamic analysis. The tissues were exposed to 0.0003, 0.003; 0.03; 0.3 or 3 □g/cm2 of FLAMOD apically 2 h/day, for 5 days. For all models, trans-epithelial electric resistance (TEER), cytotoxicity (LDH), pro-inflammatory cytokines (IL-8) and a panel of genes were evaluated. Additionally, on upper respiratory tract models, Cilia beating frequency (CBF) and Mucociliary clearance (MCC) were assessed. Although no effect on TEER, CBF, MCC and cytotoxicity was observed for all tested conditions, FLAMOD did induce a dose- dependent (i) mild increase of the cytokine IL-8 starting at 0.03 ug/cm2 and (ii) upregulation of expression of genes coding CCL4, TNF, IL-1B, CSF3, or CCL20 with a plateau obtained at 0.03 ug/cm2. Altogether, FLAMOD was well tolerated by nasal, bronchial, small airway and alveolar epithelia. Apical exposure induced biomarkers upregulation, thus highlighting FLAMOD's immunomodulation potential all along the respiratory and lung mucosa.

P-2a-3

Investigation on the reproducibility of the DIO1 inhibition in vitro method based on human liver microsomes
ABSTRACT #200

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Thyroid hormones primarily influence metabolic rate and protein synthesis and impairment of their metabolic homeostasis has been associated with several adverse effects. Increased regulatory requirements combined with the need to identify different mode of actions (MoA) impacting thyroid hormone signaling pathways will result in increased animal testing. To reduce this need as well as to establish screening methods for compound development, relevant in vitro methods to identify thyroid active substances are needed. The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) is evaluating and validating numerous in vitro methods focusing on different MoA by cooperating with a network of EU laboratories (EU-NETVAL). One of the regulators of thyroid hormone balance are deiodinases (DIO) by activation of T4 to T3 and degradation of thyroid hormones via deiodination. DIO1, one of the

three isoforms, serves as one main source for circulating T3 via deiodination of T4 in liver, kidney and thyroid. Jointly with the method developer (Charité Universitätsmedizin Berlin, Germany) a non-radioactive approach to determine substance-induced DIO1 inhibition based on iodide release in human liver microsomes was established. The released iodide was measured via colorimetric change in the Sandell-Kolthoff reaction. A concentration dependent decrease in DIO activity after incubation with the noncompetitive, specific DIO1 inhibitor 6-Propyl-2-thiouracil (IC50: 3,72 µM) and DIO inhibitor Aurothioglucose (IC50: 0,78 µM) was shown. A reproducibility assessment consisting of five different DIO1 inhibitors in five independent runs led to the calculation of respective IC50's as well determination of acceptance criteria for the validity of the method. The optimised and standardised human liver microsome based DIO inhibition method is a suitable medium-throughput method for testing the DIO-inhibiting properties of chemicals. 40 validation chemicals are being selected by a thyroid expert group, led by ECVAM, to investigate the mechanistical predictivity and the added value for regulatory purposes.

P-2a-4

A closer look at the evaluation criteria of the H295R In vitro Steroidogenesis Assay test guideline
ABSTRACT #290

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The in vitro steroidogenesis assay, performed according to OECD TG 456 and US-EPA OPPTS 890.1550 is used to evaluate interference of substances with the production of estradiol and testosterone. The evaluation criteria for active substances of this TG are based only on statistical significance. A 1.5-fold threshold used in the validation of the assay [1] has not been adopted in the TG. To address this discrepancy, negative chemicals (benomyl, ethanedimethanesulfonate (EDS), and dinitrophenol (DNP)) were tested according to the validation study. The sensitivity of the test system was demonstrated with the positive test chemicals prochloraz, forskolin, atrazine, aminoglutethimide and bisphenol A. The response of these proficiency chemicals in the steroidogenesis assay was evaluated based the criteria of the TGs or those of the assay validation study [1] and the results compared. In each experiment a quality control plate was tested including the controls forskolin (inducer) and prochloraz (inhibitor). Hormone concentrations were measured using online-SPE-HPLC-MS/MS. The positive chemicals were classified correctly, and the lowest observed effect concentrations were within the TG acceptance criteria for both assessments. However, for negative chemicals benomyl, EDS and DNP statistically significant effects were found at relative fold-change below 1.5-fold. Following the current TG criteria benomyl, EDS and DNP would have been incorrectly classified as positive or equivocal. The concentration response curves of all tested substances were very similar to the ones observed during assay validation. The results of this study demonstrate that the presence of statistically significant changes as a sole criterion for overall evaluation of a test substance is not appropriate. Inclusion of the magnitude of change (threshold 1.5-fold) and scientific judgment (e.g., concentration-dependent effects, borderline cytotoxicity) are imperative to obtain a correct classification without compromising sensitivity. Therefore, adoption of these criteria used in assay validation into the TGs is necessary.

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P-2a-5

LC-MS based analysis of substance-induced displacement of thyroid hormone from its serum binding protein
ABSTRACT #291

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Thyroid hormones (TH) play a crucial role in metabolism, growth, cell differentiation and neurodevelopment. In the blood circulation, >99% of TH is bound to the respective carrier proteins, mainly thyroxine-binding globulin, transthyretin (TTR), and serum albumin. These binding proteins consequently play an important role in the distribution of TH and in maintaining the extrathyroidal TH pool. Some chemicals might competitively displace the TH thyroxine (T4) from its binding proteins in serum, representing a possible mode of action (MoA) of substance-induced disruption of TH signaling. Here, a novel method assessing the displacement of T4 at TH binding proteins was developed. This method requires an incubation step with TTR, T4, and the test item to generate an affinity-based binding equilibrium. Protein-bound and unbound T4 are separated by chromatography and protein-bound T4 is dissociated from the proteins by ethanol extraction. The T4 concentration of the resulting solution is then quantified by LC-MS analysis. Protein-response and T4-response curves were derived using human TTR. The method was characterized regarding solvent (DMSO) concentration and storage stability of extracted samples. Tetrabromobisphenol A (TBBPA), described to competitively bind to TTR [1], showed a full dose-response activity in the assay (IC50: 215nM). Known T4-displacers (e.g., Pentachlorophenol) as well as potential negative test items with and without thyroid related MoA were also tested. In this context, the TH receptor antagonist Diclazuril was identified showing T4-displacing activity (IC50: 625nM). This assay showed reliable and reproducible substance-induced displacement of T4 from TTR. Testing of serum as a source for TH binding protein will further promote the significance of the method by integrating in vivo characteristics like plasma protein binding. This method allows to investigate the MoA of chemicals by using the respective proteins and to finally assess the biological relevance of this MoA by using rat/human serum.

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P-2a-6

Impact of interference with retinoid signaling on in vitro differentiation in human neural stem cell-based model ABSTRACT #445

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The development of human cell-based model for chemical safety assessment of (developmental) neurotoxicity is a recognized research need to further implement international efforts to reduce, replace, and refine animal testing, as well as to increase generation of human-relevant data for hazard assessment. The retinoid and thyroid hormone signaling pathways are important target pathways for endocrine disruption that have been implicated in adverse neurological outcomes during (early) development, as well as in adults. Their disruption can lead to impaired neural cells development. Here we present results from an implementation of a human neural stem cell (NSC) model, that can differentiate in vitro into neurons and (astro)glial cells. We used prototypical activators of retinoid signaling, retinoic acid receptor (RAR) agonist all-trans retinoic acid (atRA), retinoid X receptor (RXR) agonist 9-cis retinoic acid (9cRA), and model inducers of neural differentiation to identify effects of interference with retinoid signaling and to explore potential cross-talk with thyroid hormone receptor (TR) regulation. Upon initial attachment and conditioning, cells were exposed after 4 days in vitro (DIV4) to 8-1000 nM of each chemical for up to 18 days (DIV22). We monitored gene and protein expression; network patterning was characterized by immunocytochemical staining. Exposure to retinoid acids induced their metabolism via CYP26A1, and decreased expression of RARα and RARβ. The exposure caused only minor effects on markers involved in TR signaling. While NSC biomarkers were decreased upon exposure to RAs, no increase in neural markers was detected compared to solvent control. However, an increase in the early astroglial marker S100β was confirmed. Increasing concentrations of retinoic acid

decreased the degree of network patterning. Overall, exposure to 8-1000 nM retinoic acids resulted in a phenotype resembling non-differentiated neural stem cells at the highest concentration on all levels (gene expression, protein expression, cell culture morphology/network pattern).

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P-2a-7

In Vitro pharmacologic profiling For Cosmetic Chemical Systemic Toxicity Safety Testing: A case study on Homosalate

ABSTRACT #336

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When performing safety assessment of cosmetic ingredients, the evaluation of systemic toxicity based only on non-animal approaches is a challenging objective. Cosmetics Europe Systemic Toxicity Task Force has been developing a toolbox including in vitro Pharmacological profiling that enables use of next generation risk assessment (NGRA) in the safety assessment of cosmetic ingredients. Experience with drugs has proved that Human Adverse Reactions can arise via interactions of pharmaceuticals with defined tissue target macromolecules: enzymes, receptors, ion channels, … The hypothesis was made that screening for these interactions could expand the coverage of biological space when assessing systemic toxicity. The CE case study on homosalate (UV filter), was designed to assess the added value of New Approach Methodologies in safety assessments based on read across. A tiered approach is used for the in vitro pharmacology profiling. Stability and solubility tests confirmed that homosalate -the target- and its analogs were stable and soluble at 10 µM. For homosalate and four of its analogs, Dopamine transporter and serotonin HT2B receptor were the predominant hits (>75%). Estrogen receptor, Norepinephrine transporter, Melatonin receptors, Angiotensin-Converting Enzyme and Cholecystokinin A receptor targeted between 50 and 75% inhibition. Follow on dose response testing will be undertaken, to quantify potency in target assays where >50 % effect was observed. These results will have to be analysed together with the other data collected: PBK Modelling, transcriptomics, cell stress and endocrine activity. These preliminary results demonstrate that In vitro pharmacology profiling could be a valuable tool to combine with other data for assessing systemic toxicity and for providing quantitative data on an in vitro bioactivity Points of Departure that can aid for NGRA.

P-2a-8

Test Guideline No. 248 (XETA): a new OCDE approved alternative method for the identification of thyroid active chemicals

ABSTRACT #52

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The XETA is an in vivo assay using xenopus eleutheroembryos to detect thyroid active compounds. The test relies on transgenic eleutheroembryos bearing a genetic construct where Green Fluorescent Protein expression is controlled by the thyroid pathway. Thyroid

active molecules, hormones and endocrine disrupters induce increases or decreases of fluorescence, depending on their mode of action. We conducted an international OECD validation of the method during 8 years (1) and the XETA was published as the OECD test guideline n°248 (2) in spring 2019. The eleutheroembryonic life stage is post-hatch, but before the start of independent feeding on exogenous food supplies. In some regulatory jurisdictions, including Europe, the eleutheroembryonic period is regarded as a non-protected life stage, in this context the XETA could be used as an alternative method for the assessment of thyroid disruption in vivo. By nature, the XETA provides a degree of information about the likely mode of action of thyroid active chemicals. Each chemical is tested either in the presence (T3 spiked mode) or absence of T3 (unspiked mode), interpreting the results obtained in the two modes helps to hypothesised on the mode of action. Thyroid receptor agonists induce an increase of fluorescence in both modes while thyroid receptor antagonists induce a decrease in T3 spiked mode. Substances modulating the clearance or metabolism of the hormones modify the fluorescence only in spiked mode. This presentation will focus on the validation of this new test guideline, its current uses in the context of the actual reglementation on endocrine disrupters (3) and the new developments to the method that are currently made in the ATHENA (H2020) and PERIAMAR (COST action) projects.

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P-2a-9

Relevant impact on the endocrine activity potential by modification of classical in vitro transactivation assays – exemplary case studies with two UV filters

ABSTRACT #127

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A next generation risk assessment (NGRA) evaluating the systemic toxicity of cosmetic ingredients without generating animal data needs to include robust and relevant new approach methodologies (NAMs), which also cover potential endocrine activity. To this end, we developed two new assays based on classical transactivation assays (TA) from the in vitro endocrine activity toolbox, namely (1) MetTA: a method incorporating rat liver and human skin S9 fractions supplemented with phase I and/or phase II cofactors into the current gene reporter TA, and (2) a co-culture TA-3D assay, combining an EpiSkinTM reconstructed human epidermis as a skin barrier model with TA cell lines, thereby modeling consumer-relevant topical application of the test chemical. In collaboration with Cosmetics Europe, case studies with two cosmetic ingredients were performed using the whole in vitro endocrine activity toolbox, including our newly developed assays. The estrogenic activity of the UV filters, homosalate and benzophenone 3, observed in the classical TA was no longer detected in the presence of S9 fractions and phase I and II cofactors, indicating that metabolism prevented endocrine activity. In the TA-3D assay, the endocrine activities of both chemicals were significantly decreased due to the skin barrier model from EpiSkinTM, indicating that a lower bioavailability results in lower endocrine activity. Overall, these case studies demonstrate that incorporating metabolic competence and topical application into classical in vitro assays may lead to a considerable refinement of the endocrine activity potential, thus, achieving results which

better resemble real-life consumer conditions.

P-2a-10

INCORPORATING A METABOLIZING SYSTEM AND TOPICAL APPLICATION TO IMPROVE THE VALUE OF IN VITRO ENDOCRINE DISRUPTION ASSAYS ABSTRACT #181

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Regulatory bodies emphasized the need for developing appropriate metabolically competent in vitro assays mainly for the assessment of potential endocrine active substances. In an effort to comply with these recommendations, we developed our toolbox with two new assays (metabolically competent transactivation assay, MetTA and co-culture TA-3D). It is recognized that metabolically competent in vitro cell-based assays are required for the assessment of xenobiotics, in order to 1) detect potentially toxic metabolites, and 2) detect if the parent compound is detoxified. MetTA is a method incorporating S9 fractions (rat liver and human skin) into the classical gene reporter transactivation assays (TA) for estrogenic and androgenic activities assessment (T47D-kBluc and MDA-KB2 cell lines). To refine the assessment of cosmetics ingredients, we developed a co-culture TA-3D assay allowing a topical application of the compound of interest. It combined an EpiSkinTM reconstructed human epidermis, as a skin barrier model, directly above the T47D-kBluc or MDA-KB2 cell lines. To validate the methods, we used well-known estrogenic and androgenic active compounds, such as Methoxychlor, Bisphenol A (BPA), etc…. As expected, with the MetTA, estrogenic activity of Methoxychlor significantly increased in the presence of S9 liver fraction and phase I cofactors whereas no more estrogenic activity was observed using S9 fractions and phase I & II cofactors for BPA. The formation of metabolites was confirmed by HPLC or GC-Mass Spectroscopy. With the TA-3D assay, the estrogenic or anti-androgenic activities of the selected compounds after a topical exposure was decreased thanks to the skin barrier model from EpiSkinTM, compared to a classical systemic exposure. This study demonstrated the feasibility and the relevance of the incorporation of metabolic systems and the topical application of reference compounds into in vitro assays, in order to optimize the characterization of a potential endocrine activity of new chemical entities.

P-2a-11

The agonistic bioanalytical equivalent concentration: A novel tool for assessing the endocrine activity of environmental mixtures ABSTRACT #381

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Background and Objectives Cell-based bioassays are becoming increasingly popular in environmental toxicology. These assays are very sensitive and allow integrative effect screening of the whole environmental sample, which is usually composed of a mixture of agonists and antagonists. Total toxicity is usually expressed as a bioanalytical equivalent concentration, and both agonists and antagonists in the mixture are involved in this characteristic. So far, it is not possible to distinguish which part of this value is caused by the agonists and which by the antagonists. Material and Methods The estrogenic activity of the single estrogens and serial dilutions of the artificially prepared mixtures of estrogens and antiestrogens has been measured by standardised recombinant yeast assay BMAEREluc/Erα (Leskinen et al., 2005). A mathematical model has been derived from previously published models of mixture toxicity (Ezechiáš and Cajthaml, 2016). Results We present a simple method to analyze the dose-response curve of a mixture and to determine an agonistic bioanalytical equivalent concentration: a concentration of a reference chemical that would elicit the same effect as do only agonists in an unknown mixture. The method has been validated using several artificially prepared mixtures of agonists and

competitive antagonists measured in a recombinant yeast assay. No difference was observed between the calculated equivalent concentrations and the used concentrations of the agonist. Discussion and Conclusion The presented methodology could "filter" the effect of antagonists in the mixture and allow quantification of the contribution of agonists to the overall toxicity. It provides an additional view on unknown mixtures and improves the analysis of environmental samples

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P-2a-12

ENDOCRINE DISRUPTION – BREAKING FREE FROM IN VIVO TESTING ORTHODOXY
ABSTRACT #180

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Efforts to identify endocrine disruptors under EU chemical safety regulations currently rely heavily on the use of in vivo data. Protocols have been established for identifying endocrine disruptors in plant protection products and biocides and a similar approach may soon be expected for substances addressed by the REACH regulation. In vivo test methods for providing either mechanistic or adverse effect information have a number of serious shortcomings, not least because of the issue of species difference which calls into question the utility of such methods for predicting adverse effects in exposed populations. We explore the potential of non-animal methods for providing both mechanistic information and supporting adverse-effect determinations, and question the orthodoxy that effective regulation of endocrine active substances necessitates in vivo testing. We will present an analysis of the WHO definition of an endocrine disruptor, look at the drivers for the use of non-animal methods and ask the question: what more could in vitro and in silico methods contribute for the effective regulation of endocrine disruptors?

P-2a-13

PERFORMANCE LIMITATION OF OECD TEST NO. 455 ESTROGEN RECEPTOR TRANSACTIVATION ASSAY CLASSIFICATION CRITERIA USING VM7LUC4E2 CELL LINE.
ABSTRACT #327

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Concept Life Sciences (CLS) offer validated assays within Good Laboratory Practice (GLP) accredited facility, to assess endocrine disruptors, including various OECD tier-2 assays(1). The OECD test guideline 455(2) (TG455) Estrogen-Receptor Transactivation assay (ERTA) is a performance based in-vitro assay used to investigate the ability of a chemical to modulate the estrogen receptor. We describe herein our validation of the VM7Luc4E2 ERTA assay. Internal validation of ERTA in both agonism and antagonism modes at CLS was performed using the stably transfected VM7Luc4E2 cell line following the OECD TG455 recommendations. Competence of the laboratory to run the assay was demonstrated by evaluating a set of chemicals (fourteen and ten proficiency substances for the agonist and antagonist, respectively). The ratios of plates meeting acceptance criteria, vehicle control luminescence and fold induction statistics were comparable with the ICCVAM validation(3). All proficiency items for ERTA antagonism were classified correctly as per the criteria set by TG455. In the ERTA agonism mode of the assay, all positive proficiency items were classified correctly. Negative proficiency items provided consistent data across experiments indicating the robustness and reproducibility of the assay. However, application of TG455 classification criteria for the ERTA agonism led to negative proficiency item being classified as inadequate. In contrast application of classification criteria described in the ICCVAM validation report allowed negative proficiency item to be classified correctly. The VM7Luc4E2 ERTA assay displays robust

accuracy against a set of proficiency items when using ICCVAM validation criteria. This suggests that a re-evaluation of TG455 classification criteria for this VM7Luc4E2 cell line would improve the performance of the ERTA agonism mode of the assay.

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P-2a-14

A metabolomics approach to investigate in vitro the hepatotoxicity of drugs and the mechanisms so far involved

ABSTRACT #323

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Hepatotoxicity studies, are key throughout preclinical stages of drug development to minimize undesired toxic effects that might later appear in the course of the clinical use of a new drug. In vitro models and in particular cultured hepatocytes, represents an easy and robust alternative to animal drug hepatotoxicity testing for predicting human risk. Here, we describe an innovative strategy to identify potential hepatotoxic drugs and their mechanism of action by means of HPLC coupled to mass spectrometry metabolomics analysis of exposed cells. As a training set, we used 25 hepatotoxins and 4 non hepatotoxins and incubated HepG2 cells for 24 h at a concentration IC10 and IC50. Changes in the metabolome were investigated and prediction models were built for the main known hepatotoxicity mechanisms, namely oxidative stress, mitochondrial disruption, apoptosis, steatosis, and cholestasis as well as global toxicity. Thereafter, a test set of 72 chemicals with known toxicity and predominant mechanism of toxicity and 14 non-hepatotoxic compounds were analyzed at 1, 10, 100 and 1000 µM. Based on the prediction models developed, we could quantify the magnitude and extent of the injury and uncover the mechanisms of toxicity so far involved for each compound. This information is visualized in an easily interpretable spider diagrams. This analysis allowed to account for the participation of the different mechanisms in the global hepatotoxicity of a drug and their interrelation giving a better holistic approach to the phenomenon of hepatotoxicity and disclosing interesting discrepancies with current literature data. The accuracy of the different mechanistic models is somewhat lower than that of the model for global hepatotoxicity prediction, but sufficient to estimate the involvement of the different mechanisms. The relevance of the metabolites identified as hepatotoxicity biomarkers was reinforced by the metabolic pathway analysis.

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P-2a-15

Validation of Endocrine Disrupter Assays at EURL ECVAM ABSTRACT #194

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The European Union Reference Laboratory for Alternatives to Animal testing (EURL ECVAM) has been active for several years in the field of Estrogen, Androgen, Thyroid hormone and Steroidogenesis (EATS). One of the important activities concerns the validation of endocrine disrupting (ED) assays, thus contributing to the development of OECD Test Guidelines (TGs) and supporting the European Commission's community strategy on EDs. OECD has established a conceptual framework (CF) for ED TGs available, under development or methods proposed to become a TG (1). EURL ECVAM contributed to the TG for Estrogen Receptor Transactivation assays (ERTA assays, TG455, 2009) and took the lead in drafting a TG on Androgen Receptor Transactivation assays (ARTA assays, TG458, 2020). EURL ECVAM coordinated the validation of an ARTA assay, the AR-CALUX method. Three validated methods were combined in one TG (AR STTA using the EcoScreen cell line, AR STTA using the 22MTV GR- cell line and the AR-CALUX method). This TG was adopted at OECD in 2020. More recently, EURL ECVAM embarked on a large scale validation study of 17 mechanistically informative assays to detect chemicals with thyroid hormone disruption activity. This set was identified through the OECD scoping paper (2) and feedback from various expert group meetings. With the collaboration of 14 facilities of the European Union Network of Laboratories for the Validation of Alternative Methods (EU NETVAL) and 13 test method developers, the methods will be assessed in parallel for reproducibility and relevance. The validation aims at identifying a battery of methods that can indicate thyroid disruption activity. Effort is being invested to carry out this validation study in accordance with 2 international guidance documents issued by OECD: Good in vitro Method Practices (GIVIMP) and Guiding principles on good practices on protected elements in OECD TGs (4).

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P-2a-16

THE ADIPOGENESIS MODEL OF HUMAN MESENCHYMAL STEM CELLS FOR THE DETERMINATION OF OBESOGENIC POTENTIALS OF SUNSCREENS ABSTRACT #73

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Background and Objectives A chemical obesogen is an endocrine-disrupting compound that disturbs normal metabolic homeostasis. Obesognes lead to abnormal lipid accumulation, weight gain, and obesity in human. Adipogenesis of human bone marrow mesenchymal stem cells (hBM-MSCs) has been exploited as one of in vitro cell-based assays to evaluate obesogenic potentials. Molecular targets of many obesogens are nuclear hormone receptors such as peroxisome proliferator-activated receptors (PPARs), estrogen receptors (ERs), and glucocorticoid receptor (GR). This study was aimed to elucidate obesogenic potentials of extensively

prescribed organic ultraviolet (UV) filter chemicals such as avobenzone, benzophenone-3, benzophenone-8, and octocrylene during adipogenesis in hBM-MSCs. Materials and Methods To investigate obesogenic potentials of sunscreens, avobenzone, benzophenone-3, benzophenone-8, and octocrylene, the adipogenesis model of hBM-MSC was used. To identify molecular targets of obesogenic sunscreens, the time-resolved fluorescence resonance energy transfer (TR-FRET)-based receptor binding or coactivation assays were performed. Results Avobenzone, benzophenone-3, benzophenone-8, and octocrylene significantly increased lipid accumulation during adipogenesis in hBM-MSCs compared to that of the control. In TR-FRET-based PPARgamma binding analysis, benzophenone-3, benzophenone-8, and octocrylene diretly bound to PPARgamma whereas avobenzone had no effect on PPARgamma. they increased the gene transcription of three PPARgamma subtypes and major lipid metabolism-associated enzyme genes in human epidermal keratinocytes, which are the main target sites of UV filters in human skin. Discussion and Conclusion Recently, many countries have banned sunscreens containing benzophenone-3 or octocrylene because they lead to toxic outcomes of aquatic lives. This study highlights the effects of sunscreen ingredients avobenzone, benzophenone-3, benzophenone-8, and octocrylene as a major environmental obesogen similar to phthalates, bisphenols, and organotins.

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P-2a-17

INCREASING THE NUMBER OF AVAILABLE METHODS FOR THE DETECTION OF ENDOCRINE DISRUPTORS BEYOND EATS ABSTRACT #297

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The lack of regulatory methods that allow to identify Endocrine Disruptors (EDs), has promoted the creation of the Public-private platform for the pre-validation of endocrine disruptors characterization methods (PEPPER). Although many assays for EDs identification exist, very few are mature enough to be presented for a validation process (OECD, ISO, etc.). PEPPER provides expertise and financial aid to identify those methods, and then facilitate and accelerate their validation process. Additionally, the aim is to go further the Estrogen, Androgen, Thyroid, and Steroidogenesis (EATS) pathways, for which assays already exist for regulatory use. PEPPER's Relevance Committee (researchers and representants of regulatory authorities, NGOs and society) selects each year, from a preset, 3 methods, to be supported. Currently 6 methods have started the pre-validation process, from which 5 are in vitro. Two of them have recently been accepted by the OECD for inclusion on its workplan. The first one, "hPlacenTox-ED testing" informs on potential cellular deleterious effects and hormonal alteration of JEG-3-tox (placental) cells by measuring P2X7 receptor activation and hormones (Progesterone, Estradiol, human Placental Lactogen and hyperglycosylated human Chorionic Gonadotropin) secretion. The second is a human glucocorticoid receptor (hGR) transcriptional activation assay that detects the agonistic and antagonist activity of chemicals using a stably transfected cell line HMLN-hGR expressing the luciferase reporter gene. These methods have been successfully transferred, to 2 and 3 "naïve" laboratories respectively, showing good intra and inter laboratories repeatability. In a second phase, 12 and 35 blinded substances respectively, where chosen by a group of independent experts to further assess the reliability and relevance of the methods, and evaluate their predictive capacity. The statistical analysis of

the results showed a high level of robustness and reliability of both methods to characterize EDs. The built up processes for those achievements will be described with their successes and weaknesses.

P-2a-18

EVALUATION OF THE CYTOTOXICITY OF TETRAHYDROCANNABINOL,CANNABINOL AND CANNABIDIOL IN PRECURSOR CELLS OF THE GLIA ABSTRACT #112

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Cannabis is the most commonly consumed illicit substance worldwide. In Chile, its consumption has been dramatically favored; due to current regulations and laws that favor the cultivation, possession and personal consumption for medical purposes that are totally objectionable and debatable due to the possible adverse health effects of their consumption (1,2). Currently, there is a lack of knowledge about the danger of consumption in humans of cannabinol (CBN), cannabidiol (CBD) and tetrahydrocannabinol (THC), main cannabinoids. Additionally, it should be noted that a high percentage of cannabis consumed in Chile is of transgenic origin, where the concentration of the main cannabinoids are exacerbated. This study's objective was to evaluate the cytotoxicity of THC, CBN and CBD; in human embryonic cells of glia SVG-P12, through the use of Resazurin cell viability assay and evaluation of cells by inverted microscope. Our results have shown that within 24 hours of exposure, the cell viability of the SVG-P12 line decreased by around 30% (≥ 20.0 µM for THC, CBN and CBD), effect more accentuated for CBD; which is related to the smallest number of cells observed by microscopy. From this study it can be concluded that effects are not limited only to THC consumption, on the contrary, the individual exposure of each cannabinoid evaluated in the decrease in cell viability, occur at very low concentrations; more pronounced effect for CBD; and that it is of interest to evaluate the effect of the mixtures CBN + THC, CBD + THC and CBD + CBN + THC usually present in cannabis.

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P-2a-19

Grouping of chemicals into mode of action classes by automated effect pattern analysis using the zebrafish embryo toxicity test ABSTRACT #324

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Background: A central element of high throughput screens for chemical effect assessment using zebrafish is the assessment and quantification of phenotypic changes. By application of an automated and more unbiased analysis of these changes using image analysis, patterns of phenotypes may be associated with the chemical mode of action (MoA). Our aim was to explore to what extent compounds can be grouped according to their anticipated toxicological/pharmacological mode of action using an automated quantitative multi-endpoint zebrafish test. Methods: Morphological and behavioural features were annotated using FishInspector and video analysis software. Based on these features about 30 different endpoints were calculated and used to derive Chemical-response signatures of 25 chemicals assigned to 8 broad

MoA classes. Effects were related to mortality and baseline toxicity to compensate for difference in toxicokinetics and secondary effects related to mortality. Cluster analysis and partial least-squares discriminant analysis (PLS-DA) were applied using effect ratios as unsupervised and supervised statistical techniques. The capacity for discriminating MoAs was also benchmarked to the available in vitro toxicity data obtained from ToxCast library. Results: Unsupervised clustering of the profiling data demonstrated that chemicals were partially grouped by their main MoA. Analysis with a supervised clustering technique allowed to identify markers with a strong potential to discriminate between MoAs. Discussion: Variability in patterns may be explained by (1) discrepancies between the pharmacological mode of action used for grouping of chemicals and the MoA provoking the developmental phenotype or (2) differences in toxicokinetic properties of chemicals belonging to the same MoA group. The capability of the zebrafish to recognize consistent patterns of developmental toxicity was demonstrated for certain MoAs. Moreover, the comparative assessment with ToxCast data showed that the zebrafish embryo test with multiple endpoints and high-content assessment has a similar discriminating capacity as a test battery of multiple in vitro assays.

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P-2a-20

Study of Endocrine disruptors-related lipid and carbohydrate metabolism disorders in human hepatocytes ABSTRACT #419

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There is an urgent need for robust and reliable tests to decipher and understand the underlying mechanisms of action by which endocrine disruptor compounds (EDCs) are responsible for promoting metabolic disorders in humans. These include steatohepatitis, obesity, diabetes and more generally Metabolic-Associated Fatty Liver Disease (MAFLD)1. Using differentiated 2D and 3D HepaRG cells culture models 2, we assessed lipid and carbohydrate metabolism of 10 selected EDCs suspected of having obesogenic properties. The compounds of interest belong to various chemical families such as bisphenols, phthalates, butyl-paraben, perfluoroalkylated substances, organochlorides (DDE) and metals (Cd). Using non-cytotoxic concentrations, gene expressions involved in lipid and carbohydrate metabolism were analyzed by qRT-PCR. Phenotypic profiling of cells, such as lipid droplets and triglyceride accumulation, was characterized after acute treatment in 2D cultures and after long-term treatment in the 3D model along with triglyceride content with or without fatty acid supplementation. Only cadmium revealed cytotoxicity after acute and long-term treatment in both 2D and 3D cell cultures. Furthermore, genes involved in lipid (APOA4, FABP, CD36, CYP4A11) and carbohydrate metabolism (GLUT2, G6PC and GYS2) and xenobiotic metabolism (CYP3A4, 1A2) were altered by these contaminants, especially with DDE, BPA, and PFOA. Lipid droplets quantification revealed an increase in lipid content in 2D cells after DDE & DEHP at 25µM supplemented with fatty acids. For 3D cells, lipid content was increased after treatment with BPA, DEHP, DDE, and PFOA at 10µM. Additionally BPF, DDE and butyl-paraben at 10µM increase triglyceride content in the cells, which is more potentiated with the fatty acid supplementation. Taken together, these results demonstrate that EDCs deregulate lipid and carbohydrate metabolism in human hepatocytes, which may trigger several hepatic diseases like MAFLD.

These outcomes will be used to develop robust and reliable tests to characterize the ability of EDCs to disrupt human liver metabolism.

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P-2a-21

OXYSTEROLS PROFILE IN ZEBRAFISH EMBRYOS UPON EXPOSURE TO BISPHENOL A (BPA) AT 8 AND 24 HOURS POST-FERTILISATION ABSTRACT #439

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Background and objectives. Oxysterols are cholesterol oxidation products and bioactive lipids involved in developmental signaling pathways, embryonic and postembryonic tissue patterning and homeostasis. The embryonic period is a very sensitive window of exposure to bisphenol A (BPA), hence the role of BPA on the levels of oxysterols in the very early stages of zebrafish embryogenesis is a relevant novel field of investigation. Hence, the main aim of the study has been to compare the role of BPA on oxysterols levels in zebrafish embryos at 8 and 24 hours post fertilization (hpf) with cytochromes P450 (CYPs)-modulating chemicals (carbamazepine, ketoconazole, and hydrogen peroxide). Materials and methods. Upon a dose range finding, zebrafish embryos were exposed to environmentally relevant (0.04µM) and toxicological (17.5µM) BPA concentrations. Seven oxysterols were profiled by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Results. Similarly to the CYPs-modulating chemicals, BPA caused: i) no significant changes at 8 hpf and ii) a dose-dependent increase of total oxysterols at 24 hpf, with 27-hydroxycholesterol as the most regulated oxysterol. Discussion and conclusions: In the first day post-fertilization of the zebrafish embryos, the role of BPA alike a CYPs-modulating chemical was confirmed by the similar oxysterol changes observed with the already known CYPs-modulating chemicals.

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P-2a-22

HIGH CONTENT SCREENING OF BISPHENOLS AND THEIR MIXTURES UNDER CONDITIONS OF LOW-INTENSITY ADIPOGENESIS OF HUMAN MESENCHYMAL STEM CELLS (hMSCs) ABSTRACT #282

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Background In vitro models of adipogenesis are phenotypic assays that most closely mimic the increase of adipose tissue in obesity. Current models, however, often lack throughput and sensitivity and even report conflicting data regarding adipogenic potencies of many chemicals, with bisphenol A (BPA) being one such example. Methods Here, we describe a 10-day long adipogenesis model using high content analysis readouts on primary human mesenchymal stem cells (MSC) sensitive enough to compare eight bisphenol A derivatives and their mixtures quantitatively in a robust and high throughput manner. Endpoints from automated image analysis represent the number of adipocytes and adipocyte size, total

lipid content in culture, as well as the number of cells. Tested bisphenols include BPA and its replacement chemicals: bisphenol B, bisphenol C, bisphenol F, bisphenol AF, and structural analogues: tetrabromobisphenol A and tetrachlorobisphenol A, all tested at 0.1–100 µM. Results All eight bisphenols were proven adipogens in hMSCs. Tetrabromobisphenol A and tetrachlorobisphenol A were the most potent and bisphenol F the least potent among the tested compounds. The number of adipocytes was the most sensitive endpoint capable of detecting changes of 20% and was used to develop a benchmark concentration model (BMC) to quantitatively compare eight bisphenols. The model was applied to evaluate mixtures of bisphenols obtaining the first experimental evidence of their additive effect on human MSC adipogenesis. Using the relative potency factors (RPFs), we show how a mixture of bisphenols at their sub-active concentrations induces a significant adipogenic effect due to its additive nature. The final active concentrations of bisphenols in tested mixtures reached below 1 µM, which is within the concentration range observed in humans. Conclusion These results point to the need to consider the toxicity of chemical mixtures and the importance of quantitative in vitro methods for the risk assessment of chemicals.

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P-2a-23

Reproductive and developmental adverse effects of complex real-life mixtures in human-relevant cell-based assays

ABSTRACT #486

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Background and Objectives: Many chemicals found in food and the environment have a negative impact on human health. Risk assessment of chemicals has been mainly performed on a single-substance basis. However, humans are exposed to complex mixtures of chemicals at any given time. As a part of the Green Deal Project “PANORAMIX” (1), we aimed at determining the reproductive and developmental effects of real-life mixtures, extracted from pooled samples of water, fish, milk and human blood, using human-relevant cell-based assays. Material and Methods: Enriched chemical mixtures, extracted from pooled samples provided by multiple European countries, were screened in three luciferase reporter gene bioassays. For the assessment of (cardio-)developmental adverse effects, a 3D model of human induced pluripotent stem cell-derived embryoid bodies (EBs) mimicking the human blastocyst (2) was exposed to each mixture during differentiation into cardiomyocytes. Potential antagonistic/agonistic interactions of these mixtures on human androgen and estrogen receptors were assessed in stably transfected cell lines, namely AR-EcoScreenTM and VM7Luc4E2, respectively (3). To establish non-cytotoxic levels to further assess each endpoint, cytotoxicity of all mixtures was evaluated beforehand in each cell model. Results: Some complex extracts displayed a negative impact on EBs, as demonstrated by the decrease in luminescence readout in the PluriLum assay. Several extracts were also shown to detain substantial endocrine disrupting potential at non-cytotoxic levels, displaying mainly estrogen receptor agonism, and either androgen receptor agonism or antagonism. Discussion and Conclusion: This work highlights the potential of in vitro reporter gene assays as tools for the assessment of human developmental and reproductive adverse effects of real-life chemical mixtures. Our results further emphasize the potential impact of chemical mixtures on human health, and the need to advance regulatory risk assessment in order to take mixture effects into consideration.

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P-2a-24

Teratogenic potential of Cosmetics: Building and Optimize a toolbox to develop an ITS
ABSTRACT #488

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New approach methods to assess the teratogenic potential of substances have been developed in recent years. In the cosmetic domain, the full implementation of animal testing ban in the European Union in 2013 has urged these developments to keep high standards of knowledge and safety, allowing the use of new ingredients. Loreal's Research Department on alternative methods is committed to developing alternative methods for evaluating the impact of cosmetic products on humans and ecosystems. The Eco-Model laboratory develops and carries out ecotoxicity tests on different organisms representative of aquatic food chains: bacteria, micro-algae, invertebrates and also fish embryos that are specially providing relevant information for assessing human effects. As part of an internal initiative, reprotoxicity/teratogenicity is being explored. The overall objective is the development and the evaluation of an ITS (Integrated tested Strategy) and a Teratogen Index tool based on non-animal methods. Three existing tests are being used to assess the teratogenic potential: i. An test on human Induced Pluripotent Stem cells (devTOX quickPredictTM, Stemina) ii. A test on Fish Embryo models: Zebrafish embryos (Biobide) iii. In Silico (DART ontology) prediction as weight of evidence for biological coverage. Assessment of the complementarity (endpoint gap, performance, statistics,…) and/or improvement of the properties considered (in silico physchem, metabolism, human and animal plasma concentration, …) are in progress to better cover the human relevance. In conclusion, the intermediate analysis of the complementarity of data from Biobide, Stemina and DART showed good sensitivity and specificity and appears to be an interesting predictive approach. The progressive addition of substances in the tests' battery as well as the implementation of new methods would allow the refinement of the results to obtain a robust ITS for evaluating the teratogenic potential of substances. A qualitative decision tree with an evaluation of the accuracy of the tests is proposed.

P-2a-25

The role of retinoids in disturbing neural rosette formation
ABSTRACT #491

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

Session: 2b. Challenges in cosmetics safety

P-2b-2

ToxTool: an innovative database for the cosmetic regulatory affair ABSTRACT #316

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Background and Objectives ToxTool is an innovative database collecting all of available toxicological information on chemicals used for cosmetic application. This instrument can support cosmetic companies to increase their competitiveness in the development of new products. Material and methods ToxTool shows accurate descriptions both from regulatory and scientific point of view. For every compound basic detail such as CAS and EINECS numbers, the functionality in the cosmetic fields and updated regulatory information are reported in order to get a general overview. Moreover, chemical-physical characteristic useful to presume the lipophilicity or hydrophilicity and then define the bioavailability of every compound are deeply described. The toxicologic data take into account several endpoints such as: skin and eyes irritation, skin sensitisation, acute and repeated toxicity data with relative experimental value from the literature or predicted conclusion and values according in silico methods. For every cosmetic formulation is possible to estimate the margin of safety (MoS) according to the most recent European guidelines to support the risk assessment of the finished cosmetic product. In this work ToxTool is used in the evaluation of different type of products for different application using the same cluster of ingredients. Results Using ToxTool a complete overview is provided in a single glance helping the gathering of information essential for a conscious risk assessment. This is a valuable support during the decision making process leading to the cosmetic safety assessment, a scientific act mandatory for each cosmetic product intended to be put on the European market. Discussion and Conclusion The innovation in this database is the match of continuous regulatory updates and the support of the scientific data, an essential point to support the competitiveness and the qualification for every cosmetic companies.

P-2b-3

Too many Animal Tests on Cosmetic Ingredients for REACH in the EU: actual situation and future prospects ABSTRACT #375

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Contrary to political will and large parts of citizens and cosmetic industries, the animal testing ban of cosmetic products and their ingredients is often disregarded. To understand the extent of this statement, we scouted the ECHA database of the REACH registered substances looking for chemicals whose only reported use was cosmetics and determine the extent of new in vivo testing caused by REACH. We found 3,206 dossiers containing cosmetics as a reported use with 419 where cosmetics was the only use. Among them, 63 had in vivo tests completed after the Cosmetic Regulation ban on in vivo testing. A detailed analysis revealed that registrants largely used alternative, non-animal methods, but some still conducted new in vivo tests even when not really necessary. In some cases, during the evaluation procedure, ECHA rejected registrants' alternative methods as insufficient and required new in vivo tests. As ECHA continues to evaluate dossiers, more requests for in vivo tests are likely and others are expected due to the revision of REACH which is enlarging demand for REACH registration dossiers. Next year, a revision of the EU Cosmetic Regulation is also under discussion. This means that there is now a unique to implement a new vision with respect to the incorporation of new approach Methodologies (NAMs) as the sole information requirement into regulatory application. This is possible through a formal exchange of views between scientists, industry, the general public/NGOs and legal experts. This discussion has started in other area, in the EU and worldwide, and the cosmetic area could represent the area where this change will become effective. Our intention is to create the think tank through working groups and yearly

Action Conferences to address legislative solutions for gaps for worker exposure, environmental endpoints and similar legislative roadblocks in the phasing out of animal tests.

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P-2b-4

The study on EDs and the application of the NGRA according to the guidelines SCCS NoG XI Ed: two overlapping challenges. ABSTRACT #338

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Background and Objectives The European regulatory framework for cosmetic products described in the EU 1223/2009 Regulation is well established, and the scientific pathway is subject to continuous evolution. In recent years, the issue of ED has become increasingly urgent, as evidenced by the 3 Forums organized by the EU Commission (2019, 2020, 2021), and by the 28 substances currently under examination to evaluate their effects on the endocrine system. In the latest edition (XI) of the SCCS NoG guideline, the chapter concerning EDs has been implemented and numerous have been highlighted. In the same SCCS NoG, XI Ed, an innovative method for risk assessment was introduced, the Next Generation Risk Assessment, an innovative approach that requires the examination of the exposure scenario, the determination of the interaction between substances and the organism, the generation of a hypothesis which in subsequent tiers is confirmed by in vitro studies, also determining the degree of uncertainty. The innovative (and challenging) modality of NGRA is applied, in this study, to EDs, and it is based on Mode of Action (MOA) and Adverse Outcome Patway (AOP) using New Analysis Methodologies (NAM), computational models, in silico and omics, in application of the ab initio approach, confirmed by in vitro testing. Materials and methods Computational modeling, in silico studies, in vitro tests. Results The tiered, iterative strategy suggested by NGRA involves computational modeling, identification of the AOP and MOA, in silico tools. Hypothesis testing and determination of the degree of uncertainty are performed by in vitro tests. Discussion and conclusion The NGRA constitutes an important challenge for Safety Assessors. Identifying the real impact of potentially ED substances represents an equally ambitious challenge, and the two issues, which emerged in the same period, can be advantageously combined.

