22ND INTERNATIONAL CONGRESS

ESTIV

ABSTRACT BOOK

PRAGUE 2024
3 – 6 JUNE

22ND INTERNATIONAL CONGRESS
ESTIV
PRAGUE - CZECH REPUBLIC
3 - 6 JUNE 2024
Applications of NAMs in Drug Discovery and Drug and Chemical Safety Assessment

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WELCOME ADDRESS

Dear colleagues and delegates,

The 22nd International Congress of the European Society of Toxicology In Vitro (ESTIV 2024) is set to convene in the historic and picturesque city of Prague from June 3-6, 2024. Centered around the pivotal theme, 'The Application of NAMs in Drug Discovery and Drug and Chemical Safety Assessment,' the congress promises to be a comprehensive platform for professionals to showcase and discuss the latest advancements and research in the field.

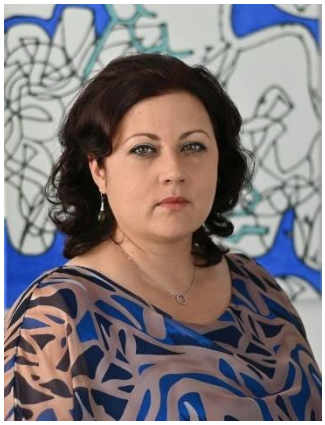
The congress will commence with a pre-congress workshop titled 'How to write and manage a successful EU Horizon proposal: From initial idea to execution.' This session, organized in collaboration with the EU H2020 granted project ONTOX, aims to provide invaluable insights and guidance on navigating the complexities of EU Horizon proposals.

Attendees can look forward to an extensive scientific program featuring over 28 scientific sessions, complemented by industry-sponsored sessions, and an insightful post-congress workshop facilitated by AFSA Masterclass and EPISKIN Academy. These sessions are meticulously designed to cater to a wide spectrum of interests and specializations within the realm of in vitro toxicology.

Participants and interested parties are encouraged to visit the ESTIV 2024 Congress web page for a detailed overview of the program, alongside pertinent information regarding the registration process. The ESTIV 2024 Congress in Prague stands as a significant occasion for professionals, researchers, and industry leaders to converge, share knowledge, and collaboratively advance the application of non-animal methodologies in drug discovery and safety assessment.

The Congress Committees of the ESTIV 2024 Congress warmly welcome you in Prague and look forward to seeing many colleagues working in the area of *in vitro* and *in silico* toxicology and pharmacology reuniting!

Helena Kandarova and Clive Roper



Acknowledgements

The ESTIV 2024 congress would not have been possible without the logistic assistance of the conference organiser Klinkhamer | conferences & events as well as the financial support of the ESTIV supporters and sponsors.

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KEYNOTE SPEAKERS AND DEBATERS

KEYNOTE SPEAKERS AND DEBATERS

Monday, 3 June 2024
14:20 - 15:00

KEYNOTE LECTURE I & DEBATER I

PD Dr. Robert Landsiedel

Dr. Robert Landsiedel is Vice President of special toxicology at BASF SE in Ludwigshafen am Rhein, Germany. He had previously worked for BASF in development and management in the USA and in Japan. He is an associate professor (Privatdozent) at the Free University of Berlin and has further teaching positions in Leipzig and Landau. He is also the chairman of the German Toxicology Society (GT) and President of the German Society for Experimental and Clinical Pharmacology and Toxicology (DGPT). His team at BASF is performing more than 500 regulatory toxicological studies per year under GLP, GIVIMP and ISO17020 as well as screenings for product development. In addition, they are developing new toxicological methods and testing strategies. They have received more than 20 external grants (German and EU-funded). Their work has been recognized by several awards, including the German Research Award for the development of alternative methods, the German GT-Toxicology Award, the Responsible Care Award of the European Chemical Industry Council (Cefic) and the Herbert E. Stokinger Awards of the American Conference of Governmental Industrial Hygienists (ACGIH). Robert received a postgraduate doctorate degree in chemistry (Dr. rer. nat.), a postgraduate degree in toxicology, and a habilitation in pharmacology and toxicology. He is a Diplomate of the American Board of Toxicology (DABT) and a Fellow of the American Academy of Toxicological Sciences (FATS). He was appointed member of the European Commission's Scientific Committee for Occupational Exposure Levels (SCOEL) where he chaired the methodology working group until the Committee's decommissioning in 2019. Currently, he is the chair for human toxicology of the German National Hub within the "Partnership for the Assessment of Risk from Chemicals (PARC).