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P-2b-5

Zebrafish: Alternative Model For Cosmetic Testing ABSTRACT #480

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Before the marketing and sale of cosmetic products, they must be safe for human use. There is a legal obligation to fulfill terms of adherence to certain safety and toxicity regulations, which has led to a need for alternative non-animal-based cosmetic testing methods to be developed and validated. Using alternative models can be more efficient than testing on animals, as a result, can be developed in a shorter time. It is also more cost-efficient than testing on live animals, as the cost of breeding, feeding, housing, and carrying out the tests is often lower. These alternative models include computer models, in vitro testing, and the zebrafish animal model. As zebrafish larvae are considered an in vitro

assay when used under 5 days post-fertilization, this model complies with the above EU Directive. Thanks to their ability to breed quickly and produce hundreds of larvae at a time, zebrafish are a cost-effective choice for cosmetic testing. Of particular note, is nanotoxicity testing on skincare products due to damage from the harmful rays of the sun, regeneration, or whitening. But also for ecotoxicity assays, including zebrafish in the OECD Guidelines for ecotoxicity needs. Alternative models for cosmetic testing are a particularly suitable solution for research on cost savings and effectiveness since more research can be undertaken. This is a breakthrough for the scientific field, especially as cosmetic testing is essential for human safety. We show some of those applications of zebrafish for cosmetics safety, ecotoxicity, and efficacy applications

Session: 3a. Models, biomarkers and assays for systemic and immune toxicity

P-3a-1

Analysis of the predictive capacity of the SENS-IS assay to define the skin sensitization potency on 186 chemicals
ABSTRACT #162

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Background and objectives In skin sensitization, the ability to measure the potency of chemicals in vitro for most of the assays is still a challenge. The SENS-IS assay, an in vitro alternative method combining a 3D human reconstituted epidermis (Episkin) and the quantitative measurement of 62 specific gene expression biomarkers, is able to determine the lowest positive sensitization dose and is used to determine the potency of pure chemicals but also mixtures. However, the poor quality of the database for skin sensitization potency determined either using the LLNA or human data make it difficult to calculate the exact predictive capacity of NAMs. Material and Methods, Starting from blindly tested 186 chemicals we selected only chemicals for which both referenced LLNA and human data were available. We then selected only chemicals for which the determination of the sensitization potency (CAT1A, CAT1B or no CAT) was exactly the same for human and LLNA data. We end-up with 100 chemicals to be use for prediction capacity. Results Out of 24 CAT1A chemicals, 22 were correctly classified. Our of 53 CAT1B chemicals 49 were correctly classified and all the 25 non sensitizers were correctly classified by the SENS-IS assay. Therefore the overall capacity was calculated to be 94% with a kappa of 0.905. For the 3 different classes the specificity and sensitivity were respectively for CAT1A 91% and 95%, for CAT1B 92% and 96% and for non sensitizers 100% and 89%. Discussion Conclusion These results show that when using a curated database for which the sensitization potency is determined using 2 different approaches (LLNA and human data), the predictive capacity of the SENS-IS assay is very high. This selection did not result in “easy” to test chemicals since the predictive capacity for the determination of hazard was below 80% for both DPRA and keratinosens.

P-3a-2

Potential beneficial effects of selenium nanoparticles in the adjuvant anticancer therapy
ABSTRACT #10

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Background and Objectives Multiple therapeutic benefits suggested through in silico, in vitro and in vivo studies for selenium nanoparticles (SeNPs), qualified them as a potential remedy for metabolic, cancerous or infectious diseases. We aimed to evaluate the biological effects of chemogenic SeNPs on normal (MRC-5) and malignant (PANC-1) cell lines to assess their therapeutic potential. Material and Methods The SeNPs were synthetized by reducing sodium selenite with glutathione and stabilized with bovine serum albumin. Their cytotoxicity at 0.1-25 µg/mL doses was evaluated in vitro after 24 h by LDH and MTT assays. Antioxidant capacity of SeNPs was assessed by measuring the variation of hyperglycemia-induced intracellular

ROS level using 2′,7′-dichlorodihydrofluorescein diacetate. Additionally, sodium selenite (containing 10 times less Se than SeNPs) effect was tested. Results The results indicated a slight decrease of LDH level after exposure of cancerous cells to SeNPs, proportionally with dose, suggesting insignificant cell membrane damage compared to control. However, a dose-dependent decrease of cell viability was registered in PANC-1 cells - 42% viability for the highest dose (25 µg/mL SeNPs) compared to control. Similar effects were obtained after cell exposure to sodium selenite. In normal cells, SeNPs up to 5 µg/mL and sodium selenite induced no significant changes on LDH level or cell viability up to 5 µg/mL SeNPs. A slight increase of LDH level and a decrease by 43% of cell viability were observed only at the highest dose. The ROS level slightly varied for the applied doses, which could be interpreted as a self-limitation of SeNPs harmful effects that would enable the benefit/risk balance control. Discussion and Conclusion SeNPs, as well as sodium selenite, exerted no damaging effect on normal cells, but they reduced significantly the cancerous cell viability. Taking into account our results, SeNPs could represent a promising potential adjuvant treatment for malignant disease.

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P-3a-3

Extending the boundaries of OECD DPRA test N°442C towards complex substances and mixtures: an innovative feat in HPLC-MS/MS and skin sensitization testing
ABSTRACT #121

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Skin sensitization is a key endpoint for cosmetic ingredients. Several in vitro alternatives to animal testing have been developed recently. In particular, OECD Test Guideline N°442C Direct Peptide Reactivity Assay (DPRA) assesses the first key event involved in skin sensitization, following the exposure to a test chemical. While this HPLC-UV based technique has a predictivity rate of approximatively 80% towards dermal sensitizers, DPRA still faces challenges relating to the accurate classification of certain chemicals and its inadaptability to complex mixtures. Specificity and selectivity are two major advantages for tandem mass spectrometry (MS/MS) as a detection method over UV, allowing HPLC-MS/MS based DPRA to overcome the hurdle of complex mixtures. We hereby present this adapted technique and demonstrate its compliance with respect to the OECD TG 442C requirements. The analysis methods for the detection of DPRA cysteine and lysine heptapeptides have been validated in a variety of solvents according to ICH guidelines. The subsequent proficiency study on ten reference substances provided results in total accordance with the prediction model of sensitizer and non-sensitizer classification for both peptides. 2019 saw this ground-breaking advancement in complex mixture characterization granted the GLP compliant status by the French Agency for the Safety of Drugs and Health Products (ANSM). Consequently, HPLC-MS/MS based DPRA opens new fields of application including but not limited to botanical extracts, fragrance compositions and complex ingredient assemblages.

P-3a-4

Target organs in cosmetics: which in vitro and in silico models to move towards to increase expertise in systemic toxicity?
ABSTRACT #333

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A major challenge in the safety evaluation of cosmetic ingredients includes oral repeated dose toxicity testing, which is intended to address the most complex human endpoints. Nine years after the full implementation of the animal testing ban for cosmetic ingredients in the EU, no alternative methods are available of fully replacing oral repeated dose toxicity testing. In the New Alternative Methods and Next Generation Risk Assessment race, one of the main purpose is to determine the fate of chemicals after they potentially reach the systemic circulation and hence to identify specific target organs in which toxicities could arise from a repeated exposure. In this context, it seems important for cosmetic industries to prioritize the development of alternative methods on specific organs that are mainly impacted and to develop promising tools for Generic Effects contextualization at the organ/tissue level. From the literature, and different tools such as Toxicity Reference Database (ToxRefDB), Cosmos, HESS-DB and internal legacy data, a meta-analysis has been performed using in vivo primary studies (subacute, chronic,…) as well as in vivo secondary studies (developmental and reproductive toxicity studies, Carcinogenicity and genotoxicity studies,…). The preliminary analysis demonstrates that the top three most targeted organs are the liver, the haematological/spleen system as well as kidneys although with markedly lower occurrence. Based on the toxic effect of cosmetics in animal studies, we were able to provide data on (i) the occurrence of target organs and their order of importance (ii) the impact of oral gavage versus oral diet (iii) the influence of cosmetic category on target organs (iv) the impact of in vivo study duration on the toxicity. These results could be used to oriented models' development from both in vitro and in silico tools to increase expertise in systemic toxicity and sustain decision making for safety evaluation of raw materials.

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P-3a-5

Safety of cosmetic perfumes screened in chemico and in vitro in combination with targeted chemical analysis ABSTRACT #58

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Background: Animal testing is prohibited in the EU for cosmetic ingredients and final products, thus only in vitro and in chemico bioassays and studies on human volunteers are available for safety assessment of cosmetics. Methods: 10 samples of commercially available deodorants, EDT and EDP were tested using a combination of bioassays suitable for detection of cytotoxicity, skin sensitization in chemico and in vitro, genotoxicity and endocrine activity. Results: Four samples exhibited the highest cytotoxicity. Skin sensitization potential was identified in chemico using DPRA method (OECD TG 442C) and in vitro using LuSens method (OECD TG 442D). Comet assay detected a concentration dependent DNA fragmentation. In vitro mammalian chromosome aberration test (OECD TG 473) did not detect any potential of genotoxicity. In YES/YAS assay (Xenometrix®), most samples anti-androgenic endocrine activity. 24 allergens (INCI: D-limonene, linalool, benzyl alcohol, citronellol, methyl 2-octynoate, geraniol, citral, hydroxycitronellal, cinnamal, anise alcohol, cinnamyl alcohol, eugenol, alpha-isomethyl ionone, isoeugenol, butylphenyl methylpropional, coumarin, farnesol, amyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxaldehyde, amylcinnamyl alcohol, hexyl cinnamal, benzyl benzoate, benzyl salicylate, benzyl cinnamate) were determined by GC/MS. NOAEL values of the finished products were estimated according to a published procedure

based on the content of the detected allergens. Conclusion: The NOAEL values of the finished products were estimated based on the NOAEL values of individual allergens detected in the products. In vitro/in chemico assays and targeted chemical analysis combined with safety evaluation based on the individual NOAEL values seems to be a promising approach for safety evaluation of mixtures, including cosmetics, for which animal testing ban should be applied. The work was supported from ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860) and MH CZ – DRO ("National Institute of Public Health – NIPH, IN:75010330").

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P-3a-7

A predictive 3D liver spheroid model for the assessment of liver repeated dose toxicity

ABSTRACT #308

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Background and objectives Concerns about long-term repeated-dose toxicity assessment have been raised since the ban on animal testing for cosmetic ingredients. Therefore, the development of new approaches methodologies that include in vitro models able to reproduce the physiological functionality of specific human organs has become essential and must continue to address current challenges for risk assessment purposes. Screening of legacy data showed that liver was the most targeted organ by cosmetic ingredients in 90-days repeated dose toxicity studies in vivo after oral exposure [1] Materials & Methods We developed a 3D-spheroid assay based on HepG2 cells that we manage to maintain for more than 28 days showing an increase of liver-specific markers expression and metabolic activities as compared to 2D cell culture. To optimize the exposure duration of the 3D-spheroid model to chemicals, the treatment protocol was refined to 4 daily repeated exposure, monitoring albumin secretion and cell viability after 96h. Training set for validation of the model was made of compounds known to be (31) or not (13) hepatotoxicant in vivo. Results Detailed analysis regarding different mechanisms of hepatotoxicity has been conducted, to evaluate the level of predictivity of the model for each of them. Overall predictivity was of 75.4%, with higher power (80%) for compounds inducing steatosis, but much lower (62%) for cholestasis. Efforts are underway to better predict cholestatic compounds, quantifying expression of specific genes being one of the tracks to follow [2]. Moreover, a statistical data processing refinement of albumin secretion and ATP quantification results is ongoing to better identify hepatotoxicants. Conclusion This cost-effective model is promising for further screening new cosmetic ingredients for long-term hepatotoxicity hazard characterization.

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P-3a-8

Developing an innervated skin model for predicting neuroinflammatory and neurosensory effect of cosmetic compounds

ABSTRACT #139

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Introduction: Stinging sensation involves two major cell types, keratinocytes and fibers of sensory neurons found in the epidermis of skin. Current tissue-engineered models lack any sensory neurons. In order to mimic the effect of 'stinging' compounds on skin. To aid this we developed a new human skin equivalent (HSE) using neuronal cells (SH-SY5Y) embedded into the fibroblast-populated dermis. Objectives: The objective of this study is to develop an innervated skin model that can define crosstalk between skin and neuronal cells to predict stinging and neurosensory effect of cosmetic compounds. Materials and Methods: Skin equivalents were generated with the addition of 3 x104 SH-SY5Y cells added to the collagen mixture at the same time as the human dermal fibroblast (HDF) Results: Tissue samples were prepared, sectioned and stained. Neuronal cells were observed in groups or bundles within the HDF-populated dermis. Skin physiology of innervated HSE was identical to ex vivo skin. We have applied Lactic acid and other surfactants that commonly exert stinging effect. Topical application of stinging compounds released neuropeptides like substance-P and CGRP in the culture medium of HSE. All the stinging compounds induced stress response and alarmin genes such as HSPA1A, IL-1α, IL1β in mRNA level after 24 hours. We have compared skin model with neuronal cells and without neuronal cells. Skin model comprising of neuronal cells tend to produce and secret higher neuroinflammatory and neurosensory genes. A subset of stinging genes reported earlier by our group have been used to validate using innervated HSE and compared to ex vivo skin collected from skin biopsies. No significant differences exist between our innervated HSE and ex vivo skin. Conclusion: This study developed an innervated skin model and an in vitro method with a panel of genes that can be used for detecting neuroinflammatory and neurosensory effect of cosmetic compounds.

P-3a-9

EFFECTS OF PER- AND POLYFLUOROALKYL SUBSTANCES IN HUMAN B AND T CELL LINES

ABSTRACT #408

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Background and Objectives: Exposure to PFASs has been linked to several adverse effects, including immunotoxicity. Reported immunotoxic effects of PFOA and PFOS include a decreased antibody response in experimental animals and humans, but so far little is known regarding underlying mechanisms and immunotoxicity data are only available for a limited number of PFASs. Materials and Methods: The in vitro immunotoxicity effects of four PFASs (PFOA, PFOS, PFNA, PFHxS) were studied in the human B lymphoma cell line Namalwa (genome-wide transcriptome analysis using RNA sequencing for PFOA and targeted RT-qPCR analysis for all PFASs) and in the human Jurkat T cell line (containing a reporter gene driven by the human IL-2 promotor). Results: In the Namalwa B cell line, transcriptome analysis revealed that PFOA affected various cellular processes, including 'B Cell Development' and 'Primary Immunodeficiency Signaling'. Interestingly, PFOA downregulated expression of recombinant activating gene 1 (RAG1) and RAG2. Subsequent qPCR analysis of Namalwa cells exposed to the four PFASs showed time- and concentration-dependent decrease in RAG1 and RAG2 expression. In Jurkat T cells, all four PFASs decreased IL-2 promotor-dependent reporter gene expression in a concentration-dependent fashion. Discussion and Conclusion: RAG1 and RAG2 have an essential role in V(D)J gene recombination, a process required to obtain a diverse repertoire of antibodies for antigen recognition. Therefore, the effects observed in Namalwa cells may point to a PFAS-induced impairment of generating a diverse set of B cells adequate for antigen recognition. The effects observed in the reporter T cell line may indicate a possible PFAS-induced decrease of T cell activation, which may play a role in the decreased T-cell dependent antibody response. Altogether, the present study provides mechanistic insights in the reported PFAS-induced decreased antibody response and the in vitro models presented here represent useful tools to screen other PFASs

for their immunotoxicity potential.

P-3a-10

IN VITRO 3D KIDNEY MODEL CO-CULTURED WITH HUMAN IMMUNE CELLS INVESTIGATING IMMUNE HYPERACTIVITY ASSOCIATED RENAL INJURY
ABSTRACT #260

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Background and Objectives: Much attention has been paid to establishing an in vitro model to elucidate interactions between immune cells and renal tissue.Despite of the previous achievements, majority of them still have relied on two-dimensional (2D) cultures, which are not sufficient to recapitulate human physiological conditions. Here, we formed 3D renal proximal tubules and co-cultured them with human immune cells (peripheral blood monocytes, PBMC) to investigate relationships between inflammatory reaction and renal damages in more details. Ultimate aim of this study is to provide an in vitro 3D kidney model for screening biopharmaceuticals with potential immune-associated renal toxicity. Material and Methods: 8x10^4 cells of RPTEC/TERT1 were encapsulated in Matrigel, then cultured on 24 well transwell insert (0.4 μm pore)for 16 days for 3D tubule formation. 5x10^4 cells of PBMC were added underneath of the insert for the co-culture system.For inducing immune hyperactivity in the system PBMC were treated with LPS (1 μg/mL) or polyI:C (20 μg/mL) for 24 hrs, respectively. Results: The formed 3D tubules showed cilium-like structures and increased expression level of kidney functional markers compared to 2D cultured cells.The drug induced immune hyperactivity on the system not only increased the inflammatory responses in the 3D tubules, but also caused notable down regulations on several proteins related to ciliary functions via progression of signaling pathways as “inflammatory response → programmed cell death → Hedgehog signaling → cilium development inhibition → Ciliopathies”. Discussion: To the best of our knowledge, this is the first 3D in vitro model describing how immune hyperactivity results in renal damage. This model suggests that overactivated human immunity possibly caused by biopharmaceuticals inhibits cilium development and results in kidney damage. Conclusion: The model is a promising tool to screen immune-associated renal impairment by biopharmaceuticals that potentially induce immune hyperactivity.

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P-3a-11

Establishment of a complex commensal 3-D skin model for toxicity testing
ABSTRACT #383

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The skin is our outer barrier and as such in direct contact with our environment. It also is subject to a diverse microbial colonization. Given the low availability of nutrients on the skin the use of xenobiotics can provide a selective advantage for the respective microbes1. Preliminary work at the German Federal Institute for Risk Assessment (BfR) has shown that microbial metabolism of polycyclic aromatic hydrocarbons is indeed a ubiquitous feature of the human skin microbiome. Using batch enrichment and skin swabs a total of 21 pure cultures were isolated, all of which use Benzo[a]pyrene (B[a]P) as their sole source of carbon and energy 2. Based on these results a microbially competent skin co-culture was established to study the respective microbial

B[a]P metabolism and toxification in vitro and to evaluate potential host effects and associated microbiome-host interactions in situ. This model consisted of MatTek's EpidermFT™ 3-D skin model colonized with B[a]P degraders M. luteus, P. oleovorans, or a mixture thereof. The model was shown to have stable colony counts allowing for a reliable study of the effects caused by the microbe on the host. Microbe-host interaction as such resulted in strong changes in the skin particularly in strengthening of the epidermal barrier with effects on B[a]P metabolite concentrations as well as on the formation of BPDE-DNA adducts3. We are now extending this co-culture in order to generate a model that better represents human skin. To this end we are creating a defined bacterial community from skin isolates that we use to colonize the models. This community consist of up to 8 different bacterial species co-occurring on skin. We also will use total skin swabs for comparison. The models will be used to determine the influence of the skin microbiome on the metabolism of different environmental chemicals such as pesticides.

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P-3a-12

EXPLORING CAUSAL RELATIONSHIPS BETWEEN PFAS EXPOSURE AND LIPID HOMEOSTASIS BY THE USE OF A HUMAN LIVER SPHEROID MODEL ABSTRACT #188

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Per- and polyfluoroalkyl substances (PFASs) are ubiquitous in our environment and several have long half-lives in humans. Two PFASs commonly found at high concentrations in humans are PFOS and PFOA. PFASs have been associated with immune modulation and increases in serum total cholesterol in humans. Both chronic low-grade inflammation and elevated serum cholesterol are established risk factors for cardiovascular disease (CVD). However, the association between PFAS exposure and CVD in humans is not clear and there is an uncertainty as to whether differences in cholesterol reabsorption rates may contribute to the PFAS-cholesterol association observed. The objective of the present study was to determine if PFOA and/or PFOS could have direct effects on inflammatory responses and lipid homeostasis, by the use of a human liver spheroid model. The model was selected as the liver plays a central role in lipid homeostasis. Liver spheroids (from InSphero AG, Switzerland) were used for these experiments. The spheroids consist of primary human hepatocytes from multiple donors, Kupffer cells and liver endothelial cells. The spheroids were exposed to human relevant concentrations of PFOA or PFOS for a period of 10-11 days. For analysis of potential modulation of inflammatory responses, the spheroids were also exposed to the immune stimulant LPS. Multiplex analysis of secreted inflammation markers and apolipoproteins were performed. In this study, we show that PFOA and/or PFOS exposure modulate expression of inflammation markers and affects the secretion of certain apolipoproteins, including the CVD risk factor ApoCIII. Furthermore, the identified markers in the spheroid study are currently being explored in a human biomonitoring study. This research was supported by the Norwegian Environment Agency (project no 18087021).

P-3a-13

In vitro study of biosafety of ZnO nanoparticles: coagulation assay and protein corona. ABSTRACT #151

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Background and Objectives: ZnO nanoparticles exhibit promising biomedical applications based on its biological activities (1). Nevertheless, when in contact with living organisms, nanoparticles interact with proteins, bind them to form the protein corona and their biological activity is modulated (2). In vitro methods offer the capacity to evaluate the potential hazards related to the administration of nanoparticles and understand the mechanism of adverse effects. In this sense, we have studied the influence of different particle size (micrometric, <50nm and <100 nm) of commercially available ZnO on the formation of protein corona and coagulation assay. Material and Methods: 1mg/ml of particles were incubated in the presence of albumin or fibrinogen (2h and 24h). After centrifugation, the amount of protein was determined by Bradford assay. For coagulations assay, particles were incubated in human fresh plasma during 30 minutes and posteriorly prothrombin and activated partial thromboplastin time were determined (3). Protein adsorption was quantitatively and qualitatively evaluated by Bradford assay, SDS-PAGE and TEM. Results: Our results showed that protein adsorption to ZnO particles surface increases with time especially in the case of fibrinogen, thus we hypothesised that potential alterations in coagulation process can succeed. A dose-response delay in plasma clotting in presence of ZnO particles for both assays and all materials was recorded. Protein determination, SDS-PAGE and MET showed higher protein adsorption for ZnO <50nm followed by <100nm. Finally, SDS-PAGE shows that fibrinogen adsorbs to ZnO particles with greater affinity than albumin. Discussion and Conclusion: Hypothesis that coagulation process can be modified by the presence of particles have been corroborated. Moreover, protein determination indicated that fibrinogen is part of the protein corona; however, further investigation should be performed. In conclusion, the methods used here to characterize ZnO nanoparticles demonstrated the importance of protein corona to explain the biological activity of nanoparticles.

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P-3a-14

Evaluation of the use of in vitro methods to study the general toxic effect of cosmetic products ABSTRACT #226

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In accordance with the requirements of CU Technical Regulation 009/2011 "On the safety of cosmetic products" in the Republic of Belarus, all cosmetic products must be evaluated for toxicological indicators (including general toxic effect). The gradual transition to non-animals testing is prescribed to assess the safety of cosmetic products. The aim of this work was to evaluate the effectiveness of in vitro methods for testing the safety of various types of cosmetic products. In the work, various types of cosmetic products (products on a liquid, solid, fat basis) were investigated. The application of three different methods for studying the general toxic effect was evaluated: MTT cytotoxicity assay on the culture of embryonic musculocutaneous fibroblasts (in accordance with ISO 10993-5), luminescent bacterial test (on the luminometer “Biotox-10”), assessment of mortality (mobility) of Tetrahymena pyriformis. It was shown that MTT cytotoxicity assay on a culture of embryonic musculocutaneous fibroblasts (exposure 4 and 24 hours) cannot be used to assess the safety of all types of cosmetic products. The reproducibility of the results for this test was 87%. The luminescent test is applicable to all

studied types of cosmetic products (relevant results were obtained). But the test had low reproducibility. The toxicity index values for cosmetic samples in repeated experiments may differ by more than 35%. The highest reproducibility (83%) and accuracy of the results (93%) in the study were in the lethality test (mobility) of Tetrahymena pyriformis (exposure 3 hours). It is shown that Tetrahymena pyriformis is an applicable test object for all types of cosmetic products. Thus, the lethality (mobility) assay of Tetrahymena pyriformis is a prognostically effective test model for evaluating the general toxic effect of various types of cosmetic products. The test can be used as an alternative method for assessing the safety of cosmetic products.

P-3a-15

LIVER METABOLOMICS IN VITRO– A MINIATURIZED SCREENING APPROACH TO PREDICT THE MODE OF ACTION OF LIVER TOXICANTS IN HEPG2 CELLS
ABSTRACT #364

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in vitro Metabolomics enables to identify and predict the mode of action (MoA) of liver toxicants in HepG2 cells. The previously used method at BASF [1], predictive for different MoA, was costly and time consuming hence not appropriate for screening purposes. The aim of the study was to miniaturize the method on a 96-well plate (µMIV). 7 substances (aroclor, pendimethalin, B-naphthoflavone, WY-14643, acifluorfen, bezafibrate, ketoconazole) known to cause liver toxicity through 3 different MoAs (Peroxisome proliferation (PP), liver enzyme induction (EIND) and liver enzyme inhibition (EINH)) were tested. The analytical method, cell seeding number, passage number and cytotoxicity testing were optimized. To define IC concentrations, the cytotoxicity assay was established by multiplexing ATP measurement and membrane integrity assessment. 15.000 HepG2 cells/well were cultivated in 96 well plates (TPP), the substances were added after 24 hours in 5 concentrations (IC1, IC5, IC15 IC50 IC85). 48 hours later, the assay was stopped by quenching with isopropanol 80% and freezing at -80°C. The plates were analyzed by LC-MS/MS for metabolic profiling. 243 unique metabolites were annotated. The PCA analysis of the metabolic profiles showed a clear clustering by the specific MoA including a dose response. A common impact in the lipid metabolism was shared by the 3 MoAs (general liver toxicity). A set of specific metabolite changes was identified to be specific for each MoA: PP showed a downregulation of fatty acid oxidation metabolites (FAOM), glycerolipids, sphingolipids and antioxidants and upregulation of phospholipids (PL) and triacylglycerol's (TAGs). EINDs were characterized by an upregulation of redox carriers (RC), Lysoglycerophospholipids and TAGs and downregulation of FAOM. EINHs exhibited a downregulation of amino acids and related metabolites, RC, acylglycerols, ceramides and cholesterol and upregulation of sphingolipids and FAOM. These data demonstrate successfully the applicability of the miniaturized method for investigation of MoA of liver toxicants.

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P-3a-16

IMPLEMENTING AN IN VITRO METABOLOMICS SCREENING METHOD TO STUDY LIVER TOXICANTS IN HEPG2 CELLS- A CASE STUDY WITH NITROFURANTOIN
ABSTRACT #365

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We have recently established a miniaturized liver metabolomics in vitro screening method (µMIV). The µMIV method differentiates between different MoAs and provides mechanistic information about the affected pathways in a fast, cost effective and high throughput manner. The 96- well plate system offers the advantage of simultaneously testing several concentrations and time points. The aim of this study was to test the model compound nitrofurantoin at five different concentrations (IC1, IC15, IC25 IC50 IC85) and four time points (3, 6, 24 and 48h) to evaluate the suitability of the µMIV method to generate a metabolomics-based dose and time response. For the dose selection, a pre-test was carried out by multiplexing ATP measurement and membrane integrity assessment. Based on the cytotoxicity results, dose response curves were built, and IC concentrations were derived. 15.000 HepG2 cells/well were cultivated in 96 well plates (TPP), the substances were added by reverse application after 24, 48, 66 and 69 hours post seeding in 5 concentrations (IC1, IC15, IC25 IC50 IC85). 72 hours later, the assay was stopped by quenching all the plates with isopropanol 80% and freezing at -80°C. The plates were analyzed by LC-MS/MS for metabolic profiling. 256 metabolites were measured together with the intracellular concentrations of the test substance and glutathione (GSH). The measurement of the intracellular concentrations confirmed the substance uptake into the cells and a concentration increase over time in a dose dependent manner. GSH levels were influenced by the time of exposure and nitrofurantoin concentrations were in line with previous reports. The PCA analysis of the metabolic profiles revealed a clear nitrofurantoin dose and time response. Metabolite changes were observed mainly in the energy metabolism, triacylglycerols and metabolites associated with the antioxidant response.Our µMIV approach allows a metabolome-based adverse outcome prediction including determination of Point of Departure and kinetic information.

P-3a-17

IMPACT OF BENZO[A]PYRENE ON MICRORNAS PROFILES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS AND THEIR DISCHARGED EXTRACELLULAR VESICLES ABSTRACT #331

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Background and objectives: Polycyclic Aromatic Hydrocarbons (PAHs) are major environmental pollutants with toxic effects on human health. The headmaster PAH, benzo[a]pyrene (B[a]P), not only presents carcinogenic effects, but also immunotoxic effects in humans. MicroRNAs (miRNAs), involved in major cellular processes have been recently linked to environmental pollutants. Lately, extracellular vesicles (EVs) and their miRNA cargo are suggested as novel sources of biomarkers for pollutant exposure. The present study aims to investigate the effect of B[a]P on peripheral blood mononuclear cells (PBMCs), known targets of PAHs, on their ability to modify miRNA expression in EVs and in their parent PBMCs. Material and methods: PBMCs were isolated from different blood donors and then exposed to 2 µM of B[a]P for 48 hours. EVs were isolated by ultracentrifugation and characterized. Small RNA extracted from PBMCs and their EVs were subjected to a high-throughput RNA sequencing analysis followed by an ontology study. Results: A principal component analysis on our RNA-seq data revealed clear separation between miRNA profiles in PBMCs and those in their secreted EVs after B[a]P exposure. Gene ontology analysis indicated that these miRNAs and their targeted genes participate in several cellular pathways, including cell death and survival, cellular movement and maintenance and potentially contribute to cancer development and immunological diseases. We picked the most highly expressed and regulated miRNAs for validation by RT-qPCR in both

PBMCs and their secreted EVs. Then, we characterized cell death and survival, the principal pathway regulated by miRNAs using gene and protein expression studies and hoescht and sytox staining in PBMCs. For the miRNAs identified in EVs, we looked for them in the circulating blood of rodents treated with B[a]P. Discussion and conclusion: This study indicate that miRNAs can serve as potential biomarkers for a better understanding of the mechanisms of diseases linked to environmental exposure.

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P-3a-18

Update and optimization of an adverse outcome pathway network of chemical-induced cholestasis
ABSTRACT #356

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Background and Objectives: Cholestasis denotes any situation of impaired bile secretion with concomitant accumulation of bile acids in the liver or in the blood circulation and may be induced by various chemicals. Our group previously introduced an adverse outcome pathway (AOP) network mechanistically describing key events (KEs) and their relationships driving chemical-induced cholestatic liver injury1. The aim of the present work was to update and optimize this AOP network in line with guidelines issued by the Organization for Economic Co-operation and Development (OECD)2. Material and methods: PubMed was queried for studies of chemical-induced cholestasis using a list of predefined key words and several known KE-related terms. SysRev, a newly developed computational tool for systematic reviewing and data extraction, was employed during the abstract screening and full-text screening. The tailored Bradford-Hill criteria, described by the OECD guidelines, were used in the weight-of-evidence assessment of the KEs and KE relationships. Results: A total of 6572 articles was retrieved from PubMed and uploaded to SysRev. An initial abstract-screening resulted in a total of 544 papers eligible for data extraction in the full-text screening process. Discussion and Conclusion: Extracted data are used for the assessment of already defined KEs and KE relationships, but also for the identification of potential new KEs, resulting in an updated AOP network on chemical-induced cholestatic liver injury. The fully assessed AOP network will serve as the conceptual basis for setting up an in vitro test battery to identify cholestatic chemicals, consisting of a series of assays that each monitor an individual KE.

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P-3a-19

An adverse outcome pathway network for liver steatosis induced by chemicals
ABSTRACT #350

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Background and Objectives: Adverse outcome pathways (AOP) are frameworks depicting existing information on causal linkages (i.e., key event relationships (KER)) between measurable biological changes (i.e., key events (KE)) leading to an adverse outcome (AO). To better represent complex interactions within organisms, different AOPs sharing one or more KEs are brought together in an “AOP network” (1). The aim of this research was to update the current AOP network on liver steatosis(2), with a focus on chemical-induced liver steatosis. Furthermore, to weigh the evidence between KEs, the updated AOP network was also assessed in accordance with the specific guidelines from the Organization for Economic Co-operation and Development. Material and Methods: PubMed was used to collect publications on chemical-induced liver steatosis published after 2016. The key search terms included steatosis, specific nuclear receptors as molecular initiating events of the AO as well as KE-associated keywords. A first title/abstract screening of all collected papers was performed with SysRev (i.e., a computational tool for systematic reviewing and data extraction) using a labelling strategy to include/exclude papers. With the application of a second labelling strategy, data regarding essentiality, biological plausibility/applicability and empirical support of KEs and KERs were manually extracted during full-text screening of included papers. Subsequently, data was used to assess the level of confidence in the updated AOP network on liver steatosis according to the tailored Bradford-Hill Criteria. Results: The PubMed search resulted in 12,478 papers. The title/abstract screening resulted in 1,626 papers eligible for data extraction in the full-text screening phase. Discussion and Conclusion: Extracted data was used to assess the level of confidence in previous described KEs and KER. In addition, data was used to identify potential novel KEs. The updated AOP network on liver steatosis will serve as a basis for the development of animal-free methods for toxicity testing purposes.

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Session: 3b. Organ-on-a-chip & Microphysiological Systems

P-3b-1

On the Real-Time Oxygen Consumption of Hepatocytes in a Microphysiological System
ABSTRACT #175

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Establishing methods for toxicity prediction of chemicals and drugs on organ-like structures is demanding and demonstrating their significance is a major challenge. An important strategy for the improvement of toxicity testing is based on the development of approaches that provide information on molecular mechanisms at the cellular level. Such information is crucial for a fundamental understanding of the effects of toxic compounds. However, from most methods currently available, detailed insights are difficult

to obtain as they rely on end-point measurements. This approach has considerable limitations, as it does not provide dynamic information as obtained by real-time measurements. Here we present our microphysiological system, which we have equipped with optical micro- or nanosensors for oxygen detection, to investigate the effects of different substances on hepatocytes viability in real time. This setup is a further development from a previous cooperation [1,2]. It allows long-term cultivation of cells, spheroids and organoids embedded in a 3D-matrix together with optical sensor particles under continuous medium perfusion. These micro- and nanoparticles contain chromophores whose phosphorescence lifetime depends on the local oxygen concentration. This allows the metabolic activity of cells in the system to be noninvasively monitored over weeks with high temporal resolution under near-physiological conditions [3].

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P-3b-2

PROPOSAL FOR A HUMAN FOETO-PLACENTAL ORGAN-A-CHIP FOR TESTING DEVELOPMENTAL TOXICITY ABSTRACT #340

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Developmental toxicity of chemicals causes high socio-economical and health concern. Developmental toxicity has to be tested in vivo using the teratogenicity assays in rats or rabbits by performing the OECD TG 414. The estimated number of animals required for a single test varies between 150 and 800; while the economic cost ranges between 63.100 and 92.500 € per test for rats and rabbits; respectively. In addition to the bioethical and economic issues raised by application of OECD TG 414 test on animals, there are also scientific concerns related to the extrapolation to humans of results obtained in animals. Relevant differences in the foetal-placenta system exist across species, in particular due to the role exerted by placenta, which acts as a barrier organ involved in biotransformation of chemicals, endocrine regulation and exchanging surface for nutrients and gases. We propose the development of an organ-a-chip platform based on human immortalised cells in which chemicals would interact first with placenta cells and downstream with foetal and amniotic cells. This system would mimic physiologically relevant conditions and would allow to assess the effects of chemicals, also at human relevant doses, with a number of read-outs as omics, microscopy, electrophysiology, biochemistry, etc. This platform would: i) reduce the economic costs of the current testing developmental toxicity; ii) overcome the scientific uncertainties of transferring observations from animals to humans; iii) potentiate the 3R paradigm by limiting animal experimentation as dictated by Directive 2010/63/EU; iv) support regulatory decisions based on grouping of chemicals or read-across; v) address gender-specific effects by using XX and XY cells; and, vi) help the development and implementation of Adverse Outcome Pathways.

P-3b-3

ALL-IN-ONE MICROFLUIDIC-BASED ROBOTIC PLATFORM FOR AUTOMATED TOX SCREENINGS IN C. ELEGANS. ABSTRACT #413

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Background and Objectives: The current alternative methods to animal testing are typically based on cellular models. Nematode Caenorhabditis elegans constitutes a valuable alternative in predictive toxicology studies, that can complement in vitro models to better predict the outcomes in mammals. This microscopic worm gained popularity due to its small size, short life cycle, ease of cultivation and propagation and a powerful genetic toolkit. However, the current methods for C. elegans experimentation often lack automation and standardization, representing the main limitation for their wider use in screenings. Material and Methods: We developed a microfluidic-based robotic platform that automates the entire process of culture, treatment, imaging and high-content phenotypic analysis of C. elegans, and executes different types of toxicology assays. The imaging potential of the platform is further extended by a possibility to acquire fluorescent pictures, allowing to benefit from the existing large collection of reporter strains. Results: We assessed the effects on reproduction and development of twenty benchmark chemicals, amongst which were bisphenol A, thalidomide, hydroxyurea, paraquat, busulfan, 5-fluorouracil. Synchronized populations of worms were chronically exposed to 5 doses of test compounds starting from the last larval stage (L4). The images of each worm were recorded every hour and time-resolved phenotypic readouts were then extracted from the collected images, including growth dynamics, sexual maturity, fertility, embryonic viability, progeny accumulation rate and survival rate. Out of the tested compounds methotrexate showed the most pronounced adverse effects on embryonic viability, while bisphenol A strongly impacted the development of the mothers. Discussion and conclusion: We propose an innovative solution for rapid identification of toxic compounds and their potential mechanism of toxicity, using a biological model that perfectly bridges the gap between in vitro and in vivo assays. Our technology allows not only to perform endpoint measurements, but to monitor the dynamics of biological responses.

P-3b-4

Mutagenicity and genotoxicity assessment of a new biopreservative product rich in Enterocin AS-48 ABSTRACT #171

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Background and Objectives: At present we are developing a biopreservative derived from the fermentation of a dairy byproduct, carried out by Enterococcus faecalis UGRA10 strain. This product possesses a strong and wide spectrum of antibacterial activity mainly due to the presence of Enterocin AS-48 in its composition. The present work aims to assess the safety of the dairy byproduct enterocin AS-48 as potential additive. Material and Methods: by meaning of the bacterial reverse-mutation assay in Salmonella typhimurium TA97A, TA98, TA100, TA102, TA1535 strains (Ames test, OECD 471) and the in vitro mammalian cell micronucleus (MN) test in the L5178Y Tk+/−cell line (MN test, OECD 476). After exposure to the byproduct (at a range of 6.75-100 µg/plate Enterocin AS-48 concentrations). Results: in the Ames test, with absence and presence of the S9 metabolic activation system, the results revealed that the product under assay was not mutagenic at the conditions tested. In the MN assay, the cell line L5178Y was seeded and exposed to different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 µg/µL of Enterocin AS-48) in the absence or presence of S9. Mytomicyn C (0.0625µg/mL) and cyclophosphamide (0.125 µg/mL) were

selected as positive genotoxicity controls, for the Ames and MN tests, respectively. In the same way, in the MN test, the lactic fermentation byproduct did not show genotoxic effects on L5178Y Tk+/− cells after 4 h (with S9) and 24 h (without S9) of exposure. Discussion and conclusion: These results point out the good safety profile of the product and support its use as additive regarding muta/genotoxicity. However, further studies are required before its commercial application. Acknowledgments: Spanish Ministry of Economy and Competitiveness for financing the project (RTC-2017-6199-2, MINECO / FEDER, UE). Antonio Cascajosa Lira thanks the Spanish Ministerio de Universidades for funding FPU grant (FPU2019-01247).

P-3b-5

A multiorgan-on chip platform for the in vitro investigation of off-target cardiotoxicity of liver-metabolized anticancer drugs
ABSTRACT #444

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Background and Objectives: Off-target cardiotoxicity is one of the main causes of drug withdrawal from the market. Multiorgan-on-chip (MOoC) platforms represent a disruptive solution to predict liver metabolism on off-target organs to ultimately improve drug safety testing during drug development [1]. Here we integrate liver and cardiac models in a compartmentalized valve-based MOoC and we show its application in studying the effects of liver-metabolized Terfenadine on cardiac microtissues. Material and Methods: The two-compartment MOoC integrates a liver micropatterned co-culture (MPCC) of HepG2 and fibroblasts [2] with a 3D mechanically stimulated cardiac microtissue generated from neonatal rat cardiomyocytes [3]. Communication between compartments is achieved through a system of normally-closed valves, whose aperture is controlled via an overlaying vacuum-activated control layer. Numerical and experimental simulations were conducted to evaluate the dynamics of drug diffusion across the compartments. Medium supplemented with 10μM Terfenadine was administered to the liver; once metabolized (after 24h), cardiac microtissue viability and functionality were assessed by means of an integrated electrical recording system upon valve aperture [3]. Results: The platform was validated for pharmacokinetic-based drug screenings by measuring the effect of the anticancer drug Terfenadine after metabolism by MPCCs in the liver compartment. While non-metabolized Terfenadine caused a significant decrease in cardiac cell viability and an increase in field potential duration (FDP), its non-toxic metabolite Fexofenadine did not cause any significant alteration in cardiac microtissues. Discussion and Conclusion: We developed a valve-based MOoC for liver-heart compartmentalized cultures. The system allows for controlled diffusion of liver-metabolized drugs (e.g., Terfenadine) to a cardiac compartment while excluding cell-cell interactions and eliminating convective transport, demonstrating the potential for studying drugs off-target cardiotoxicity upon liver metabolism.

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P-3b-6

3D human vasculature-on-a-chip: the biological effect of combustible cigarette smoke and vapor from three heated tobacco products on monocyte adhesion to vessels comprising coronary artery endothelial cells
ABSTRACT #261

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Background and Objectives: Heated tobacco products (HTPs) are potentially reduced-risk products because the aerosol contains fewer harmful constituents than cigarette smoke. Although research on the reduced-risk potential of HTPs has mainly focused on respiratory tract, the effect on cardiovascular system

should also be investigated. In this study, we used a human-mimic microfluidic vascular-on-a-chip model to assess the effect of HTP aerosol on monocyte adhesion, which is an early stage biological event of atherosclerosis. Material and Methods: Human primary coronary artery endothelial cells (HCAECs) were cultured on OrganoPlates®, a microfluidic tubular cell culture platform. Human monocytic leukemia cells (THP-1) were differentiated into macrophages by stimulating with 300 nM PMA for 48 h and then incubated in fresh medium for 16 h. Media containing particle phase extracts of a 1R6F reference cigarette smoke and three commercially available HTPs vapors, including our proprietary DT3.0a, were applied to the macrophages. After 1 h of exposure, the medium was refreshed and incubated for 3 h to prepare the conditioned medium, which was then applied to HCAEC tubules, and incubated for 24 h. To assess monocyte adhesion, fluorescent-labeled monocytic THP-1 was applied to the HCAEC tubules under flow conditions for 1 h and the number of THP-1 adhered to the endothelial surface was counted. The expression of intracellular adhesion molecules (ICAM)-1 was separately evaluated by immunostaining. Results: All HTP vapors showed a similar lower effect on the ICAM expression and monocyte adhesion than 1R6F cigarette smoke, even each HTP employs different heating mechanisms to generate the vapor. Discussion and Conclusion: The vapor of DT3.0a as well as other on-market HTPs are less pronounced in early stage biological events of atherosclerosis. However, because of the complex pathogenesis of atherosclerosis, biological assessment of later-stage atherosclerosis is necessary to better understand the reduced health risk potential of HTPs.

P-3b-7

What are the barriers to adoption of new approach methodologies for gastrointestinal toxicity testing? A systematic review of in vitro models of gastrointestinal toxicity
ABSTRACT #347

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Over the last decade, progress has been made in the development of microphysiological systems (MPS) for absorption, distribution, metabolism, and excretion (ADME) applications. Central to the assessment of new molecular entities (NMEs) for oral applications is the intestine that mediates drug absorption. However, there are limited preclinical methods used to predict absorptive properties of drug candidates available (Vancamelbeke and Vermeire, 2017). Moreover, the impact of adverse drug reactions within the GI tract (such as bleeding and diarrhea) is often not well addressed leading to the attrition of the drug during development or post-marketing surveillance. Here, we present a systematic review and assessment of the available approaches to study gastrointestinal toxicity (GIT) in vitro which provide evidence for scalability and applicability of MPS to drug discovery and development. This approach was selected to ensure that our findings are as objective and reproducible as possible in order to inform best practice for this seeking to validate novel MPS. The search strategy returned a total of 6,088 results and secondary sources contributed another 387 articles. After removal of duplicates (n=99), 6,376 articles abstracts were screened of which 40 were included for final analysis. A previously published study quality checklist, (Irvine et al., 2021) composed of key insights from regulatory and industrial stakeholders, was utilized to assess MPS models meeting the inclusion criteria. The highly diverse range of cell lines and modelling approaches generated prevented a statistical comparison through a meta-analysis. It was observed that more complex models (n= 11) scored better against traditional 2D cell culture models (n=23) highlighting the potential of such models to predict GIT. In the final analysis, we distilled the key outcomes and physiological features required to qualify a novel MPS seeking to address patient variability, mimicking the microphysiology of the gut, and accurately predicting drug-induced GIT.

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821-834.

P-3b-8

A systematic review of in vitro models of drug-induced liver injury ABSTRACT #348

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Drug induced liver injury (DILI) is the leading cause of drug withdrawals from the market and is a major strain on R&D resources. Therefore there is a high demand for more human-relevant, high throughput solutions. Coordination by bodies such as the OECD and regulators to stimulate approaches that adhere to the 3R's (reduce, refine, and replace) has motivated development of new human-relevant in vitro solutions. Yet, no systematic overview and evaluation of such new approach methodologies (NAMs) exists, which hampers their pre-clinical adoption. Here we aim to close this gap by comparing, cataloguing and evaluating the many different in vitro solutions to predict DILI. We prespecified a protocol as per a standard Cochrane review of medical interventions, including search strategies for Pubmed and Embase databases, exclusion and inclusion criteria and methods for meta- and qualitative analyses. Our search strategy returned 5279 articles, of which abstracts were independently screened by two researchers. We are currently resolving conflicting decisions and expect close to 300 abstracts to meet the inclusion criteria for full text analysis. Full text analysis and data extraction will be followed by meta-data analysis and qualitative evaluation where applicable. Initial impressions indicate that selected studies can be divided into simplistic and complex study groups. The former will likely include studies employing one cell type and read-out only, such as acetaminophen induced liver injury through HepG2 viability. In contrast complex studies will likely include multi-cellular systems in combination with data on adverse outcome pathways. Qualitative and meta-analyses will provide an overview of high potential NAMs and shortcomings in current study designs. Finally, we will propose necessary changes in study designs to allow pre-clinical adoption of new in vitro solutions to predict DILI in the future.

P-3b-9

USE OF MICROFLUIDIC CHAMBERS IN ENVIRONMENTAL TOXICITY STUDIES USING FISH CELLS AS A MODEL ABSTRACT #410

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It cannot be denied that there has been an increase on the production and use of chemical compounds, such as pesticides, drugs or metals, in the past decades. This has led to the exposure of aquatic organisms to a wide variety of chemicals and therefore the need to investigate and understand their toxicity. Even though there are some guidelines to study fish toxicity (1), almost all of them still require in vivo methodologies. Lately, the interest to develop in vitro alternatives to avoid animal testing has increased. One example is the development of microfluidic chambers as they provide an environment that can be controlled, the reagent consumption is low and can be automated (2, 3). This work focuses on the attempt to seed zebrafish liver cells (ZFL cells) into PDMS microfluidic devices as a model to assess potential fish toxicity. To do that, the first step was to evaluate the cell viability in the device at different time points using a live-dead staining (Calcein-AM, Ethidium homodimer-1). Cells can grow in the devices for up to 7-days (high viability) allowing the performance of long (chronic) exposure experiments. High mortality was detected 12-days after seeding. The generation of oxidative stress by model chemicals was assessed using CellROX Deep

Red dye, and the induction of cyp1a using 7-ethoxyresorufin as substrate. Overall, ZFL cells showed high attachment and viability in the microfluidic devices for at least 7 days after seeding, and promising results were obtained to support the automation and application of the devices as an alternative method in environmental toxicity studies.

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P-3b-10

SOLUBLE FACTORS ARE INVOLVED IN THE PROPAGATION OF LIVER FIBROSIS IN VITRO
ABSTRACT #339

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Background: The development of in vitro models of the liver able to recapitulate key features of hepatic diseases has made great progress in recent years. However, it is still difficult to transfer the in vitro data to humans. Here, we aim to develop a suitable human model that can accurately reproduce the multicellular processes leading to liver fibrosis. The model is based on the three major cellular players in liver fibrosis: hepatocytes, Kupffer cells and hepatic stellate cells, identified as drivers in the liver fibrosis adverse outcome pathway (AOP). We aim to investigate if solely soluble factors or direct cell-cell contacts are involved in promoting the sequence of events that ultimately lead to liver fibrosis. Methods: Human cell lines representing hepatocytes (HepaRG), Kupffer cells (THP-1) and hepatic stellate cells (hTERT-HSC) were cultured in a contact-free co-culture in a specially designed chip. The cells were treated with profibrotic compounds such as transforming growth factor β1 (TGF-β1) or methotrexate (MTX) or the acute hepatotoxicant acetaminophen (APAP) for seven days. The response of the different cell types was evaluated by measuring viability (ATP content), albumin production, total glutathione, and expression of profibrotic markers (e.g. αSMA). Results: Albumin expression decreased in HepaRG exposed to APAP and TGF-β1, while ATP content and total glutathione was reduced by all compounds. Increased αSMA deposition in TGF-β1- or MTX-treated cells indicated activation of hTERT-HSC, a hallmark of fibrosis. In contrast, hTERT-HSC exposed separately to MTX were not directly activated by the compound. Conclusion: Our preliminary experiments demonstrate that cell-cell communication mediated by soluble factors released probably by the HepaRG cells can trigger activation of hepatic stellate cells that is not elicited directly by the compounds. Identification and quantification of the responsible factors will be key for a more quantitative approach for the study of AOPs in vitro.

P-3b-11

A microfluidic-based in vitro reconstruction of synaptic circuits as an alternative model for pharmacologic research
ABSTRACT #493

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Biomedical research and scientific authorities are currently placing rising pressure to Reduce, Refine and Replace (3R) the use of animals for research and validation processes. In order to improve the ethical landscape of preclinical research while maintaining the quality of scientific data, new technologies are required to model physiologic and pathologic environments. In vitro alternative systems have evolved over years and are in continuous development since they allow quick and high throughput drug screening to investigate the efficacy and toxicology of different compounds, before moving to in vivo applications. In this context, we set-up a brain-on-chip, an in vitro microfluidic device that combines different human-derived cell types simultaneously, to recreate brain circuits on a dish with a more physiological arrangement. The new platform is intended to generate a more reliable human-related model using neurons induced from human pluripotent stem cells (hPSCs). This system integrates multiple interacting human-derived cell types, improving the pertinence and validity of drug screening or toxicity assays. To generate our brain-on-chip we established robust protocols to differentiate pluripotent and somatic human stem cells into mature neurons from different brain regions to be integrated, onto high-quality standard operating procedures (SOP), complying with the guidelines set by UNE-EN-ISO 9001. As part of Avantdrug, the platform created with the Production and Validation Center for Advanced Therapies (Creatio) and the Research Centre on Toxicology (CERETOX) through the Unit of Experimental Toxicology and Ecotoxicology (UTOX), we aim to get ongoing improvements and use our device to offer trials based on in vitro differentiated human neuron systems The prospect of recreating synaptic connections and testing multiple molecular compounds with no need for animal use is very promising for studying neuronal interactions, screening drugs, and their secondary effects. Overall, it represents the new generation of models that is pushing forward pharmacologic research for humans.

P-3b-12

A microfluidic, patient-derived tumor-on chip platform for therapeutic efficacy and safety evaluation of CAR-T cell products

ABSTRACT #496

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CAR-T-cell immunotherapies in the individualized treatment of patients with solid tumors are not very effective to date. Despite improvements in toxicity management, there is still a lack of understanding of the mode of action and side effects. To overcome these obstacles of cell-based cancer immunotherapies, we need physiologically relevant test systems that can mimic the patient's tumor and complex human processes

including vascularization outside the human body. For this aim, we established a patient-derived tumor-on-chip-system consisting of patient-derived microtumors (PDM) integrated into a microfluidic platform to enable assessment of personalized CAR-T cell product efficacy and safety. Here, PDM are isolated from primary tumors (e.g. cholangiocarcinoma, breast-carcinoma) using limited enzymatic tissue digestion and subsequent culture in serum-free media. We have shown that PDM display a heterogeneous cellular composition and complexity by means of extracellular matrix and tumor microenvironmental components similar to the corresponding patient tumor tissue. Here, PDM were embedded in a customized microfluidic chip within a Dextran-Hydrogel. To create a physical barrier for CAR-T cells migrating towards PDM, the perfusion channel was covered with human primary microvascular endothelial cells (mvECs). We established a reproducible protocol for injection of PDM and mvECs, which enabled the on-chip-culture and perfusion with stable viability for > 15 days. Following perfusion of these chips with CD276-targeting CAR-T-cells the therapeutic efficacy was assessed by microscopy and LDH-release cytotoxicity assay. Efficient CAR-T-cell products were identified by a significant increase in cytotoxic microtumor killing. Furthermore, CAR-T cell activation in result of target antigen recognition during co-culture was monitored by quantification of IFN-gamma from chip-perfused media. Integrating PDM as tumor model into a customized microfluidic chip system equipped with an endothelial cell barrier and vasculature-like perfusion enables the study of immune-cell-cancer interaction and provides a new platform for preclinical efficacy and safety assessment of cell-based cancer immunotherapy products.

Session: 4a. Computational toxicology – in silico modelling, read-across, artificial intelligence and machine learning

P-4a-1

Adverse Outcome Network For Obesity Initiated By Endocrine Disruptors developed using the AOP-helpFinder tool
ABSTRACT #16

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Background and objectives: Adverse outcome pathways (AOP) are a conceptual framework that support the use of alternative toxicological approaches in risk assessment. To help the development of AOPs and AONs (AOP networks), which are a combination of several AOPs sharing at least one biological event, we have developed a tool named AOP-helpFinder. AOP-helpFinder is based on artificial intelligence to systematically and rapidly explore all available abstracts stored in the PubMed database. It identifies and extract known and dispersed associations between stressors of interest and KEs, therefore supporting the development of AOPs and AONs. Material and Methods: In the context of the H2020 OBERON project, we investigated the applicability of using the AOP-helpFinder tool to develop an AON related to obesity, adverse health outcome known to be associated to endocrine disruptors (EDs). Co-occurrence between EDs and biological events (from the AOP-Wiki database and from in house experts to complete and refined precise cellular targets) were automatically screened against abstracts from the PubMed database. Results: A total of more than 15000 articles mentioning at least one of the 12 studied EDs was selected. The text mining allowed to automatically screened these articles against more than 1600 events related to metabolism. Then a manual curation by experts was performed to refine the identified knowledge, and to select the most relevant publications. All information for liver, adipose tissue and pancreas were kept and integrated to establish an AON for obesity based on scientific evidence. Discussion and conclusion: The presented text-mining approach for AON development, illustrated with obesity, can assist research community in gathering existing information, assessing weight of evidence, and also in identifying existing gaps regarding the modes of action of chemical substances. It can accelerate and improve ED testing and, consequently, support future regulatory recommendations in decision making process.

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P-4a-2

Development of a easy-to-use, semantic technology-based knowledgebase containing toxicological information of cosmetic ingredients to assist animal-free risk assessment

ABSTRACT #330

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There are no validated animal-free replacement methods to assess repeated dose toxicity. This poses problems for developing new compounds across various sectors, mainly cosmetics, where animal testing is banned in the EU. Since biological responses in animals cannot be reflected using single non-animal methods, it is necessary to combine these into Integrated Approaches to Testing and Assessment (IATA) relying on an integrated analysis of existing information coupled with the generation of new information using non-testing (grouping, read-across) and testing (in vitro) methods. This study aims to develop an easy-to-use, semantic technology-based knowledgebase (KB) in which existing safety data of cosmetic ingredients is gathered and maintained assisting non-animal systemic toxicity assessment. First data set to create the KB, 93 opinions (pdf) issued by the Scientific Committee on Consumer Safety (SCCS) between 2008-2019 dealing with 88 ingredients are processed and the information is stored in Excel files. These are converted automatically into RDF (Resource Description Framework) format. For the data scheme of the KB, officially adopted OECD testing guidelines have been profiled for acute, repeated dose toxicity, and toxicokinetics & skin absorption endpoints. SMILES is included for machine-readable purposes and Klimisch scores evaluating the reliability of the studies. When needed, expert opinions are consulted to ensure the accuracy of the observed effects in laboratory animals. To date, 76 of the 88 cosmetic ingredients, have been processed into Excel files. The results are used to improve the structure of the KB and the further automatic conversion of the remaining ≈520 opinions (1998-2021) to complete the KB. The KB is developed to facilitate the search for existing information on cosmetic ingredients, including, e.g., in vivo indications of organ and systemic toxicity. When completed, it will serve for setting up hypothesis-driven case studies on the use of IATAs for systemic toxicity assessment of cosmetic ingredients.

P-4a-3

Three steps to select analogues for skin sensitization prediction using read-across: an exemplary case study with vanillin

ABSTRACT #305

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The Next Generation Risk Assessment (NGRA) framework for the evaluation of the skin sensitization potential of ingredients uses an exposure-led weight of evidence (WoE) approach which includes new approach methodologies (NAMs) and the application of read-across for hazard characterization. As previously illustrated, read-across may be a key component to increase confidence in the NGRA for skin sensitization [1]. However, it requires an

explicit description of the analogue identification process as well as a transparent justification of the final analogue selection. This study illustrates the 3-step process underlying the selection of analogues for the read-across approach to support the skin sensitization potential prediction of vanillin: Step1: A search was performed using the alerts identified for vanillin in structure-activity relationship (SAR) mechanistic profilers related to skin sensitization combined with common structural features, and search for skin sensitization data. For vanillin, 7 possible structural analogues with skin sensitization data were retrieved. However, the analogues revealed large inconsistencies in the skin sensitization profiles ranging from strong, to non-sensitizer. The aromatic aldehyde alert observed for vanillin and all possible analogues is characterized by a variety of skin sensitization outcome across the chemical class. Therefore, all chemicals are kept despite the shortcomings observed in the skin sensitization data for the two strongest sensitizers. Step2: The profiling was extended using predictions of physicochemical properties, similarity score and simulated autoxidation/skin metabolism, but failed in refining the selection of the analogues. Step 3: The analogue selection process can be completed either by application of the most conservative approach, i.e., selection of the strongest skin sensitizer chemicals, or by exclusion of chemicals with limitations in the skin sensitization studies or by integration of in vitro data strengthening the chemical grouping. This 3-step process enabled the identification of appropriate analogues as basis for an acceptable NGRA conclusion of vanillin.

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P-4a-4

Elucidating the Inhibition Mechanism of six FDA-Approved Drugs on P-glycoprotein (P-gp) Transporter by Molecular Docking Simulation

ABSTRACT #368

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Background: P-glycoprotein (MDR1) is an efflux transporter that restricts the distribution of its substrates into organs, and it eliminates its substrates from excretory organs, mediating both biliary and renal excretion, and occasionally direct gut secretion. Impaired P-gp activity due to action of drugs can lead to the Drug-Induced Liver Injury (DILI)[1-2]. Therefore, it is always recommended to understand the interaction of the drugs with the P-gp. Objectives and Method: However, the mechanistic pathway of inhibition of P-gp is not clear to date, therefore in this study, we aim to look mechanistic insights into the inhibitory effects of six P-gp inhibitors using the molecular docking approach. Results: Our molecular docking results revealed that Elacridar (IC50: 0.05µM, -10.70 kcal/mol, no. of interactions: 11, Figure 1 (A)) and Zosuquidar (IC50: 0.18µM, -10.50 kcal/mol, no. of interactions: 11, Figure 1(B)), with low IC50, had greater affinities. While Quinidine (IC50: 51.3µM, -8.40 kcal/mol, no. of interactions: 7, Figure 1(C)), with high IC50, had the lower affinity for P-gp [3]. Discussion and Conclusion: Our in silico approach furnishes a deep understanding of the interaction of inhibitor compounds with the P-gp protein. Therefore, our in silico approach can perhaps be helpful in designing in vitro clinical trials using P-gp protein in order to estimate minutely the contribution of inhibitor compounds in hepatotoxicity.

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P-4a-5

ORBITOX – A COMPUTATIONAL TRANSLATIONAL DISCOVERY PLATFORM FOR DATA MINING AND READ-ACROSS
ABSTRACT #380

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Background/Objective: As the reliance on traditional animal testing is phasing out, new approach methodologies generate massive amount of detailed data which are often difficult to utilize efficiently. Using novel data clustering, machine learning and visualization techniques, we have developed an interactive 3D environment which houses millions of scientific data points connected per known or predicted relationships among them, thus enabling easy access and toxicologically relevant knowledge extraction all in one intuitive user interface. Methods: OrbiTox houses curated data from 900,000 substances, 22,000 human targets, 1,500 biological pathways, and over 100 test organisms. Data objects (chemicals, genes, pathways, organisms) are organized in concentric 3D globes. Experimental toxicity data are stored as 400,000 connections that represent human and rodent carcinogenicity, bacterial mutagenicity, acute toxicity, and various bioactivities. OrbiTox also houses a panel of QSAR models for over 40 Tox21 assays and bacterial mutagenicity tests in various strains and conditions. Results: The connectivity among data in OrbiTox allows querying from any domain. For any given chemical query, OrbiTox identifies chemistry-aware similar chemicals using our recently published Saagar substructures (doi:10.1021/acs.chemrestox.0c00464). In one test study, we were able to query the fructose metabolism pathway, identify member genes of that pathway and identify inhibitors of a member gene AKR1B1 with their IC50 values – all in one view. The complete path-mapping functionality of OrbiTox is unique in helping toxicologists automatically map several search queries (chemical substances, gene/protein names) via existing experimental connections or predicted relationships (QSAR models, chemical/biological similarities). Such a suggested path can be viewed as a hypothesis for functional or mechanistic relationship between searched entities. The technology underlying OrbiTox allows real-time interaction with the data and is readily scalable to over 2 million data points. To our knowledge, the technology and functionality of OrbiTox is not available in any other existing data graph-visualizing frameworks.

References
NONE

P-4a-6

Use of text-mining and chemoinformatics as additional decision-making tools for the chemical selection process of the EU-NETVAL thyroid validation study
ABSTRACT #199

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The European Commission Joint Research Centre's European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) has launched a validation study to assess 17 mechanistic methods for the detection of chemicals disrupting thyroid function. Central to the validation exercise is the selection of the chemical validation set. It is common practice in the field (Pazos et al., 2010) to define chemical validation sets based on expert judgement and availability of reference data. For this reason, an expert meeting was organised in November 2019 to propose a potential validation set of chemicals (OECD 2018) with known activity in at least one of the methods/mechanisms of action. The expert group proposed a list of 51 chemicals that needs to be reduced to a final set of 30 chemicals. This reduction was informed by the use of chemoinformatics tools and Artificial Intelligence based (AI) tools, i.e. machine learning and text analytics. Chemoinformatics was used to determine the chemical space covered by the selected chemicals, compare them to a space covered by different types of chemicals such as REACH registered substances, approved drugs or pesticides, and select those chemicals that expanded the space and were not redundant. In parallel, text analytics and machine learning were used to assign each chemical to a set of groups (i.e. annotate), which were defined solely from the text of the publications that were used by the experts to show the mechanism of action of each chemical. The use of these available tools complementing knowledge received from experts with additional chemical space, allows more solid and less human-biased decision-making in the selection of a validation set of 30 chemicals aimed to assess the relevance of the suit of mechanistic methods part of the validation study.

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P-4a-7

IMPLEMENTATION OF IN SILICO-BASED READ-ACROSS ASSESSMENT FOR GENOTOXICITY FOR PESTICIDE RESIDUES UNDER EU LEGISLATION AND KEY CHALLENGES ABSTRACT #21

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In accordance with Regulation (EC) No 1107/2009 and Commission Regulation (EU) No 283/2013, the establishment of residue definitions for dietary risk assessment of pesticide residues requires scientifically robust toxicological assessment of all identified metabolites in plants, livestock and groundwater. This poster focuses on our internal procedure for the selection of metabolites that require toxicological assessment and the methodology for in silico-based genotoxicity screening of metabolites with case studies. Genotoxicity is the first stage in the toxicological assessment of metabolites as set out in the EFSA guidance document [1]. Metabolites are grouped using a combination of in silico genotoxicity predictions (Leadscope (a statistical-based model; [2]) and Derek Nexus (a knowledge-based model; [3])), read-across (using relevant profilers in the OECD QSAR Toolbox [4]) and expert analysis. Grouping can be further refined using structural similarity, ADME information and other toxicological profiles for general toxicity. Existing experimental data or genotoxicity conclusions for the parent and any metabolites can be used to conclude on genotoxicity for other metabolites within the same group. If a genotoxicity conclusion cannot be made for a group of metabolites using an in silico approach and the TTC is exceeded, a representative metabolite within the group is selected for testing. If a metabolite is present in rat urine (or urine plus bile, or plasma) at levels above 10% of the administered or absorbed dose, then the toxicity of the metabolite is considered to be covered by the toxicity data for parent and a separate genotoxicity assessment is not required. Metabolites that occur naturally can generally also be excluded from the

toxicological assessment, however consideration should be given to the concentration of metabolites arising from use of a pesticide compared to the levels occurring naturally in food and feedstuffs, as these metabolites may still attract regulatory scrutiny if present at significant levels.

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P-4a-10

Combining gene expressions and imaged-based morphological features for chemical-phenotype profiles ABSTRACT #281

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Objectives: As part of the RISK-HUNT3R project and based on a previous study we performed [1], a computational study was intended to integrate molecules that induced transcriptomic perturbations and cellular morphological changes into a biological network in order to assess chemical-phenotypic relationships. Such network was enriched with existing disease information in an effort to suggest molecular and cellular mechanisms of action leading to diseases. Material and Methods: Two datasets were used for this study. Firstly, we used the “Cell Painting morphological profiling assay” dataset, composed of 30,000 compounds tested on osteosarcoma cells (U-2OS)[2]. Secondly, we used the “L1000 mRNA profiling assay” (LINCS) dataset [3], a collection of transcriptional expression data from cultured human cells treated with approximately 20,000 bioactive small molecules. Furthermore, pathways, GO terms and disease enrichment were performed on the transcriptomics data. Results: Our study allowed to develop a biological network combining chemical-genes-pathways-morphological perturbations and disease relationships. It contains an ensemble of 9,989 chemicals, 732 significant morphological features that concern 3 cells compartment: cytoplasm (257), nucleus (229) and the cell itself (246); 12,328 highly deregulated genes by at least one compound. The network is enriched with 1185 GO terms, 323 KEGG pathways and 855 Disease Ontology terms. As an example, our network was used with a set of 287 Drug-Induced Liver Injury. We found that some sets of drugs shared similar genes and morphological perturbation which suggest possible links between molecular and cellular perturbations. Discussion and Conclusion: Although the study is based on the assumption that the cellular behavior in presence of a chemical is similar independently of the cell type, some extrapolation about genes-cellular features and diseases relationships can be performed. Our network could be enriched with other types of phenotypic screening, transcriptomic information, based for example on the RNAseq technologies, and chemical-disease or toxicity annotations.

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P-4a-12

CONTRIBUTION MAPPING: USING STRUCTURE–TOXICITY RELATIONSHIPS (STR) AND MECHANISTIC INTERPRETABILITY OF IN SILICO MODELS TO ASSESS DEVELOPMENTAL TOXICITY AND ENDOCRINE-DISRUPTING POTENTIAL OF TWELVE UV FILTERS
ABSTRACT #220

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In the last years, the safety of some UV filters has been questioned about association with percutaneous permeation into the circulatory system that could lead to hormone diseases. Recently European Commission has opened a call to provide information on endocrine-disrupting (ED) potential of some cosmetic ingredients, aiming a higher level of protection from hazardous chemicals. Predicting ED of compounds is a complex task and should consider 14 different human nuclear receptors that regulate reproduction, behavior, development, metabolism and immune system. One of the challenges on the safety assessment is to develop predictive and unambiguous approaches with mechanistic interpretability. In silico methods have been proposed as key components of a future testing paradigm, providing mechanistic domains for both tested and non-tested molecules. This study aimed to assess the ED potential of twelve UV filters, identifying compounds potentially active (binders, agonists, and antagonists) to in vitro estrogen receptors (ER) and in vivo developmental toxicity. Ingredients were assessed by the tool DevTox-iS, using five Quantitative structure-toxicity relationship (QSTR) models developed to identify potentially active binders, agonists, and antagonists to in vitro ER and in vivo toxicants for the development of mammals. For each model, we explored the contribution mapping with Structure–Toxicity relationships (STR), trying to check fragments that could decrease toxicity/activity (-) (red) or increase toxicity/activity (+) (green), resulting in hypotheses and mechanistic interpretations (OECD Principle 5). Considering the animal testing ban under the Cosmetic Regulation, this study allowed to integrate QSAR docking and computational system biology tools to predict suspects of ED activity as an approach for evaluation to cosmetic ingredients. Integrated in silico strategy with STR mapping can drive the safety assessment, not only eliminating potentially toxic ingredients, but providing mechanistically targeted results for weight of evidence (WoE) of experimental data and predictions in the safety assessment of cosmetic ingredients.

P-4a-13

Physiological map to study kidney toxicity in the ONTOX project
ABSTRACT #428

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Background and Objectives Continuous improvements of computational approaches also increase the predictive performances of toxicological in silico models [1]. However, being mainly based on animal test data, these computational models lack a good correlation with human toxicity, and, being often based uniquely on chemical structures, they are unable to explain toxicological processes. To overcome these limitations, we have developed a new semi-automated strategy to build a Physiological Map (PM), a framework to study human toxicological mechanisms. Materials and Methods Our method is useful to build a PM or to validate an existing PM. To retrieve information, a manual literature review was accompanied by computational interrogation of ontologies (e.g. Gene Ontology), thus creating a network of genes, proteins, molecules and phenotypes [2]. The network was converted

manually into a PM using the CellDesigner software and visualized on the web using the MINERVA platform. The entire procedure was supported and revised by field experts. Results We present here the human kidney PM, developed in the framework of ONTOX, a European project aimed at improving risk assessment avoiding the use of animal tests [3]. With the purpose to better understand tubular necrosis and nephrolithiasis, the PM represents the normal physiology in proximal tubule, the loop of Henle, distal tubule, and collecting duct cells, displaying the vitamin D metabolism and the urine production processes: filtration, reabsorption and secretion. Discussion and Conclusions Our method assists the user to build a PM even starting from limited data. The PM is initially a static representation of physiological processes, also useful to study and develop new adverse outcome pathways. Subsequently, we could add kinetic parameters, transforming the PM into a dynamic model able to represent cellular perturbations. This approach presents an opportunity to investigate human toxicities, improving the toxicological predictions from a qualitative and quantitative perspective.

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P-4a-14

QUANTITATIVE ADVERSE OUTCOME PATHWAY MODELING FOR CHRONIC TOXICITY
ABSTRACT #255

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Background and objectives Quantitative adverse outcome pathway (qAOP) – a mathematical representation of an AOP — can be used for risk assessment. Problematically, only a limited number of qAOPs are devoted to chronic toxicity. To address this gap, we developed a hypothetical qAOP for chronic toxicity using Bayesian network (BN) formalism. Our method is flexible, can be used to estimate probability of an adverse outcome and can be generalized to other scenarios. Methods We built a hypothetical AOP with four acute phase MIE and KEs, five chronic phase KEs, and an AO (biomarkers). Accounting for inter-donor difference in primary cells, we generated a primary dataset of each biomarker for a total of eight donors, multiple exposure repetition and dosing. We resampled the primary dataset to build a replicate level virtual dataset. The virtual dataset was then subjected to a dynamic BN modeling for the quantitative risk assessment. We further explored data-driven AOP restructuring using lasso regularization. Results Using our model, we estimated probability of AO based on activation of upstream events at a previous exposure. We found that the strength of interaction changed over time, especially between acute and chronic phase KEs. Our analysis further revealed that some acute-phase KEs were not predictive of AO, suggesting that our model was able to capture when the response transitioned from an acute to a chronic phase. Discussion and conclusion In sum, we developed a BN-based qAOP framework that can be used for risk assessment. Although we used virtual dataset, we believe that this methodology can be applied directly to real data including data from in vitro NAMs. Our results show that cumulative effects from repeated exposures are important and structure of an AOP is dynamic. This formalism can be used retrospectively to gain mechanistic insight.

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Manuscript in preparation

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Applying machine-learning approaches to identify key genes associated with drug-induced cholestasis
ABSTRACT #349

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Background and Objectives: Drug-induced cholestasis (DIC) is one of the most severe manifestations of adverse drug reactions, constituting a major subgroup (up to 50%) of total drug-induced liver injury (DILI) cases 1. Due to its complex process, early detection of DIC during drug development remains challenging. Preclinical animal studies, a standard model in drug safety evaluation, often fail to detect DIC in humans mainly due to interspecies differences 2. Recently, toxicogenomics in vitro assays, especially based on human liver cells, have become a more convenient and practical approach for the prediction of human-relevant DILI. Over the past decade, the established large-scale databases, combined with machine-learning (ML) approaches, give us the opportunity to identify transcriptome signatures of DILI. In the present study, we leveraged the publicly available database, Open TG-GATEs3, for the identification of transcriptomic signatures of DIC. Material and Methods: We retrieved toxicogenomics data derived from in vitro cultured primary human and rat hepatocytes following exposure to 18 compounds (9 cholestatic compounds and 9 non-cholestatic compounds). These transcriptome profiles were measured at two time points (8 and 24h) following a single exposure to a given compound at three dosages (control, middle and high) with two biological replicates. Due to the mechanistic complexity of DIC, the model cholestatic compounds were selected because of their potential to cause cholestatic hepatotoxicity through diverse toxic mechanisms. Several supervised ML approaches, including Random Forest, Support Vector Machine and Logistic Regression, were applied to the human liver TG-GATEs dataset to develop a prediction model. Results: We identified a signature consisting of 20 genes that predicted cholestatic hepatic injury with high specificity and selectivity. The selected feature genes and model were validated using the in vitro rat TG-GATEs dataset. Discussion and Conclusion: Our transcriptomic signature has yielded high accuracy in the identification of potential cholestasis-inducing compounds.

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P-4a-17

A COMPARATIVE COMPUTATIONAL PREDICTION OF THE BINDING OF ANDROGEN- AND ESTROGEN-LIKE FLAVONOIDS TO THEIR COGNATE (NON)NUCLEAR RECEPTORS
ABSTRACT #434

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Background and objectives. Flavonoids are hormone-like polyphenols showing a chemical similarity to the endogenous sex steroids, i.e. 17β-estradiol and testosterone, and estrogen- and androgen-like activity(ies). Flavonoids binding to nuclear receptors (NRs) and the significance of the underlying activated sex steroid signaling have been studied since many years, focused in particular on estrogen receptor alpha (ERα), while less attention has been paid to other nuclear and non-nuclear membrane androgen and estrogen receptors. Here, six plant-derivedflavonoids – apigenin, genistein, luteolin, naringenin, quercetin, and resveratrol – have been investigated by computational docking simulations to predict their capability of binding NRs such as estrogen receptors ERα and ERβ, estrogen-related receptors (ERRs) ERRβ and ERRγ, androgen receptor (AR), and its variant ART877A as well as androgen membrane receptors, i.e., ZIP9, GPRC6A, OXER1, TRPM8, and estrogen membrane receptor, i.e., G Protein-Coupled Estrogen Receptor (GPER). Materials and methods. Homology modelling was performed using the Swiss-Model Protein Modelling Server (https://swissmodel.expasy.org/) with the proper selected PDB IDs for the above mentioned ten receptors. Flavonoids and 3 steroids (E2, T, DHT) were submitted to docking calculations towards each of 10 receptors once protein structures were prepared by the Protein Preparation Wizard in Maestro and ionized at pH 7.5 using PROPKA. When available, the co-crystallized ligands were used as the centroid of the receptor grid. Results. According to literature data, our results confirm how these flavonoids show a relevant degree of complementarity with both estrogen and androgen NR binding sites, likely triggering genomic-mediated effects. It is noteworthy that reliable protein-ligand complexes and estimated interaction energies were also obtained for some suggested estrogen and androgen membrane receptors, indicating that flavonoids could also exert non-genomic actions. Discussion and conclusions. Further investigations are needed to clarify flavonoid multiple genomic and non-genomic effects although flavonoids binding to ARs appear functionally as relevant as to ERs.

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P-4a-18

Designing physiological maps as a tool to study liver toxicology
ABSTRACT #388

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Background and Objectives: Physiological Maps (PMs) are conceptual constructs that

incorporate information as mechanistic representations of biological processes1. PMs can be used qualitatively and quantitatively as a mechanistic basis for improving Adverse Outcome Pathways (AOP) and supporting model rationale for several purposes. Within the ONTOX project, we have created two PMs to study the following chemical-induced liver diseases: steatosis and cholestasis. The purpose of the PMs is to improve current AOP networks and develop ontologies to support liver toxicity prediction. Material and Methods: We adapted the workflow from the Disease Maps project2 to construct the maps. First, relevant physiological literature was curated with the support of domain experts. Then, we listed fundamental mechanisms to be mapped and screened online databases for previously described pathways. Finally, we integrated pathways and literature data using the CellDesigner software and displayed them using the MINERVA platform3. Results: The maps include all the processes known in the current state of the art to trigger the corresponding AOP network on cholestasis and steatosis. However, they are not restricted to the currently available AOPs but also include other physiological processes vital to liver physiological functioning and homeostasis. Moreover, these maps expand beyond the liver, encompassing routes in different compartments critical for understanding liver mechanistic processes. Discussion and Conclusion: We designed these maps using expert-curated literature and previously available community-developed pathways, focusing on physiological functions and the human genome, transcriptomics, and proteome. They must be constantly updated to serve the community as a dynamic tool. Besides, PMs will become a more robust and multi-layered tool with the incorporation of quantitative kinetic and chemical information in future versions, which will be developed further as chemical-induced disease ontologies1. Such tools will offer a more comprehensive understanding of the liver-specific pathways regulated by chemical compounds, allowing for future toxicity prediction.

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P-4a-19

Toxicity prediction of mycotoxins by in silico modeling

ABSTRACT #411

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Background and Objectives: Mycotoxins are toxic secondary metabolites produced by fungi. They are generated in crops and can seriously affect human health through the food chain.1 Up to date, many hundreds of different mycotoxins have been described; however, a regulatory legislation only exists for a few of them, due to the lack of toxicity data. In this context, the aim of our work is the generation of toxicity data of a wide range of mycotoxins by in silico modeling, to provide useful data for their regulation.2 Material and Methods: For the generation of the models, extensive data from different databases and literature sources of mycotoxins and other natural products from the same chemical space have been collected. Different QSAR models have then been generated, testing several descriptor selections and modeling algorithms, and the best models have been selected after the evaluation of their accuracy, sensitivity and specificity, as well as their application to a set of compounds reserved for validation purposes. Results: Robust models have been developed for the prediction of mutagenicity, cytotoxicity, genotoxicity and oral acute toxicity of mycotoxins, with values of accuracy, specificity and sensitivity of over 80% in most of cases. Discussion and Conclusion: Our results provide a useful, simple and economic tool way to predict toxicity data of mycotoxins. These

models will be implemented in a technological platform intended to help industry and authorities in optimization and regulation purposes, respectively.

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P-4a-20

Evaluation of state-of-the-art in silico testing methods to fill physico-chemical and toxicokinetic data gaps within the ONTOX project
ABSTRACT #355

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Background and objectives The ONTOX project aims at developing new approach methodologies (NAMs) in order to address systemic repeated dose toxicity effects related to six toxicological adversities in the liver, kidney and developing brain. NAMs will be based on artificial intelligence (AI) –based systems fed by available biological, mechanistic, toxicological, epidemiological, physico-chemical and kinetic data. Within ONTOX, WP3 particularly focus on the identification of state-of-the-art in silico tools that will be used to fill data gaps resulting from experimental physico-chemical (PhCh) and toxicokinetic (TK) data. Methods QSAR models were identified from the literature and open-source software for the prediction of a series of PhCh and TK relevant properties. Datasets including experimental data for the above mentioned properties were gathered from the literature and manually curated in order to perform a comprehensive external validation of the QSARs and to identify optimal models for each considered property. When possible, the inclusion of chemicals outside the training set and the applicability domain assessment of the QSARs were kept into consideration. Results A total of 68 QSARs for 31 properties were evaluated on 50 experimental datasets, returning for some properties high predictive performance (i.e. R2 > 0.90 for regression models and Balanced Accuracy > 0.80 for classification models). Discussion QSARs selected from this validation are used to predict chemicals that were selected within the project as relevant for the six adversities. Predictions are used to fill experimental data gaps if present. Data distributions for properties of chemicals with positive and negative responses for each adversity will be compared to identify possible relationships between the adversities and the single PhCh and TK properties. Conclusions Selected QSARs for properties identified as relevant will be integrated in the computational AI model that will be produced as a result of the ONTOX project.

P-4a-21

Development new web-tool for phototoxicity prediction on the base of machine learning approach
ABSTRACT #430

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Background and Objectives: Diverse organic chemicals, including drugs, pesticides, cosmetics and fragrance materials, may induce photo-sensitisation and phototoxicity. Topical and systemic exposures to photoreactive compounds and subsequent exposure to sunlight may produce severe adverse reactions in both humans and animals. The in vitro 3T3 (NRU) phototoxicity test (OECD_TG_432), the reconstructed human epidermis phototoxicity assay (OECD_TG_498), and the ROS Assay (OECD_TG_495) are often used in combination to confirm phototoxicity of a chemical experimentally. In contrast, the computation

methods based on the structure-activity relationships (SARs, QSARs) can represent a faster and cheaper approach for screening phototoxic potential and for improving the overall risk and safety assessment of the materials of concern. Given the increasing attention of the regulators toward the potential phototoxicity of cosmetics, pharmaceuticals and pesticides, the main goal of this study was to build a freely available and accurate QSAR tool that will enhance the tiered testing strategies for the phototoxicity testing. Material and Methods: 546 compounds have been used to create the QSAR. We compiled highly diverse chemical data sets from 3T3 NRU phototoxicity and 3D model reports. Descriptors were calculated by the open-source cheminformatics tool RDkit. Various descriptors such as the cycle counts, Gasteiger charges, lipophilicity or CSP3 were employed along with physicochemical descriptors to construct useful QSAR model compliant with OECD's QSAR guideline (ENV/JM/MONO(2007)2). Our dataset was randomly separated into 436 compounds (the training set) and 110 compounds (the test set). Results and Conclusions: The sensitivity and specificity of the newly-developed QSAR model were 88% and 75%, respectively. This new QSAR model is freely available on the web platform mltox.online to assist the safety assessors in the more accurate risk assessment of chemical compounds. The developers will further upgrade the software to provide advanced functionalities and summary reports. Acknowledgement: This work was supported by VEGA 2/0087/22

P-4a-22

QSAR MODEL FOR PREDICTING MYCOTOXIN MUTAGENICITY ABSTRACT #377

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Background and objectives Mycotoxins are common contaminants in a wide variety of food matrices, thus representing a threat to Public Health. About 600 mycotoxins have been described in literature, but only a few of them are regulated, because of the lack of data regarding their toxicity and mechanisms of action. Thus, it is necessary to evaluate the toxicity of some mycotoxins commonly found in foodstuffs but not yet regulated. In this sense, in silico toxicology (IST) approaches can be used to rapidly assess chemical hazard and thus, computational methods, such as QSAR models, can be used for predicting toxicological endpoints. The main objective of the present study was the development of scientifically valid QSAR models for predicting the mutagenicity of mycotoxins. Those models can be used later by the industry and the regulatory organisms, providing a way to determine reliable safety levels for consumers, while reducing the use of in vivo assays. Material and Methods A survey of scientific literature on mutagenicity of mycotoxins has been done. Most of data for bacterial mutagenicity have been generated with the bacterial reverse mutation assay (Ames test), that relies primarily on Salmonella typhimurium tester strains. Data has been extracted from different sources (Pubchem, EPA, ECVAM, EFSA OpenFoodTox), considering both the amount and quality of the data (homogeneity, reliability). A set of more than 4,000 chemical descriptors has been calculated for all of the compounds, and different QSAR strategies were followed to compare and select the best model. Results The final dataset was composed by 654 mycotoxins, and the best model was able to correctly classify approximately 80% of them in both, the training and the validation set (75% - 25% respectively). Discussion and Conclusion The selected model is a valuable tool for screening mutagenicity of non-regulated mycotoxins, and prioritizing mycotoxins for regulatory purposes.

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P-4a-23

Computational modelling of neural tube closure defects
ABSTRACT #458

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Closure of the caudal neural tube is a critical event that occurs early in development, around day 27 of human gestation. Failure of neural tube closure results in severe birth defects, such as spina bifida. These neural tube defects (NTD) are among the most prevalent human congenital malformations, which warrants specific attention in chemical safety assessment. Computational models of biological processes are likely to revolutionize chemical safety assessment in the near future. Such models can be used to predict the effect of chemical-induced gene expression changes and provide a template for establishing quantitative adverse outcome pathway networks. This study aims to develop an in silico model of the human neural tube closure, which will be applied to predict chemical-induced NTDs. By extensively mining the developmental biology and toxicology literature, we first created a physiological map of human neural tube closure. Based on the physiological map, we built a multicellular agent-based model using CompuCell3D. The constructed physiological map depicts the all-trans-retinoic acid (ATRA) related molecular pathways linked to the various cell types in which they occur, and their morphogenetic consequences, that lead to closure of the neural tube. The morphogenetic events driven by gene expression changes are visualized by the computational model. We simulated in silico the complex biological process of neural tube closure, in order to demonstrate the feasibility of this approach. At a later stage in the project, the computational model will be applied to predict chemical-induced changes in gene expression and cell characteristics. The predictions of the model will be validated using a set of dedicated in vitro assays in conjunction with existing knowledge on in vivo developmental neurotoxicity. Such computational models may ultimately provide an alternative in silico approach for chemical safety assessment without the use of animals.

P-4a-24

ASSESSING A BATTERY OF IN SILICO MODELS AS PREDICTION TOOL FOR COMPOUNDS EXERTING REPRODUCTIVE HEALTH EFFECTS
ABSTRACT #485

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Given the high attention to endocrine disrupting chemicals, 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 [1]. This study aimed at the assessment of the correlations between the prediction results of a battery of in silico tools and the reported observed adverse effects from in vivo reproductive toxicity studies. We used OpenVirtualToxLab (OVTL) software and the EndocrineDisruptome online tool [2,3] to model the binding affinities to nuclear receptors of 17 pesticides, 7 of which classified as reprotoxic substances under CLP. Then, we aligned the results of the in silico modelling with data from ToxCast assays and in vivo reproductive toxicity studies. Results from in vivo studies conducted according to OECD 415 and/or 443 modified with the aim of refinement and reduction of use of animals were retrieved from the archive of our GLP laboratory. 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 molecular modelling of binding to nuclear receptors can be useful for the prediction of potentially reprotoxic compounds. Detailed results will be provided in the poster.

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P-4a-25

Facilitating modern toxicology with Natural Language Processing ABSTRACT #492

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Background and Objectives With the current trend towards the reduction of animal testing, modern toxicology relies on the gathering of multiple streams of evidence and information, from available literature to knowledge databases. This information is both tedious and increasingly time-consuming to process manually with the growing volume of data available. The advancement of machine learning provides alternative solutions to this challenge. To extract and organize information from relevant sources, we want to take advantage of the power of Natural Language Processing (NLP) techniques. We show a proof-of-concept example of how NLP can support the extraction of toxicological information from scientific text by identifying biological entities of interest and the relationships between them. Material and Methods We collected a sample corpus of PubMed literature, consisting of articles obtained from querying each chemical from a list compiled by the ASPIS cluster(1). We analyzed this corpus with the help of a trained toxicology-oriented Natural Language Processing model and organized the results in a graph database for further processing with graph algorithms. Results We automatically gathered multiple phenotypes at various biological scales and were able to associate them with a diversity of chemicals. Conversely, we were also able to extract groups of chemicals triggering or linked to adverse outcomes of interest. Discussion and Conclusion Natural Language Processing can support the extraction of knowledge from scientific text, in particular toxicological information. We foresee multiple applications, from chemical selection for testing new approach methodologies to gathering weight of evidence for the establishment of novel Adverse Outcomes Pathways (AOPs), or to reconstruct and strengthen evidence of existing AOPs(2).

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P-4a-26

Using molecular docking simulations to elucidate molecular initiating event (MIE) interactions of neonicotinoid pesticides and human nicotinic acetylcholine receptors (nAChRs) ABSTRACT #494

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Neonicotinoids are still globally used as pesticides for agricultural and urban uses. Initially, they have been designed to achieve species-selectivity on insect nicotinic acetylcholine receptors (nAChRs). However, recent studies have shown that a subgroup of neonicotinoids activate human nAChRs as well, which reinforces concerns about their species selectivity. Moreover, latest findings indicate

that nicotinoid metabolites are even more potent than their parent insecticide structure. In accordance to the OECD and EFSA recommendations for an integrated approach for testing and assessment (IATA), animal-free toxicity risk assessment is of high importance in this field. Therefore, NAMs (new approach methods), amongst them computational methods like molecular docking and calculations for binding energy approximates, as well as cell-based assays are used to generate insights. It is suggested that the MIE (molecular initiating event) triggers several measurable key events which eventually lead to an adverse outcome. The AOP (adverse outcome pathway) involves activation and/or desensitization of the target protein the nicotinic acetylcholine receptors (nAChRs). This assumption implements that nervous system development is affected when key biological processes are disturbed. It is supposed that the neonicotinoids share their target protein with the prototype agonist nicotine, the CNS-located nAChRs, which most abundantly occurs as subtypes α7, α3β4 and α4β2. Therefore, protein-ligand interactions are modelled that could possibly be critical for receptor binding. For this purpose, molecular docking simulations are performed on the latest published cryo-EM structures of the human nAChR structures, including different conformations of those. Furthermore, several scoring functions are compared to experimental values derived from functional binding assays and reveal a positive correlation of the activity ranking. Additionally, the docked complexes are analysed according to protein-ligand-interaction fingerprints.