Star Wars on Ceres. Episode I – The irreproducibility menace

ABSTRACT #357

PD Dr. Robert Landsiedel[1]

[1]*DABT, FATS*

The ongoing transition from chemical hazard and risk assessment based on animal studies to assessment relying mostly on non-animal data, requires a multitude of novel experimental methods, and this means that guidance on the validation and standardisation of test methods intended for international applicability and acceptance, needs to be updated. These so-called new approach methodologies (NAMs) must be applicable to the chemical regulatory domain and provide reliable data which are relevant to hazard and risk assessment. Confidence in and use of NAMs will depend on their reliability and relevance, and both are thoroughly assessed by validation. Validation is, however, a time and resource- demanding process. As updates on validation guidance are conducted, the valuable components must be kept: Reliable data are and will remain fundamental. In 2016 the scientific community was made aware of the general crisis in scientific reproducibility - validated methods must not fall into this. In this commentary, we emphasise the central importance of ring trials in the validation of experimental methods. Ring trials are sometimes considered to be a major hold-up with little value added to the validation. Here we clarify that ring trials are indispensable to demonstrate the robustness and reproducibility of a new method. Further, that

methods do fail in method transfer and ring trials due to different stumbling blocks, but these provide learnings to ensure the robustness of new methods. At the same time, we identify what it would take to perform ring trials more efficiently, and how ring trials fit into the much-needed update to the guidance on the validation of NAMs.

Tuesday, 4 June 2024
08:30 - 09:00

KEYNOTE LECTURE II

Prof. Dr. Theo M. de Kok, ERT

Prof. Dr. Theo M. de Kok graduated in 1988 as a biologist at the Catholic University of Nijmegen, The Netherlands, with Microbiology and Toxicology as majors. He received his PhD in 1992 at the University of Limburg, after 4 years of investigating the relationship between dietary habits, the endogenous formation of carcinogenic compounds and colorectal cancer risk. From 1992 until 1997 he was appointed at the Open University of the Netherlands and was at the same time involved in several research projects at Maastricht University in the field of environmental health sciences and Toxicology. In 1997, he was appointed as an assistant professor at this University and became coordinator of the Environmental Health Sciences programme. He continued his research in the field of genetic toxicology and gene-environment interactions. In 2008, he was appointed as an Associate Professor at the Department of Health Risk Analysis and Toxicology of Maastricht University. Currently, he is appointed as a full Professor at the Department of Toxicogenomics and focussing on the application of systems toxicology in human population studies, as well as the development of in vitro cellular systems as an alternative for animal tests applied for the prediction of toxicity of chemical exposures.
He has been the coordinator of the FP7 project PHYTOME, focusing on the prevention of human colorectal cancer risk by the introduction of innovative meat processing methodologies. Furthermore, he has been involved in a significant number of EU projects, including ECNIS, NewGeneris, BRAFO, CarcinoGENOMICS, EnviroGenoMarkers, DETECTIVE, EXPOsOMICS, TransQST and ONTOX. As such he is well experienced and familiar with the tasks and responsibilities that come along with the coordination of (larger) research consortia. He has also been involved in the setup of the interfaculty Maastricht Centre for Systems Biology (MaCSBio) as coordinator of a research programme on Systems Biology Approaches for Personalized Prevention, which started in September 2014. He is currently supervising 9 PhD-students.

Safety assessment of food additives without animal testing: the case study of E171 (titanium dioxide)
ABSTRACT #360

T.M. de Kok[1], N.S. Bischoff[1], J.J. Briede[1], S.G. Van Breda[1], D.J. Sijm[2]
[1]*Department of Toxicogenomics, Maastricht University, GROW Institute of Oncology and Developmental Biology*
[2]*Department or Pharmacology and Toxicology Maastricht University.*