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Session: 4b. Local toxicity testing (safety and efficacy)

P-4b-1

Concentration, typology of surfactants, in vitro and clinical ocular tolerance studies: a multi-parameterized approach for foaming cosmetics intended to claim “do not sting the eyes”

ABSTRACT #109

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Background and Objectives: The ocular tolerance linked to the claim “do not sting the eyes” represent a challenge for foaming products specifically intended for infants and children because of the risk of running into the eyes. The aim of this study was to predict the clinical ocular tolerance using both the surfactants’ composition and in-vitro results. Material and Methods: 22 foaming cosmetics divided into 3 categories(CAT) according to their concentration(C) of surfactants’ actives ingredients(SAI) were studied: CAT1;N=6:C(SAI)≤8%;[5.57%-8%] CAT2:N=15:8%<C(SAI)≤15%;[9.09%-14.75%] CAT3;N=1:C(SAI)>15%;(15.35%) The ocular tolerance was studied at 3 dilutions[1%/5%/10%] on two in-vitro models: HET-CAM(1) and Reconstructed Human Corneal Epithelial(2) tests. Moreover, clinical specific ocular tolerance study(TOS) was performed. It consisted of a single application of the product diluted at 5% on the top of each subject’s eyelid, with a stinging potential (limit authorized score<70) and an ophthalmological assessment. Results: A 4-level classification system was established:4=Non-irritant;3=slightly-irritant;2=moderately-irritant;1=irritant CAT1: in-vitro results were 4-

3(1%), 4-2(5%) and 3-1(10%). 100% of the TOS's conclusions were good to very good;scores<70. CAT2: 73.3%(N=11) containing from 9.09% to 14.75% of SAI, obtained in-vitro results 4-3(1%), 4-2(5%) and 3-1(10%). 100% of the TOS's conclusions were good to very good;scores<70. However, 26.7%(N=4) containing from 10.13% to 11.73% of SAI, obtained in-vitro results 4-2(1%), 2-1(5%) and 1(10%). TOS concluded to a moderate tolerance; scores≥70(N=3);<70(N=1). CAT3: in-vitro results were 4-2(1%) and 1(5%/10%). Therefore, no TOS was performed. Discussion and conclusion: The correlation TOS/in-vitro results is good to very good at 1%/5% dilutions and poor to moderate at 10%. Thus, it appears that the C(SAI) influences the ocular tolerance. However, the CAT2 analysis illustrates that others parameters like the SAI typology (anionic/amphoteric/non-ionic) can also impact the results. This study shows that a multi-parameterized approach can predict the clinical ocular tolerance, which will be useful for the screening of new foaming cosmetics intended for family target.

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P-4b-2

Effect of a modulator on the skin sensitization potency of cosmetic fragrance formulations
ABSTRACT #275

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Perfume long lasting is an important concern widely addressed in fragrance research and innovation. There is a need for new technologies to prolong the perception and intensity of fragrances over time. The intended function of fragrance modulators is to slow down the evaporation rate of some perfume raw materials, with a potential impact on the toxicological profile of the final formulation. This work evaluates the impact of a fragrance modulator on the skin sensitizing potency of fragrance formulations. In the context of Integrated Approaches to Testing and Assessment, the intention is to address several key events of the skin sensitization Adverse Outcome Pathway by applying the GARDskin Dose-Response assay. This assay is a modification of the validated GARDskin protocol (OECD TGP 4.106) that incorporates dose-response analysis. The readout is a cDV0 value, describing the lowest concentration required to generate a positive classification. This value correlates with potency and can be used to rank test items by their relative sensitizing potency. This assay allows to evaluate mixtures and delivers continuous potency predictions, crucial point for comparing the modulator's effect on the formulation's skin sensitizing potency. The project examines the effect on skin sensitization potency when a modulator is spiked or not into fragrance formulations. Testing performed using GARDskin Dose-Response, and the derived cDV0 values were compared using 95% confidence intervals. Results are hitherto available for one fragrance formulation. Both conditions, with and without modulator, gave rise to monotonically increasing dose-response curves. The cDV0 value for the formulation containing the modulator was significantly higher (458ppm, 95% CI: 332-626) compared with the cDV0 value for the one without (268ppm, 95% CI: 248-292). In conclusion, based on preliminary data reported in this study, the fragrance modulator appears to reduce the sensitization potency of cosmetic formulations based on a fragrance mixture.

P-4b-3

Comparison between HET-CAM protocols and a product use clinical study for eye irritation evaluation of personal care products according to their surfactant composition
ABSTRACT #207

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The hen's egg test on chorioallantoic membrane (HET-CAM) is one of the most frequently used alternative tests for prediction of ocular irritation of cosmetic products. There are different HET-CAM protocols widely accepted, but there is no information about which of the protocols better correlates with the results obtained in product use clinical study under the conditions of use. Two Fix Time Methods (FTM) -Lüepke and the ICCVAM guideline - and two Reaction Time Methods (RTM) -ECVAM DBALM Prot. No. 47 and No. 96- were employed to test 18 cosmetic products. Simultaneously, they were evaluated by an ophthalmological clinical test. A unified classification system was used, and products were classified into four irritation levels: non-irritant, weak, moderate and severe irritant. The duration of use (rinse-off or leave-on), and the concentration and type of surfactants were taken into account in the analysis. All the products that were classified as non-irritant by any HET-CAM protocols were also safe in the product use clinical study. The product that was found to be non-safe in the product use clinical evaluation was also unsuitable by most of the HET-CAM protocols. All protocols resulted appropriate to evaluate 10% dilutions of rinse-off products with a high content of non-ionic surfactants as shampoos. However, HET-CAM is not recommended to test products containing high concentration of anionic and cationic surfactants due to the highly irritating reaction obtained. These results were employed to develop an algorithm that allows selecting the appropriate HET-CAM protocol for each type of product to be tested. Applying the algorithm, a protocol is chosen taking into account the following points: 1-weight of the evidence, 2-duration of use, 3- surfactant concentration and 4- surfactant type.

References

ocular irritation cosmetics

P-4b-4

DECISION TREE FOR OCULAR SAFETY ASSESSMENT OF AGROCHEMICAL FORMULATIONS ABSTRACT #208

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For the registration of agrochemical formulations, acute eye toxicity assessment is required by regulatory agencies. The Draize rabbit eye test (OECD TG 405) has worldwide acceptance to assess eye irritation, even it has been increasingly questioned. The test distinguishes four categories considering reversible and non-reversible ocular lesions according to UN GHS Categories 1 (severe eye damage), 2A and 2B (reversible eye damage) and No Category (minimal effects). Bovine Corneal Opacity and Permeability (BCOP) and Short Time Exposure (STE) are methods for identifying Cat. 1 and No Cat. products, according to OECD TG 437 and TG 491 respectively. Histopathological analysis of the corneas, after the BCOP test, is recommended to identify the depth of the damage as complementary information. As the limitation of these alternative methods is to classify in the middle-range categories (2A and 2B), the aim of this work was to create a strategy to identify these categories. The STE and BCOP methods were used to test 17 pesticides manufactured by ATANOR SCA. These products had been previously classified in categories 1, 2A, 2B or No Cat. using the Draize eye irritation test. By STE test, all formulations were tested at 5 and 0.05%. By the BCOP test, liquid products were tested neat and at 10% and solids at 10%. After BCOP, an histopathological analysis was performed and 3 different models were used to analyse: depth of injury, stromal thickness, and total histopathological damage. Following a 4-step decision tree, we obtained the same classification for 14 of the 17 agrochemicals. An analysis by category shows: Cat. 1 100% accuracy and specificity; Cat 2A 82.4% accuracy and 86.7% specificity; Cat 2B 88.2% precision and 90.9% specificity and No Cat. 94.1% precision and 100% specificity. Finally,

we successfully established an in-house strategy for testing agrochemicals eye irritation.

References
eye damage pesticides SIRC cell line

P-4b-5

THE RECONSTRUCTED HUMAN EPIDERMIS (RHE) EFFICIENCY IN THE CLASSIFICATION OF BIOLOGICAL PRODUCT BY THE IN VITRO IRRITATION AND CORROSION TESTS (OECD 439 AND 431). ABSTRACT #258

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Background and Objectives: Following the use of alternative methods and especially Art 6 of RDC 294 (July 29, 2019) from Brazilian Regulation, we evaluated the in vitro irritation (OECD 439) and corrosion (OECD 431) methods to classify biological products, using reconstructed human epidermis (RHE, SkinEthicTM) provided by Episkin Brasil. This model represents the in vitro target organ, which consists of normal human keratinocytes cultured to maturity of the epidermis. Some care must be taken when handling the biological product in order to avoid cross contamination or loss of effectiveness of the product. Material and Methods: A biological product has been tested according to OECD 439 and OECD 431. Cell viability in RHE model was measured by enzymatic conversion of the vital dye MTT, into a blue formazan salt that was quantitatively measured after extraction from tissues after biological product exposure. Results: Viability results obtained in vitro (mean ± SD) were NC: 100 ± 3.27; PC: 1.92 ± 0.07; Biological Product: 104.05 ± 7.92 for the irritation test. For corrosion, were NC: 100 ± 7.90; Biological Product: 99.62 ± 1.19 for 3 minutes of exposure and NC: 100 ± 1.65; PC: 2.75 ± 0.13; Biological Product: 94.63 ± 0.46 for 60 minutes of exposure. Our results were within the acceptance criteria. Discussion and Conclusion: As expected, the positive control demonstrated a significant relative cell viability reduction when compared to the negative in both tests. In addition, according to the results obtained, the Biological Product was classified according to UN GHS Category as “No Category” and “Non-Corrosive”, corroborating with in vivo classification tests. Therefore, the RHE could be used for classification of biological products, as long as proper precautions are taken to obtain quality results.

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P-4b-6

PROFICIENCY DEMONSTRATION OF MERIEUX NUTRISCIENCE (BIOAGRI LABORATÓRIOS LTDA) ON IN VITRO EYE IRRITATION AND SERIOUS EYE DAMAGE IMPLEMENTATION ABSTRACT #259

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Background and Objectives: The purpose of proficiency study is to provide efficiency and precision of a specific method from a particular laboratory. In this sense, our laboratory (Bioagri Laboratórios Ltda – Piracicaba) tested references items with different categories described in the literature using Reconstructed Human Cornea-Like Epithelium (RhCE, SkinEthicTM HCE). This model represents the in vitro target organ, i.e., human immortalized corneal epithelial cells, therefore similar to the in vivo corneal epithelium three-dimensional structure. Material and Methods: Six solid reference items were chosen according to the OECD 492 (2,5-Dimethyl-2,5-hexanediol; Sodium oxalate; Sodium benzoate; 1-(4-Chlorophenyl)-3- (3,4-dichlorophenyl) urea; 2,2'-Methylene-bis-(6-(2Hbenzotriazol-2- yl)- 4-(1,1,3,3-tetramethylbutyl)- phenol); Potassium tetrafluoroborate). The assay is based on its ability to induce cytotoxicity in RhCE tissue construct, as measured by the MTT assay. The tissue viability following exposure is determined in relation to negative control substance and then used to predict the eye hazard potential. Results: Viability results obtained in vitro (mean % in relation of negative control ± SD) were NC: 100 ± 0.41; PC: 13.81 ± 2.01; 2,5-Dimethyl-2,5-hexanediol: 0.78 ± 0.02; Sodium oxalate: 2.58 ± 2.01; Sodium benzoate: 1.36 ± 0.16; 1-(4-Chlorophenyl)-3- (3,4-dichlorophenyl) urea: 121.26 ± 2.47; 2,2'-Methylene-bis-(6-(2Hbenzotriazol-2- yl)- 4-(1,1,3,3-tetramethylbutyl)- phenol): 107.15 ± 0.84; Potassium tetrafluoroborate: 109.18 ± 5.16. Our results were within the acceptance criteria. Discussion and Conclusion: As expected, the positive control demonstrated a significant relative cell viability reduction when compared to the negative. All reference items demonstrated viability values that allowed your classification according to UN GHS Category (OECD 492), reproducing the findings of others laboratories. Together, the findings have shown that reference items presented within the category described in the OECD 492 (2019), consequently the proficiency study conducted with solid item test was able to deliver reliable and adequate results. Therefore, the laboratory has conditions to operate it properly.

References

OECD Test Guideline No. 492 Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, June 18th 2019.

P-4b-7

SkinEthic™ HCE Time-to-Toxicity : World's first adopted new approach methodology on its own for eye hazard identification adopted by OECD ABSTRACT #296

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Background and Objectives: None of the in vitro methods implemented into regulations is able to replace in vivo rabbit eye irritation test due to the complexity of the endpoint and the classification schemes applied by the regulation under the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS). Taking into account our expertise on the SkinEthic™ Human Corneal Epithelium model and our knowledge on protocols, the SkinEthic™ HCE Time-to-Toxicity test method was established. Materials and methods: The method consists of two protocols, one for liquids and one for solids. Based on the viability observed for the different exposure periods (from 5 to 120-min) a classification is assigned. Results: The method was developed with 74 training chemicals and

challenged with 52 test chemicals selected on the basis of the main in vivo drivers of classification. 75% UN GHS Cat.1, 68% Cat.2 and 74.9% No Cat. chemicals were correctly identified. The relevance and reliability of both protocols have been also assessed on 40 coded chemicals in three laboratories with an overall within and between laboratory reproducibility of 95%. When considering all 151 tested chemicals, the test method has a balanced accuracy of 74% with correct predictions of 79% for Cat 1, 69% for Cat 2 and 75% for No Cat. Furthermore, none of the UN GHS Cat. 1 were identified as false negative leading to a conservative approach. Overall, these studies provide evidence that the test method is capable of distinguishing between the three UN GHS categories. Conclusion: The SkinEthic™ HCE Time To Toxicity was recommended as a full replacement to the in vivo Draize acute eye irritation test for classification of substances and mixtures and therefore becomes the World's first adopted new approach methodology on its own for eye hazard identification adopted by OECD.

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P-4b-8

COMPOUND A-111 – A NOVEL SMALL MOLECULE CANDIDATE FOR THE TOPICAL TREATMENT OF HYPERPIGMENTATION
ABSTRACT #399

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Background and Objectives: Hyperpigmentation disorders constitute remarkable medical and esthetical problems. They result from the incorrect deposition and/or the increased production of melanin [1]. There are known some treatment options for hyperpigmentation, however, they require long duration to show visible results and often cause adverse effects [2,3]. In the search of new melanogenesis inhibitors we identified a novel small molecule candidate for the topical treatment of hyperpigmentation - compound A-111. Material and Methods: The structure and purity of the synthesized compound were confirmed by LC-MS, 1H and 13C NMR. Mushroom tyrosinase was used in enzymatic assays for the evaluation of monophenolase (substrate: L-tyrosine) and diphenolase (substrate: L-DOPA) inhibitory activity. Mouse melanoma cell line B16-F10 was used in melanogenesis inhibition assay with two different melanogenesis inducers: α-melanocyte-stimulating hormone (α-MSH) and 3-isobutyl-1-methylxanthine (IBMX). Cytotoxicity was evaluated in BJ and HaCaT, hepatocytotoxicity in HepG2, and neurocytotoxicity in SH-SY5Y cell lines. Skin irritation and skin permeation studies were performed in reconstructed human epidermis (EpiskinTM). Results: Compound A-111 was initially identified as a tyrosinase inhibitor in the screening of a series of its 40 homologues and analogues. Its IC50s are 36.98±1.07 μM and 146.71±16.82 μM for mono- and diphenolase activity, respectively. Kinetic studies showed that it is a mixed-type tyrosinase inhibitor. It inhibited melanin synthesis induced by α-MSH and IBMX in B16F10 cells at the concentrations range 200-25 μM. Cytotoxicity evaluation in cell lines proved its safety in concentrations up to 250 μM. Tests in reconstructed human epidermis showed no skin irritation potential.

Compound A-111 was able to cross epidermis barrier with 44 µg/cm2 permeated after 24 h exposure. Discussion and Conclusion: Our studies provided sufficient evidence for the in vitro melanogenesis inhibition potential of compound A-111. Moreover, it showed beneficial in vitro safety profile and proved sufficient bioavailability.

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P-4b-9

Corneal-derived biomolecular solution: application as an in chemico method for ocular toxicity assessment
ABSTRACT #446

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Background and Objectives: New methodologies for assessing eye damage should be developed to reproduce results more similar to those found in humans and replace the in vivo Draize eye test. Ocular opacity is a crucial endpoint of eye damage, and it is related to the interaction of corneal extracellular matrix biomolecules, mainly collagen and glycosaminoglycans, with eye toxicants. This endpoint can be addressed by employing the Bovine Corneal Opacity and Permeability (BCOP) (OECD TG 437). However, some issues involving access and preservation of bovine cornea and the specific equipment make this test less widespread. This study aims to propose an accessible and low-cost method, based on the bovine cornea-derived decellularized extracellular matrix (dECM), for application as an in chemico pre-screening strategy for identifying GHS Category 1 and uncategorized chemicals. Material and methods: The biomolecular solution was prepared from the solubilization of cornea-dECM. Subsequently, the biomolecular solution was exposed to different eye irritants in a 3D printed device coupled with a cellulose membrane. The substances were suspended and placed in contact with the matrix in a controlled manner for 24 hours at room temperature. Then, the complex turbidity was measured in a spectrophotometer at 405nm and compared with matrixes exposed to PBS (Phosphate-buffered Saline) as a negative control. Category 1, uncategorized substances, and pesticides were used as test materials. Results: Results showed a change in turbidity after exposure to category 1 substances and pesticides compared to the negative control and uncategorized substances. Discussion and conclusion: In conclusion, the test can be used as a pre-screening method for detecting substances that induce severe eye damage (Category 1), as well as no categorized substances and pesticides, featuring a cheaper and accessible test that does not have specific inputs to be performed as the BCOP test.

P-4b-10

A new approach for Eye Hazard Assessment of surfactants based on in vitro Test Methods
ABSTRACT #334

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Background and Objectives: Two defined approaches (DAs) for non-surfactant liquids have been accepted and were integrated in a new OECD guideline for eye hazard assessment. Currently, no single in vitro

method or DA has been developed to assess eye hazard potential of surfactants across the 3 UN GHS categories. Rrecently, a DA was developed to predict this endpoint for liquid, semi-solid and solid chemicals having surfactant properties. The DA is based on the combination of Reconstructed human Cornea-like Epithelium test methods (RhCE, OECD TG 492, EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the Short Time Exposure test method (STE, OECD TG 491). Materials and methods: The reference set used to develop the DA represented different surfactant families and the most important drivers of in vivo Cat. 1 and Cat. 2 classification. The 2 main subgroups for No Cat. (CO=0 and CO>0) were also included. A RhCE method is used in a first tier to distinguish No Cat. from classified substances. In case of a positive call, the STE method is used to further sub-categorize into Cat. 1 (viability < 20% at 5% and 0.5% w/v) or Cat. 2 (all other combinations). Results: The balanced accuracy of the DA was 79.3% (N=40), 83.3% of Cat. 1 (N=18), 77.8% of Cat. 2 (N=9) and 76.9% of No Cat. (N=13) were correctly identified. The performance of the DA was greater than the proposed minimum values of 75% for Cat. 1, 50% for Cat. 2 and 70% for No Cat. agreed by the OECD experts. Conclusion: Combination of a RhCE method with the STE method demonstrates their potential to successfully distinguish between the 3 UN GHS categories for eye hazard identification. This DA, applicable to surfactants, will fit in a OECD guideline.

P-4b-11

DEVELOPMENT OF AN IN VITRO WOUND HEALING MODEL USING LIVE-CELL IMAGING: APPLICATION IN DERMATOLOGY AND COMSETIC FIELDS
ABSTRACT #343

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Background and Objectives The wound healing assay is a standard in vitro technique to study cell migration. It is based on a scratch that creates a gap on a cell monolayer and capturing images at regular intervals. Although the assay is straightforward and widely applied, it lacks standardization in its application. The objective of the study was to develop a standardized in vitro wound healing assay using live-cell imaging applied in the field of dermatology and cosmetics. Materials and Methods Human dermal fibroblasts were cultured in Ibidi® culture-inserts creating reproducible wounds. At confluency, inserts were removed, and cells were treated with TGF-beta (known to promote the migration and invasion of tumor cells) or taxol (known to inhibit cell migration). Untreated cells were used as control. Live-cell imaging of the wounded area and quantifying the dynamics of cell migration were done using CytoSMART Omni brightfield device operating from inside CO2-incubator. Acquisition of time-lapse images was performed during 24 hours and wounding area was measured by the algorithm of the system. Results As expected, the cell migration rate was significantly lower in cells treated with taxol than in control untreated cells, indicating that taxol slowed down cell migration. On the other hand, the cell migration rate was significantly higher in cells treated with TGF-beta than in control untreated cells, indicating that TGF-beta accelerated cell migration. These results show that our model is sensitive enough to discriminate between two different treatments with the advantage of real-time visualizing and recording of cell migration and wound closure. Discussion and Conclusion This standardized in vitro assay is a simple, versatile and cost-effective method to study collective cell migration and wound healing and helps screening new compounds with wound healing properties. In addition, this assay can be applied on both fibroblasts and keratinocytes.

P-4b-12

A NEW HUMAN IN VITRO SKIN MODEL OF EPIDERMAL BARRIER DAMAGE
ABSTRACT #346

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Background and Objectives The recommended hygiene procedures for the fight against COVID-19 caused by SARS-CoV-2 virus include repeated hand washing, and frequent use of hand sanitizers that can disrupt the skin barrier integrity. We hypothesized that compromised skin could represent a potential transmission route for SARS-CoV-2, especially since we showed the expression of key receptors of the virus in skin models. The aim of this project was to develop an in vitro skin model with epidermal barrier defect. Once validated, this model will be used for testing new products to prevent or repair skin damage. Materials and Methods Fresh human skin samples from three different donors were treated with sodium dodecyl sulfate (SDS) to induce epidermal barrier damage. Different concentration and treatment duration were tested. The skin barrier function was evaluated by measuring the TransEpithelial Electrical Resistance (TEER). On the molecular level, mRNA expression of specific biomarkers of epidermal barrier and inflammation was measured by quantitative real-time RT-PCR. Results SDS treatment induced significant decrease of TEER, indicating that epidermal barrier was compromised. Moreover, mRNA expression level of Filaggrin was down-regulated, indicating a disruption of skin barrier integrity. In addition, SDS treatment induced an up-regulation of interleukin-8 (IL-8), a pro-inflammatory chemokine. Discussion and Conclusion The different end points allowed us to validate this in vitro model of damaged skin. This model is of great interest for testing products to prevent or repair damaged skin. In addition, this model will be used to validate the interaction/binding/entry of a pseudovirus expressing the spike protein.

P-4b-13

Study of the efficacy and safety on in vitro human skin models of a Curcumin emulsion for skin pathologies treatment

ABSTRACT #392

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Curcumin is the bioactive component of the spice turmeric. This polyphenolic compound has been widely used in ancient oriental medicine for its healing properties. Due to curcumin properties, such as antimicrobial, antioxidant, anti-inflammatory and antiproliferative, this compound might be a good candidate for the treatment of different diseases, including skin disorders. However, curcumin has poor absorption, fast metabolism and rapid systemic clearance, thus limited tissue distribution. To overcome this fact, this study focused on the development, safety and efficacy of encapsulated curcumin topical delivery by means of different skin ex vivo and in vitro models. In order to assess the safety of the curcumin formulation, skin irritation, skin absorption (franz cells), EpiDerm phototoxicity, mutagenicity and sensitization were evaluated. All the safety assays followed OECD guidelines. Curcumin emulsion efficacy was evaluated by assessing cell proliferation and inflammatory response in an ex vivo model with an etiology similar to psoriasis. The skin irritation test showed that the viability of the emulsions was above 90%, so it was classified as non-irritant. The same experimental system was used in the phototoxicity tests, where the product showed no toxicity related to UV irradiation The sensitization test followed the 2 out of 3 strategy and no sensitization effects were observed. In order to determine if the emulsion had an effect in gene mutation, reverse bacterial mutation test was carried out and no mutagenicity was observed. The curcumin skin absorption study from the emulsion in human skin explants, rendered that quantifiable amount of curcumin penetrated the skin. Human ex vivo studies showed an improvement in the physiological status of psoriatic skin after topical application of the ointment containing encapsulated curcumin. This study concludes that the curcumin emulsion for topical administration is considered safe and could be a good candidate for the prevention and treatment of psoriasis.

P-4b-14

Cutaneous tolerance of personal care products dedicated to babies and adults with sensitive skin: in vitro/in vivo correlation
ABSTRACT #266

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Background and Objectives: Dermal irritation assessment is an essential component of personal care products' safety evaluation. The skin, which is the first protective barrier, will react differently depending on its integrity or maturity. AlternaSkin, a fully differentiated 3D epidermis equivalent, and AlternaSensiSkin, an immature reconstructed human epidermis, were developed to determine the cutaneous irritant potential of cosmetic products on normal and sensitive skin. Our study aimed to retrospectively examine the correlation between in vitro and in vivo skin tolerance results of 15 cosmetic products intended for babies or adults with sensitive skin. Material and Methods: AlternaSkin results were compared to those of clinical studies conducted under medical control on a healthy skin adult population while AlternaSensiSkin results were compared to clinical results obtained on the target population and to cosmetovigilance data. The in vitro cutaneous irritant potential of the test item was determined by measuring the viability of living cells after 20h of exposition using MTT reduction assay and expression levels of key cellular markers of inflammation (IL-1α / IL-8). A 3-level classification system was established to allow a comparison between in vitro results, clinical and cosmetovigilance dataset: 1 = irritant; 2 = moderately irritant; 3 = non-irritant. Results: The correlation between the AlternaSkin results and the tolerance under medical control is excellent (100% perfect correlation). Very good correlations are obtained between the AlternaSensiSkin results and those of clinical studies on the target population and cosmetovigilance data: 87% perfect correlation – 13% correct correlation. Discussion and Conclusion: Regarding very good predictability results of skin tolerance, this analysis highlights the interest in the use of in vitro sensitive skin models within the framework of the pre-clinical evaluation of formulations intended for sensitive skin.

P-4b-15

Retrospective review on in vitro phototoxicity data generated in 3D skin models to support development of new OECD test guideline
ABSTRACT #234

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Phototoxicity (also photo-irritation), is a light-induced skin reaction that occurs when photoreactive chemicals are activated to produce cytotoxic effects. Phototoxicity testing of chemicals is mostly conducted in vitro, following the methods described in the OECD test guideline (OECD TG 432), using the Balb/C 3T3 mouse fibroblast cell line. The test is highly sensitive, but low specificity has been reported in some studies (1). The test has also limitations with testing poorly soluble compounds. The use of 3D reconstructed human epidermis (RhE) tissue models has been proposed as a second-tier in an integrated testing strategy to assess potential phototoxic activity, especially for topical exposures (e.g., chemicals, pesticides, cosmetics) (2). We collected published data on phototoxicity testing using various RhE models and protocols. The dataset contains more than 80 materials and over 800 entries utilizing five different RhE models. The analysis conducted on the datasets revealed, that despite some differences between the protocols (e.g., exposure times, dosing, solvents), the RhE models have the potential not only to distinguish between phototoxic and non-phototoxic materials but also the potential to predict phototoxic potency as demonstrated in

limited studies conducted in parallel to clinical studies in human volunteers (3). This database provides a valuable resource and supported the regulatory acceptance of the new OECD method (OECD TG 498). This project was funded in whole or in part with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C

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P-4b-16

ESTABLISHMENT OF A COMMENSAL 3D SKIN MODEL FOR STUDYING MICROBIAL MODULATION OF PESTICIDE TOXICITY ABSTRACT #387

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The human skin acts as an interface to our surrounding environment and functions as physical and metabolic barrier against exogenous impacts. A major part of this interface is the skin microbiota, which comprises millions of commensal microbes including bacteria, fungi, and viruses. Our skin microbiota is involved in many aspects critical for human health including wound healing, pathogen protection and immune regulation [1,2]. Nonetheless, their major metabolic potential might add an additional risk factor to xenobiotic substance exposure. Feeding a growing world population demands an increased use of pesticides, leading to an increased exposure of the public. Several studies indicate that pesticides could potentially disrupt the gut microbiota, which may lead to different diseases including cancer, autoimmune disorders and neurotoxicity. Moreover, it was shown that gut bacteria could metabolize pesticides modifying their toxicity and bioaviability [3]. Although the skin is a first site of exposure, toxicological studies on pesticides and the skin microbiome are scarce. In previous studies, we developed a long-term human commensal 3D full thickness skin model based on MatTek's EpiDermFT™ colonized with two bacterial skin isolates. Tests with the xenobiotic pollutant benzo[a]pyrene confirmed the model's extended metabolic capabilities [2]. We now want to investigate the role of the human skin microbiota for host health in the context of exposure to pesticides that are likely to affect the human tryptophan or tyrosine metabolism. The latter two are microbial metabolites playing a crucial role in host-microbe communication. For this, we are extending our commensal skin model by a defined bacterial community or whole skin swabs. Next, we want to investigate commensal effects on pesticide toxification and skin toxicity in situ. The analyses will focus on pesticide-induced microbial-host interactions, characterization of resulting pesticide metabolites using mass spectrometry, as well as potential cyto- and genotoxicity.

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P-4b-17

Determination and Sub-categorization of Ocular Irritants Using the EpiOcular Tissue Model - Prediction Models for

Liquids and Solids
ABSTRACT #440

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Evaluation of serious eye damage/eye irritation originally involved the use of laboratory animals (OECD TG 405).In 2015,a new test guideline (OECD TG 492) was accepted which enables the use of in vitro procedure based on reconstructed human cornea-like epithelium to distinguish between chemicals (substances and mixtures) not requiring classification and those that must be labeled for eye irritation or serious eye damage.Chemicals identified as requiring classification for eye irritation/serious eye damage must be further tested to distinguish between eye irritants and those causing serious eye damage.There have been several projects focused on the development of tiered testing strategies for eye irritation assessment which takes in account all drivers of classification.The goal of these projects has been to develop a testing strategy to sub-categorize chemicals which:a)do not require labeling for serious eye damage or eye irritancy-No Category,b)can cause serious eye damage-Cat 1,and c)are eye irritants-Cat 2[1,2]. In the current project,a set of 13 chemical,7 liquids and 6 solids,that are listed as proficiency chemicals in draft OECD TG 492B were tested using EpiOcular model.We used a testing strategy developed in CON4EI project and confirmed in ALT4EI project,which combines the most predictive time-points of EpiOcular time-to-toxicity neat and dilution protocols.Liquids and solids were test separately with different methodologies and prediction models.The set of chemicals consisted of 4 Cat 1 chemicals,5 Cat 2 chemicals and 4 No Cat chemicals.Using the proposed testing strategy,we were able to correctly identify 100% of Cat 1 chemicals (4/4),100% of Cat 2 chemicals (5/5) and 100% of No Cat chemicals (4/4). The testing strategy proposed in CON4EI and verified in ALT4EI projects to achieve optimal prediction for all three categories–prediction models for liquids and solids seems to be a very promising tool in an integrated testing strategy that can discriminate chemicals to No Cat,Cat 2 and Cat 1.

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P-4b-18

SAFETY AND EFFICACY TESTING OF COSMETIC PRODUCTS: OVERVIEW OF ESTABLISHED METHODS AND NEW MODELS.
ABSTRACT #342

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Over the past decades, considerable efforts towards the development, evaluation, and implementation of in silico and in vitro-based models have facilitated improvements in the hazard identification and risk assessment of preclinical drug candidates, and contributed to reduce, refine, and replace animal testing. While cosmetic products need to have a proven efficacy combined with a comprehensive toxicological assessment, an increasing number of alternatives to animal testing has been developed and validated for safety and efficacy testing of cosmetic products and cosmetic ingredients. For example, 2D cell culture models derived from human skin can be used to evaluate anti-inflammatory properties, or to predict skin sensitization potential. 3D human skin equivalent models are used to evaluate skin irritation potential. Excised human skin is used as the gold standard for the evaluation of dermal absorption, skin metabolism and drug transporters. An overview of the main in vitro and ex vivo alternative models and methods used in the

safety and efficacy testing of cosmetic products and cosmetic ingredients will be presented. The advantages and limitation of each model will be highlighted, with a focus on in vitro dermal absorption testing.

P-4b-19

In vitro evaluation of safety profile of a cosmetic ingredient - 4-methoxychalcone
ABSTRACT #403

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Background and Objectives: Chalcone derivatives constitute important class of compounds within modern medicinal chemistry and also cosmetic chemistry. Many biological activities was proved for various chalcone derivatives including antiproliferative, antimicrobial, antioxidant, and depigmenting properties [1,2]. 4-Methoxychalcone (CAS: 959-33-1) is indexed in an on-line cosmetic database “Cosing” [3] as an antioxidant, bleaching, and skin conditioning cosmetic products ingredient. We evaluated its in vitro safety profile to provide more data in regard of its use. Material and Methods: Tyrosinase inhibition was evaluated using commercially available tyrosinase from mushroom. Inhibition of melanin production was confirmed in mouse melanoma cell line B16-F10. Cytotoxicity was evaluated in human keratinocytes (HaCaT) and fibroblasts (BJ), hepatocytotoxicity in human hepatocellular cell line (HepG2), and neurocytotoxicity in human neuroblastoma cell line (SH-SY5Y). In vitro metabolism studies were performed in Cunninghamella model. Skin irritation and skin permeation studies were performed in reconstructed human epidermis (EpiskinTM). Experimental lipophylicity parameter (RM0) was determined by means of reverse-phase thin layer chromatography. Results: 4-Methoxychalcone inhibited melanogenesis production at safe concentation of 12.5 μM. Cytotoxicity evaluation confirmed safety at 25 μM in HepG2 and BJ as well as 10 μM in HaCaT and SH-SY5Y cel lines. Tests in reconstructed human epidermis (EpiskinTM) showed no skin irritation potential and proved dermal bioavailability. The tested compound was able to cross epidermis barrier and also accumulate inside the epidermal tissue. In vitro metabolism studies proved extensive biotransformation processes in models utilizing three Cunninghamella species. RM0 was found to be 3.41. Discussion and Conclusion: The performed research confirmed depigmenting potential of the tested compound. The results of in vitro safety evaluation showed that 4-methoxychalcone is safe for the use in cosmetic products. However, some cytotoxicity in higher doses was observed so further tests could be performed to establish maximum safe concentration in cosmetic products.

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P-4b-20

Pre-Clinical in vitro assessment of tobacco-free nicotine pouch products
ABSTRACT #269

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Background and Objectives: Smoking combustible tobacco products like cigarettes is

known to cause serious disease in smokers, including lung cancer, heart disease and emphysema. Recent advances in technology have facilitated the creation of a range of new and innovative tobacco-free nicotine pouch (TFNP) products with the potential to be significantly less harmful than cigarettes. Typically, TFNPs are small pouches containing high-purity pharmaceutical-grade nicotine within a plant fibre-based substrate alongside other high-quality ingredients such as flavourings. The objective of this study was to characterise the in vitro toxicological activity of two commercially available TFNP extracts and where relevant compare any biological responses to cigarette smoke. Material and Methods: An extraction method based on the International Organization of Standardization 10993-12 was utilised to generate test articles with nicotine in phosphate-buffered saline, quantified as a dosimetry marker. Extracts were assessed using regulatory in vitro assays (Neutral Red Uptake, Ames and In Vitro Micronucleus tests), GARDSkin® sensitisation assay, and a 3D human oral epithelium tissue (Endpoints measured: cytotoxicity – LDH & MTT, barrier integrity - TEER and histology – H&E & Tunnel). Results: TFNPs were negative in both Ames and IVM tests, and demonstrated weak cytotoxicity in the NRU assay (a 99% reduction in cytotoxicity when compared to cigarette total particulate matter), under the conditions of the tests. Additionally, TFNPs were classified as non-skin sensitisers in the GARDSkin® assay and showed minimal to no cytotoxic or barrier damaging activity in the 3D human epithelium tissue model when compared to controls. Discussion and Conclusion: The data presented adds to the emerging body of evidence that TFNPs have low biological activity compared to combustible cigarettes and thus have the potential to make a meaningful contribution to harm reduction.

P-4b-21

Skin sensitization of “challenging” compounds: in vitro strategy applied to bio-based ingredients

ABSTRACT #130

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Assessment of skin sensitization potential is mandatory for any ingredient dedicated to topical applications. Since the ban on animal testing, a battery of in vitro tests covering the key steps of the Adverse Outcome Pathway for skin sensitization shall be implemented. From this perspective, different in vitro models were combined with a two-fold goal: - Develop and validate a testing strategy suitable for “challenging” ingredients (i.e. poorly water-soluble, surfactants, complex substances) - Explore the applicability of the selected strategy to other complex ingredients, focusing on market demand for bio-based compounds. Two validated methods were used: 442D (ARE-Nrf2 Luciferase Test Method- Keratinosens), 442E (human Cell Line Activation Test h-CLAT) and the Sens-Is assay, currently in the work plan of the OECD. The results demonstrated different capability of the models to discriminate sensitizers from non-sensitizers. Therefore, a sequential strategy was set up combining 1 to 3 models, depending on the results, with Sens-Is as a starting test (1:Sens-Is; 2:h-CLAT; 3:KeratinoSens). This experimental strategy reached 88% accuracy with a minimum risk of a false negative conclusion. In a second step, the strategy was experimented on diverse ingredients categories, derived from green chemistry or plant extraction: emulsifier, emollient, texturizing agent, active ingredient, fragrance. The results on known natural sensitizers confirmed the reliability of the testing strategy and led to adoption of this approach in current development projects. For new ingredients candidates, positive answers prompted to discard them while negative answers allowed to move to final safety evaluation on human volunteers. Based on overall experience, the scope of the methodology was enlarged to various ingredient categories and chemical natures, contributing to place on the market new ingredients, safe for workers and end-users.

P-4b-22

Measurement and culture method of organoid for toxicity assessment of nanomaterials

ABSTRACT #468

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Over the past several decades, increased applicability of nanoparticles (NPs) in agriculture, medicine, cosmetics, and other fields, gives rise to questions regarding their safety assessment and possible toxic effects. Presently, cell-based assessments are the standard form of biomaterial toxicity evaluation amid a persistent lacking of in vitro–in vivo cross talks or in vitro data integrity and robustness. To overcome the shortcoming of conventional methods, organoids are suggested as a promising tool to offer an alternative and efficient assay for evaluating toxicity caused by NPs. However, the organoids, the 3-dimensional multicellular structure, are not grow equally and they require the solid extracellular matrix such as Matrigel®, which has poor penetration of nanoparticle. In this study, we develop the organoid-based toxicity testing of nanoparticle. We found the culture method using minimized Matrigel® for penetration of nanoparticle within organoids. After Matrigel®-embedded culture, the Matrigel® dome were cracked by gently pipetting to separate the organoids from Matrigel®. The separated organoids from Matrigel® maintained their three-dimensional structure over 2 weeks in media containing low concentration of Matrigel®. To test the uptake of nanoparticles, the organoids were exposed to nanoparticles for 24 hours. The results showed that the nanoparticles were significant internalized into the organoids cultured with our method compared to the Matrigel®-embedded organoids. We believe that our method will utilize for organoid-based toxicity testing of nanomaterials.

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P-4b-23

New based on cell assay to evaluate eye stinging potential of chemicals and cosmetic formulations
ABSTRACT #477

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Background and Objectives: The Transient Receptor Potential Vanilloid type 1 (TRPV1) is one of the most well characterized pain-inducing receptors and has been recently identified as a valuable tool to predict eye stinging potential of cosmetic formulations. In this study, using the HEK-293 with high expression of TRPV1 receptor, we sought to predict eye stinging of chemicals and based cosmetic formulations. Material and Methods: The TRPV1 expressing cells was exposed to test substance (stinging and non- stinging) and, after that, TRPV1 activity was evaluated by measuring acute increases in intracellular calcium. Also, the capsaicin, a classical compound inductor of stinging in human was used as a positive control. To confirm the specific activation of the TRPV1 channel, it was used the antagonist of capsaicin, the capsazepine compund. Results: The results showed that this new model was able to distinguish between stinging compounds from non- stinging. In addition, formulations that induced stinging in the human test were also positive using this new cell based assay. Discussion and Conclusion: Our data support that this assay may be a valuable in vitro tool to predict human eye stinging sensation for cosmetic formulations, as well as confirm that the TRPV1 channel is a principal mediator of eye-stinging sensation induced by surfactant-based formulations.

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P-4b-24

Cytotoxicity Profile and Prooxidant Effects of the New Ruthenium Complex HE-10 in Human Skin Fibroblast Cells ABSTRACT #495

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Nitric oxide is an important biological signaling agent that is produced endogenously and it is involved in various physiological processes. The effects of NO on vascular relaxation, synaptic plasticity, autophagy, and apoptosis pathways, as well as its potential therapeutic applications in the regulation of wound healing and anti-inflammatory processes, lead to the search for donors that can deliver NO to desired areas. Transition metal nitrosyls such as ruthenium complexes, which can release NO via light induction, have become a new target for NO donors. In this context, we synthesized a ruthenium (II) nitrosyl-complex incorporating 4'-phenyl-terpyridine and benzoquinone diimine ([Ru(ptp)(o-bqdi)NO](PF6)3), called HE10. Our aim was to investigate the possible prooxidant, and cytotoxic effects of HE-10, which can release NO by white LED light irradiation using VH10 human skin fibroblasts as a model. Cytotoxicity assessment of the treated groups was performed by MTT assay. Nitrite (NO2-) levels as an indirect marker of NO formation in the cell media were measured by Griess assay. Cell cycle analysis, intracellular reactive oxygen species (ROS) / reactive nitrogen species (RNS) levels, and endogenous NO production were measured by flow cytometry. The acridine orange/ethidium bromide staining method was used to observe the type of cell death. Protein expression levels for measuring protein nitration, DNA damage, autophagy flux, proteasome, and type I collagen were analyzed using western blotting. We confirmed that the toxic effect of HE-10 started after the dose of 7.5 μM at both light and dark conditions, and high HE-10 concentrations affected the cell cycle phase distribution and increased intracellular ROS/RNS. Unexpectedly, intracellular NO depletion was observed in the light-triggered group. Nevertheless, we concluded that the prooxidant effects of HE-10 may increase in the presence of light and induce oxidative damage and cytotoxicity in the VH10 cells. [Supported by APVV-18-0336 and LogicLab MSC-ITN [GA No.813920]]

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P-4b-25

Application of fluorescence-based methods for in chemico and in vitro detection of photoreactive chemicals ABSTRACT #504

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Background and objectives: Phototoxicity assessment is mandatory for registering several products/substances. It is well established that reactive oxygen species (ROS) generation is a crucial aspect of a phototoxic dermal response. Thus, methods that address the ROS generation in UV light exposed systems are valuable for photosafety assessment. In this work, we proposed two fluorescence-based in chemico and in vitro assays for quantifying the ROS production by UVA-exposed chemicals. We employed the 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) probe, in presence or absence of cells, consisting as a screening tool to identify

photoreactive substances. Material and methods: We evaluated eighteen chemicals, including phototoxic and non-phototoxic substances. First, chemicals were prepared in Hank's Balanced Salt Solution (HBSS) in three different concentrations (1, 0.5 and 0.25mM) and exposed to 9.6J/cm2 of UVA radiation. Then, solutions were kept in presence or absence of antioxidants for 1 hour and mixed with DCFH-DA solution at 50µg/mL, following a 30 minutes incubation and fluorescence measurement. Moreover, UVA-exposed chemical solutions were also transferred for 96-well plates containing HaCaT keratinocytes (3x10^4 cells/well) in three different concentrations (50 µg / mL, 100 µg / mL and 200 µg / mL), following an 1 hour incubation. Then, cells were washed, incubated with DCFH-DA solution for 30 minutes, and lysed for further fluorescence measurement using a fluorimeter. Results: The results showed that phototoxic chemicals promoted a significant increase in ROS generation in both in chemico and in vitro models by increasing the fluorescence signal due to DCFH-DA oxidation. This effect was significantly neutralized by antioxidant presence. Discussion and conclusion: We showed that applying a low-cost fluorescence-based method was useful for identifying photorreactive chemicals. Besides, we demonstrated that ROS generation occurs in both intracellular and extracellular compartments after chemical UVA-exposure, contributing to the mode of action (MoA) elucidation of phototoxic materials.

Session: 5a. Toxicokinetics and in vitro – in vivo extrapolation

P-5a-1

In vitro biotransformation of propyl-propane-thiosulfonate (PTSO): Identification and characterization of metabolites

ABSTRACT #435

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Background and Objectives: Propyl-propane-thiosulfonate (PTSO) is an organosulfur compound present in Allium species. Several applications in the agri-food sector have been proposed for PTSO, such as food additive in active packaging, reducing oxidation and microbiological contamination of foods. However, the authorization of its use depends on its toxicity evaluation. Therefore, as a part of its toxicology evaluation, in this work we performed a pilot-study of toxicokinetic profile of PTSO joining in silico and in vitro procedures. Material and Methods: An analytical method to determinate the original compounds and the metabolites by UPLC-MS/MS has been developed and validated following EURACHEM and AOAC standards. Concerning phase I of metabolism, PTSO molecular structure have been submitted in RS-WebPredictor 1.0 in order to predict CYP450 mediated metabolism and region selectivity of their several isoforms. Additionally, PTSO has been incubated with rat (male and female) and human liver microsomes and cofactors to later be analyzed by UPLC-MS/MS. On the other hand, in phase II metabolism PTSO has been incubated with microsomes and Glutathione (GSH) or uridine 5'- diphosphoglucuronic acid (UDPGA). Results: The analysis by UPLC-MS/MS discovered the presence of two metabolites originated by hydrolysis and redox reaction in phase I: Dipropyldisulfure (DPDS) and Metil-propane-thiosulfonate (MPTSO). In addition, two conjugates: S-propylmercaptoglutation (GSSP) and s-propylmercaptocysteine (CSSP) in phase II. However, conjugation with glucuronic acid does not seem to be a metabolic pathway for this additive. Discussion and Conclusion: We can establish a metabolic pathway for PTSO before its commercialization.
Acknowledgements: Junta de Andalucía (Project P18-TP-2147) for its financial support. Antonio Cascajosa Lira thanks the Spanish Ministerio de Universidades for funding FPU grant (FPU2019-01247).

P-5a-3

Preliminary results to the determination of dermal toxicological reference values for a neurotoxic organophosphorus agent

ABSTRACT #362

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Dermal penetration assessment for chemical warfare agents is currently very sparse even if it can largely contribute to the systemic toxicity of those chemicals especially with certain very persistent and non-volatile organophosphates. The CBRN French Toxicology Department is developing new approaches to assess the contribution of skin penetration of organophosphorus compounds to their systemic toxicity by combining an experimental in vitro approach and a mathematical modelisation approach. The in vitro assays use the Franz-type diffusion cell with a piece of human skin placed between two glass compartments one of which is the receiver solvent compartment where the dermal penetrated toxic can be sampled as frequently as wanted. Penetration rates through the skin surface up to the blood compartment are modeled using internet available software developed by the American Industrial Hygiene Association and which is called IH Skin Perm. The mathematical model is being validated for VX and gives very predictive results for amounts of product in the skin compartment and the receiver solvent compartment over hours after deposition. The model highlights the importance of surface of exposure to the penetration rate of VX although this parameter is rarely taken into account for dermal risk assessment. This observation has an impact on the skin decontamination delay (determination of the skin surface amounts profile) and on the adverse effects appearance delay (determination of the blood amounts profile).

P-5a-4

PBK Modelling Of In Vivo Distribution Kinetics In The ONTOX Project

ABSTRACT #295

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Background and Objectives: PK-Sim® and MoBi® are well-established Physiologically Based Kinetic (PBK) modelling and simulation tools which are continuously developed and qualified under the Open- Systems-Pharmacology Suite (OSPS[1]). They enable PBK modelling of ONTOX relevant target organs (liver, kidney and developing brain) as well as the necessary flexibility to include relevant absorption, distribution, metabolism and excretion (ADME) processes. The aim of this work was to evaluate and use these tools for the purposes of the EU- funded ONTOX project to simulate in vivo distribution kinetics of selected case study chemicals. Material and Methods: Using the EPAA-LJMU PBK[2,3] model database and scientific research engines (e.g. PubMed), we screened the literature for existing PBK models, relevant input parameters and information to support model development and evaluation. Several peer-reviewed PBK models of ONTOX case study chemicals had already been implemented in PK- Sim®/MoBi®, we used, reproduced and evaluated those using in vivo PK data (e.g. Deltamethrin, Caffeine, VPA). We simulated whole-body distribution down to the target organ level for relevant external exposure scenarios. We evaluated those simulations to inform in vitro testing strategies. Results: Both tools were explored and found to be suitable for the needs of the PBK modelling framework of the ONTOX project. This work will present the ONTOX PBK modelling strategy, PBK models of case study chemicals selected by the consortium, how they were generated, and how PBK simulations can be used within the ONTOX project. Discussion: First, in vivo PBK simulations will be used to inform in vitro testing strategies. In a later phase, they can be leveraged to develop a framework for ab initio prediction of PK of new compounds. Further, they will enable probabilistic risk assessment by relating predicted target organ concentrations to toxic effect models, for example, based on the quantitative adverse outcome pathway concept.

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P-5a-5

Developing toxicokinetic models for chemical risk assessment of Homosalate using MOIE - a Cosmetics Europe Case Study
ABSTRACT #482

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Innovation in developing cosmetics has become a huge challenge since the implementation of animal testing ban for Cosmetics in Europe in 2013. Indeed, the ban on generating new animal data for the safety evaluation of cosmetic ingredients has pushed the cosmetics sector to develop a new toolbox and novel approaches in risk assessment. Several aspects covering the route of administration, species, and pharmacokinetics (PK) have been conceived. Within the Next Generation Risk Assessment (NGRA) and read-across approach for the human health risk assessment, PK data are essential for the prediction of systemic concentrations and to refine the safety margin. By building Physiologically Based Kinetic (PBK) models, we aimed to calculate the Margin of Internal Exposure (MOIE) for Homosalate (a UV filter) i.e. the ratio of animal plasmatic concentrations (Cmax or AUC) at the NOAEL and human plasmatic concentration at the estimated exposure dose. To achieve this, we identified the rat NOAEL of Octisalate and Cyclohexyl Salicylate, read-across chemical analogues. Clinical data are available for Homosalate; therefore, it was possible to validate the human PBK model. However, the validation step for the rat PBK model lacked sufficient in vivo data. Therefore, we could investigate two options for overcoming this issue using analogues with available in vivo PK data. According to OECD guidance 331, we could use a read-across approach with a chemical identified as a PK analogue, i.e. analogues with similar in vivo PK parameters to those predicted for the target chemical. Otherwise, we could use chemicals structural analogues having different PK parameters. In conclusion, a validated human PBK dermal model was generated. The development of the rat PBK models are in progress and this approach will pave the way for risk assessment using MOIE calculations in the absence of in vivo legacy data for the target chemical.

Session: 5b. In vitro systems to assess respiratory toxicity

P-5b-1

INHALATION TOXICITY ASSESSMENT OF AN AEROSOLIZED SUNSCREEN PRODUCT WITH AN IN VITRO PULMONARY MODEL
ABSTRACT #46

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Background and Objectives: Evaluation of the toxicity of cosmetic products generating a cloud of particles and its ingredients must consider the different exposure routes. Although the main exposure route is dermal, inhalation is a potential one for this type of cosmetics. Previously, the systemic toxicity assessment after exposure by inhalation to a cosmetic product was carried out. Thus, it was interesting to complete this study by assessing local toxicity in the upper respiratory tract using an in vitro pulmonary model. The aim was to assess the repeated dose toxicity of an aerosolized sunscreen product on a three-dimensional model of fully differentiated human upper airway epithelium: MucilAir™. Material and Methods: The potential toxic effects after two weeks of daily exposure were evaluated on an epithelium reconstructed from a pool of nasal donors. The cells were exposed to the tested product at the apical level, at 4 concentrations: 1%, 10%, 50% and 100%. Several endpoints were analyzed: cytotoxicity, epithelial functions (cilia beating frequency, mucociliary clearance), airway inflammation/remodeling (IL-8/MMP9) and histology. Results and Discussion: Exposure at 50% and 100% of sunscreen for two weeks has induced dose- and time-dependent cytotoxicity. The IL-8 and MMP9 release was also altered. Exposure at 10% didn't affect the cell viability and airway

epithelial functions. The IL-8 and MMP9 release and mucus production didn't increase. However, epithelium reshaping and vacuole formation were observed. The solar spray was well tolerated at 1% (tissue structure similar to control). Although this exposure is the closest to real-life conditions, it remains a conservative situation (6.6 times higher than 0.15% of respirable particles released by the sunscreen). Conclusion: The study showed that exposure to the sunscreen diluted at 1% didn't induce local and systemic toxicity in the upper respiratory tract. The epithelium was therefore functional, able to exercise his clearance and defense activity.

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P-5b-2

Novel fully primary human airway epithelium-alveolar macrophages in vitro co-cultures models to study host pathogen interactions
ABSTRACT #116

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Being the first line of defense of the organism against airborne pathogens like bacteria and viruses, the respiratory epithelium acts as a physical barrier as well as an efficiency mucociliary escalator. Furthermore, the airway epithelium is also a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections. Many animal models have been used to study lung infections, but the relevance and predictability of animal models are still questionable. Here we established a new co-culture model using well characterized, standardized human airway epithelium such as MucilAir™, SmallAir™ and human lung macrophages (CD45+,HLA-DR+, CD206+, CD11b+and CD14-) for studying bacterial and viral infections. The alveolar macrophages were not only able to adhere to the epithelial cells, but also functional: The macrophages were capable of phagocytosis, evaluated using pHrodo™ Red (S cerevisiae Bio-particles Conjugate). Moreover, the co-culture models respond to pro-inflammatory stimuli such as LPS, TNF-A and Poly(I:C) with an increased IL-8 secretion. Upon bacterial infection with methicillin-susceptible Staphylococcus aureus strain (MSSA), compared to MucilAir™ monocultures, MucilAir™-macrophages showed stronger immune responses: (i) a reduction of bacterial growth (up to 1.5Log10 CFU) and (ii) decreased upregulation of IL-8 and B-defensin-2 secretions. Interestingly, greater difference was observed for Streptococcus pneumonia (Sp19F): The presence of macrophages led to a decrease of 3.5Log10 CFU after 24 hours of culture (N=12) versus MucilAir™ alone. These novel in vitro models might find applications in understanding the role of immune-epithelial cell interactions in infection diseases

P-5b-3

Utilisation of human 3D bronchial tissues for e-cigarette assessment
ABSTRACT #250

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Background and Objectives: With the rise in popularity of e-cigarettes with adult smokers as a potentially less harmful alternative to combustible cigarettes, there is a need for better understanding of the potential biological impact of these products. In vitro techniques fulfil that need, allowing rapid and robust assessments. Here we describe the results from a range of studies utilising human 3D bronchial tissues for the assessment of e-cigarettes. Methods and Results: Initial investigations acutely exposed EpiAirwayTM tissues to 400 puffs of e-cigarette aerosol, resulting in no disruption to barrier function, cellular viability, DNA integrity (γ-H2AX) or cytokine secretion compared to air controls.

Conversely, cigarette smoke impaired barrier function, induced a strong cytokine response and reduced cell viability to ~30% after 45 puffs. Building on this study, and to obtain a deeper mechanistic insight, the transcriptomic responses of MucilAirTM tissues exposed to matched non-cytotoxic concentrations of either cigarette smoke or e-cigarette aerosol were assessed. Exposure to cigarette smoke resulted in a transcriptomic response at 4 hours, with 2199 differentially expressed genes, which reduced by 50% after 48 hours recovery. E-Cigarette aerosol exposure had a limited impact on the transcriptomic response with only 28 differentially expressed genes, with this response reducing by 50% at 48 hours recovery. Finally, MucilAirTM tissues were repeatedly exposed to either cigarette smoke or e-cigarette aerosol over 28-days. Cigarette smoke caused a marked dose response, with significant changes to tissue morphology, proinflammatory response (IL-1β, IL-8, MMP-1, MMP-3, MMP-9 and TNF-α) and decreases in cilia activity. Tissues exposed to e-cigarette aerosol were indistinguishable from air control tissues across multiple endpoints, under the conditions of test. Discussion and Conclusion: The 3D tissues used in these studies show great versatility for a range of different endpoints and exposures. They may be a considered a key component of product assessment strategies.

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P-5b-5

IN VITRO AIR-LIQUID INTERFACE EXPOSURE OF LUNG CELLS TO THERAPEUTIC AEROSOLS FOR PRECLINCAL DRUG DEVELOPMENT ABSTRACT #148

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Until now, in preclinical in vitro lung research, inhalable drugs are mainly dispersed and pipetted into cell culture medium or onto the lung cell surface layer to study e.g. safety/toxicity, uptake, and/or efficacy. However, treatment of lung diseases is predominantly performed using aerosols and not by liquid application. Delivery of a therapeutic aerosol to lung cells cultivated and exposed at the air-liquid interface (ALI) is a more realistic way for testing inhalable drugs. The objective of this study was to validate an ALI exposure system for safety testing of inhalable pharmaceutical compounds. As a first case study, the antiviral effect of inhaled Remdesivir was investigated and compared with classical liquid application. VITO has a platform with three commercial ALI systems (VITROCELL® 6/4, 24/48 and Cloud 12) and one in-house developed system (NAVETTA). This allows us to choose the most relevant system depending on the characteristics of the inhalable drug. Dry powder formulations applied in e.g. MDI and DPI's can be exposed to lung cells at the ALI using the Precise Inhale and 6/4, 24/48 or NAVETTA exposure system. Nebulized drugs can be exposed to lungs cells at the ALI using the Cloud system. It is equipped with a clinically relevant nebulizer (Aerogen; Ireland) providing a similar particle size and dose rate within the same exposure time (minutes) as in clinical settings. The deposition efficiency (~60%) of the Cloud was tested using fluorescein in stainless steel inserts. Furthermore, model compounds i.e. lactose (negative control), amine-modified polystyrene nanoparticles (positive control cell

viability), and lipopolysaccharide (positive control inflammatory response) were tested in A549 cells. Remdesivir (GS-5734, MedChemExpress, HY-104077) is tested in 3D fully differentiated human bronchial epithelial mono-donor MucilAir™ tissues (Epithelix Sàrl, Swiss). Exposing lung cells at the ALI to therapeutic aerosols is a promising technology for safety testing of inhalable pharmaceutical compounds.

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P-5b-6

New concepts in inhalation toxicology: The in vitro approach ABSTRACT #182

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An advanced procedure for in vitro inhalation testing was developed based on an optimized exposure device (P.R.I.T.® ExpoCube®). The general applicability of the P.R.I.T.® ExpoCube® for acute lung toxicity testing was assessed with five commercial fungicides and sodium-dodecyl sulfate (SDS) as test items and positive control, respectively. Highly concentrated aerosols were generated from small amounts of powders using the PreciseInhaleTM device and utilized to expose human lung alveolar epithelial (A549) cells. Cytotoxicty was measured by means of WST assay and dose-response curves were established based on mass per cell surface based dosimetry considerations. EC50-values from in vitro testing were correlated to LD50 values from acute rat inhalation in vivo testing and showed good predictivity. In order to estimate the extent of systemic absorption upon inhalation, apparent permeability coefficients (Papp) were determined for the inhalable antibiotic ciprofloxacin hydrochloride monohydrate (CHM) in human lung barrier models and used as input parameters for our in-house developed lung PBPK model. Two different cell models were used to account for different lung regions: The Calu-3 cell line producing features of differentiated small airway epithelial cells and functionally immortalized human alveolar epithelial cells (CI-hAELVi), resembling alveolar type 1 cells. Both barrier models were exposed to CHM aerosol under ALI conditions and the concentration of CHM was determined in medium and cell samples by LC-MS/MS. Papp coefficients obtained from these experiments were used to predict human blood levels. Blood levels of Ciprofloxacin calculated by the PBPK model showed the same concentration-time course and concentration range as measured in human patients. In conclusion, our findings suggest that a combined approach utilizing in vitro data and in silico modelling is able to predict acute local lung toxicity as well as the bioavailable dose after inhalation exposure in humans. Future studies will extend the work to toxicity after repeated exposure.

P-5b-7

UNDERSTANDING THE TOXICITY OF A BTEX MIXTURE: AIR/LIQUID INTERFACE EXPOSURE OF ORGANOTYPIC LUNG CULTURES ABSTRACT #310

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Background and Objectives: Several epidemiological studies have shown an increase in the prevalence of respiratory and allergic pathologies linked to exposure to volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene and xylenes (the so-called BTEX mixture). Very few experimental studies have focused on the harmfulness of the quaternary mixture. The majority of the studies focus on the toxicity of a single compound or binary mixtures. However, actual exposure to these compounds is most often multiple. As a result, data are incomplete, particularly in terms of each compound's contribution to overall toxicity. Our study aims to answer the following questions: What is the pulmonary toxicity of BTEX whether used alone or in mixtures? What are the mechanisms of action involved in the toxicity of BTEX in the contribution to respiratory pathologies? Materials and Methods: Based on occupational and indoor air exposures, a PBPK model was used to determine realistic doses of exposure to BTEX. An organotypic culture model of human respiratory epithelium (MucilAir™) was exposed 8h/day in air/liquid interface (ALI), acutely and repeatedly during one week to BTEX individually and in quaternary mixture. Phenotypic markers (cytotoxicity, mucins and cytokines production), transcriptome and proteome were characterized. Results: Exposure to BTEX induced changes in cytokine production and proteome for both exposures. Significantly, repeated exposure allowed to show a decrease in cell wall integrity (TEER) and an increase of mucin production. Discussion and Conclusion: Our findings showed that 3-D in vitro tissue model is well suited to study the effects of repeated exposures. This model can help in investigating the cocktail effect observed when exposed to a complex environmental mixture. The goal is then to identify the initiating and key events allowing to complete existing adverse outcome pathways (AOP).

P-5b-8

Fine dust toxicity evaluation on uterus using endometrial stem cells ABSTRACT #267

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Background and Objectives Fine dust which has small diameter characteristics is one of the causes of air pollution, and it caused toxic effects on cell levels. Many studies suggested that female fertility could be associated with fine dust exposure. However, it is not easy to evaluate the toxic effects of fine dust on the reproductive system. Organ-specific toxicity evaluation has been dependent on in vivo tests. In vitro evaluation, however, is necessary for technical, economical, and ethical reasons. One of the novel toxicity evaluation ways is stem cell evaluation, and we isolated endometrial stem cells from the uterus for organ-specific evaluation. The objective of this study is to evaluate fine dust toxicity in the uterus using endometrial stem cells. Material and Methods Stem cell viability was tested using an MTT assay. Migration ability was evaluated by the transwell system and western blot of MMP2, 9. Cell morphology was observed with immunofluorescent stain. Cell damage was evaluated by DAPI stain and western blot of capase3. Cell aging was observed with β-galactosidase activity assay and real-time PCR of P16, P18, P21, and IL6. Stem cell differentiation ability was tested using adipogenic differentiation and osteogenic differentiation assay. Fine dust was melted in cell culture media. Results The fine dust declined the cell viability dose-dependently. Cell migration ability is also decreased by fine dust. The morphology of the stem cell was changed to a polygonal shape in the fine dust treated group. Cleaved apoptotic nuclear and cleaved caspase3 were increased in the treated group. β-galactosidase and the genetic markers for aging, P16, p18, p21 and IL6 was increased by fine dust. Adipogenic and osteogenic differentiation ability of endometrial stem cells was decreased in the fine dust treated group. Discussion and Conclusion Overall, the fine dust mixed media had toxic effects on endometrial stem cells.

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P-5b-9

Flavors in e-cigarettes - a tasty hazard? ABSTRACT #385

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Background and Objectives: The amount of toxic substances in e-cigarette aerosols is reduced compared to traditional tobacco cigarette smoke. Flavors used in e-liquids represent a substantial contribution to the attractiveness of e-cigarettes. Although, the flavors are proven safe for oral ingestion, major concerns are the uncertainties regarding inhalational toxicity. The objective of this work was to optimize an in vitro method for the investigation of flavors in reference e-liquids, without requiring complex and expensive equipment. Materials and Methods: Reference e-liquids containing either vanillin, limonene, linalool, eugenol or cinnamaldehyde were prepared according to ISO 17375 at three concentrations and were vaped with a linear LM4E Borgwaldt vaping machine. One hundred puffs were collected in impingers, with a 55 mL puff volume, 3 s duration and a 30 s frequency. The conditioned medium (CM) was analyzed for flavor concentrations using headspace-GC/MS and used for a 24 h exposure of A549 cells. Further, pure flavors in cell culture medium at expected concentrations as in CM were used for a 24 h exposure of A549 cells. MTT and Lactate dehydrogenase (LDH) assays were performed following exposure. Results: A concentration-dependent decrease of metabolic activity was observed for all flavors except linalool when tested as pure flavor. No effects on metabolic activity in response to CM were observed for any flavor. Initial results of the analytical data show that CM contains lower flavor concentrations as in original reference e-liquids. These results need finalization. Discussion and Conclusion: Cytotoxic effects by pure flavor compounds compared to CM indicate a higher hazard potential. This could be due to low solubility of flavors in cell culture medium leading to low concentrations in CM. On the other hand, unidentified chemical reactions could occur during vaping leading to lower concentrations of analyzed flavors in CM. Therefore, the vaping method needs further optimization.

P-5b-10

Dosimetry in inhalation: an in vitro inhalation model to study the relevance of dose parameters related to particle mass and particle number ABSTRACT #384

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An ongoing discussion exists on the relevant metric relating dose and biological effects regarding the toxicity of inhaled particles. Deposited particle surface versus number or mass are under discussion correlated to different types of aerosols (e.g. poorly soluble, combustion, cigarette smoke). An experimental model was established to study the otherwise unchanged toxicological properties of an aerosol in defined states regarding particle size, mass- and number concentration, giving the unique opportunity to test the most relevant dose-metric. The test setup consisted in a source of highly concentrated aerosol of ultrafine particles (2-stroke engine exhaust) and an ageing unit based on Brownian coagulation allowing for variation of number and mass concentration independently from each other. Acute local biological effects were studied by application of an optimized exposure device for air-lifted interface cultures (ALI, P.R.I.T.® ExpoCube®), a human lung cell line (A549) and read-outs for viability (WST-1), mitochondrial membrane potential (JC-1) and Interleukin-8 release. Four different conditions were generated, representing different number concentrations and mean particle sizes at comparable aerosol mass concentrations. Dose-response relationships were established after 30- or 60 minutes exposure. Particle deposition was validated analytically, exposures to filtered aerosol enabled discrimination between particle- and gas-phase related effects. Results clearly demonstrated a strong correlation between particle mass dose and biological effect, not particle number dose. It is expected that these characteristics are a consequence of the physico-chemical

characteristics of the aerosol particles (oily) and therefore are in good agreement with classification of the relevant dose metrics which are under discussion for other aerosol types. The results indicated a significant characterization of complex relationships regarding dosimetry in inhalation by application of the in vitro inhalation model. By this way, it may be possible to get more insight and experimental evidence to find most appropriate dose metrics also for other types of aerosols.

P-5b-12

Ex vivo porcine precision cut lung slices (PCLS) for pulmonary toxicity assessment
ABSTRACT #478

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Background and Objectives: traditionally tests to assess inhalation toxicity are performed in animals, with no alternative method to animal experimentation validated and available for this purpose. Ex vivo porcine lungs, residues from alimentary industry, has been used to produce PCLS (Precision Cut Lung Slices), showing similar characteristics of human's lung, with a high potential for preliminary tests in the evaluation of toxicity. Therefore, the objective of this work was to characterize an ex vivo porcine model of PCLS to evaluate pulmonary toxicity mechanisms. Material and methods: The PCLS were prepared using the Tissue Slicer DTK-3000W equipment. To analyze the tissue viability, the tetrazolium reduction method was used. Tissues were processed and stained with hematoxylin-eosin for histological analysis. The tissues were challenged at different concentrations of aerosolized paraformaldehyde by nebulization in the Vitrocell® Cloud 12 exposure chamber on the fifth day of cultivation. The production of ROS was measured by DCFH-DA staining and assessment of mitochondrial activity was measured by MITOTRACKER® staining. Caspase was evaluated by Utilizing indirect immunofluorescence technique. Results: Preliminary results suggest that the ex vivo porcine PCLS responds to paraformaldehyde exposure by reducing cell viability, in addition to producing ROS, reducing mitochondrial activity, and increasing caspase. Discussion and conclusion: The ex vivo porcine model of PCLS can be an important tool for the assessment of pulmonary toxicity, reproducible and with low cost.

Session: 6a. In vitro methods for safety testing of biopharmaceuticals/biotherapies/ vaccines

P-6a-1

Liver spheroid co-cultures with fresh or cryopreserved hepatocytes and endothelial cells as tool to investigate metabolism and hepatotoxicity
ABSTRACT #1

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Background and Objectives Primary human and animal liver cells are the gold standard for all pharmacological-toxicological in vitro studies during drug development. Three-dimensional (3D) cultures became more popular in recent years since they might mimic the in vivo cell morphology, polarity and cell-cell interactions better than traditional two-dimensional (2D) cultures. Due to better access and continuous availability, cryopreserved cells become more popular, but functional differences to fresh hepatocytes might occur. Material and Methods Here, 3D spheroids were generated in U-bottom ULA (ultra-low attachment) plates with fresh and cryopreserved human hepatocytes as single culture, and in coculture with liver endothelial cells to display an even more physiological situation. The metabolic activity of different Cytochrome P450 activities and the acute toxicity for known substances like acetaminophen were tested and compared for fresh and cryopreserved cells. Results Spheroids from fresh and cryopreserved human hepatocytes were inducible for CYP

activity, but a strong variability of basal CYP activity and CYP-induction potential was detected between different donors especially in fresh hepatocytes. A higher CYP activity and CYP inducibility was determined in 3D-cultures compared to 2D. In total, CYP inductions demonstrated a better and more stable inducibility in spheroids from cryopreserved cells. The ATP assay displayed almost the same concentration dependent toxicity for acetaminophen in 3D and 2D cultures. In contrast, the AlamarBlue assay didn't show this dependency for spheroids, here only the highest concentration led to a decrease in viability. Discussion and Conclusion Our results indicate that differences may exist between 3D-cultures with fresh and cryopreserved hepatocytes and in comparison to standard 2D-culture models. These differences may lead to different and conflicting results in the assessment of drug toxicity and drug-drug interaction.

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P-6a-2

A novel polymer generates cell repellent surfaces and allows 3D cell culture

ABSTRACT #23

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Background and Objectives: Once isolated, primary cells are usually placed in an artificial environment in protein coated plastic or glass containers, where they assemble as 2D layer, reshaping their morphology and behavior. Limitations in such 2D cultures have been increasingly recognized and different systems have been established which allow growing primary cell as 3D spheroid. Low attached surfaces are chemical defined and can beeasily handled, but remaining interaction of cell and surface coating may impact the morphology and behavior of the cell spheroid. Methods: Here, a cell repellent surface with Biofloat solution was generated in uncoated non-tissue culture treated U-bottom plates. The plates were used to assemble 3D spheroids with cryopreserved non-human primate hepatocytes. These cultures were compared to spheroid cultures in U-bottom ULA plates available on the market. Time for spheroid formation and functionality of 3D cultures in terms of metabolic CYP P450 activities and their inducibility were investigated. Results: Round spheroids with clear borders were formed. Clear differences could be found in shape and kinetic of spheroid formation for the different products. Pre-coated benchmarks tend to form spheroids, which were less regular and needed more time to form a compact aggregate. This is likely due to remaining adhesion points and interaction with the plastic surface on plates which are not fully inert. One should carefully choose the equipment as this has a major impact on quality of the cell aggregates. Spheroids from cryopreserved Cynomolgus hepatocytes were inducible for CYP activity in a similar extent in Biofloat plate and all other tested ULA plates. Conclusion: These results indicate that Biofloat is an optimal polymer formulation to generate cell repellent surfaces and thereby making 3D cultures possible. The coating provides the possibility to attach functional groups anchored in a fully inert background which are usable for other cell culture purposes.

P-6a-4

Validation of the ToxProfiler reporter assay and its application in mechanistic toxicity testing

ABSTRACT #417

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The in vitro chemical safety assessment mostly rely on general cytotoxicity endpoints, but with the new approach methodologies (NAMs) more information can be provided on the toxicological mode-of-action (MoA) which improve in vitro toxicity predictions. Here we present ToxProfiler, a new reporter based in vitro assay and NAM, that can accurately quantify seven key toxicological relevant endpoints: oxidative stress, genetic stress, endoplasmic reticulum stress, ion stress, protein stress, autophagy, and inflammation. Activation of these fluorescent reporters is visualized and quantified by high content imaging to derive a quantitative toxicological profile of test substances. Here ToxProfiler was validated using a selection of 40 reference chemicals with known toxicological MoAs. Concentration response curves for each endpoint were generated, from which point-of-departure (PoDs) were then derived. Data was visualized using heatmaps in which the hierarchical clustering was used to group chemicals with a similar biological activity. To further explore the value of this approach, we next screened a group of compounds with a similar chemical structure: the angiotensin-II-receptor antagonists Sartans. We found that these compounds mainly induced ER stress, but some members of this group induced oxidative stress and genetic stress. Since some of these chemicals are metabolized in the human body, we also performed the ToxProfiler assay in the presence of S9 (induced rat liver extract). For some Sartans (e.g. Fimasartan) we found that metabolism plays an important role and the metabolite(s) were more potent than Fimasartan itself on the ToxProfiler endpoints. The heatmap/clustering facilitated easy separation of the biological active Sartans from the less potent group members. To summarize, we validated ToxProfiler, a new imaged-based in vitro reporter assay. ToxProfiler provides relevant mechanistic toxicity information for seven endpoints to reveal the toxicological MoA. ToxProfiler can be applied in the safety assessment for compounds from the pharma and different chemical industries.

P-6a-5

Genotoxicity assessment of potentially mutagenic nucleoside analogues using ToxTracker

ABSTRACT #418

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Since the late 1950s researchers have developed nucleoside analogues to target viral replication and infections. Following the HIV/AIDS outbreak several early nucleoside analogues were employed. These compounds can interfere with viral transcription and translation events targeting DNA and RNA polymerases. The downside is that this is not necessarily restricted to the viral polymerases but can also target the host polymerases and have detrimental effects causing for instance mutations and carcinogenicity. In a retrospective study, we applied the ToxTracker® genotoxicity reporter assay to assess the potential of a selection of representative nucleoside analogues to cause genotoxicity. Among the early nucleoside analogues, several triggered a genotoxic response, in line with historical data (e.g. Zidovudine). Generally, later generation nucleoside analogues did not trigger the genotoxicity reporters in ToxTracker but in some cases the reporters for oxidative stress and protein damage were activated. Remdesivir and Molnupiravir, two nucleoside analogues that are currently being repurposed for Covid-19 treatment, were designed as pro-drugs and will after metabolization release their respective active metabolites. Neither pro-drug nor their metabolites triggered any genotoxicity biomarkers but the metabolite of Molnupiravir (EIDD-1931) did trigger oxidative stress, p53 and protein damage at concentrations relevant for human treatment. Overall, the ToxTracker data were in line with the in vivo micronucleus assay while the AMES test for the nucleoside analogues had problems to assess their mutagenic potential. Nucleoside analogues continue to be attractive treatment options for viral infections. ToxTracker readily distinguished between the genotoxic analogues and those with different profiles and provides a basis for clustering and potency ranking, offering a comprehensive tool to assess the toxicity of nucleoside analogues.

P-6a-6

Exploring the use of spheroid cultures of human liver cells for the mechanistic testing of carcinogenic compounds
ABSTRACT #270

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Background and objectives Before any new chemical can be placed on the market, its carcinogenic potential, along with many other toxicological endpoints, must be thoroughly investigated. However, the current carcinogenicity assessment has limitations in predicting the potential risk associated with non-genotoxic carcinogenic compounds. As a result, new predictive models for more efficient and reliable hazard identification are urgently needed. Spheroid cultures of liver cells have been considered promising models in this regard. Material and methods Spheroid cultures consisting of human hepatocyte C3A cells and human liver stellate LX-2 cells in ratio of 10:1 were set up. The spheroid cultures were exposed to 4 different concentrations of genotoxic liver carcinogenic compounds (benzo[a]pyrene, hydroquinone and aflatoxin B1), non-genotoxic liver carcinogenic compounds (dioctyl phthalate, acetamide and methapyrilene) and non-carcinogenic compounds (D-mannitol, tolbutamide and clonidine) for 72 hours. The spheroid cultures were monitored for oxidative stress using 2′,7′-dichlorodihydrofluorescein diacetate (Incucyte and flow cytometry), mitochondrial dysfunction using Rhodamine 123 (Incucyte and flow cytometry), endoplasmic reticulum stress using Thioflavin T (plate reader and flow cytometry), apoptosis (caspase 3/7 activity) and inflammatory cytokines (enzyme-linked immunosorbent assay). Results Hydroquinone and aflatoxin B1 consistently affected the spheroid cultures through increased production of reactive oxygen species, induced depolarization of mitochondrial membrane potential, increased endoplasmic reticulum stress and increased caspase-3/7 activity. No effects induced by other compounds in any of the parameters were observed. Discussion and conclusion There are no in vitro tests validated by the regulatory authorities for identifying the non-genotoxic carcinogenic compounds. The C3A–LX-2 spheroid model could be further explored for this purpose. Therefore, the 3D spheroid cultures of liver cells could be valuable tools for mechanistic testing of carcinogenic effects.

P-6a-7

In vitro study of the potential of new liposomal delivery systems for future medical applications in inhalation therapies
ABSTRACT #393

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Inhalation therapy is established as one of the most promising routes of administration for treating lung diseases. However, it is necessary to focus on the development of assays to evaluate the mechanisms triggered in the interaction of drug delivery systems and lung barriers, under air-liquid interface (ALI) exposure conditions, presenting more realistic physiological conditions of the lung. The objective of this study was to establish an in vitro assay to determine the differences in the deposition and internalization of PEGylated and non-PEGylated liposomes, as well as to determine the differences between the exposure in liquid-liquid interface (LLI) or ALI. The cytotoxicity generated by both PEG and non-PEGylated liposomes was characterized.

Subsequently, the differences in deposition and cellular uptake were evaluated exposing the cell models (A-549 and Calu-3) to both types of liposomes, in LLI (in submerged medium) and ALI (through Vitrocell Cloud 12 exposure system (VITROCELL® Systems GmbH)). After testing the safety of the compounds, results with A-549 cells indicate an increase in the acquisition of non-PEGylated liposomes compared to those with PEG. For Calu-3 cells, there is a longer retention time and permeability of PEGylated liposomes in the pulmonary mucus. In addition, in both cell lines, there is a greater acquisition of liposomes at the LLI compared to ALI. PEG can increase stability and penetration of the delivery system through the mucous layer. However, its presence may hinder the cellular uptake. In addition, differences in liposome delivery between LLI and ALI could be due to the delivery systems applied, as ALI requires a nebulizer or aerosol, while LLI is a liquid application. ALI exposure with nebulizer systems resembles the real physiological conditions and allows to evaluate the interaction of the compounds with the pulmonary barriers. Moreover, depending on the biomedical application, delivery systems with PEG behave differently.

P-6a-8

In vitro microenvironment modifies the neurotoxic response of SH-SY5Y cells ABSTRACT #412

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Introduction: Thirty-four percent of clinical trial safety failures are related to the brain, pointing to the lack of relevance of pre-clinical models for neurotoxicity assessment (Cool et al. 2014). Among these models, the SH-SY5Y neuroblastoma cell line is the most widely used for drug discovery (Kovalevich and Langford, 2013), but its differentiation into neurons in vitro is questioned. The objective of this study was to compared the SH-SY5Y differentiation methods in order to develop a relevant in vitro model for neurotoxicity assessment, including both mature neurons organized in 3D and the Extracellular Matrix (ECM). Material & Methods: First, we screened the 24 major differentiation media described in the literature to differentiate these cells into neurons in 2D cell culture, and selected the 3 most differentiating conditions in terms of proliferation slowdown and neurite elongation: retinoic acid, staurosporine, and cyclic Adenosine Monophosphate (cAMP) combined to B21 supplement. Then, we compared their effects on SH-SY5Y cells cultivated in 3D in an hydroscaffold mimicking the ECM, by analyzing the cell proliferation, neuronal proteins expression, and sensitivity to compounds of known toxicity. Results: Immunostainings showed that the three media decreased the cell proliferation, but only staurosporine and B21+cAMP media increased the neuronal marker expression in 3D. Both maturity and sensitivity of SH-SY5Y to the neurotoxic compound 6-hydroxydopamine were higher in B21+cAMP medium. However, cell sensitivity was lower than in 2D. Conclusion: This work highlighted that the microenvironment of neurons, including the ECM and the soluble factors, can modify the neuronal response in vitro, and should thus be considered carefully in academic research and as early as possible in the drug discovery industrial process.

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P-6a-9

THE POTENTIAL OF 3D CELL-BASED PLATFORMS FOR THE SCREENING OF NOVEL DRUG CANDIDATES TARGETING THE HEPATIC PLASMODIUM INFECTION ABSTRACT #124

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The liver-stage of Plasmodium infection is the first step of the parasite's life cycle in the mammalian host, representing an attractive target to prevent disease and achieve malaria radical cure. The asymptomatic character of this stage of infection and the limited access to Plasmodium sporozoites have hampered the development of drugs targeting hepatic parasite forms, including P. vivax or P. ovale dormant forms, hypnozoites. This gap in anti-plasmodial drug development, highlights the need for new preclinical models that better predict the Plasmodium and host responses to different interventions. Here, we present 3D human in vitro platforms suitable for the discovery of novel drugs targeting Plasmodium hepatic infection. The platform relies on human hepatic spheroids generated in stirred-tank culture systems that sustain hepatocyte identity, biosynthetic function, and polarity for at least 3 weeks in culture. We established the Plasmodium infection in hepatic spheroids, in static and agitation-based cultures, showing that P. berghei (Pb) infects and develops in spheroids in both conditions. As proof-of-concept of the platforms' applicability for drug discovery, we showed that M5717, a drug candidate under clinical development, efficiently inhibits Pb hepatic infection in this model. The dose- and time- responses obtained for M5717 corroborated this drug's inhibitory effects observed in vivo, proving the platforms' predictive capacity. Moreover, we generated novel preclinical data suggesting that a combination of M5717 with pyronaridine, a blood-stage anti-plasmodial drug, has increased M5717 activity against liver-stage parasites. Employing the developed platform to primary human hepatocytes, spheroids were generated and maintained for at least three weeks in culture. The ability of these spheroids to sustain the infection of human infectious parasites (i.e. P. vivax and P. falciparum) is currently being assessed, highlighting the potential of these tools to be employed in the preclinical development of new treatments targeting the hepatic stage of infection, including hypnozoites.

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P-6a-10

The potential of 3D reconstructed Human intestinal models for biowaiver studies and finished products testing ABSTRACT #106

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The BCS based regulatory biowaiver of clinical bioequivalence studies provides significant relief from the regulatory burden on the development of generic products as well as the ethical advantage of avoiding unnecessary exposure of healthy human volunteers. Over 50% of the world's most used and essential oral, immediate-release drugs are estimated to fall into the BCS Class I and Class III classification, representing an enormous potential for companies developing generic formulations or managing the lifecycles of existing products to save money and time. In the context of in vitro studies, Caco-2 cell lines have become the most frequently used in vitro models to perform such studies. The present work evaluates a novel primary human cell-based 3D organotypic small intestinal microtissues to be a potential pathway for evaluating in vitro bioequivalence, but also

using formulated API to estimate the impact of formulation in promotion of absorption and to make comparison of adult versus pediatric forms which are currently in development, at doses corresponding to those employed in clinics. The permeability coefficients across the microtissues were determined for a panel of benchmark drugs with known human absorption. The reference substances were accurately classified into low and high permeable drugs. The 3D organotypic Human small intestinal tissue model is eligible to elaborate a correlation curve according to BCS-based biowiavers. The predicted fraction absorbed in human determined with the EpiIntestinal model was equivalent in both tested conditions, test adult form (capsule of 500 mg) vs. pediatric form (Sachet content of 500 mg). The object of this presentation is to give an insight into the added value that could bring the 3D organotypic Human small intestinal tissue model, over Caco-2 cells, for selecting appropriate formulations to improve systemic drug exposure or anticipate the impact of a change in formulation for generics or pediatric drug products.

P-6a-11

Mechanistic Investigation of Drug-Induced Liver Toxicity using Human 3D InSight Liver Model
ABSTRACT #131

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Background and Objectives: Human 3D InSight(TM) liver model is more predictive than 2D primary hepatocyte cultures in assessing clinical liver safety of pharmaceutical compounds. Moreover, complex toxicological mechanistic investigations can also be performed in this model. Here, the mechanisms of toxicity of acetaminophen, aflatoxin B1 and trovafloxacin were evaluated in the 3D InSight liver model. Material and Methods: Using different treatments of the 3D InSight liver model, the in vivo mechanisms of toxicity of acetaminophen, aflatoxin B1 and trovafloxacin were evaluated. Results: Acetaminophen produces the toxic metabolite NAPQI when processed by CYP3A4 and CYP2E1. NAPQI is subsequently detoxified by cellular glutathione. Correspondingly, acetaminophen was more toxic in 3D InSight liver models sensitized with the glutathione synthesis inhibitor L-buthionine sulfoximine, suggesting that the in vivo role of glutathione in detoxification of acetaminophen is recapitulated in vitro. Trovafloxacin stabilizes TNF-α signaling, resulting in liver injury in otherwise benign liver inflammatory conditions. Correspondingly, trovafloxacin cytotoxicity was enhanced in 3D InSight liver models treated with lipopolysaccharides, suggesting that the in vivo synergic interplay between trovafloxacin and inflammation is recapitulated in vitro. Aflatoxin B1 is activated in vivo by CYP3A4 and CYP1A2. Correspondingly, the pan-specific cytochrome P450 inhibitor 1-aminobenzotriazole decreased aflatoxin B1-mediated cytotoxicity in 3D InSight liver model, suggesting that the in vivo activation of aflatoxin B1 by CYPs is recapitulated in vitro. Discussion and Conclusion: The in vivo mechanisms of toxicity of acetaminophen, aflatoxin B1 and trovafloxacin were recapitulated in vitro using 3D InSight liver models in combination with specific sensitizers. The physiological relevance of the 3D InSight liver model makes it an effective in vitro tool for studying toxicological mechanisms of clinical hepatotoxicants. Furthermore, the scalability of the 3D InSight liver model offers the opportunity for in vitro high throughput screening (96- and 384-well plate format) for specific in vivo mechanisms of hepatotoxicity.

P-6a-13

MTS application as alternative in vitro method to assess the toxicity of veterinary autogenous vaccines produced at Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati": preliminary data.
ABSTRACT #313

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Background and Objectives. Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (a public company operating within the National Health Service) has the authorization for the production of veterinary autogenous vaccine and auto-vaccines by a Ministerial Decree. Vaccination is a control method to prevent bacterial disease spreading and useful tool to overcome the antibiotic-resistant conditions. In vaccine production process the Quality Control (QC) testing is necessary to ensure product effectiveness and safety, for autogenous vaccines it has always been performed by Abnormal toxicity test (ATT) in vivo, involving large number of animals with significant pain and distress. Nowadays, ATT test is no longer mandatory (1) thanks to advanced technologies in QC testing and in line with 3Rs philosophy (Replacement, Reduction, and Refinement). According to these new safety rules, we evaluated the cytotoxicity of 6 veterinary autogenous vaccines produced at Pharmaceutical Unit, by a colorimetric endpoint dilution in vitro assay (MTS assay). Material and Methods. Murine fibroblast cells were incubated with vaccines/adjuvants in two-fold dilution for 24h, then MTS substrate was added for 2h and absorbance read (492nm). The percentage of metabolic active cells, compared to untreated and positive controls, was evaluated. Results. All vaccines were cytotoxic when tested undiluted; a progressively positive metabolic effect was recorded as vaccine dilutions increased, ranging from 1:64 (ovine lameness) to 1:256 (ovine colibacillosis). Discussion and Conclusion. Replacement of in vivo ATT for autogenous vaccine QC testing with in vitro methods could determine a substantial refinement and reduction in the use of animals. However, further tests combining different methods are necessary to obtain solid results and specially to relate the in vitro evidences to what happens in lab animals. The final prospective is the standardization of protocols and the application of validated alternative test. Funded by the Italian Ministry of Health, IZSUM P3Stars.