The toxicological safety evaluation of food additives, such as E171 (titanium dioxide), involves comprehensive assessments to ensure their safety for human consumption. Even though E171 has been widely used in various products, including candies, confectionaries, pharmaceuticals, and cosmetics, it has recently come under scrutiny due to emerging evidence of its genotoxic effects, alterations in the gut microbiome, and pro-inflammatory properties. This has led the European Food Safety Authority (EFSA) to re-evaluate E171 and declare it unsafe as a food additive. Most of the concerns have been generated by utilizing rodent models that demonstrated an upregulation of tumor

progression markers and demonstrated dysplastic changes in the colonic epithelium, the development of aberrant crypt foci, as well as immune system alterations. Furthermore, toxicogenomic studies showed transcriptional changes in colonic tissues that are indicative of early markers of CRC development. Despite EFSA's recommendation for E171 withdrawal, contrasting viewpoints exist. For instance, regulatory bodies like Health Canada and the Food and Drug Administration (FDA) maintain that E171 is safe in view of its interaction with food and the digestive tract that may mitigate its adverse effects. Nevertheless, a comprehensive understanding of the effects of prolonged and dynamic GI digestion on E171, particularly on its ROS-generating capabilities, remains unknown. Our research aimed to bridge this knowledge gap by deploying the in vitro TNO GI Model (TIM-1) to replicate the conditions of the upper GI tract, including the mouth, stomach, and small intestine. We found that digestion resulted in profound changes to E171's physicochemical properties, showing either an increase or decrease in E171 aggregation depending on the matrix. Using SDS-PAGE, we identified a protein corona around the particles which reduced the ROS formation in all tested conditions. Additionally, we evaluated the potential use of human colon organoids, as more advanced in vitro model compared to the Caco-2 cell line model we used previously, to study the impact of E171 on ROS formation, DNA damage induction and gene expression changes. Even though the Matrigel in which the colon organoids are cultures presents an additional barrier for particles to reach the cells, dose-dependent ROS formation and DNA strand breaks were induced. The ultimate validation of these in vitro outcomes to support the safety assessment of titanium dioxide requires a human dietary intervention study with E171, which is currently being finalized in our lab.

Wednesday, 5 June 2024
08:30 - 09:00

KEYNOTE LECTURE III

Prof. Mathieu Vinken, Ph.D., Pharm.D., E.R.T.

Mathieu Vinken is a professor affiliated with the Vrije Universiteit Brussel-Belgium. He has a background in pharmaceutical sciences (Pharm.D.), holds a doctoral degree in experimental in vitro toxicology (Ph.D.), is a European registered toxicologist (E.R.T.) and a trained chemical risk assessor. His research focus is situated in the fields of experimental hepatology (cellular communication as a drug target for the therapy of liver disease), in vitro toxicology (mechanistic modelling of liver toxicity and set-up of liver-based in vitro systems) and space toxicology (effects of space conditions on the liver). He is author of more than 230 papers published in international peer-reviewed journals (h-index 47). He is editor of 3 books. He is editor-in-chief of Toxicology, associate editor of Archives of Toxicology and European editor of Applied In Vitro Toxicology. He coordinates several national and international projects, including the European Horizon 2020 project ONTOX. He is a regularly invited speaker on international conferences in the area of toxicology. He is past-president of ESTIV, executive committee member of EUROTOX and founder of the In Vitro and In Silico Toxicology Speciality Section of EUROTOX. He has received several research grants of the excellent science pillar of the European Union's research and innovation frameworks, including from the European Research Council, the

Future and Emerging Technologies program and the Marie Skłodowska-Curie Actions program. He also received a number of scientific awards, among which the Galen Award for Pharmacology.

Ontologies as the basis for setting up animal-free test batteries: liver toxicity as a case study

ABSTRACT #273

Mathieu Vinken[1]
[1]*Vrije Universiteit Brussel*

Ontologies are gaining momentum in the field of toxicology and risk assessment. Ontologies are defined as mode-of-action frameworks qualitatively and quantitatively integrating and structuring relevant biological, toxicological, chemical and kinetic data from various sources. Ontologies have their roots in adverse outcome pathway networks, which in turn originate from physiological maps. Among other applications, ontologies can serve as the conceptual basis for setting up animal-free and human-relevant batteries for the toxicity testing of chemicals. This will be demonstrated in this presentation. Focus will be put on the liver, which is a frequent target for systemic toxicity because of its unique location and function in the organism. A tiered ontology-driven approach for the prediction of steatotic and cholestatic liver toxicity induced by chemicals and relying on combined in silico/in vitro testing as well as on expression and functional analysis will be presented.