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P-6a-14

In vitro microenvironment modifies the neurotoxic response of SH-SY5Y cells ABSTRACT #28

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Introduction: Thirty-four percent of clinical trial safety failures are related to the brain, pointing to the lack of relevance of pre-clinical models for neurotoxicity assessment (Cool et al. 2014). Among these models, the SH-SY5Y neuroblastoma cell line is the most widely used for drug discovery (Kovalevich and Langford, 2013), but its differentiation into neurons in vitro is questioned. The objective of this study was to compared the SH-SY5Y differentiation methods in order to develop a relevant in vitro model for neurotoxicity assessment, including both mature neurons organized in 3D and the Extracellular Matrix (ECM). Material & Methods: First, we screened the 24 major differentiation media described in the literature to differentiate these cells into neurons in 2D cell culture, and selected the 3 most differentiating conditions in terms of proliferation slowdown and neurite elongation: retinoic acid, staurosporine, and cyclic Adenosine Monophosphate (cAMP) combined to B21 supplement. Then, we compared their effects on SH-SY5Y cells cultivated in 3D in an hydroscaffold mimicking the ECM, by analyzing the cell proliferation, neuronal proteins expression, and sensitivity to compounds of known toxicity. Results: Immunostainings showed that the three media decreased the cell proliferation, but only staurosporine and B21+cAMP media increased the neuronal marker expression in 3D. Both maturity and sensitivity of SH-SY5Y to the neurotoxic compound 6-hydroxydopamine were higher in B21+cAMP medium. However, cell sensitivity was lower than in 2D. Conclusions: This work highlighted that the microenvironment of neurons, including the ECM and the soluble factors, can modify the neuronal response in vitro, and should thus be considered carefully in academic research and

as early as possible in the drug discovery industrial process.

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P-6a-15

Interplay between in vitro off-target pharmacological promiscuity, cytotoxicity, and in vivo tolerability in rodents to improve the safety profile of anti-malarial drug discovery ABSTRACT #294

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Within drug development, high off-target promiscuity, as well as potent cytotoxicity, is associated with a low likelihood of success. In the frame of the Medicines for Malaria Venture (MMV) partnership, UCB developed novel plasmepsin X (PMX) inhibitors for the treatment of malaria. As part of the screening cascade, a total of 249 PMX compounds were profiled both in a panel of in vitro secondary pharmacology assays containing 44 targets (SafetyScreen44™ panel from Eurofins), as well as in a cytotoxicity assay in HepG2 cells using CellTiter Glo. Eight of the lead compounds were also tested in vivo in 4-day or 7-day rat toxicology studies, and/or in cardiovascular studies conducted in anesthetized guinea pigs. Overall, cytotoxic liabilities in the HepG2 assay correlated with high promiscuity (off-target hit rate above 20%) in the SafetyScreen44 profiling and were associated with suboptimal tolerability in vivo (decedents, morbidity, adverse clinical signs or severe cardiovascular effects). Some of the side effects noted in vivo in rats or guinea pigs were putatively attributed to some of the hits identified in the secondary pharmacological profiling, such as muscarinic M1 and M2, opioid mu and/or kappa receptors or hERG/CaV1.2/Na+ channels, which were common to >50% the compounds tested in vivo. In summary, compounds showing cytotoxic liabilities associated with high promiscuity are likely to be poorly tolerated in vivo. However, such associations do not necessarily imply a causal relationship. Identifying the targets that cause these undesirable effects is key for early safety risk assessment. A tiered approach, based on several in vitro assays, helps select the right compounds to proceed to in vivo studies.

P-6a-16

USEFULNESS OF THE ZEBRAFISH EMBRYO MODEL TO EVALUATE NEW THERAPIES AGAINST MULTIDRUG-RESISTANT BACTERIA ABSTRACT #436

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Background and Objectives: The appearance of drug-resistant bacteria has made necessary the reintroduction of old antibiotics that were effective but had a narrow therapeutic window. This is the case of polymyxins, a family of old antibiotics that, due to its toxicity, is only used as a last resource against multidrug-resistant gram-negative bacteria. To reduce their toxicity, this study proposes three different strategies based on changes in chemical structure, new formulations, or on a combination with a protector. These strategies are leading to a large number of compounds to be tested, so it is necessary to find a good predicting model alternative to animal experimentation to screen and select the best compounds. Therefore, the objective of this study is to evaluate the suitability of the zebrafish embryo model to predict the toxicity associated to polymyxins. Material and Methods: General toxicity of 10 compounds was studied in zebrafish embryos from 2 to 72 h post-fertilization (hpf) and neurotoxicity was evaluated with a touch-evoked response assay at 72 hpf. The maximum tolerated concentration of 5 protectors, and co- or pre-exposure with polymyxin E (colistin) was tested to find the best protective strategy. Results: Zebrafish embryos exposed to polymyxin B or colistin showed systemic and neurotoxic effects, consistent with the in vivo literature. No significant differences were found in the derivatized compounds. However, new formulations of the drug or a pre-exposure to melatonin, or epigallocatechin-3-gallate were promising strategies to reduce neurotoxicity. Discussion and conclusion: Based on these results, zebrafish embryos are a promising model that has allowed us to evaluate the toxicity of 15 substances and 5 combinations of those. The new formulations and the administration of a protector are promising strategies to reduce the toxicity. However, further studies are needed to confirm the suitability of this model to predict the toxicity of polymyxins.

P-6a-17

Induction and evaluation of an oxidative stress response in the EpiDermFT in vitro human skin model ABSTRACT #187

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As the main barrier to the outside world, human skin is constantly impacted by environmental stressors such as air pollution. A major source of air pollution is vehicular exhaust which can cause oxidative stress and induce immune responses. Air pollution can negatively affect the skin causing premature skin aging and other skin problems. In this study, a full-thickness in vitro human skin model (EpiDermFT) was exposed topically to varying concentrations of Diesel Exhaust Particles (DEPs) for 24 hours. Tissue structure, viability, lipid oxidation, cytokine release, and gene expression were evaluated. No major effects in tissue structure or viability were observed in the DEP treated tissues. Lipid oxidation, determined by 8-Isoprostane release, increased significantly following DEP exposure by at least 2-fold. Using Clariom S Human microarrays, 213 genes were identified whose expression was upregulated or down-regulated by at least 2-fold in n=2 experiments. Genes related to immune signaling and response and cellular senescence were elevated suggesting tissue exposure to DEPs mimics human skin responses to air pollution including inflammation and skin aging. In additional experiments, tissues were pre-treated for 4 hours with the antioxidant, Resveratrol, and release of the inflammatory cytokine, IL-8, was measured. Tissues exposed to DEPs showed at least a 2-fold increase in IL-8 release, but baseline levels of IL-8 were observed when the tissue was pre-treated with Resveratrol. These findings support the utility of the EpiDermFT model to study environmental pollution responses in vitro and to evaluate active

ingredients and other molecules for the prevention of the human skin oxidative stress response.

P-6a-18

hiPSCs and hiPSCs-derived renal proximal tubular cells showed different response to nephrotoxic compounds ABSTRACT #279

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Objectives: Given that many drug candidates fail in clinical trials due to nephrotoxicity, better models are needed to identify potential toxicity early in the developing process. Human-induced pluripotent stem cells (hiPSCs) differentiating into proximal tubular epithelial cells (PTEC) could provide a 3R-conform alternative model to test and identify potential nephrotoxins. Therefore, the present study investigates the vulnerability of PTEC- like cells generated from hiPSCs towards well-known nephrotoxins. Materials and Methods: hiPSCs were differentiated into PTEC-like cells by cultivating them in renal epithelial cell growth medium supplemented with bone morphogenetic protein (BMP) 2 and 7, human epidermal growth factor (hEGF), insulin, epinephrine, and transferrin for nine days. Results: Currently, we have shown that hiPSCs, after the applied differentiation protocol exhibited PTEC-like morphology. Moreover, the cells showed increased expression of prototypical PTEC markers and transporters, while stem cell markers were downregulated. Furthermore, the PTEC-like cells were capable of megalin-dependent cubilin-mediated endocytosis of fluorescently labeled albumin, proving their functionality. hiPSCs and differentiating cells were more sensitive to the nephrotoxin cisplatin than the fully differentiated cells, which was not observe for another nephrotoxin, cyclosporin A. Differentiating cells were more susceptible to oxidative stress than hiPSCs and PTEC-like cells. Consistent with these data, these cells expressed low levels of genes involved in oxidative stress. Discussion and Conclusion: Already this small selection of compounds show the diverse reaction of cells in various differentiation states to substances with different mode of action. While it is obvious that a DNA damaging substance like cisplatin has its highest impact on fast-growing proliferating cells, the deleterious effect of oxidants on differentiating cells was unexpected. Overall, these hiPSC-derived in vitro kidney model is ready now to investigate the nephrotoxic potential of several selected compounds and the effects of these toxins on the differentiation and functional competence of derived differentiated progeny.

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P-6a-19

Low Endotoxin Recovery (LER) effect in pharmaceutical products analyzed with the Monocyte Activation Test (MAT). ABSTRACT #280

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Background and Objectives Pyrogens - endotoxin pyrogens and Non-Endotoxin Pyrogens (NEPs)- are substances that can induce an increase in body temperature, shock and death. The Limulus Amoebocyte Lysate (LAL) test is considered the gold standard to identify specifically endotoxin. It can be affected by the Low Endotoxin Recovery (LER) effect. LER is a time-dependent effect that causes a reduction in the capability to detect endotoxin in the sample, due to excipients (mainly surfactants and chelators)(1). The LER effect was observed in the Monocyte Activation Test (MAT) too. Material and Methods Reagents were supplied by MilliporeSigma: MAT kit (PyroMAT kit), monocytic cell line (Mono-Mac6), NEP controls (Heat-killed Staphylococcus aureus and Flagellin from Salmonella typhimurium), endotoxin-free water. Eleven biotech pharmaceutical formulations

were used as test items. The endotoxin standard curve was prepared with Reference Standard Endotoxin (EDQM). Each analyzed drug product was validated through a Product Specific Validation (PSV). After the PSV, a Hold-Time Study (HTS) was performed on all products, for up to 14 days of storage incubation time before the analysis. Results During the PSV, all the products resulted in a valid dilution not exceeding the maximum valid dilution and not interfering with the ELISA test. During the HTS, three out of the eleven products showed LER effect. The LER effect was confirmed since in such samples the endotoxin recovery % fell below 50% on two consecutive time points. Discussion and Conclusion The identification of the LER effect in the MAT assay was not previously reported. This effect was described for the LAL test only. One inhibited product contained Polysorbate 20, a surfactant already known for its masking effect on endotoxin detection. The remaining two products contained Benzyl Alcohol that can potentially cause the LER effect by downregulating the TLR4 signaling pathway(2) and/or destabilizing the lipopolysaccharide conformation(3).

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P-6a-20

An epidermal model containing melanocytes for skin pigmentation and lightening studies

ABSTRACT #190

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Considerable interest exists in evaluating raw materials and/or skin care formulations which cause lightening of the skin. These products are utilized to modulate one's natural skin color or to combat skin pigmentation disorders such as melasma, dark spots, solar lentigo, and other hyperpigmentation lesions. To aid in the development and testing of such products, we have developed a skin whitening protocol using the epidermal skin model, MelanoDerm™, to evaluate both raw materials and skin lightening formulations. MelanoDerm is a highly differentiated, three-dimensional tissue culture model of human epidermis that contains normal human melanocytes (NHM) and keratinocytes (NHK). Epidermal tissues have been produced containing NHM of varying skin phototypes which follow the pigmentation level of the donor tissue, i.e. black > Asian > Caucasian. For lightening studies, tissues were treated topically three times a week over a two to three week period to mimic consumer application. Several over-the-counter skin lightening products were evaluated in cultures containing NHM from black and Asian donors. Over the treatment period, negative control cultures became increasingly pigmented with retention of normal epithelial morphology. In contrast, tissues treated topically with cosmetic skin lightening agents containing tyrosinase inhibitors such as kojic acid and magnesium ascorbyl phosphate remained lighter than the control cultures. The skin lightening effect on treated tissues was quantitatively evaluated for melanin content using a Solvable melanin assay and for skin brightness (L* value) using a hand-held spectrometer. Treated tissues showed significant changes in overall melanin content and brightness compared to control tissues. These results suggest that this model can provide valuable in vitro data for screening raw materials prior to the commencement of costly clinical trials and that it will be useful to study melanogenesis, skin lightening, and other pigmentation phenomena of the skin.

P-6a-21

PITFALLS OF THE MTT ASSAY AND CYTOTOXICITY EVALUATION OF TRADITIONAL ANTIMALARIALS ABSTRACT #328

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Background and Objectives. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test has been deemed the gold standard for assessment of cell viability and proliferation denoted also in ISO 10993-5 as a recommended quantitative cytotoxicity method. According to the ISO standard, reduction of cell viability by more than 30 % is considered a cytotoxic effect. In the present work, the mechanism encompassing cytotoxicity of the boundary IC30 of chloroquine was investigated under both serum-fed and highly preferred serum-starved conditions in BV-2 microglial cells. Material and Methods. Besides the MTT assay, flow cytometry with a range of fluorescence probes, in particular, H2DCF-DA, hydroethidine, JC-1, propidium iodide, annexin V-FITC and fluorescent beads, was employed to assess H2O2, superoxide levels, mitochondrial membrane potential, cell cycle progression, apoptosis and phagocytosis, respectively. Results. Under serum deprivation, chloroquine supported H2O2 levels, mitochondrial hyperpolarization along with reduction of phagocytosis. In serum-fed cells, chloroquine induced mitochondrial depolarization, G1 cell-cycle arrest and superoxide production in normally phagocytizing cells. Under both conditions, chloroquine promoted early apoptosis. Both conditions maintained lower cytotoxicity of hydroxylated derivative compared to parent molecule. Discussion. MTT test was suggested to have limitations related to confounding effects of serum absence, proliferation or apoptosis. Thus, besides of mitochondrial activity changes, decrease in proliferation of serum-fed cells can contribute to chloroquine-induced decrease in measured O.D. values. However, MTT reduction was not shown to be reflective to early apoptosis occurrence. Immunotoxicity and prooxidant effects of the antimalarials tested seem to be underestimated under serum-fed conditions. Conclusions. The similar MTT-assay-derived data measured under different serum conditions can comprise dissimilar cytotoxic mechanisms of the compounds tested. This might be, especially, the case of lysosomotropic drugs interfering with the pro-survival autophagy pathway activated under serum starvation. Acknowledgement. This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-18-0336 and EU project ITMS2014+313021Y920.

P-6a-22

Long-term upregulation in drug metabolization and hepatic gene expression in primary human hepatocytes (PHH) after exogenous exposure to human intestinal microbiome secretome peptides ABSTRACT #404

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Background/Objectives: The human intestinal microbiome's effects on the liver are finally starting to be unveiled in health and disease. However, its outcomes on liver maturation and function are still unclear. Notably, the potential effects in the liver of the peptides generated by the gut enzymes' activity on the microbiome's secreted proteins are entirely unknown. Hence, we sought to determine their role in hepatic function and metabolism in cultured PHH in vitro for up to 3 weeks. Material/methods: Several synthetic peptides were produced after an in-silico digestion of the whole human secretome from intestinal microbiome. Preliminary work with selected synthetic peptides identified four peptides capable of inducing hPSC-derived hepatocyte differentiation and functionalization. Then, cryopreserved PHH were plated and exposed

to these peptides shown to be active in the preliminary screening. After three weeks, PHH (n=6) were tested for cell viability, morphology, drug metabolization (bupropion, midazolam, phenacetin, 7-OH coumarin) by LC-MS/MS, and gene expression (ALB, HNF4a, CYP3A4, CYP2C9, CYP2B6, GAPDH) by RT-PCR. Results: After three weeks in culture, PHH exposed to each peptide showed a slight increase in survival compared to control. Cell morphology was kept constant in all conditions, but PHH exposed to the microbiome's peptides showed higher expression of CYP3A4, CYP2C9, CYP2B6, and upregulation of ALB and HNF4a. Likewise, we determined that these peptides could significantly increase phase 1 and 2 drug metabolization in vitro, even after three weeks of culture. Conclusion: Considering that PHH lose their metabolic function rapidly in vitro, the long-term maintenance of PHH survival and function by these microbiome's secretome peptides is significant. Additional efforts are needed to unravel these mechanisms. However, it already hints at the potential that the unexplored protein fraction of the human intestinal microbiome secretome might have in hepatocyte function and regulation and its potential role in biotechnological and possible therapeutic applications.

P-6a-23

Novel in vitro approaches in safety evaluation of cemtirestat ABSTRACT #415

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Objectives: In diabetic patients, under conditions of hyperglycemia, increased flow of glucose through the polyol pathway contributes to the development of chronic diabetic complications(DCs). Glycemic control can decrease the incidence of DCs but is not adequate alone to prevent or treat the disease. Aldose reductase (ALR2), the first enzyme of the polyol pathway, thus becomes an essential target of pharmacotherapy of DCs. Several ALR2 inhibitors have been tested, yet epalrestat is the only ALR2 inhibitor used clinically. Recently designed drug cantidate Cemtirestat (3-mercapto-5H-1,2,4-triazino[5,6-b]indole-5-acetic acid) was characterized as a bifunctional agent which combines aldose reductase inhibition with antioxidant activity. The aim of the present study was to assess the general toxicity of Cemtirestat using in silico predictions and in vitro assays. Methods: Predictions were performed by ADMET_SCBDD and ProTox-II toxicity software. Different cell lines, including mouse microglia BV-2, the insulinoma pancreatic beta-cells INS-1E, the human colon cancer cells HCT116 and human epithelial endometrial cell line HIEEC and Saccharomyces cerevisiae were used for determination of the cytotoxic effect of Cemtirestat. In vitro tests using 3T3 fibroblasts (OECD-TG-432), and 3D reconstructed human tissue models EpiDerm (OECD-TG-498) and EpiOcular have been used for the assessment of phototoxicity. Results: ProTox-II toxicity prediction software gave 16“Inactive” outputs, a mild hepatotoxicity score (0.52 probability). These outputs classify Cemtirestat as a compound of acute toxicity class 3. ADMET SCBDD software predicts 30 parameters which suggest better ADMET and drug-likeness properties than clinically used epalrestat. Cell culture viability tests performed on six different cell lines proved remarkably low cytotoxicity. Tests of phototoxicity have shown that Cemtirestat is not a phototoxic compound. Discussion and Conclusion: According to in silico prediction and in vitro tests, Cemtirestat did not show significant cytotoxic and phototoxic effects. In summary, these results suggest that Cemtirestat is a safe drug that can proceed beyond preclinical studies.

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P-6a-24

Drug-induced QTc prolongation and Torsades de Pointes: evolving ICH S7B in light of emerging in silico, in vitro and in vivo data and implication for drug development

ABSTRACT #47

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Drug-induced prolongation of the QT interval of the electrocardiogram and its association with life threatening arrhythmia known as Torsades de Pointes (TdP) remains a key issue in drug discovery and development. Two guidelines promulgated in 2005 addressed the non-clinical and clinical studies (ICH S7B and E14, respectively; Anon., 2005a,b) that are required to ensure the safety of clinical trials participants and patients. Although the ICH S7B and E14 guidelines highlight the need for integration of information in a manner which is informative as a totality of evidence, in practice S7B studies have primarily informed safety before first-in-human dosing but then generally have not been considered in regulatory decision-making once drugs enter clinical development. Consequently, a set of Questions and Answers (Q&As) has been developed and just released (Anon., 2022) that focus on connecting the 2 Guidelines for scenarios where the nonclinical data are informative in clinical study implementation and evaluation. The new S7B Q&As offer best practice considerations for i) the core S7B assays (hERG in vitro electrophysiology and in vivo QTc) and additional ion channel assays that can be used as follow-up studies; ii) in vitro cardiomyocyte assays and principles for proarrhythmia models; iii) the S7B integrated risk assessment Q&As that in combination with the revised E14 Q&As describe how nonclinical data can be used to reduce the number of TQT studies and reach a low-risk determination when a TQT or equivalent cannot be performed. The integrated risk assessment also describes how follow-up studies can be used to understand and predict TdP risk of QTc-prolonging drugs, however, these are evaluated on a case-by-case basis. The communication will provide an update on the Q&As and associated clinical scenarios that could benefit from high quality non-clinical data to inform clinical QT assessment.

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P-6a-25

Novel Human-Relevant Preclinical Safety Testing Strategy for Recombinant Human Monoclonal Antibodies Directed Against Foreign Targets

ABSTRACT #469

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Background: A novel non-animal preclinical safety testing strategy was developed to address the specific toxicity risks associated with a well-characterized human recombinant monoclonal antibody (mAb) directed against a foreign antigen. Any mAb in this product class is best de-risked with modern, human-relevant techniques rather than animal models, so the strategy prioritizes human cell and tissue assays that thoroughly address the relevant safety concerns and support a single-dose, first-in-human (FIH) clinical study. Methods and Results: A detailed assessment of the potential toxicity risks specific to the target drug

class was performed using ICH guidance(1) and the growing body of data from pharmaceutical mAbs. The review indicated an overall low risk safety profile for the target drug class, and two specific risks of immunogenicity and off-target binding were identified for mitigation before an FIH trial. Animal models have been known to poorly predict these risks, but they are robustly addressed with well-established in vitro techniques. A case study candidate provides an example of the type of drug to which the non-animal preclinical strategy applies, and it allows targeted discussion with regulators as it proceeds towards FIH trials. The poster will give an overview of the candidate and the in vitro assays included within the non-animal preclinical data package, as well as a summary of how the assay data is used to determine whether the test article is acceptably safe for a human trial. Discussion and Conclusion: The regulatory requirement to evaluate new drug candidates using animal-based safety studies prior to a FIH trial may not be necessary given currently-available in vitro methods and regulatory guidance(2) that recognize the value of integrating modern science into the review process.

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P-6a-26

Hepatic 3D cell models as a cell-based biosensor-like system for the in vitro (geno)toxicity testings
ABSTRACT #473

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To better evaluate the safety of newly developed chemicals, there is a considerable need for reliable research models and approaches, particularly for determining genotoxic activity and potential adverse effects on human health. In this regard, high-content flow cytometry has emerged as a leading technology supporting many applications to study the nature of individual cells within homogeneous or mixed cell populations. In the present study, HepG2 spheroids were used as a biosensor-like model for screening. Spheroids were cultured for 3 days (static cond.) and validated by testing the activity of the indirect-acting compounds, benzo(a)pyrene (BaP; 0.1, 1, 10 and 20 µM) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP; 50, 100, 150 and 200 µM). After 24-h exposure, the effect on spheroid growth was monitored by planimetry, while live/dead cells were determined by FDA/PI staining. BaP decreased spheroid surface area at ≥ 10 µM and affected cell viability at 20 µM, while PhIP did not affect cells. The effects of BaP and PhIP on cell proliferation (Ki67 marker) and cell cycle alterations were assessed by flow cytometry, and genotoxicicity was determined by comet and γH2AX assays. BaP decreased the number of Ki67-positive cells and arrested HepG2 cells in the S phase of the cell cycle. On the contrary, PhIP had no significant effect on cell proliferation at applied exposures. BaP and PhIP induced the formation of DNA single (comet) and double (γH2AX) strand breaks. Also, at the mRNA level, both BaP and PhIP deregulated the expression of phase I (CYP1A1, CYP1A2, CYP3A4) and II (UGT1A1, SULT1A1, SULT1B1, NAT1, NAT2) enzymes, while DNA damage responsive genes (P53, GADD45α, CDKN1A) were more deregulated by BaP. In summary, the newly developed HepG2 spheroids are a suitable experimental model for genotoxicity assessment due to their improved metabolic capacity.

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P-6a-27

Development of a primary culture system to investigate compound toxicity in steatotic hepatocytes
ABSTRACT #476

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The number of non-alcoholic fatty liver diseases (NAFLD) in industrialized countries has risen sharply in recent years. Steatosis can increase the sensitivity of the liver to toxic effects of drugs. The aim was the development and prevalidation of an in vitro system using steatotic human hepatocytes to study whether and by which factor the susceptibility of steatotic versus normal liver is increased. The hepatocytes were brought into a steatotic state in culture by means of additives (free fatty acids, hormones). Immediately thereafter, these steatotic hepatocytes and control hepatocytes were incubated with xenobiotics (diclofenac, acetaminophen, ibuprofen and metformin) for 48 h under the previous cell culture conditions. Compound toxicity (EC50 values) was determined by a quantitative ATP assay. In addition to 2D culture, experiments on fatty degeneration were performed in 3D culture to investigate differences in susceptibility between 2D and 3D. The magnitude of change in EC50 value due to fatty degeneration showed a good correlation between 2D and spheroid culture for diclofenac, ibuprofen and acetaminophen. In contrast, metformin induced toxicity to a greater extent in 2D steatotic hepatocytes than in 3D cultures. Evaluation of the results from 2D and 3D culture also revealed a different susceptibility of individual lots of human hepatocytes to fatty degeneration and its effects. In summary, the results of both successfully developed culture systems showed higher susceptibility of steatotic hepatocyte cultures compared to untreated cultures. Decreased sensitivities of up to 30 % were observed. Among these, the susceptibility to drugs was higher in 3D culture than in 2D culture, giving spheroid cultures a higher potential than 2D cultures in the further development and future use of in vitro systems.

P-6a-28

LONG-TERM RECORDING OF CARDIAC ACTION POTENTIALS FOR CHRONIC CARDIOTOXICITY ASSESSMENT
ABSTRACT #498

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The process of drug discovery is an extremely long and expensive one, and cardiotoxicity is still the major cause of drug failure and withdrawn from the market. To make drug development more efficient, the reliable identification of chronic cardiotoxic effects during in vitro screenings is fundamental for filtering out toxic compounds before in vivo animal experimentation and clinical trials. Current techniques such as patch-clamp, voltage indicators, and standard microelectrode arrays (MEAs) do not offer at the same time high sensitivity for measuring transmembrane ion currents and low-invasiveness for monitoring cells over long time. Although MEAs provide non-invasive and long-term measurements of electrical signals from electrogenic cells, this technology is limited to record extracellular field potentials (FPs) instead of the intracellular action potentials (APs). Extracellular FPs occur as a biphasic signal, whereas intracellular APs waveforms display the collective activity of several ion channels, providing crucial information about the complex effects of drugs on transmembrane ionic currents. Here, we propose laser-based optoporation [1-3] applied to MEAs to measure intracellular APs from human induced pluripotent stem cells (hiPSC-CMs) for more than 1 continuous month. Thanks to an automatic poration by means of laser scanning, we obtain high-quality intracellular AP recordings from all electrodes of the multiwell 60-electrode commercial MEAs and the individual interval sessions of intracellular APs can last as long as 20-30 minutes. In particular, our approach permits the accurate electrophysiological assessment of cardiac syncytia maturation during time and provides reliable data on chronic cardiotoxic effects caused by known compounds such as pentamidine, a hERG channel trafficking inhibitor, and doxorubicin, one of the most effective chemotherapy drugs. Our results demonstrate that optoporation may be an effective in vitro strategy to detect chronic cardiotoxicity in the early phases of drug development.

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Session: 6b. Knowledge sharing and education

P-6b-2

Ukrainian National 3Rs Center. An effective tool to advance the 3Rs principles by focusing on their scientific impacts and benefits ABSTRACT #205

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At the begin of the 2020 year Ukrainian National 3Rs Center was created by group of scientists, wich work in vitro and in silico toxicology and wich work in vivo toxicology (DNT, DART etc). The result of collaboration between these two groups was the implementation of three in vitro methods (OECD 431, 439, 492) in L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health. We have tested pesticides imazamox and acifluorfen by in vitro assays as case study of the possibility to implement alternative methods. In this study three in vitro tests was used: skin irritation test, skin corrosion test and eye irritation test using human reconstructed tissues EpiDerm TM and EpiOcular TM. Each test was performed according to OECD TGs 439, 431 and 492 respectively in GLP lab. The conclusions have made according to the results obtained in this in vitro study are coincide with conclusions made from in vivo tests. These addition facts are important to the implementation of alternative methods, reduce animal use and comparability of in vitro results with animal data special during reregistration of pesticides. These activities will help save the lives of 120 animals annually. We believe that only collaboration that mode gives the effective way to use in vitro and in silico methods. In some study where animals strongly involved and alternative methods are far from the full implementation should be used next two postulates Reduction and Refinement. In this case, people who closely work in this area (in vivo) and who has knowledge in alternative methods can realize it more effective and create new approaches. 3Rs Center is tool for communication between professionals in government, industry and science which can make impact into legislation to facilitate animal protection and on legislation level accept in vitro methods.

P-6b-3

Reproducibility in Cell Culture: Replacing Fetal Bovine Serum ABSTRACT #301

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Reproducibility is key for all scientific developments and industrial production. This holds true especially in cell culture, where the smallest impurities could cause false conclusions or process malfunctions. Nonetheless, it is astonishing that one of the most ubiquitous cell culture supplements is also known to be a cause for irreproducibility: Fetal bovine/calf serum (FBS/FCS); used due to its promoting effect on cell growth and proliferation. Its undefined nature combined with high batch-to-batch variability and risk of contaminations place it as an additional uncertainty factor introduced in cell culture. In this poster we present examples for such uncertainties and potential risks arising from the

use of FBS [1]. These can be overcome by the use of replacements which paves the way towards reliable, reproducible and safe science [2]. Furthermore, due to its unsolved animal welfare issues, replacing FBS will also contribute towards cruelty-free research. Therefore, we demonstrate two cell culture supplements: Human platelet lysate (hPL) and a do-it-yourself chemically defined medium. When derived from expired blood donations, hPL can be an ethically acceptable and xeno-free replacement for animal-derived FBS, especially suitable in tissue engineering, organoids and cell therapy. As a natural product, hPL can still be a source of variability, thus for certain applications a switch to chemically defined media would be the solution. We will provide an example for L929 and Caco-2 cell lines and present the publicly available "Fetal Calf Serum-Free Database" (https://fcs-free.org/), hosted by the 3Rs-Centre at Utrecht University. FBS-free media do not (yet) exist for all cell types, so these (or a single universal medium) have to be developed [3]. Furthermore, all animal-derived materials should be completely replaced or otherwise eliminated. Transition to an animal-component-free cell culture is not only an ethical but a scientific necessity for modern science, health and production.

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P-6b-4

FUN with NAMs
ABSTRACT #500

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Education brings neither glory to the scientists nor a better h-index. Nevertheless, it is useful for multiple reasons such as knowledge sharing, capacity building and creation of an adequate ecosystem. Overall, one can admit that the education and training about 3Rs at university level has the merit to exist even if it could be possibly better advertised and communicated. The JRC launched a mapping exercise on this matter in 2018 but as far as the authors are concerned the results of the study were not published (1). A category of individuals that is rarely targeted properly is the general public as well as teaching at primary and secondary school. JRC took care of the latter by providing learning scenarios to empower the teachers (2). Moreover, organising open days as well as participating in science festivals are great venues for reaching out the general public. Still, there is space for creativity by providing other formats. At Altertox, two new concepts and formats are expected to complement the current "arsenal" of tools available. The first one is an edutainment game meant to open a conversation about NAMs (New Approach Methodologies) and validation process in a fun and convivial environment. "TATABOX" (Towards Alternatives To Animal testing) tiles are not meant to be exhaustive in terms of content as well as persona but rather a starting point for discussion with concrete items within a team on the process towards regulatory acceptance. The second one is a quiz game for youngsters (from 9+, "Little genius") meant to raise public awareness about NAMs, laboratory animals, life sciences, legislation in Europe and NAMs job. Questions for quiz were adapted to general public using true/false, multiple choices, open ended questions. Hopefully, these two new formats will provide supplementary ways for the scientific community to exchange at national level.

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P-6b-5

Plastic toxic chemical bisphenols reduce rat gut smooth muscle contractile activity in vitro
ABSTRACT #467

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Bisphenols are used in the plastic industries for manufacture of various plastic items including water bottles, baby feeding bottles and other food and beverage containers. Bisphenols are detected in body fluids and their adverse effects on endocrine, reproductive and metabolic system are well documented. However, their effects on gut smooth muscle activity are less studied, despite the fact that the primary route of entry of these chemicals is via gut. The objective of the present study was to assess the effect of bisphenols on gut smooth muscle contractile activities in vitro. Experiments were carried out after obtaining the approval from institutional ethical committee for animal experiments. Gut segments from small and large gut were prepared from overnight fasted male albino rats (adult and neonate) and recording of isometric contractions in vitro was performed with the help of a standard organ bath and digital recording systems (AD Instruments, Australia) under bath concentration (1-100 µM) of bisphenol A and Bisphenol S. In addition, various antagonists (atropine, L-NAME, Tamoxifen, hexamethonium etc.) were used to evaluate the mechanisms of action of bisphenols on contractile tension (g) and frequency (Hz) of gut segments. The results showed that both of bisphenol A and bisphenol S (1-100 µM) significantly ($p<0.05$) depress contractile tension and frequency of small and large gut segments in a dose dependent manner, in both adult and neonate rats. Further, the bisphenol-induced attenuation in contractile responses appeared to be independent of oestrogen receptors, nitric oxide and cholinergic system. The present experiments demonstrated that bisphenols reduce rat gut smooth muscle contractile activities under in vitro conditions. Thus, the toxic chemical like bisphenols may be a potential contributor to the development of gastro intestinal motility disorders, especially in view of extensive use of plastic items in our daily life.

Session: 7a. Developmental Neurotoxicity (DNT)

P-7a-1

Developmental toxicity assessment of nanoparticles: The importance of indirect placenta-mediated toxicity mechanisms
ABSTRACT #352

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Exposure to nanoparticles (NPs) is steadily increasing, which is of particular concern for vulnerable populations. Prenatal particle exposure has been associated with pregnancy complications and diseases later in life. Recent epidemiologic studies have confirmed that NPs can reach the human placenta but the mechanisms underlying NP-induced fetotxicity are largely unknown. Since materno-fetal transfer of NPs is often low or undetectable, we speculate that NP accumulation in placental tissue can interfere with the secretion of essential placental signaling factors. Here, we aimed to unravel indirect fetotoxicity pathways

elicited from NP at the human placenta. We exploited human placenta explant cultures (1st and 3rd trimester) to investigate the impact of TiO2, SiO2 NPs and diesel exhaust particles (DEPs) on i) human placental tissue viability and the release of inflammatory, vascular and endocrine factors ii) the secretion of other placental mediators by secretomics profiling and iii) the consequences of altered placental signaling on angiogenic and neurodevelopmental processes. NPs induced material- and time-dependent sublethal effects on the release of endocrine, inflammatory or vascular placental mediators. For instance, concentrations of the key pregnancy hormone hCG were significantly decreased upon exposure to SiO2 and TiO2 NPs in 1st trimester explants, while DEPs did not affect hCG levels at both pregnancy stages. Secretomics profiling exposed further potential indirect toxicity pathways (e.g. complement activation, regulation of insulin secretion). Importantly, conditioned media from NP-treated explants affected angiogenesis and neural progenitor cell proliferation. Our results show that NP can extensively interfere with the secretion of many placental signaling factors affecting angiogenesis and neurodevelopmental processes relevant to fetal wellbeing. The responses were different for 1st or 3rd trimester exposures, indicating that the exposure window has a decisive impact. Overall, our study highlights the urgent need to consider indirect placenta-mediated toxicity pathways in the future safety assessment of NP-enabled products and nanomedicines.

P-7a-2

DEVELOPMENTAL NEUROTOXICITY OF ACRYLAMIDE AND ITS METABOLITE GLYCIDAMIDE IN A HUMAN MIXED CULTURE OF NEURONS AND ASTROCYTES UNDERGOING DIFFEFRENTIATION ABSTRACT #321

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Background: There is a worldwide concern on adverse health effects of dietary exposure to acrylamide (AA) due to its presence in commonly consumed foods. When absorbed AA is metabolized mainly in the liver by CYP2E1 to glycidamide (GA). AA and GA are suggested to reach the developing foetus and infant via placental transfer and breast milk, respectively. Prenatal AA exposure in rodents and in vitro have resulted in various adverse neurodevelopmental effects. Less is known regarding possible effect of real-life relevant concentration of AA and GA on the developing human brain. Objective: To elucidate whether exposure to AA and GA may affect key neurodevelopmental processes vital for normal brain development in humans. Materials and methods: Neural stem cells (NSCs) derived from hiPSC were used to investigate effects of exposure to AA and GA on key neurodevelopmental processes assessed by gene expression (RNAseq) and protein markers (immunocytochemistry and high content imaging). Results and Discussion: AA and GA at low concentrations (10-7 M) increased markers of proliferation both in proliferating NSCs (7 days) and in maturing neurons after 14 to 28 days of differentiation. Increased expression of brain derived neurotrophic factor (BDNF) concomitant with decreased synaptogenesis were observed for GA exposure at later differentiation stages, and an increased number of astrocytes after 14 and 21 days of differentiation. AA exposure decreased differentiation. After 28 days, neurite branch points and number of neurites per neuron measured by microtubule-associated protein 2 (Map2) staining decreased, while the same neurite features measured by βIII-tubulin increased, indicating perturbation of neuronal differentiation and maturation. More mechanistic data and RNAseq results will be presented. Conclusion: Disturbance of proliferation, differentiation, maturation or gliogenesis in the developing brain may lead to impairment of cognitive processes. Thus, the

current finding raise concerns regarding possible developmental neurotoxicity of AA exposure in children.

P-7a-3

In vitro study of fumonisin B1 and ochratoxin A on undifferentiated SH-SY5Y cells and contribution of beetroot in alleviating toxic effects ABSTRACT #322

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Background: The presence of mycotoxins in food as fumonisin B (FB1) and Ochratoxin A (OTA) have shown to exert an effect over neuronal activity in vitro. Different mechanisms of action by which they exercise their cytotoxicity have been attributed to FB1 and OTA including the production of reactive oxygen species (ROS). The benefits of natural components in food rich in polyphenols, carotenoids, and betalains, are being studied as a new tendency in the food industry as up-cycling strategy and thus contributing to sustainability. Objectives: Here it is presented the assessment of cell viability on undifferentiated SH-SY5Y cells exposed to FB1, OTA, and beetroot extract (BRE) (individually and in mixtures), the production of ROS and cell cycle alterations under the same conditions. Material and Methods: Cell viability was performed by the MTT assay ([3-4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Monitorization of intracellular ROS production was carried out by the H2-DCFDA probe. Lastly, cell cycle distribution and cell proliferation was performed by flow cytometry (with propidium iodide). Results: Viability results for OTA showed an IC50 of 5.8 µM and 9.1 µM at 24 h and 48h, respectively whereas no cytotoxic effect was detected for FB1. ROS production was increased when cells were treated with FB1 and OTA; while for cell proliferation it was observed a decrease in G0/G1 phases when exposed to several concentrations of OTA. Discussion and conclusion: Oxidative stress plays an important role in the mechanism of action of the mycotoxin to the detriment of SH-SY5Y human neuroblastoma cell line affecting also its cell cycle and being OTA the most cytotoxic one. The cytoprotective effect of BRE is a promising strategy to alleviate such cytotoxic effects. This work has been supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-100.

P-7a-4

APPLIYING NAMS TO SAFETY EVALUATION OF HERBAL PRODUCTS CONSUMED BY CATALAN PREGNANT AND LACTATING WOMEN: INTERVIEW-BASED CONSUMPTION SURVEY AND DEVELOPMENTAL NEURO/TOXICITY EVALUATION IN ZEBRAFISH EMBRYOS ABSTRACT #373

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Herbal products (HPs) consumption by general population has been increasing in the last years. The belief that they are "safe" makes them also attractive for pregnant or breastfeeding women. However, many HPs are not subject to the classic risk assessment processes, and their safety during development is not well characterized. Our aim was to perform a survey of consumption among pregnant and lactating women in Catalonia and further evaluate the safety of the most consumed products in the zebrafish embryo model. A questionnaire adapted from Kennedy et al. (2013) was reviewed and validated by 10 independent experts. An online personal interview was performed to women from gestational week 22 until 6 months after delivery. Number of participants was balanced according to the number of inhabitants of the catalan health regions. The most orally consumed HPs were selected for further

evaluation of developmental neuro/toxicity in zebrafish embryos. We show that the prevalence of oral consumption of HPs is higher than 50% among Catalan pregnant and breastfeeding women. The most consumed products were ginger (Zingiber officinale Roscoe) and raspberry leaves (Rubus idaeus L.). Thyme (Thymus vulgaris L.), chamomile flower (Matricaria chamomilla L.), cranberries (Vaccinium macrocarpon Aiton), and rooibos (Aspalathus linearis (Burm.f.) R.Dahlgren) were the next group of similarly consumed HPs. The main source of recommendation of consumption was own-initiative, followed by midwives and media (internet, magazines…). A DNT effect in zebrafish embryos was identified after exposure to raspberry leaf extract depending on the time and initial stage of exposure. We have characterized for the first time the consumption of HPs among Catalan pregnant and breastfeeding women. We demonstrate that this consumption is high and therefore that it is important to assess the safety of HPs. NAMs and specifically the zebrafish embryo model is a useful tool for developmental toxicity assessment of HPs.

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P-7a-5

Application of a human in vitro testing battery for endocrine disruptor (ED)-induced developmental neurotoxicity (DNT) to refine EDC hazard assessment ABSTRACT #481

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Endocrine disrupting chemicals (EDCs) have been intensively studied regarding their harmful effects on human brain development. Despite increasing evidence that early developmental EDC exposure causes developmental neurotoxicity (DNT), regulatory EDC risk assessment does not feature DNT endpoints. Therefore, the incorporation of DNT testing strategies into the risk assessment of EDCs is urgently required. Currently, the identification of chemicals causing adverse neurodevelopmental effects is based on animal studies. However, insufficient test throughput, species differences, and ethical concerns demand alternative in vitro models with high predictivity for humans. Therefore, a DNT in vitro testing battery has been assembled including a multiplexed high-content assay based on human neural progenitor cells (hNPCs), the Neurosphere Assay. To identify hormone-sensitive neurodevelopmental key events for ED-DNT in vitro assays development, we investigated the effects of specific hormone receptor agonists and antagonists on key neurodevelopmental processes modeled within the human Neurosphere Assay including NPC proliferation, migration, and terminal differentiation into neurons and oligodendrocytes. Strikingly, oligodendrogenesis was especially sensitive to endocrine modulation, being influenced by activation of the aryl hydrocarbon receptor (AhR), liver X receptor (LXR), retinoic acid receptor (RAR), peroxisome proliferator-activated receptors (PPARs), progesterone receptor (PR), prostaglandin E2 receptor (PGE2R) and vitamin D3 receptor (VDR). By incorporation of both male and female NPCs of human and rat origin in our testing strategy, we identified several neurodevelopmental key events impacted by hormones in a species- or sex-specific manner. Since hormone-regulated neurodevelopmental processes provide putative targets for EDCs, the established ED-DNT in vitro assays will be used to screen libraries of known and putative EDCs and identify ED-induced DNT. Due to the dramatic

consequences of DNT for human development, testing putative EDCs for their developmentally neurotoxic potential is of the highest importance for several stakeholders including regulatory bodies, industry, and the civilian population.

Session: 7b. Implementation of NAMs into regulatory frameworks – establishing scientific confidence, development of standards and good practices

P-7b-1

INCREASING PREDICTIVITY OF COMPOUND MULTI-ORGAN TOIXICITY THROUGH HIGH-THROUGHPUT ZEBRAFISH ASSAYS
ABSTRACT #489

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Current preclinical animal experiments provide lower-than-expected prediction of toxic liabilities and therapeutic effects in human patients. Importantly, international organizations and agencies, among them U.S. Environmental Protection Agency, have also declared their strong intention to reduce and eventually completely eliminate animal testing during the next decade. In this landscape, new approach methodologies (NAMs) are the focus of innovative research efforts in drug discovery and regulatory hazard determination. Among NAMs, the zebrafish is one of the most promising models since it ensures at the same time high-throughput capabilities and biological relevance of a whole organism. At larval stages it is considered as a non-animal method, which constitutes zebrafish a relevant gamer for in vitro integrated approaches and 3Rs principles application. Many years ago, the National Institutes of Health already ranked the zebrafish as the second most important animal model behind the mouse. On current days, several fish toxicity tests are a standard information requirement under REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation: among them the AFT (acute fish toxicity) is one of the most spready used. Here we present, among others, an example of a teratogenic assessment assay using a deep learning algorithm, able to classify phenotypes and segment regions with good scores. Overall, we demonstrate that combining the experimental advantages of the zebrafish larval model with artificial intelligence allows for high-throughput, fully automated detection of compound teratogenicity, thus paving the ground for a faster and reliable human risk assessment based on NAMs. Together, this data demonstrates that zebrafish can complement if not replace mammalian testing, while being equally protective of human health. Furthermore, it reinforces the evidence for the zebrafish to be present in the pharmaceutical preclinical regulatory phases in addition to regulated safety studies in the chemical industry.

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P-7b-2

LARGE SCALE MANUFACTURING OF NAMS
ABSTRACT #501

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The development of standardized human-relevant NAMs of industrial interest is fundamental to improve the assessment of chemicals and cosmetics, reduce drug development failure rate, advance human benefits and protect animal welfare. The supplier industry has been diligent in the fostering of NAMs and there are many cases of

synergy with other stakeholders towards the 3Rs principle. Despite these advances, the wide adoption of alternative in vitro methods for toxicology testing is a current challenge. The effective industrial manufacturing of NAMs is critical to accelerate the in vivo to in vitro transition. To efficiently translate a NAM into regulatory, commercial, and technical requirements for large-scale manufacturing, the early engagement with a mature industrial partner is encouraged. From our 60-years of experience as plastic and membrane OEM of commercial off-the-shelf and customized NAMs, we would like to summarize some key points that we believe will help scientists to translate their ideas in line with financial and regulatory specifications. For instance, the upscaling of a new device implies an attentive evaluation of the required times and costs. Consequently, the scientific relevance, potential market size, scalability, reproducibility, and high-throughput potential must be considered in advance. In addition, elements such as material and tolerance levels, quality control, homogeneity, regulatory strategy, environmental conditions, packaging, appearance, functionality, ergonomics, ecology, shelf life, sterility, labelling and transport are of extreme importance. We would like to encourage NAMs developers to partner with experienced industry stakeholders. The supplier industry is open to nurture NAMs and it is committed to help reduce, replace, and refine the use of animals in toxicology testing, while advancing human benefit [1].

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P-7b-3

Development of a new methodology to quantify the activation of the Nrf2 (key event 2) by allergens
ABSTRACT #503

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Background and Objectives: Allergic contact dermatitis is one of the most frequent occupational diseases associated with chemical exposure. The understanding the adverse effect mechanisms (AOP) of skin sensitization allows us to develop in vitro tests as alternatives to animals tests. The second key event in this AOP takes place in the keratinocytes including gene expression associated with the activation of the Keap1-Nrf2-Are pathway. For the key event 2, the test guideline OECD TG 442D provide 2 in vitro ARE-Nrf2 luciferase test using luminescence technique, which during their implementation has shown critical aspects, such as: sensitive of the luminometer, plate format, light-cross-contamination, luciferase substrate quality and high variability. The main objective of this work was the development of a new methodology for evaluating key event 2 based on the quantification of the activation Nrf2-Are pathway, using flow cytometer. Material and Methods: 18 substances, including 14 skin sensitizers and 4 non-sensitizers, were selected according to the OECD TGs. The keratinocyte cell line HaCaT (2x105 cells/ml) was exposed to the test substances at different concentrations for 24h. After that, the cytotoxicity, was evaluated to obtain the CV75. Using the CV75 of each test substance, the cells were exposed for 24h and the quantification of the transcription factor Nrf2 (Alexa Fluor® EP1808Y), was performed by flow cytometer. Results: Preliminary results showed that this new methodology using cytometry allowed distinguishing skin sensitizers from non-skin sensitizers. Discussion and Conclusion: Non-sensitizers compounds showed low free quantity of Nrf2 in the cell, whereas classical sensitizers, such as PPD and DNCB, increased the presence of Nrf2 in the cells, suggesting the activation of the Nrf2-Are pathway. Studies are in progresses to improve the development and determine the accuracy of this new methodology.

P-7b-4

Reduction of animal use in reproductive and developmental toxicity studies - new methodology

approach
ABSTRACT #509

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Nowadays, in vivo reproductive toxicity studies can't be entirely replaced by in vitro studies. For covering bioethics and 3R principles focused on decreasing animal usage in the study, we present some approaches to evaluate reproductive toxicity and developmental toxicity (teratogenic activity) with a reduced number of animals and with a higher amount of assessment parameters for target endpoints evaluation. There are two main OECD guidelines for chemical reproductive and developmental toxicity testing: OECD 443 "Extended one-generation reproductive toxicity study" and OECD 414 "Prenatal Developmental Toxicity Study". The overall number of used animals in two of these studies is around 440 adult animals and an average of 1600 foetuses. To decrease the amount of animal usage, in some cases, we can reduce their number by combining two of these studies in one. It is two different studies with different methodological procedures. The main differences are that the duration of the exposure period in a reproductive toxicity study is much longer than in a developmental study. The next important thing that we should consider is the chemical influence on implantation when providing exposition through all periods of pregnancy, which is a critical part of the development study because it could impair the lower number of foetuses for further evaluation. All of these points should be taken into account when starting the experiment. Also, experimental animals mated with untreated intact animals can provide additional information regarding sex sensitivity in this methodology approach. At this point, we are far from a complete replacement in vivo study to in vitro. Implementing Reduction and Refinement is essential as two of the 3R principles. The crucial benefit of a combined study is animal use reduction and refinement. Only one combined study can reduce the number of animals used in an experiment to 220 adults and 400 foetuses.

Session: 8a. In vitro methods for safety assessment of medical devices

P-8a-1

Optimization of skin sensitization testing strategy in vitro for medical device extracts
ABSTRACT #24

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Before marketing, medical devices have to be tested in accordance with ISO 10993-10 to avoid skin sensitization. This standard predominantly refers to in vivo tests, however, it does not exclude the use of alternative in vitro methods, which have been sufficiently technically and scientifically validated. It is foreseen that due to the complexity of the sensitization endpoint, combination of several methods will be needed to address all key events of the skin sensitization Adverse Outcome Pathway. The objective of this study was to evaluate the sensitization potential of 97 commercially available samples of medical devices using a combination of in vivo (LLNA DA, OECD TG 442A), in chemico (DPRA, OECD TG 442C) and in vitro (LuSens, OECD TG 442D) methods with the aim to enhance the testing strategy for safety assessment of medical device extracts, to optimize the test and extraction procedures and to extend the applicability domains of separate in vitro methods recently successfully validated for chemicals. A good agreement between in vitro and in vivo results was achieved regarding the absence of skin sensitization potential; however, discrepancies in positive classifications have been recorded. The mismatch between in vitro and in vivo results might be caused by specific response of the immune system of the living organism, however, the in vitro methods are suggested as feasible for bottom-up skin sensitization testing, starting with test methods accurately identifying non-sensitizing medical device extracts. Supported by ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative

toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860) and by Ministry of Health, Czech Republic - conceptual development of research organisation („National Institute of Public Health - NIPH, IN: 75010330“).

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ISO 10993-10:2021. Biological evaluation of medical devices — Part 10: Tests for skin sensitization

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P-8a-2

In vitro method for quantitative potency assessment of skin sensitizers during development of novel materials for intended use in medical devices
ABSTRACT #307

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The use of acrylates in materials for use in medical devices can bring several advantages such as super-absorbency, transparency, flexibility, toughness and hardness. The manufacturing of acrylic materials typically involves using at least one acrylate or methacrylate monomer which react to form a polymer. The polymerization consumes the monomers, however depending on the manufacturing process, the degree of polymerization of the final material can vary. Some products therefore contain more residual monomers than others and human exposure to these monomers may increase the risk of skin sensitization and allergic contact dermatitis. In this study we explore the potential to use the GARDskin Dose-Response assay for quantitative potency assessment of two acrylate monomers to characterize their skin sensitizing potential. The GARDskin Dose-Response assay is a modification of the GARDskin (OECD TGP 4.106) protocol and provides a cDV0 value as readout, which corresponds to the lowest concentration required to exceed a binary classification threshold in GARDskin. This concentration correlates significantly with LLNA EC3 and human NOEL values and linear regression models have been established to exploit these relationships for potency predictions. The two acrylate monomers were both classified as skin sensitizers in the GARDskin Dose-response assay with predicted LLNA EC3 values and human NOEL values of 0.848% and 22.4%, and 230 µg/cm2and 12200 µg/cm2, resulting in final classifications as a strong to moderate skin sensitizer (HP 2) and a moderate to weak sensitizer (HP 5), respectively. The results obtained in this study agreed with information in the ECHA registration dossiers and with gathered human data evidence for the respective monomers, illustrating that GARDskin Dose-Response has the potential to replace the in vivo LLNA method for quantitative potency assessment of potential skin sensitizers during development of novel materials for use in medical devices.

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P-8a-3

Development, pre-validation and validation of the EpiDerm in vitro skin irritation protocol for the medical devices extracts
ABSTRACT #172

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Evaluation of dermal irritation is an essential com¬ponent of the safety evaluation of medical devices. Reconstructed human epidermis (RhE) models have replaced rabbit skin irritation testing for neat chemicals (OECD TG 439). However, medical device (MD) extracts are dilute solutions with low irritation potential, there¬fore the validated RhE-methods needed to be modified to reflect needs of ISO 10993. A protocol employing RhE EpiDerm was optimized in 2013 using known irritants and spiked polymers (1). After the suc¬cessful

transfer and standardization of the protocol, 17 laboratories worldwide were trained in the use of the protocol in the preparation for the validation. All laboratories produced data with almost 100% agreement of predictions for the selected references (2). Moreover, several medical devices benchmark materials (5 irritants and 2 vehicles) were evaluated in the controlled human patch testing (4 h and 18 h) and in EpiDerm in vitro skin irritation protocol, results were then compared to existing rabbit skin irritation test data. In 2016, an international round robin validation study was conducted to confirm the ability of the RhE models to correctly predict the intra-cutaneous irrita¬tion of extracts from MDs. Four irritant polymers and three non-irritant controls were tested. Blinded polymer samples were extracted with sesame oil and saline per ISO 10993-12. Positive and negative solvent controls were included (3). EpiDerm tissues were able to correctly identify virtually all of the irritant polymer samples either in the saline or in the sesame oil or in both solvent extracts. Our results indicate that RhE tissue models can detect the presence of skin irritants at low concentrations in dilute medical device polymer extracts (3). The use of the reconstructed tissue models, as replacements for the rabbit intra-cutaneus test is implemented into the ISO 10993 standards used to evaluate medical device biocompatibility.

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P-8a-4

Bio-compatibility assessment of medical devices using reconstructed in vitro 3D human cornea-like tissue model

ABSTRACT #231

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Medical devices are increasingly used in the population to cope with degenerative diseases, treat traumatic injuries or in personal and dental care. Each medical device used in a patient/consumer must be subjected to a structured biological evaluation program within a risk management process described by ISO standards. In recent years, the ISO standard implemented a series of highly sensitive in vitro methods that help the safety assessors in the screening of potential health hazards of medical devices even before conducting animal studies or clinical trials (1). Significant success has been achieved with the development and validation of in vitro protocol for sub-cutaneous irritation testing of medical devices (2,3). The outcome led to the development of a new ISO standard 10993-23. Building on the experience obtained in that project, an in vitro protocol for ocular irritation testing has been developed using in vitro 3D reconstructed cornea-like tissue model. Six benchmark materials, representing standard biocompatible polymers as well as impurities from the production process of medical devices, have been tested to evaluate the sensitivity and specificity of the proposed in vitro test. The accuracy of the test towards these materials was 100%. In the next phase of the project, materials representing medical devices used in ophthalmology will be tested to challenge the in vitro predictions and protocol. The development of a protocol for early detection of toxic materials and impurities posing a health risk will help to increase the safety of medical devices for patients. At the same time, in vitro methods will help to reduce or even eliminate the need for follow-up tests on animals in selected medical device categories. This project is supported by the Scientific Grant Agency of the Slovak republic VEGA, grant number 2/0153/20, APVV Grant APVV-19-0591 and DS-FR-2019-0048

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Session: 8b. In vitro COVID-19 research / Lung and cardiovascular models

P-8b-1

COMPARISON OF EXPRESSION PROFILE OF SARS-COV-2 KEY RECEPTORS IN SKIN AND LUNG IN VITRO MODELS
ABSTRACT #345

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Background and Objectives The entry of SARS-CoV-2 in host cells depends on the availability of virus receptors on the surface of host cells. We hypothesized that damaged skin could represent a potential entry route for SARS-CoV-2. The objective of this work was to measure mRNA expression of SARS-CoV-2 key receptors in different human in vitro skin and lung models. Materials and Methods Constitutive expression of ACE2, TMPRSS2, NRP1 (neropilin-1) and AR (androgen receptor) was measured by quantitative RT-PCR in human epidermal keratinocytes in primary culture, 3D-reconstructed human epidermis (RHE) model, human skin biopsies, Calu-3 human lung adenocarcinoma cell line and 3D-mucociliary tissue model EpiAirway. Moreover, modulation of the expression of ACE2 was measured in 3D skin and lung models challenged with lipopolysaccharide and treated with dexamethasone. Results ACE2, TMPRSS2, NRP1 as well as AR are expressed in keratinocytes, RHE and skin biopsies. Expression level is generally higher in RHE and skin biopsies than in keratinocytes. The four key receptors of SARS-CoV-2 are also expressed in Calu-3 cells and EpiAirway tissues. Expression level is generally higher in 3D-lung model than in Calu-3 cells. The expression level of ACE2 and TMPRSS2 is higher in lung models than in skin models, that of NRP1 is similar in both models, and that of AR is very high in skin biopsies. In 3D skin and lung tissues challenged with lipopolysaccharide, treatment with dexamethasone induced a marked downregulation of ACE2 expression in 3D-skin model and no changes in 3D-lung model. Discussion and Conclusion This study shows that SARS-CoV-2 receptors are expressed in skin models, and ACE2 mRNA expression can be modulated by dexamethasone. This finding is of great interest as it indicates that the skin could represent a potential entry route for SARS-CoV-2, especially when skin barrier integrity is damaged.

P-8b-2

INTESTINAL ADVERSE OUTCOMES IN COVID-19: CURRENT EVIDENCE AND UNCERTAINTIES USING THE ADVERSE OUTCOME PATHWAY FRAMEWORK.
ABSTRACT #455

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Background and Objectives. COVID-19 patients could experience gastrointestinal

disorders and alteration of gut microbiota. Besides, the SARS-CoV-2 receptor ACE2 is highly expressed in enterocytes. Thus, it has been proposed that SARS-CoV-2 enteric infection leads to intestinal barrier disruption, inflammation, and dysbiosis. However, the underlying mechanisms are still poorly understood. Methods. Here, we applied the Adverse Outcome Pathway (AOP) framework to explore existing evidence supporting the sequence of events of proposed pathways depicting the mechanisms behind SARS-CoV-2 mediated gut pathophysiology. Results. One AOP outlines SARS-CoV-2 enteric infection leading to intestinal barrier dysfunction via cytopathic effects. Studies with human enterocytes in vitro demonstrate SARS-CoV-2 infection. However, evidence for the viral replication in vivo in animals and in (healthy) human gut is unclear, either due to timely interferon response limiting viral replication or the multiple layered barrier. While the biological plausibility is high, currently, there is not enough evidence to support enterocyte massive cell death due to SARS-CoV-2 infection. Besides, ACE2 plays a key role in intestinal homeostasis, notably in dietary amino acids uptake, such as tryptophan. Evidence supports high plausibility for intestinal ACE2 dysregulation due to spike (S) protein binding, but further examination is needed to distinguish between the direct effect of the spike protein ACE2 binding and subsequent replication. In addition, more evidence is required to understand the role of observed tryptophan alteration which regulates the secretion of antimicrobial peptides, altering the gut microbiota. Lastly, another putative AOP proposes a new mechanism for COVID-19 transmission mediated by gut bacteria that may be transducing infective SARS-CoV-2 components. Current inconsistencies regarding detection of replicating SARS-CoV-2 in feces calls for additional research. Discussion. This AOP-aligned approach highlights current significant inconsistencies in the evidence and knowledge gaps that can guide future research. In addition, it facilitates synergy from different disciplines to address health issues.

P-8b-3

COVID-19 through the lens of the Adverse Outcome Pathways: What can we learn from the view on the relationship between ACE2 dysfunction and its interaction with SARS-COV2 ABSTRACT #432

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Understanding the key mechanistic perturbations of COVID-19 pathogenesis is still needed to better inform testing and targeting adverse outcomes of this complex disease. The CIAO1 Project aims to model the pathways of key mechanistic perturbations driving COVID-19 pathogenesis using the Adverse Outcome Pathways (AOPs), an approach originated in regulatory toxicology to facilitate the use of New Approach Methodologies (NAMs). Representing evidence/knowledge as sequences/pathways of key events (KEs) is an established scientific practice. The AOP framework adds value by providing a structured and transparent approach to the analysis of the weight of evidence for causality of the relationships between KEs, the Key Event Relationships (KERs). Thus, AOPs facilitate a shift of the focus from the reliance on individual nodes (KEs) to edges (KERs) as critical “glue” elements to establish a credible KE sequence i.e. pathway leading to adverse outcomes, including disease. An AOP-driven approach is presented that evaluates the evidence for ACE2 dysregulation as a result of its interaction with SARS-COV2, addressing the dominant hypothesis that following interaction with the viral S-protein, ACE2 is downregulated which leads to most AOs of COVID-19. The evaluation is presented in the AOP Wiki(1) (the main tool for AOP development) KER23112) and indicates that ACE2 dysregulation exhibits a complex temporal, cell/tissue and even test system specific pattern which requires further detailed examination. An integrative approach to evidence analysis within a single KER in the context of pathways relevant to different organ systems may facilitate understanding of this and other complex interconnected relationships. An AOP approach with the WoE analysis of KERs as the main focus may provide an enhanced context for evaluating the significance/relevance of the NAM measurements of specific nodes/KE for the final outcome of a particular pathway that would also

be informative in the identification and early evaluation of therapeutic targets.

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P-8b-5

ESTROGENIC ACTIVITY RELATED TO THE PRESENCE OF SARS-COV-2 IN WASTEWATER
ABSTRACT #319

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Background and objectives The scientific community has, on the basis of epidemiological data, highlighted differences between two sexes in becoming infected with SARS-CoV-2 and developing the disease defined as Covid-19. These differences can be explained by biological, sexual and hormonal mechanisms. The exposure to environmental estrogens could alter the SARS-CoV-2 susceptibility. This study investigates estrogenic activity in wastewater samples from urban wastewater treatment plants in relationship with Covid-19 pandemic trend. Material and Methods The E-SCREEN assay is able to detect any estrogenic activity in environmental chemicals on the basis of the proliferative effect of estrogens on their target cells as an end point. It is a quantitative test that compares the number of cells obtained from similar inocula of MCF-7 (breast cancer) cells in the absence of estrogen (negative control) and in the presence of 17 beta-estradiol (positive control) and a range of concentrations of chemicals suspected to be estrogenic. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) test was used as a measure of cell proliferation. The same samples analyzed by E-SCREEN underwent SARS-CoV-2 quantification by qRTPCR for the monitoring of the trend of Covid-19 in Regione Liguria by following the “Sorveglianza di SARS-CoV-2 in reflui urbani” (SARI) project protocol coordinated by Istituto Superiore di Sanità. Results We tested in all locations both estrogenic and anti-estrogenic potency and these effected were correlated with the SARS-CoV-2 pandemic trend. Preliminary analyses have been presented. Discussion and Conclusion The estrogenic activity of sewage in Genova was evaluated after treated. Since, this sex-dependent susceptibility has also been observed in humans and thus identifies the protective effects of estrogen receptor results for patients with infections such as SARS-CoV-2, we are going to test for the first time the estrogenic potency in sewers, in order to assess whether it is informative with respect to the pandemic trend.

Session: Models and methods (formerly called OTHER)

P-MODELS-1

APPLICATION OF BENCHMARK DOSE APPROACH FOR ASSESSMENT OF EFFECTS ON RED BLOOD CELLS REVEALED DURING NON-CLINICAL TOXICITY STUDY OF DRUG CANDIDATE
ABSTRACT #81

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Despite growing interest in benchmark dose (BMD) analysis, it is not so often used for drug candidates safety assessment. Nevertheless, the approach may provide more information from the same number of animals and may be considered as one of the elements of the 3Rs. The study aimed to assess potentially harmful effects on red blood cells revealed during non-clinical toxicity study of potential drug using the BMD method following NOAEL methodology. Materials and methods: Individual data sets were extracted from toxicity study of potential pharmaceutical agent diethyxime, administered i.m. to rats females (n=6) during 10-days at doses 0-10-50-100-200 mg/kg. Continuous data sets for red blood endpoints (HCT, RBC, HGB, MCV, MCH, MCHC) were analyzed.

Dose dependence analysis performed using EFSA web-tool PROAST66.24, critical effect size – 10 %. Benchmark dose lower levels (BMDL), and upper levels (BMDU), and their ratios were considered following NOAEL analysis. Results. Toxicity/safety assessment of diethyxime revealed red blood endpoints affection considered as an adverse effect, occurring at low levels of dosing. Different NOAELs were established for HCT (10 mg/kg), RBC (50 mg/kg), HGB (100mg/kg), MCH (100mg/kg). Statistical deviations unrelated to dose were found for MCHC. The changes of red blood cells were considered as suitable for following BMDL analysis requirements. The conducted analysis resulted in similar BMDL for HCT, RBC, and HGB (11-16 mg/kg), in contrast to different NOAELs (10-100 mg/kg), defined for these endpoints. BMDU/BMDL ratios reflecting the probabilistic nature of BMD, calculated for HCT, RBC, and HGB were as well similar in the study, reflecting interrelation of these parameter changes. The observed statistically significant increase of MCHC (BMDL–84mg/kg, BMDLU/BMDL–47), and MCH (BMDL–225mg/kg, BMDLU/BMDL–116) may reflect the compensatory reaction of organism, secondary to anemia. MBD method, in this case, offered more relevant information about the hemolytic effect of the studied substance.

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P-MODELS-4

PANORAMIX: Providing risk assessments of complex real-life mixtures for the protection of Europe's citizens and the environment ABSTRACT #401

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The impact of chemical mixtures' exposure is a matter of public concern. However, mixtures are only slowly making their way into regulatory risk assessment. Critical knowledge gaps are which chemicals drive mixture effects and the validity of the dose addition principle for complex mixtures of chemicals at low concentrations. Advances in modern animal-free high-throughput toxicology can provide novel tools for evaluating chemical mixture exposures in our society. This presentation will give an overview of the EU-funded Green Deal project, PANORAMIX, active since Nov 2021, its partners and objectives (https://panoramix-h2020.eu/). The consortium plans to overcome the limitations of existing mixture approaches by developing a novel experimental path based

on whole mixture assessments. This project focuses on identifying and quantifying the risk associated with chemical mixtures deriving from real-life samples representing environment, food, and humans. Moreover, we will present the ready-to-use and practical tools for mixture risk assessment of chemicals that PANORAMIX plans to develop. We will outline the methodologies used in the project and how they combine in an innovative manner. These techniques include in vitro assays, effect-directed analysis and suspect and non-targeted chemical profiling. Additionally, the project takes advantage of the "Odense Child Cohort", which contains more than 2500 samples of cord blood and donors' health information, to link developmental adverse health outcomes with chemical mixtures exposure. PANORAMIX will also use mixture modelling, case studies and experimental data to develop the "Chemical Mixture Calculator 2.0", a web-based interface for calculating risks associated with chemical mixtures. Furthermore, we will develop effect-based trigger values for in vitro effects to be directly measured in water, food, and blood to identify mixtures exposure's health threats. Lastly, we will show how the project involves regulatory and scientific stakeholders to facilitate implementing new mixtures risk assessment and identify the most critical environmental and human health exposures.

P-MODELS-5

IN VITRO MUTAGENICITY AND GENOTOXICITY OF PURE ANATOXIN-A ABSTRACT #433

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Background and objectives: Water eutrophication and climate change have contributed to the proliferation of cyanobacterial species, leading to an increasing occurrence of cyanotoxins. Anatoxin-a (ATX-a) is a globally distributed freshwater neurotoxin that has been linked to human and animal poisonings. Unfortunately, to date, the toxicological knowledge of ATX-a is limited. For this reason, the aim of this study was to investigate the mutagenicity and genotoxicity potential of pure ATX-a. Material and methods: ATX-a was assessed by two different in vitro tests recommended by the European Food Safety Authority (EFSA). Concentrations ranged from 0.125 to 20 µg/mL were selected to perform the bacterial reverse-mutation assay in Salmonella typhimurium (Ames test, OECD 471) in absence and presence of metabolic fraction S9 and the mammalian cell micronucleus test (OECD 487) on L5178YTk± cells in presence of S9. The exposure periods ranged between 4 and 72 h depending on the assay. Results: There were no effects after bacteria exposure to ATX-a in a wide range of concentrations with or without metabolic activation. Similarly, genotoxicity was not observed in micronucleus test in presence of S9 at any toxin concentration employed. Discussion and conclusion: Previous studies reported genotoxic effects of cyanobacterial extracts containing ATX-a, but these results suggest that ATX-a is not the responsible compound. This data could contribute to a better risk assessment of this cyanotoxin. Nevertheless, further studies are needed to elucidate the toxicity profile of ATX-a. Acknowledgments: The authors would like to thank the Spanish Ministerio de Economía y Competitividad (PID2019-104890RB-I00 MICIN/ AEI /10.13039/501100011033) and Junta de Andalucía (PREDOC_00447) for the financial support for this study, as well as the Microscopy and Biology Services of CITIUS (University of Seville) for the technical assistance offered.

P-MODELS-6

Mutagenenicity and genotoxicity evaluation of reduced graphene oxide by the mouse lymphoma assay and standard and enzyme- modified comet assays
ABSTRACT #438

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Background and Objectives Graphene materials are of great interest for their promising applications in many fields, including food packaging. However, these materials may induce genotoxicity. The aim of this work was to

explore the mutagenicity and genotoxicity of reduced graphene oxide (rGO) using the mouse lymphoma assay (MLA) and the standard and enzyme- modified comet assays in the Caco-2 cell line. Materials and Methods L5178Y Tk+/- cells were used for MLA. Cells were exposed to 0-250 µg/mL rGO for 4 and 24 h. RPMI medium was used as a negative control, and methylmethanesulfonate (MMS 10 µg/mL) as positive control. For the comet assay, Caco-2 cells were incubated with rGO for 24 h (176.3, 88.2, and 44.1 µg/mL) and 48 h (166.5, 83.3, and 41.6 µg/mL), equivalent to mean effective concentration (EC50), EC50/2 and EC50/4. Medium was used as negative control and H2O2 as positive control. The Endonuclease III (Endo III) and formamidopyrimidine-DNA glycosylase (Fpg) enzymes were selected for the enzyme-modified comet assay. Results For MLA, rGO increased the frequency of mutation at 125 and 250 µg/mL after 4 h of exposure. No mutagenic effects were observed at any concentration tested after 24 h of exposure. In the standard comet assay, Caco-2 cells did not undergo DNA breaks after 24 h and 48 h of exposure at any concentration assayed. rGO did not induce oxidative DNA damage. Discussion and Conclusion Our results evidence that rGO has a good toxicity profile for its potential applications in the food packaging industry, but further toxicological tests are required. Acknowledgement: Fondo Europeo de Desarrollo Regional (FEDER) and Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía, within the Programa Operativo FEDER 2014–2020 for the project US-1259106. And project P18-RT1993 (PAIDI-2020/FEDER, Consejería de Transformacion Economica, Industria, Conocimiento y Universidades, Junta de Andalucía).

P-MODELS-9

Development of an adverse outcome pathway for kidney tubular necrosis ABSTRACT #359

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Background and Objectives Adverse outcome pathway (AOP) networks combine AOPs that share one or more key events (KEs). Our aim is to develop an AOP network determining KEs and the relationships that drive chemical-induced kidney tubular necrosis (TN). To weigh the evidence between KEs, the network will be assessed in accordance with guidelines from the Organization for Economic Co-operation and Development (OECD) (1). The objective is to develop an ontological knowledge framework that integrates biological, toxicological, and chemical data toward predicting systemic repeated dose toxicity effects of nephrotoxic chemicals associated with kidney TN (2). Materials and Methods Embase was used to search for literature on chemical-induced kidney TN using key search terms relevant to clinical presentations, biochemistry, histology, and chemically applicable, data-rich nephrotoxic compounds. Initial title/abstract screening of papers employed SysRev, a computational tool for systematic reviewing and data extraction, using labeling strategies for inclusion/exclusion criteria. Tailored Bradford-Hill criteria described in OECD guidelines will assess confidence levels and weight of evidence for KEs within the AOP network. Kidney physiological maps were designed to establish mechanisms contributing to TN, with systemic mapping of currently reported AOPs involving nephrotoxicity identifying relevant MIEs and KEs. Results The Embase search retrieved 2735 papers to upload to SysRev. The title/abstract screening would further identify papers eligible for data extraction in the full-text screening process. A total of 19 existing AOPs related to kidney dysfunction were identified and analyzed to support the implementation of additional in vitro endpoints for TN. Discussion and Conclusion Data extracted will assess confidence levels in previously described KEs and KERs and identify potential new KEs. The AOP network will form the conceptual basis for establishing a test battery of in vitro assays to characterize nephrotoxic

chemicals by measuring individual KEs for the generation and evaluation of AOPs of TN-related kidney failure.

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P-MODELS-10

Neurotoxic effect of potential countermeasures in case of nerve agent poisoning
ABSTRACT #249

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Nerve agents (NAs), the deadliest organophosphates, act as inhibitors of cholinesterase enzymes that are vital in the cholinergic system. Phosphylation of acetylcholinesterase (AChE), a pivotal enzyme in hydrolysis of the neurotransmitter acetylcholine, and its related enzyme butyrylcholinesterase (BChE) leads to a cholinergic crisis that could ultimately result in death. Medical countermeasures include compounds, containing oxime group with the ability for nucleophilic reactivation of phosphylated cholinesterase, which until now have shown limited potency in reactivation of inhibited AChE in the central nervous system (CNS). With that purpose, in previous studies, we have designed and defined several pyridinium oximes with high potency in reactivation of inhibited BChE that can act as a protector of AChE in CNS. Now through a combination of in silico, in vitro, ex vivo results we want to demonstrate a feasible approach to develop a safe oxime-assisted bioscavenger of NAs based on the efficient reactivation of BChE. Selected oximes have shown to be the most prominent reactivators of BChE inhibited with nerve agent cyclosarin through their overall kinetic reactivation rate. Tested oximes also showed no effect on the viability of neuroblastoma cells upon 4-hours treatment, contrary to cyclosarin exposure. Furthermore, the cytotoxicity profiles on neural cells were determent for oxime-assisted catalytic BChE degradation of cyclosarin, wherein case of post-treatment approach 50 % of neural cells had preserved, while in pre-treatment almost all of the cells have been protected. The most promising bioscavenger combination was then evaluated in ex vivo conditions of human blood resulting in up to 80% of restored phosphylated cholinesterase activity within a short time. Taken all together our findings offer a platform for further safe and promising antidote development in case of NAs exposure. Acknowledgments: The Croatian Science Foundation (IP-2018-01-7683) supported this work.

P-MODELS-11

New Salmonella strains resistant to sulfonylurea and triazole-pyrimidine herbicides and their use in the Ames test
ABSTRACT #201

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Cytotoxicity of some pesticide technical grade active ingredients (TGAIs) is a drawback of Sallmonella/microsome assay with regard to objective assessment of their equivalence to the original products. The impossibility of testing for genotoxicity of some TGAIs, e.g. sulfonylureas and triazole-pyrimidines, at high concentration due to their cytotoxicity makes difficult to detect low-level mutagenic impurities. Selection based on the cultivation of S.typhimurium TA100 with thifensulfuronmethyl was applied to obtain a mutant insusceptible to sulfonylurea toxic effect. We obtained the strains resistant not only to sulfonylureas but also triazole-pyrimidines that may be mediated the same mechanism of action of the pesticides from these classes – inhibition of acetohydroxyacid synthase. The first mutant

strain (deposited as S. typhimurium VKPM B-14099 in the Russian National Collection of Industrial Microorganisms) demonstrated the TA100 phenotypic characteristics: hisG46, rfa del, uvrB-bio, pKm101 Ap-r. The second strain showed the TA1535 phenotypic characteristics and probably lost the R-factor due to the selection using the poor Gm-media with thifensulfuronmethyl. Electron microscopy of the ultrathin sections of cells indicated the difference in the structure of the cell wall and cytoplasm of mutant and parental cultures. The maximum non-cytotoxic concentrations of sulfonylureas and triazole-pyrimidines for the parent strains did not exceed 0.05-0.125 mg/plate, while no evidence of cytotoxicity was observed for the mutants up to 5.0 mg/plate. The number of spontaneous revertants of new strains was within the historical laboratory control limits obtained for the parent cultures. Positive controls caused pronounced mutagenic effects (±S9). Thus, the mutants did not lose the ability to response to induction of the reverse gene mutations. The acquired resistance to sulfonylureas and triazole-pyrimidines expands the possibilities to reveal mutagenic impurities that may occur in TGAIs in the small amounts

P-MODELS-12

BPS DISPLAYS SIMILAR PHENOTYPIC AND METABOLIC RESPONSES TO BPA IN HEPG2 AND INS-1E CELLS ABSTRACT #218

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Bisphenol A (BPA) and bisphenol S (BPS) are metabolism disrupting chemicals that can lead to impairment of glucose homeostasis and alterations in lipid metabolism, however not much is known about potential mechanisms of toxicity associated with these metabolic alterations. This study aimed to investigate potential cellular targets regulated by both compounds in hepatocarcinoma (HepG2) and insulinoma (INS-1E) cells, that could impair their development, function, and ultimately dysregulate energy homeostasis. HepG2 and INS-1E were monitored for 72 hours under exposure to a range of BPA or BPS concentrations (100, 10, 1, 0.1, 0.01 and 0.001 µM) for assessment of cellular growth, proliferation and viability. Additionally, in order to investigate the molecular changes triggered by BPA or BPS (10 – 0.001 µM) after 24, 48, and 72 hours, the baseline metabolic characterization of intracellular polar extracts obtained from both strains was acquired through non-targeted metabolomic analyses by FIA-qTOF. Metabolites with 1.5-fold mean changes in abundance between the experimental groups were considered differentially expressed. Growth curves, rates and cellular viability analyses highlighted antiproliferative and cytotoxic effects in HepG2 cells after exposure to the highest and lowest-concentrations of the contaminants (nonlinear dose-response curves), while the same exposure regimens produced milder effects on INS-1E proliferation and viability, except for the highest concentration of both contaminants. HepG2 and INS-1E metabolomics analyses revealed time- and dose-dependent molecular changes and that BPA and BPS could share similar pathways of toxicity, upregulating levels of metabolites which are mediators in inflammatory processes (linolenic and linoleic acid metabolic pathway) in both cell lines. In summary, this study evidences the importance to conduct in vitro toxicological tests with BPA and BPS using different cell lines and exposure regimens and the relevance of using metabolomics approaches to unveil potential mechanisms of toxicity.This work was supported by FAPESP (Grant n. 2018/19554-0).

P-MODELS-13

Comparison of a heated tobacco stick product and a combustible cigarette: chemical analysis and in vitro toxicological evaluation ABSTRACT #257

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Background and objectives Previous studies have shown that heated tobacco products (HTPs) emit aerosols with fewer harmful and potentially harmful constituents (HPHCs), leading to less biological activity in vitro and lower levels of biomarkers of exposure in clinical studies. However, various heating systems are used in different HTPs; thus, it is desirable to understand whether different heating mechanisms affect the biological properties of the aerosol. Here, the chemical and in vitro toxicological properties of aerosols from DT3.0a, a heated tobacco stick product with a distinctive heating mechanism, were compared with 1R6F cigarette smoke (CS). Materials and Methods Two flavors (regular and menthol) of DT3.0a, and the 1R6F cigarette were tested. The extracts of the particulate and gas-vapor phases of the aerosols were analyzed. Chemical analysis was conducted for analysis of HPHCs. In addition to the battery assays (Ames, micronucleus, and neutral red uptake assays), biological analysis was conducted using the ToxTracker assay that enabled mechanism-based genotoxicity assessment and using the 2D/3D cell culture assays of human bronchial epithelial cells that enabled analysis of oxidative stress and inflammatory responses. Results The levels of HPHCs found in the DT3.0a aerosols were lower compared with those in 1R6F CS. All the genotoxicity-related assays indicated that DT3.0a aerosols were not genotoxic, regardless of metabolic activation. In the other biological assays, DT3.0a aerosols showed the reduced induction of cytotoxicity, oxidative stress, and inflammatory response compared with 1R6F CS. The results were confirmed for both regular and menthol types of DT3.0a. Conclusions Similar to the results reported for HTPs with internal or external heating mechanisms, the results of the present study indicated that the chemical and biological properties of the DT3.0a aerosols were likely to be less harmful than 1R6F CS. These results are anticipated to be reflected in future clinical outcomes.

P-MODELS-16

CHRONIC LOW-DOSE EXPOSURE TO DIBUTYL PHTHALATE AFFECTS NO PRODUCTION, CELL MIGRATION, AND ANGIOGENESIS IN HUMAN ENDOTHELIAL CELL LINE EA.HY926 ABSTRACT #293

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Although epidemiological studies suggest a possible association between exposure to dibutyl phthalate (DBP), a chemical widely used in various products of human use, and cardiovascular diseases, the impact of DBP on endothelial cells (ECs) remains unclear. In particular, very few in vitro studies that mimic “real-life” exposure scenario have been conducted to date. Here, we sought to investigate nitric oxide (NO) production, cell migration, and angiogenesis after long-term exposure of human ECs to environmentally-relevant concentrations of DBP. EA.hy926 cells were repeatedly exposed to either vehicle (0.05% DMSO) or three different concentrations of DBP (1, 10, and 100 nM named DBP-1, DBP-10, and DBP-100 groups, respectively) during 12 weeks. After 3, 6, 9, and 12 weeks, we assessed NO production using the Griess’ method, cell migration using the modified Boyden chamber assay, and angiogenesis using the endothelial tube formation assay in growth factor-reduced basement membrane extract-loaded cell culture plates. NO production was unaffected after 3 weeks; however, an increase was evident in DBP-100 group after 6, 9, and 12 weeks and in DBP-10 group after 12 weeks of exposure. Cell migration was diminished in all DBP groups after 3 weeks and increased in DBP-10 and DBP-100 groups after 9 weeks. Angiogenesis was diminished in DBP-10 and in DBP-10 and DBP-100 groups after 3 and 6 weeks, respectively, whereas significant increase was detected in DBP-100 and in DBP-10 and DBP-100 groups after 9 and 12 weeks, respectively. Obtained results indicate that long-term exposure of EA.hy926 cells to low doses of DBP promotes oscillatory changes in physiologically synchronized functions – NO production, cell migration, and angiogenesis. These findings are important since they represent the first evaluation of the direct effect of DBP on endothelial tube formation, showing

that angiogenesis of human ECs may be the target for DBP with potential pathophysiological consequences.

P-MODELS-17

Mixture effects of extracts from environment, food and blood on neurite outgrowth compared to cytotoxicity in SH-SY5Y cells
ABSTRACT #367

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Background and Objectives Developmental neurotoxicity (DNT) is raising concern for human health. DNT in vitro testing battery has been established for testing single chemicals, however, it has not been explored whether mixtures of chemicals found in the environment and in people can elicit DNT. Here we present first data on the DNT of mixtures of chemicals extracted from representative pooled samples along the continuum "environment – food – human". This study is part of the H2020 Project "PANORAMIX– Providing risk assessments of complex real-life mixtures for the protection of Europe's citizens and the environment." Material and Methods Complex mixtures of organic chemicals in pooled samples from up to 10 European countries of diverse types of water (wastewater, surface water, drinking water), fish and milk as proxies of food, and pooled human blood from adults and umbilical cords were extracted and enriched by collaboration partners of the PANORAMIX project. Human neuroblastoma SH-SY5Y cells were used to test effects of extracts on neurite outgrowth, which is one of key DNT endpoints, based on imaging system. Neuronal cytotoxicity was measured together to quantify specificity of the effects on neurite outgrowth. Genotoxic effects were quantified with the Histone H2AX phosphorylation (gamma-H2AX) assay in SH-SY5Y cells. Results Inhibition in neurite outgrowth and cytotoxic effects were detectable in many of the sample extracts. The effect concentrations for causing inhibition in neurite outgrowth and cytotoxic effects varied between and within matrix types. For example, extracts of wastewater elicited high effects that were reduced by wastewater treatment plants and low but measurable in surface water. Specific effects on neurite outgrowth compared to cytotoxicity were observed in several extracts. Discussion and Conclusion We demonstrated the applicability of this neuronal-cell assay for screening effects of diverse mixtures for DNT across a diverse type of environmental samples and blood.

P-MODELS-18

Connexin-based channel activity is not specifically altered by hepatocarcinogenic chemicals
ABSTRACT #251

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Background: In contrast to genotoxic carcinogenic (GTX) compounds, non-genotoxic carcinogenic (NGTX) compounds can only be detected by ethically unfavourable and costly 2-year rodent assays. To identify and distinguish NGTX from GTX in vitro, focus has been put on the effect of NGTX on cellular communication, in particular gap junction intercellular communication (GJIC). In the presented study, the effect of NGTX, GTX and non-carcinogenic chemicals on connexin expression and functionality has been evaluated in a

differentiated human hepatocellular carcinoma HepaRG cell model. Methods: Real-time reverse transcription quantitative polymerase chain reaction analysis (Rt-qPCR), immunoblot analysis and in situ immunostaining were used to quantify connexin mRNA and protein levels, respectively. GJIC was monitored with a scrape-loading/dye transfer assay while connexin hemichannel opening was evaluated by measuring the extracellular adenosine triphosphate release. Results: RT-qPCR analysis and immunoblot analysis showed alterations in connexin26, connexin32 and connexin43 expression for various chemicals. Three non-carcinogenic compounds inhibited GJIC, while hemichannel activity was lowered in response to certain carcinogenic compounds. Conclusion: Connexin32 protein expression was negatively affected by both NGTX and GTX compounds, but no other specific effects could be attributed to a chemical type at the expression or functional level.

P-MODELS-19

STUDY OF CITRININ TOXIC EFFECTS ON A 3D NEUROBLASTOMA MODEL: CYTOTOXICITY, OXIDATIVE STRESS AND CELL DEATH
ABSTRACT #371

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Background and Objectives: Citrinin (CIT) is a mycotoxin produced by fungi of the genera Aspergillus, Penicillium and Monascus and is mainly found in cereals. While its role in causing damage to the kidneys has been extensively studied, its effects at the neuronal level are poorly known. The aim of this work is to study the effects of CIT on 3D cultures (spheroids) of a human neuroblastoma cell line (SH-SY5Y) to identify its toxicity mechanisms. Material and Methods: We evaluated IC50 using MTT, ATP and Presto Blue viability assays at 24, 48 and 72h post exposure. A Live & Dead test was used to evaluate cell viability. ROS generation, alterations in mitochondrial membrane potential (MMP) and apoptosis and necrosis processes were also assessed. Results and Discussion: We showed that IC50 ranged from 83.14±5.70 to 46.73±8.61 µM. We exposed spheroids to CIT 25 and 50 µM for 48h. A decrease in MMP was observed at the lowest dose, while no significant differences with respect to control were recorded after exposure to 50 µM. This may be due to the activation of compensatory mechanisms in the cells. Regarding the generation of ROS, no significant increase was observed at any of the selected concentrations. Finally, an increase in late apoptosis and necrosis processes was observed in cells exposed to CIT compared to control at both concentrations although more at 50 µM. Conclusion: Our findings indicate that CIT causes cytotoxic effects by inducing apoptosis and necrosis and altering MMP. Further studies will better detail these toxicity mechanisms. Acknowledgements: This work was funded by the Spanish Ministry of Science and Innovation (PID2020-115871RB-100). The work was also supported by the European Research Council Starting Grant (ERC-StG) MICRONEX (UERI17, PI: E. Cimetta). In addition, FJ Martí-Quijal thanks the pre-doctoral grant "Atracció de Talent" from the Universitat de València.

P-MODELS-20

THE ROLE OF ENVIRONMENTAL CONTAMINANTS DETECTABLE IN WASTE OF ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) PLANTS IN A549 AND HEPG2 CELL LINES – THE VAISAL PROJECT
ABSTRACT #441

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Background and objectives. Waste of electrical and electronic equipment (WEEE) is a valuable source of secondary raw material for recovery and recycling as well as an emerging threat for environmental and human health due to the occurrence of toxic organic and inorganic chemicals in e-waste components. Suspended particles arising from WEEE recycling operations are a specific concern. Hence, the VAISAL project (granted by INAIL BRiC 2019 - ID13) aims to identify potential toxic inorganic and organic compounds, including emerging organic pollutants in WEEE treatment plants, and investigating toxicological and metabolic responses also taking into account the synergistic effects of different classes of pollutants, such as per- and poly-fluoroalkyl substances (PFAS), brominated flame retardants and organophosphates. Materials and methods. Particle matters (PM) samples, collected in three WEEE plants by 14-stage cascade impactors, have been characterized for their content of inorganic and organic compounds (23 and 149 molecules, respectively). In addition, non-targeted analysis methods were applied to the same PM extracts using liquid-chromatography coupled to high-resolution mass spectrometry in order to detect further compounds referable to the classes of PFAS. Overall, some of the targeted and non-targeted PFAS have been subjected to dose-dependent cytotoxicity by MTS assay, metabolic degradation by liver microsomes and lipidomic analysis in two human-derived cell lines, A549 (epithelial bronchial cells) and HepG2 (hepatocytes). Results. Cytotoxicity studies by MTS assay as well as lipidomic analysis were performed at 1pM-10□M range of tested concentrations: an impact of some PFAS on cell viability at concentrations ≥ 1 □M were detected. Discussion and conclusions. The role of some PFAS (e.g. PFAS, PFOA, PFBS, GenX) detected in three different Italian WEEE plants have been studied in human derived cell lines (A549 and HepG2) representatives of two PM routes of exposure. Obtained data will be presented and discussed.