Thursday, 6 June 2024
08:30 - 09:00

KEYNOTE LECTURE IV

Prof. Thomas Hartung

Thomas Hartung, MD PhD, is the Doerenkamp-Zbinden-Chair for Evidence-based Toxicology in the Department of Environmental Health and Engineering at Johns Hopkins Bloomberg School of Public Health and the Whiting School of Engineering, Baltimore. He also holds a joint appointment for Molecular Microbiology and Immunology at the Bloomberg School. He is adjunct affiliate professor at Georgetown University, Washington D.C.. In addition, 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. CAAT hosts the secretariat of the Evidence-based Toxicology Collaboration (http://www.ebtox.org) and manages collaborative programs on Good Read-Across Practice, Good Cell Culture Practice, Green Toxicology, Developmental Neurotoxicity, Developmental Immunotoxicity, Microphysiological Systems and Refinement. As PI, he headed the Human Toxome project funded as an NIH Transformative Research Grant and the series of annual Microphysiological Systems World Summits starting in 2022 by 60+ organizations. He is Field Chief Editor of Frontiers in Artificial Intelligence. 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 675 scientific publications with more than 47,000

citations (h-index 115). His toxicology classes on COURSERA had more than 19,000 active learners.

Probabilistic Risk Assessment
ABSTRACT #353

Thomas Hartung[1]
[1]*Johns Hopkins CAAT*

"Probability is the very guide of life" (Cicero, 106-43 B.C.) and in the future of toxicology. Uncertainty and probability are 2 sides of the same coin. Taking a probabilistic approach to risk assessment, however, was always hampered by the considerable effort associated and often a lack of mathematical literacy. The possible game changer is the advent of artificial intelligence. In fact, probabilistic risk assessment only becomes beautiful by artificial intelligence. It excels on data retrieval and integration based on pattern recognitions allowing the most probable evidence integration. Not pretending any certainty in suggested classification, but explicitly expressing the probability of an accurate result, it can serve as a co-pilot to risk assessments. The currently ongoing European project ONTOX aims to design exactly this, a prototype of a predictive tool, which can be benchmarked for further optimization.

Wednesday, 5 June 2024
15:00 - 16:00

DEBATER II

Dr. João Barroso , PhD

João Barroso is a Scientific Officer at the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the European Commission's Joint Research Centre (JRC). He graduated in 1999 as a biochemist at the University of Coimbra, Portugal, and holds a doctoral degree in Biochemistry and Molecular Biology since 2005. He joined EURL ECVAM in 2008, where he stayed for three years, returning in November 2013 as a permanent official. Between 2011 and 2013, João worked as a senior toxicologist consultant at SeCAM Services & Consultation on Alternative Methods and acted as Cosmetics Europe Project Manager on Alternatives to Animal Testing, being responsible for managing research activities and implementation of New Approach Methodologies (NAMs) in the areas of eye irritation, genotoxicity, skin tolerance and systemic toxicity. At EURL ECVAM, he coordinates and conducts scientific and policy support activities in the area of non-animal approaches, including the identification and validation of promising NAMs for regulatory and biomedical applications and their translation into EU legislation and international standards (e.g., OECD Test Guidelines, UN GHS). João also coordinates the ESAC, a scientific advisory committee responsible for conducting independent scientific reviews on non-animal methods and approaches on request of EURL ECVAM, as well as the issuing of EURL ECVAM Recommendations on the basis of ESAC Opinions. João is currently co-leading (on behalf of the EU and together with the Netherlands and the US) the revision of OECD Guidance Document 34 on the validation and international acceptance of methodologies for hazard and risk assessment. In 2009 and 2016, João was presented with JRC Awards for Excellence in the category Policy Impact in recognition of significant scientific achievement. He was Vice-President of ESTIV between 2018 and 2022.