P-MODELS-21

PK-driven drug test for the evaluation of the efficacy of anti-cancer treatment regimens
ABSTRACT #443

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Cancer remains the second major cause of death in the world. Molecular analysis of patients' tumors paved the way for the design of accurate markers for sensitivity to various treatment regimens. Unfortunately, if the actionable tumor targets are not available, there is no functional drug test that is clinically tested to predict the most effective treatment for an individual cancer patient. Primary tumor cell culture technologies provided the opportunity to perform individual drug testing for almost every cancer patient1. However even utilization of these next-generation cancer cell models is non-sufficient to predict clinical response to certain therapy using standard drug testing approaches such as GI50 and the development of more precise methods is needed2. We have developed an algorithm which transforms in vivo pharmacokinetics of a therapeutic scheme or PK/PD modeling data into in vitro testing protocol. This method is designed to evaluate the efficacy of treatment regimens and recapitulates concentration-time profiles, sequence of administration, and simultaneous presence of drugs in vitro. Using our approach, we tested the susceptibility of primary colorectal cancer cell cultures to FOLFOX, XELOX and FOLFIRI protocols and compared the results with the treatment history. The new test results were concordant with the clinical response in all cases. GI50-based test results were not univocal for different drugs of the regimens and were completely inaccurate in 25% of cases as well as for peak plasma concentration method3. Notably our approach was the only one to demonstrate the equivalence of the FOLFOX and XELOX protocols known to be of similar efficacy. Taken together our results indicates that utilization of this approach could be a beneficial for the assessment of efficacy and toxicity of drugs as well as for the comparison of different dosing schemes in an animal-free

high-throughput setup.

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P-MODELS-23

IDENTIFICATION OF A NOVEL MODE OF ACTION OF VANILLIN DERIVATIVE COMPOUND VERATRALDEHYDE. ABSTRACT #466

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Compounds that test positive in in vitro micronucleus tests (MNTs) need to be followed up with in vivo MNTs. However, it is common for these compounds to test negative in in vivo MNTs, known as “misleading positives.” To reduce in vivo follow-up tests, we developed an in vitro mode of action (MoA) analyzing platform which consists of ToxTracker assay and high-content imaging analysis (HCA) to classify MN-inducing compounds according to their MoA: clastogenic, aneugenic, or reactive oxygen species–generating. To demonstrate the utility of our platform, we analyzed the MoA of a flavoring compound veratraldehyde (VER), a derivative of vanillin in which the hydroxyl group of vanillin is replaced with a methoxy group. Both VER and vanillin are known to be misleading positives in in vitro MNTs. To reveal the MoA of these compounds, we employed the ToxTracker assay which uses flow cytometer to analyze six reporters. The results showed that VER activated one of the two reporters associated with DNA damage, characteristic of aneuploidy, whereas vanillin did not activate any of these six reporters. A subsequent ToxTracker ACE (Aneugen Clastogen Evaluation) assay showed that VER induced cell cycle defects, indicative of aneugenicity. HCA combined with nucleus staining and gamma-H2AX immunostaining revealed that VER also induced an abnormal nuclear morphology but minimal DNA damage. Additionally, staining microtubules with fluorescent dye resulted in a decrease in the fluorescence signals, indicating modulation of microtubule dynamics. From a perspective of chemical structure, we considered that VER interacts with the colchicine binding pocket of tubulin to modify its polymerization because VER has adjacent methoxy groups, similar to colchicine. A novel MoA by which VER induces micronuclei in vitro was revealed through the collective results of this study, thus demonstrating the utility of our platform that combines ToxTracker assays with HCA.

P-MODELS-24

Using an in vitro inflamed intestinal model to study the effect of deoxynivalenol on primary bile acid malabsorption in human ABSTRACT #474

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The human small intestine is continuously exposed to xenobiotics and pathogenic organisms, which can result in disrupted immune homeostasis and an inflammatory state of the small intestine. Chronic intestinal inflammation can develop into inflammatory bowel disease, which is defined as intestinal epithelial damage and increased intestinal permeability. Current in vitro models of an inflamed intestine are characterized by intestinal epithelium leakage. However, in the initial state of the inflammation, the in vivo intestinal epithelial integrity is not compromised. Inflamed cells, send local and systemic signals, which in turn may alter intestinal absorptive functions. We developed an in vitro intestinal model with controlled inflammation, using LPS-stimulated THP-1 and differentiated Caco-2 cells layers in Transwells. This model preserves the intact intestinal epithelium, allowing to study the intestinal absorption function in inflamed states. Bile acids are synthesized in liver and subsequently

secrete into the small intestinal lumen from which they are reabsorbed and transported back to liver. The fungal secondary metabolite deoxynivalenol (DON) not only induces intestinal inflammation but also reduces intestinal bile acids absorption. We found that the transport of primary bile acid mixtures decreased to 4.8 ± 0.5 nmol in inflamed intestinal co-cultures compared to 8.3 ± 2.0 nmol in healthy co-cultures. In the inflamed co-cultures, DON exposure further reduced the transport of these primary bile acids to 2.8 ± 0.5 nmol. DON exposure enhanced the secretion of pro-inflammatory cytokines IL-1β and TNFα 1812.6 ± 163.7 and 885.9 ± 2.6 pg/ml in inflamed co-cultures. The secretion of IL-1β and TNFα was concentration-dependently decreased to 1512.6 ± 38.0 and 760.5 ± 52.1 pg/ml by pre-exposing 125 µM primary bile acids to the inflamed co-cultures when DON was present. The results reveal that DON affects the bile acid intestinal kinetics in an inflamed intestine, thereby accelerating the process of inflammation in the intestine

P-MODELS-25

Use of RNA seq-based gene expression signatures of pesticide active substances in human kidney cells to support definition of cumulative assessment groups (CAGs) for risk assessment
ABSTRACT #483

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Typically, safety assessment of chemicals is performed for exposure to single chemicals. In recent years, more attention is directed to the possible adverse effects of combined exposure to multiple chemicals. To allow assessment of health risks upon combined exposure to pesticide active substances (cumulative risk assessment), the European Food Safety Authority (EFSA) works on the grouping of chemicals in so-called cumulative assessment groups (CAGs). To that end, compounds are identified that exhibit similar toxicological properties in a specific organ or system. So far, EFSA's Panel on Plant Protection Products and their Residues (PPR) has applied this methodology to define groups of pesticides that are toxic to the thyroid and central nervous systems, and currently CAGs are being defined for other targets. The present study aims to use an in vitro approach to support definition of CAGs related to kidney toxicity. To that end, human kidney cells (RPTEC/TERT1) were exposed to a series of pesticide active substances reported to cause adverse effects to the kidney (fludioxonil, pyrimethanil, pyraclostrobin thiabendazole, pyriproxyfen, carbendazim, 2,4-dichlorophenoxyacetic acid (2,4-D)) and their effects on the transcriptome were assessed using RNA seq. The data were used to assess similarities and differences in mechanisms of kidney toxicity in vitro, data that may be of use for deciding on inclusion or exclusion of chemicals in a certain CAG. In future studies, the effects of mixtures of these pesticides will be studied to assess whether effects upon combined exposure follow principles of dose addition. Altogether, in vitro mechanistic data on effects of pesticide active substances in human cell-based test systems are promising to support definition of CAGs, contributing to cumulative risk assessment.

P-MODELS-26

Receptor-mediated activities of 4- and 5-ring unsubstituted and methylated polycyclic aromatic hydrocarbons (PAHs) in relation to their developmental toxicity potency
ABSTRACT #484

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Our previous study using the zebrafish embryotoxicity test (ZET) demonstrated that the presence of a methyl substituent on different positions of the aromatic ring of 4- and 5-ring polycyclic aromatic hydrocarbons (PAHs) can either increase or decrease their in vitro developmental toxicity. However, the underlying mechanism of some PAHs in inducing the observed developmental toxicity in the ZET remains unclear. In light of this, the role of the aryl hydrocarbon receptor (AhR), estrogen receptor alpha (ER-α), and retinoic acid receptor (RAR) in mediating the

developmental toxicity of some (un)substituted PAHs in the ZET was investigated. Nine PAHs were tested including benzo[a]anthracene (BaA) and three monomethylated BaAs, benzo[a]pyrene (BaP) and three monomethylated BaPs, and dibenz[a,h]anthracene (DBA). The results show that all tested PAHs were AhR agonists, and all monomethylated PAHs showed higher potency in activating the AhR than their corresponding unsubstituted PAHs. Only two PAHs: BaA and 8-methyl-BaA, were ER-α agonists. No ER-α antagonist or RAR (ant)agonist activity was observed upon exposure to the tested PAHs. Co-exposure of zebrafish embryos to the PAHs and the AhR antagonist (CH223191) counteracted embryo lethality and developmental retardations such as yolk sac edema, pericardial edema, and deformed body shape, of all tested PAHs, except for 8-methyl-BaP. Fulvestrant (ER-α antagonist) counteracted the embryo lethality induced by BaA and developmental retardations such as craniofacial deformities and kinked tails by 8-MeBaA. To conclude, our findings show that methylation of PAHs influences their ability to interact with certain receptors relevant to developmental toxicity including the AhR and/or ER-α, and that the RAR may not be relevant for PAH-induced developmental toxicity.

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P-MODELS-27

Validation of skin and ocular irritation and severe damage (corrosion) tests as non-animal alternatives using OECD guideline evaluation substances. ABSTRACT #487

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Corrosive or irritating chemicals can pose significant risks to human health if there is sufficient exposure to concentrated chemicals or mixtures. Safety assessments are focussed on routes of intended and likely exposure, which for industrial, cosmetic and agrochemical materials typically result in the assessment of both skin and ocular toxicity potential being required. Historically, these endpoints have been assessed using in vivo methods such as the acute dermal irritation/corrosion and Draize eye tests using rabbits, which now have limited use given legislation changes and societal pressures. Following the development of non-animal models for dermal and ocular irritation and corrosivity, many have now been validated with OECD guidelines adopted. Gentronix has establishment and generated proficiency data for the in vitro skin irritation test (OECD test guideline 439), reconstructed in vitro skin corrosion test (OECD 431) using the EpiDerm™ reconstructed human epidermis model, EpiOcularTM eye irritation test (OECD 492), and Bovine Corneal Opacity and Permeability test (BCOP 437), to differentiate chemicals with ocular irritation and severe eye damage potential from those not requiring classification. Testing within the skin corrosion and irritation endpoints showed excellent agreement with the expected outcomes (12 out of 12 and 10 out of 10 substances were overall correctly predicted for irritation and corrosion, respectively), with low incorrect classification predictions using the established prediction models (1 out of 70 experimental runs). In the case of the EpidermTM irritation test, uneven application of bromohexane to the test system was resolved by use of spreading aids resulting in 3 out of 4 runs producing the expected irritant prediction. Proficiency testing of all skin and ocular irritation and corrosion tests achieved the expected outcomes for the OECD guideline recommended proficiency substances. This exercise supports the adoption of a skin and ocular toxicology workflow, providing comprehensive safety assessment approaches using non-animal test methodologies.

P-MODELS-28

Vital human material as an innovative approach to move towards human-based science without animal research - What are the challenges ? ABSTRACT #490

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Animals are often used in scientific research as models for safety, pharmacokinetics and efficacy testing. Although humans and animals share similarities, there are major species differences that can contribute to differences in responses to compounds. One of the main underlying questions is the translational value of animal tests to human physiology. Scientists are in favor of using data derived from human material, because the use of human material might meet the scientific and ethical constraints regarding the use of animals for scientific research. The use of human tissues, provided that the tissue is fresh, viable and metabolically active, represents a sensible opportunity to provide better safety and efficacy outcomes to humans. APPROACH In order to prove these opportunities researchers should be facilitated to develop new methods demonstrating their utility. However, in most (European) countries there is no transparent research infrastructure to get access to viable human material. Additionally, there are several technical, ethical and scientific issues to encounter when considering the use of human material: Accessibility and matching: How to obtain and provide human material? Who will get access to human material? Vitality and Quality: How to preserve viable tissues? Safety: Biohazard and personal data security such as GDPR. Logistics: Transport of the material/tissues? Variability between human donors. Ethical & Cultural barriers incl. engagement with patients and general public. EXPECTED RESULTS - OUTPUT A guidance paper, that will detail the challenges faced to use vital human tissue that contribute to the transition to animal-free science, and a roadmap to solve these challenges specifically in the food and beverage sector, that can be used as an example to follow.

P-MODELS-29

Perspective multi-modal acting compounds against SARS-COV-2 virus ABSTRACT #502

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Objectives: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known to cause coronavirus disease 2019 (COVID-19), a highly transmissible respiratory disease, which has considerably affected global public health. Therefore, there has been an urgent need for seeking efficient antiviral strategies to combat COVID-19. Main protease (Mpro) belongs to the viral nonstructural proteins, which has been included among attractive viral targets due to its importance in SARS-CoV-2 replication and its high conservation among various coronaviruses. Several compounds have been found to possess inhibitory potential against Mpro, however, only a few of them managed to enter clinical trials. Development of systemic inflammatory response and bacterial co-infection in patients belong to severe frequent complications of COVID-19. Thus, we focused on the evaluation of anti-inflammatory and antibacterial activities of the SARS-CoV-2 Mpro inhibitors. Methods: Literature survey was done using PubMed, Scopus, and SciFinder® scientific search tools. Oral acute toxicity (rodent) was predicted using a virtual lab, ProTox-II (https://tox-new.charite.de/protox_II/). Selected molecular obesity parameters were calculated. Results and Discussion: We systematically screened the scientific databases for compounds with the activity against SARS-CoV-2 Mpro protease, anti-inflammatory, and antibacterial properties. We collected a structurally diverse group of Mpro inhibitors, pointing to a multimodal mechanism of their action. Estimates of drug-likeness as well as prediction of oral toxicity of the compounds were evaluated. The most potent multitarget candidates with low toxicity were identified. These included both the natural compounds, such as baicalein, amentoflavone, kaempferol, rhoifolin, quercetin-3-β-galactoside, rutin or tannic acid, as well as synthetic drugs, such as dipyridamole, ebselen

or eltrombopag olamine, etc. Perspective substances will be further examined with respect to their toxicity and efficacy in follow-up in vitro studies. Conclusion: Antiviral strategy against SARS-CoV-2 Mpro combined with anti-inflammatory and antibacterial efficacy may represent an appealing approach in the management of severe and long-COVID-19 cases.

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P-MODELS-30

THE RENAL PROXIMAL TUBULE TXG-MAPR: SAFETY ASSESSMENT BASED ON QUANTITATIVE GENE NETWORK ANALYSIS.
ABSTRACT #507

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

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Session: Other - Nanoparticle

P-NANO-1

ASSOCIATION OF DOXORUBICIN AND pH-SENSITIVE NANOPARTICLES CONTAINING AN ORGANOSELENIUM COMPOUND AS AN INNOVATIVE APPROACH TO SENSITIZE MDR CELLS: AN IN VITRO STUDY
ABSTRACT #5

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Background and Objectives: Cancer incidence and mortality is rapidly growing worldwide; on the other side, the traditional chemotherapy present serious adverse outcomes due to its poor specificity by the tumor site. In this context, some approaches such as nano-based drug delivery systems and combination therapy have showed a great potential to improve cancer treatment efficacy. Thus, in this study we proposed PLGA nanoparticles as carriers of the new organoselenium compound 5'-Se-(phenyl)-3-(amino)-thymidine (ACAT-Se). Moreover, we evaluated the synergic antitumor effects of the association of ACAT-Se nanoparticles with the antitumor drug doxorubicin (DOX). Material and Methods: Nanoprecipitation method were used to obtain the nanoparticles (ACAT-Se-NPs) and the pH-responsive lysine-based surfactant (77KL) was used to confer them a pH-dependent behavior. The in vitro antitumor activity was evaluated using a sensitive (human breast cancer, MCF-7) and a resistant (human ovarian cancer cells, NCI/ADR-RES) tumor cell line. Results: The nanoparticles present suitable physicochemical properties. ACAT-Se-NPs presented higher in vitro antitumor activity than free ACAT-Se in MCF-7 tumor cells. Moreover, the association of DOX and the ACAT-Se-NPs resulted in synergism in MCF-7 and NCI/ADR-RES tumor cells, being especially able to successfully sensitize the MDR cells. Conversely, the association of free ACAT-Se and DOX was not effective, and resulted mostly antagonistic. Discussion and Conclusion: The pH-sensitive ACAT-Se-NPs were successfully prepared. The benefits of nanoencapsulation was evidenced by the increase of ACAT-Se antitumor activity in MCF-7 cells after its nanoencapsulation. In addition, the association of the ACAT-Se-NPs and DOX resulted in synergistic effects in both sensitive and resistant tumor cells; this approach was able to sensitize the NCI/ADR-RES tumor cells, suggesting that this combination can overcome the cells resistance mechanisms. Therefore, our results suggest that ACAT-Se-NPs present a great potential to a more efficient antitumor therapy and its association with DOX can be an alternative to overcome MDR.

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P-NANO-2

Toxicity of polystyrene micro- and nano-plastics in human A549 lung cells ABSTRACT #103

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Micro- and nanoplastics (MPs/NPs) are ubiquitous in the environment, but the magnitude of human exposure and risks to human health are far from understood. Recent studies have identified the presence of microplastics (MPs) in lungs and quantified its levels in blood at levels of 1.6 to 12 µg/mL1,2. Being inhalation an important source of exposure to MPs/NPs to the human body, this work investigates the toxicity of polystyrene MPs (1 µm) and NPs (52 nm) in human lung (A459) cells. Exposure to MPs/NPs spheres (3, 50, 130 µg/mL) for 24 h did not alter cell viability (Alarmar Blue). However, when CFDA-AM was used as a probe for membrane integrity, a small depletion (20%) of cell viability was detected after exposure to NPs (130 µg/mL). Being lipids major components of cell membranes, further experiments were conducted to investigate changes in the lipidome of A549 cells after exposure to MPs/NPs. Lipids were analyzed by UHPLC coupled to a Q-TOF ESI+ mass spectrometer. Alterations in membrane lipids were detected after exposure to MPs (130 µg/mL), namely a down-regulation of phosphatidylinositols (PIs) and triglycerides (TGs), and an up-regulation ether lipids, mainly

phosphatidylcholines (PC-Os 32:0, 32:1, 36:0, 38:0). These changes are indicative of a significant rearrangement of membrane lipids after exposure to MPs. A disregulation of membrane lipids was also observed after exposure to NPs (3 µg/mL). In addition, the highest concentration of NPs strongly depleted cellular lipids and induced the formation of micronuclei, a marker of cancer development. Overall, this work contributes to unraveling the mechanisms of toxic action of MPs/NPs in lung cells. Further studies are needed to accurately estimate internal concentrations of small size MPs/NPs in human lung/blood in order to refine exposure experiments and to understand the relevance of these findings.

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P-NANO-3

NEUROTOXICITY ASSESSMENT OF Cd/Se QUANTUM DOTS NANOPARTICLES IN T98G HUMAN GLIOBLASTOMA CELLS THROUGH A TRANSCRIPTOMIC APPROACH
ABSTRACT #341

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Background and Objectives: Quantum dots (QDs) conveniently functionalized are very promising nanoparticles (NPs) to be employed with biomedical applications such as imaging diagnosis, immunoassay, single-molecule detection or targeted drug delivering. The objective of this work is to assess potential neurotoxicity of Cd/Se QDs NPs. Material and Methods: We exposed T98G human glioblastoma cells to 40 µg/mL Cd/Se QDs NPs for 72 hours (the highest exposure that causes no significant cytotoxicity) and afterwards we analyses transcriptome of these cells through an experiment of massively parallel sequencing of the RNA. Results: Transcriptome of exposed cells were altered in biological processes and molecular pathways addressed mainly to neuroinflammation and hormonal control of hypothalamus via the gonadotropin-releasing hormone receptor. Discussion: The biological significance of neuroinflammation alterations is still unclear since the expression of several markers is downregulated rather than upregulated. The disruption of the hypothalamic-pituitary-gonadal axis controlled by the gonadotropin-releasing hormone could potentially lead to hypogonadotropic hypogonadism. Conclusion: The inhibition in the expression of pro-inflammatory interleukins suggests that the use of these NPs for alleviation of inflammation in diseases as Parkinson deserves further attention. On the opposite, the hormonal control alterations of the hypothalamus pose a new concern about a potential adverse effect of QDs on fertility and development. Funding: This research was funded by Ramón Areces Foundation, grant number CIVP18A3939.

P-NANO-4

New development for studying the genotoxicity of nanomaterials in liver cell models
ABSTRACT #452

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Nanomaterials (NMs) are materials that have one or more dimensions in the nanometer range (≤ 100 nm), leading to new physico-chemical properties that are valorized in numerous industrial applications of chemistry, electronic, medicine, food, cosmetics etc. However, the potential toxicity of these NMs is not always well established and toxicological testing of NMs remains a major challenge for toxicologists. Along with the development of new alternative methodologies (NAMs) for hazard assessment, and in order to reduce animal use, the development of more realistic in vitro models provides new opportunities for NMs testing. For this purpose, the genotoxicity of three NMs, titanium dioxide and 2 zinc

oxides, was investigated and compared on the human liver cell line HepaRG grown in 2 or 3 dimensional conditions. Genotoxicity was assessed with the alkaline comet assay with and without the Fpg enzyme for detection of oxidative DNA base lesions. For high throughput purposes, the Comet Chip version was also used. While TiO2 had no genotoxic effect in either model, the two ZnO induced DNA damage. Although some differences between NMs were noted, genotoxicity was observed essentially at ZnO cytotoxic concentrations in the 2D model but also at sublethal concentrations in the 3D model. Similar results were obtained when using the Comet chip although the genotoxic response with ZnO was observed with lower, non-cytotoxic concentrations for the two ZnO NMs. Therefore, new in vitro models can improve the prediction of in vivo outcomes while developing Comet chip systems can be a promising tool for setting up a high throughput strategy for genotoxicity testing.

P-NANO-6

Cytotoxicity of nanomixtures: effects of silver and polystyrene nanoparticles on human macrophages

ABSTRACT #256

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Background and Objectives The growing importance of nanotechnology and use of nano-enabled products increases the possibility of releasing nanoparticles (NPs) into the environment, which can further lead to accidental human exposure [1]. While effects of exposure to single NPs type have received significant attention [2], co-exposure to different NPs has been poorly explored, and data for reliable risk assessment of nanomixures are missing. Here, we present results on co-exposure of human macrophages to silver (AgNP) and polystyrene (PSNP) nanoparticles. Materials and Methods Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) were used for NPs physico-chemical characterization. Cell viability, nanoparticle localization, induction of oxidative stress, cytokine expression, DNA damage and induction of apoptosis were assessed for THP-1 cells treated with AgNP, PSNP or their mixture. Additionally, nano-mechanical properties of THP-1 cells were analyzed with AFM. Results Both AgNP and PSNP induced oxidative stress, pro-inflammatory cytokine expression, DNA damage, apoptosis and cell death. However, nanomixtures showed greater impact on viability THP-1 cells under in vitro settings compared to individual NPs. Remarkably, combinations of non-cytotoxic AgNP and PSNP concentrations significantly reduced THP-1 cell viability. Localization experiments showed that AgNP and PSNP entered both cytosol and nuclei, and did not aggregate inside cells. Conclusions Results that are presented here demonstrate additive detrimental effects of nanomaterial mixtures on THP-1 cell metabolism and survival compared to individual nanoparticle types, even in non-toxic doses. We thereby highlight the potential health hazard of co-exposure to different nanomaterials that are released into the environment, and present an in vitro experimental platform to screen for ROS-induced mechanism of NP toxicity.

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P-NANO-7

Erythrocyte photoassay as a preliminary tool to evaluate potential photoprotective activity of guarana-loaded nanosomes

ABSTRACT #143

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Background and Objectives: Nanotechnology research is rising in pharmaceutical and cosmetic industries because of the advantages of encapsulation over free actives [1]. Therefore, nanomaterials have to be evaluated to demonstrate low toxicity and safety. The present work evaluates the photoprotective potential in vitro of guarana-loaded nanosomes as a part of a global project focused in improving the therapeutic activity of guarana [2], obtained from Paullinia cupana. The main objective of the project was to evaluate the photoprotective potential of novel guarana–loaded nanosomes by the photohaemolysis assay. Materials and Methods: Guarana-loaded nanosomes were prepared by reverse phase evaporation method [3] with Ethanol, Tween 80®, Lipoid S 100®, Cholesterol and Vitamin E as components. Potential phototoxicity and photoprotection of guarana-loaded and free nanosomes was assessed by the induction of photohaemolysis and haemoglobin oxidation using rat blood cell suspension. Samples were exposed 5 J•cm-2 of UVA light and compared to those remained at dark. Photoprotection was studied in front of a well-known phototoxic drug (chlorpromazine). Finally, the effect of each nanosome component was also evaluated. Results: Our data indicated that both free and encapsulated guarana do not induce haemolysis when irradiated (5J/cm2) and protect from photohemolysis induced by chlorpromazine. However, guarana-loaded nanosomes induce haemoglobin oxidation and only free guarana prevents from haemoglobin oxidation induced by chlorpromazine. Regarding nanosome components, Tween 80® presents some degree of haemolysis with and without irradiation, while cholesterol shows haemoglobin oxidation activity. Discussion: According to our observations of photohaemolytic and photoprotective activity of free and encapsulated guarana, nanosome formulation do not increase the efficacy of potential beneficial effects of guarana. The oxidative behaviour of nanosomes over haemoglobin is attributed to cholesterol. Conclusion: Photohaemolysis assay and haemoglobin oxidation are a good in vitro tools for both screening potential photobiological activity of new formulation and ingredients.

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P-NANO-8

Exposure to polystyrene micro- and nano-plastics modifies the lipidome of zebrafish liver cells.
ABSTRACT #104

Tiantian Wang[1], Cinta Porte[1]

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Pollution by micro- and nano-plastics (MPs, NPs) in the aquatic environment and their possible adverse effects on aquatic organisms has become a major scientific and societal concern. The fate and impact of micro (>10 µm) and mesoplastics has been investigated, but the impact of smaller MPs and NPs is still unknown. This work investigates the toxicity of polystyrene MPs (1 µm) and NPs (52 nm) using zebrafish liver (ZFL) cells as a model. Exposure to MPs/NPs (3, 50, 130 µg/mL) for 24 h did not alter cell viability (Alarmar Blue). However, when CFDA-AM was used as a probe for membrane integrity, a small depletion (20%) of cell viability was detected after exposure to NPs (130µg/mL). Being lipids major components of cell membranes, further experiments were conducted to investigate changes in the lipidome of ZFL cells after exposure to MPs/NPs. Lipids were analyzed by UHPLC coupled to a Q-TOF ESI+ mass spectrometer. Alterations in membrane lipids were detected after exposure to MPs (130 µg/mL), namely a down-regulation of phosphatidylinositols (PI 36:4, 38:3, 38:4, 40:3) and a concomitant increase in phosphatidylserines. These alterations in membrane lipids were more evident after exposure to NPs. At the lowest concentration tested, a strong up-regulation of phosphatidylcholine-plasmalogens was observed. These lipids are precursors for

inflammatory mediators, but also play a role as modulators of membrane fluidity, and have antioxidant properties. When ZFL cells were exposed to a higher concentration (50 μg NPs/mL), an additional decrease of PIs occurred. PIs are minor components on the cytosolic side of eukaryotic membranes with multiple functions in cell signaling and serve as a source of arachidonic acid. Overall, this study evidences the modification of the cellular lipidome after exposure to MPs and NPs, and the greater capacity of NPs to alter the metabolism of lipids in ZFL cells.

P-NANO-9

In vitro phototoxicity assessment of nanoparticles
ABSTRACT #470

Min Beom Heo[1], In Young Kim[1], Tae Geol Lee[1]

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Background and Objectives Today, our society enjoys considerable convenience due to nano products. However, there is a safety issue for nanomaterials that fall off from nanoproducts. It can cause problems for human health and environmental pollution. Therefore, scientific knowledge to evaluate the safety of nanomaterials is continuously improving. Each year, progressive assays and predictive models are being developed. Titanium dioxide nanoparticles have a small size and white color, and thus have been widely applied commercially. However, the harmfulness of TiO2 nanomaterials can be confirmed through various research results. Material and Methods In this study, we measured TiO2 phototoxicity in human keratinocyte HaCaT cells, mouse fibroblast NIH 3T3 Cells, human lung adenocarcinoma A549 cells, and chinese hamster ovary CHO-K1 cells. Cell viability was verified using the MTS method. Results In all cells, there was no effect on cell viability in the absence of UVA irradiation, but it was found that cell death was increased in a time-dependent manner when UVA was irradiated. In our previous study, we observed the generation of reactive oxygen species (ROS) and the generation of lysosomal membrane permeability (LMP) by simultaneous exposure of TiO2 and UVA. Discussion and Conclusion Through this study, we intend to present a systematic phototoxicity test protocol that can be applied to various nanomaterials including TiO2. This test method serves as a standard for determining whether or not phototoxicity of various nanomaterials contained in cosmetics or sunscreens.

References

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P-NANO-10

Effects of gold nanoparticles with protein corona on immune cell heterogeneity and cellular differentiation : A single cell based, high-dimensional mass cytometry study
ABSTRACT #471

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•Background and Objectives Understanding how nanoparticles interact with biological systems is important in many biomedical research areas. However, the heterogeneous nature of biological systems, including the existence of numerous cell types and multitudes of key environmental factors, makes these interactions extremely challenging to investigate precisely. Here, by using single-cell-based, high-dimensional mass cytometry approach, we demonstrated that the presence of protein corona has significant influences on the cellular associations and cytotoxicity of gold nanoparticles for human immune cells, and those effects vary significantly with the types of immune cells and their subsets. • Material and Methods Cells were incubated in RPMI containing gold nanoparticles and different FBS concentrations and collected after 3 hours. After cell surface marker staining, mass cytometry was used to assess the cellular interaction of gold nanoparticles with various protein corona. • Results The altered surface functionality of protein corona reduced the

cytotoxicity and cellular association of gold nanoparticles in most cell types (e.g. monocytes, dendritic cells, B cells, NK cells, and T cells), and those immune cells select different endocytosis pathways such as receptor-mediated endocytosis, phagocytosis, and micropinocytosis. However, even slight alterations in the major cell type (phagocytic cells and non-phagocytic cells) and T cell subsets (e.g. memory and naive T cells) resulted in significant protein corona-dependent variations in their cellular dose of gold nanoparticles. Especially, naive T killer cells exhibited additional heterogeneity than memory T killer cells, with clusters exhibiting distinct cellular association patterns in single-cell contour plots. • Discussion and Conclusion This multi-parametric analysis of mass cytometry data established a conceptual framework for a more holistic understanding of how the human immune system responds to external stimuli, paving the way for the application of precisely engineered nanoparticles as promising tools of nanomedicine under various clinical settings, including targeted drug delivery and vaccine development.

ADDITIONAL ABSTRACTS

ADDITIONAL ABSTRACTS

Session: ILSI-Europe lunch session

Introduction to Thresholds of Toxicological Concern Concept and how in vitro methods and TTC complement each other
ABSTRACT #252

Heli Miriam Hollnagel[1]

[1]*Dow Europe GmbH*

During the past decade, many basic and some highly sophisticated in vitro models were developed to depict specific molecular, cellular and tissue effects of chemicals. Most in vitro models, and in silico models alike, are useful to investigate specific modes of action, but cannot provide general safety assurance in the absence of information on modes of action and toxicokinetics and –dynamics of a given compound throughout the multitude of tissues of an integrated organism. This is the point in animal data-free risk assessment where the value of read-across and Thresholds of Toxicological Concern (TTC) approaches becomes apparent. TTC thresholds are based on large datasets of oral cancer and non-cancer repeated dose in vivo toxicity data and describe de minimis exposures below which there is a low risk of any appreciable risk to human health. At this point in time, the TTC concept and read-across are the only options to perform repeated dose systemic risk assessments when there is a lack of time, resources and /or legal acceptance to run animal studies on specific natural or man-made chemicals. Therefore, it is important to understand its scientific basis, where it can be applied with confidence, where improvements are being developed and for which cases it should not be applied currently. In vitro methods can be particularly useful to identify the latter, provided that they are sufficiently sensitive. Ongoing collaborative research to derive TTC thresholds based on in vivo no/low-effect plasma concentrations (internal TTCs) will result in a large dataset with useful information for In-Vitro-In-Vivo-Extrapolation.

Session: JSAAE lunch session

An introduction to the 3rd Asian Congress for Alternatives to Animal Experiments (ACAAE) and the proceeding process of ‘Act on promotion of development·dissemination and use of alternative test methods’ in Korea
ABSTRACT #254

Kwang-Mahn Kim[12]

[1]*YONSEI UNIVERSITY*
[2]*KOREAN SOCIETY FOR ALTERNATIVES TO ANIMAL EXPERIMENTS*

The 3rd Asian Congress of Alternatives to Animal Experiments (ACAAE) will be held in Jeju Island, Republic of Korea from December 14 to 16, 2022. This is the third conference after Japan and China. In addition, South Korea has been actively adopting the validated OECD test guidelines for toxicology testing, including those for non-animal test methods. There is also active interest in expanding the research support into pathway-based approaches and using computational tools and technologies like microphysiological systems that are increasingly receiving academic attention. In order to coordinate and support development and implementation of non-animal studies, a new legislative initiative was proposed in Republic of Korea. The proposed Act on the Promotion of Development, Dissemination and Use of Alternatives to Animal Testing Methods would prioritize human-mimetic technologies to modernize and improve human health research and product safety testing. It is equally important to note that communication between developers and regulators is crucial for the success of disseminating new methods. Therefore, the new bill seeks to foster the necessary multidisciplinary cooperation by establishing an expert committee and providing support for trainings. With more proactive discussions among scientific and regulatory communities, Korea is well positioned to drive technology advancement as well as regulatory update of those methods. Therefore, I would like to introduce these two.

Session: ILSI-Europe lunch session

High-Throughput-Screening: Challenges and Opportunities in food safety risk assessment

ABSTRACT #262

Ans Punt[1]

[1]*Wageningen Food Safety Research*

High-throughput in vitro screening plays an important role in next generation (non-animal) toxicity testing strategies. The ILSI Europe ToxCast Expert Group aimed to explore how mechanistic insights in the biological targets of food-relevant chemicals can be obtained from high-throughput ToxCast results. Starting point are the 556 direct additives that have been identified in the ToxCast database. These different chemicals were subdivided into structurally related chemical groups and functional use classes according to EU regulation (e.g. E-numbers, nutrients, flavourings, regulatory-restricted chemicals). Relevant biological targets of a chemical group were considered those toward which a high percentage of chemicals within a group are active. The most obvious activity identified was the estrogen receptor-mediated actions of the chemical group containing parabens and structurally related gallates, as well the chemical group of pyranones, containing genistein and daidzein (Punt et al., 2020). In a second step, the possibilities of using the ToxCast data of the defined chemical groups for read-across purposes were evaluated (Firman et al., 2021). These results revealed that Tox21/ToxCast are particularly useful to discriminate out-of-domain structural relatives (i.e. structural relatives that are active towards a certain target vs those that are not). Despite the opportunities of utility of HTS for read-across purposes, challenges exist with respect to linking the activated pathways towards apical toxicological endpoints. Examples of these possibilities and challenges will be presented.

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Session: JSAAE lunch session

Latest activities of JSAAE toward establishment of Asian Federation
ABSTRACT #263

Yasuyuki Sakai[1], Hajime Kojima[2], Yasunari Kanda[3], Masato Hatao[4]

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[4] *Japan Cosmetic Industry Association (JCIA), Japan*

After the establishment in 1989, the Japanese Society for Alternatives to Animal Experiments (JSAAE) has been promoting 3Rs research in Japan through a wide variety of activities. Simultaneously, we have been making serious efforts to international cooperation and contributions to 3Rs, particularly in Asian region, while extending the cooperation with ESTIV, EUSAAT and ASCCT. One of the important next steps is to establish Asian Federation in alternatives research to broaden the 3Rs concept and research in Asian region. Until now, we initiated the Asian Congress series in Karatsu, Japan, in 2016, which was continued by Chinese Society in Guangzhou, China in 2018, and then will be hosted by KSAAE in this year, December 14-16, 2022 in Cheju Island in Korea. Primary mission of Asian Federation is to support this series of regional conferences. The relatively-old societies such as JSAAE, KSAAE or TATT (Society of Toxicological Alternatives and Translational Toxicology, Chinese Society of Toxicology) and TTAM (Society of Toxicity Testing and Alternative Methods (TTAM), Chinese Environmental Mutagen Society) in China, strongly wish to have such Federation, as well as other new Asian societies such as SAAE-I (Society for Alternatives to Animal Experiments-India) and SAAT-SL (Society for Alternatives to Animal Testing in Sri Lanka) do. Currently, we are discussing by-laws and possible organization structure of the Federation. We hope we can have a signing ceremony in coming 3rd Asian congress in this December in Korea for establishment of the Asian Federation. Another important mission is to establish an official scientific journal of the

Asian Federation. This is strongly expected by Asian alternatives researchers. Also, we highly recognize the importance of young generations and their communication across the Asian region to ensure the sustainable development of alternatives research and their social implementations in this area.

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Development of SARS-CoV-2 infection model using human iPSC technology
ABSTRACT #304

Yasunari Kanda[1]

[1]*National Institute of Health Sciences*

COVID-19 are known to infect various organs, including lung, cardiomyocytes, and central nervous system and complications and long-term COVID-19 are urgent issues. It is important to establish human infection models for elucidation of its pathophysiological mechanisms and COVID-19 treatments. Based on 3Rs principles, human stem cell technology holds great promise as in vitro human relevant models for drug development. We have made various organ models from human iPSC and evaluated their usefulness as SARS-CoV-2 infection models. We found that SARS-CoV-2 infected human iPSC-derived cardiomyocytes efficiently. SARS-CoV-2 caused arrhythmia and contractile dysfunction in iPSC-cardiomyocytes. Imaging analysis revealed that SARS-CoV-2 induced contractile dysfunction in iPSC-cardiomyocytes. Next-generation sequencing analysis revealed that SARS-CoV-2 reduced the expression of proteins related to contractility, suggesting that SARS-CoV-2 directly infects the cardiomyocytes and induces dysfunction. In addition to cardiomyocytes, SARS-CoV-2 infected alveolar epithelial cells, brain blood barrier, and small intestinal epithelial cells from human iPS cells. Taken together, human iPS cell-derived differentiated cells can be a useful model for the development of COVID-19 therapeutics and the elucidation of pathological mechanisms.

Session: ILSI-Europe lunch session

Non-animal methodologies for food safety risk assessment
ABSTRACT #329

Bas Blaauboer[1]

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Over the past two decades we could witness major changes in the way the area of toxicology is practiced. The traditional way of practicing toxicology with its strong focus on animal models and the use of apical endpoint of toxicity has become increasingly criticised for ethical as well as scientific reasons. Since the 80s of the previous century there has been a search for methods to determine the toxicity potential of compounds with alternatives, now referred to as New Approach Methodologies (NAMs). Many of these approaches consist of a combination of in vitro and in silico methodologies. This allows more emphasis on the mechanisms of toxicity rather than apical endpoints. It also incorporates biokinetics making use of physiologically-based biokinetic (PBBK) modelling, ideally allowing a quantitative in vitro-in vivo extrapolation of data (QIVIVE). ILSI Europe developed a roadmap implementing these approaches for the evaluation of food and food ingredients safety.

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Session: Sponsored session 1 and 2

Research support to replace animal models: the Alternatives Research & Development Foundation (ARDF) Annual Open grant program
ABSTRACT #400

Angela Hvitved[1]

[1]*Alternatives Research & Development Foundation*

In 1993, the Alternatives Research & Development Foundation (ARDF) Annual Open grant program was established to fund research developing innovative methods to replace or reduce the use of animals in research, testing, and education. One of the longest-running programs of its kind, ARDF's Annual Open grant program funds investigator-initiated research across a broad—and evolving—range of scientific areas with a focus on projects with high scientific merit and the greatest potential to contribute to the replacement of animal models. Toxicology, as a discipline, has historically played an important role in the development of new approach methodologies (NAMs) and toxicologists have served as leaders in this exciting and rapidly developing field. The Annual Open program invites proposals from non-profit educational or research institutions worldwide. Current awards are $40,000 and the program had an average funding rate of 21.5% for 2015-2020. Since 1993, the program has provided over $4 million in funding for research projects across 31 states in the United States and five other countries. The types of projects funded have shifted over the past 28 years, along with the shifting scientific landscape. ARDF relies on a diverse and dedicated cohort of external reviewers from industry, academia, and government agencies who provide expert assessments based on the program's review criteria. This presentation will provide an overview of ARDF's Annual Open grant program, including information for potential applicants along with a summary of awarded projects in recent years. We hope interested attendees will be provided the information necessary to engage with the program, either as potential applicants or expert reviewers.

AN ANIMAL FREE, DEFINED WORKFLOW FOR HUMAN INDUCED PLURIPOTENT STEM CELLS ABSTRACT #510

Yas Heidari[1]

[1]*Bio-Techne*

Human induced pluripotent stem cells (iPSC) are critical for ex vivo models for studying development, disease mechanisms, performing drug discovery and other evolving stem cell applications. However, many iPSC workflows involve undefined culture surfaces and cell medium with lot-to-lot variability and animal components that can limit data reproducibility or hamper transition to the clinic. It is critical to overcome these obstacles by creating an animal free and fully defined workflow that ensures validity, reproducibility of results and easier compliance with cGMP standards. In this work, an animal component free ExCellerate™ iPSC medium was made to support the proliferation and growth iPSC. Human iPSC grown with animal-free Vitronectin matrix and the media retained their pluripotency by successfully differentiating to multiple cell types including different germ layer lineages. Finally, we created animal free neuronal and dopaminergic (DA) neural precursor differentiation processes and successfully obtained neurons and DA progenitors using the aforementioned vitronectin, iPSC media and animal-free supplements. These results demonstrate the feasibility of a standardized defined, animal free, and reproducible process for iPSCs research and other relating applications.

Session: BEMF Award Lecture

Development and validation of 3D tissue models-based assays for topical toxicity testing - BEMA Lecture ABSTRACT #414

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[2]*Institute of Biochemistry and Microbiology Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia*

Development, validation and consequent regulatory acceptance of reconstructed 3D human tissue models-based (3D RHTM) assays for topical toxicity testing proved, that reduction and replacement of animal models in toxicology testing is possible.

The technologies leading to the development of reconstructed human three-dimensional tissue models (3D RHTM) have been known for more than 40 years. The first tissue models served mainly for research interests to study the cell-cell interactions, tissue morphology and

physiology and later on for e.g. treatment of burned patients. However, as the tissue engineering technology progressed, 3D RHTM models started to be used for broader purposes. These included safety and efficacy testing of cosmetic ingredients and products, as well as hazard assessment of chemicals and pesticides.

Four OECD Test Guidelines (TGs) refer explicitly to the use of 3D skin or cornea-like models: OECD TG 431 for skin corrosion testing, OECD TG 439 for skin irritation testing, OECD TG 492 for eye irritation testing and OECD TG 498 for assessment of phototoxicity.

The use of 3D RHTM has been implemented in the biocompatibility testing of medical devices (ISO 10993:23) and for the preclinical testing of drugs (ICH S10). Validation studies with skin models have been completed for genotoxicity testing using the Comet and Micronucleus assays. 3D RHTM has found usability in both regulatory as well as non-regulatory testing areas including:

- safety and efficacy assessments of raw cosmetic/pharmaceutical materials and formulations,
- hazard and risk assessment of chemicals or formulations with accidental contact with human eyes or skin (regulated, e.g. by REACH and other chemical legislations) and help to address occupational safety,
- mechanistic information that can be utilised e.g. to determine whether a molecule or compound can be altered to reduce toxicity without loss of efficacy,
- prevention or enhancement of the penetration of a substance via target tissue(s).

The 3D RHTM models also found their use in the pre-clinical testing of drug candidates and medical devices (ISO 10993-23). Following the standardisation of tissue models production by commercial developers and thanks to the extensive international validation studies, reconstructed human skin and cornea-like models are nowadays used globally by industrial and academic research laboratories to assess the local effects of topically applied chemicals and formulations in vitro.

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