Wednesday, 5 June 2024
15:00 - 16:00

DEBATER III

Prof. Dr. Paul Carmichael

Prof. Dr. Paul Carmichael has worked in the Safety & Environmental Assurance Centre (SEAC) of Unilever in the UK since 2004, where he is responsible for the development and implementation of novel non-animal-based approaches for assuring human and environmental health; that is to say, how can consumer and environmental safety be assured without using and harming animals? He has over thirty years' experience in toxicology and cancer research, largely in the academic arena, and prior to Unilever, he was a Senior Lecturer at Imperial College London in the Faculty of Medicine, where he taught pharmacology and toxicology and conducted research into advances in toxicology. A graduate of Surrey University with a B.Sc. in Biochemistry/Toxicology and a Ph.D. from King's College London, he was a postdoctoral scientist at the Institute of Cancer Research (Royal Marsden Hospital) for seven years, exploring mechanisms of chemical genotoxicity and carcinogenicity. He currently has close academic links with Peking University in China (the School of Public Health) and is an Adjunct Assistant Professor of Pathology and Laboratory Medicine at Brown University in the USA. He has been an Endowed Professor at Wageningen University, Division of Toxicology in the Netherlands, since March 2020.

He has published over one hundred research papers in peer-reviewed scientific journals and has served or serves on many external committees in the UK, US, EU and China. His passion is the advancement of new safety assessment approaches using the inspiration of 'Toxicity Testing in the 21st Century' – this can be termed 'next generation risk assessments' or NGRA. https://doi.org/10.14573/altex.2204281

Wednesday, 5 June 2024
15:00 - 16:00

DEBATER IV

Anne Gourmelon

Anne Gourmelon is the principal administrator of the Test Guidelines Programme, within the Environment Directorate at the Organisation for Economic Cooperation and Development. Anne started working at the OECD in 2002, initially on projects related to endocrine disrupters testing and assessment methodologies. She also worked for some years on the OECD Cooperative Chemicals Assessment Programme. She took over the lead of the Test Guidelines Programme in 2013. Many Test Guidelines using alternatives to animal testing have been developed in the last two decades, to meet evolving regulatory needs and address ethical questions, but many challenges remain to improve chemical safety and ensure sufficient protection of human health and the environment. Prior to OECD, she worked at the UN Food and Agriculture Organisation (FAO) in Rome from 2000 to 2002. She has Master's degree in environmental sciences from the University of Wageningen in the Netherlands, obtained in 1999.

Wednesday, 5 June 2024
15:00 - 16:00

DEBATER V

Erin Hill

Erin Hill, President and CEO, comes to ICCS with over 30 years of experience promoting the widespread use and acceptance of animal-free safety testing methods. She has fostered collaborations with industry, animal protection organizations, and regulatory agencies, both foreign and domestic, to help coordinate efforts to advance the use of New Approach Methodologies (NAMs) in decision-making. Erin is widely known for co-founding the non-profit Institute for In Vitro Sciences (IIVS) in 1997, where she most recently served as president. In addition to her pioneering work in the field, Erin is actively involved in various boards and associations dedicated to NAMS and reducing reliance on animal testing methods. She sits on numerous boards and committees, including ESTIV and ASCCT, leveraging her expertise and experience to further the adoption and implementation of ethical testing practices. Her contributions and dedication to the cause have earned her numerous awards and recognition within the scientific and animal protection communities.

ORAL PRESENTATIONS

ALL ORAL PRESENTATIONS

Monday, 3 June 2024
16:30 - 18:30

Session 1: ECHA, EMA and EFSA perspectives on the use of NAMs / Funding the development, validation, and implementation of NAMs

Chairs: *Helena Kandarova (CEM SAS, Bratislava, Slovakia) & Costanza Rovida (CAAT-Europe)*

O-1-1
Counting the number of animals that REACH and CLP are requesting as the first step to start accepting NAMs
ABSTRACT #231

Costanza Rovida[1]
[1]*CAAT-Europe*

Background and Objectives REACH (Regulation EC 1907/2006) has the aim of promoting alternative methods. In spite of that, risk assessment of chemicals is based on in vivo tests, also with the need to define the classification according to CLP (Regulation EC 1272/2008). Actually, calculation of the resulting animal use is not available. Material and Methods The dossiers of the registered substances are public available in the ECHA dissemination platform. A Python program was applied to scan these data and retrieve information on the number of animals that were used in new in vivo tests performed for REACH purposes, focusing on repeated dose toxicity, reproductive toxicity and developmental toxicity. Other assumptions lead to the estimation of the number of animals that have been used for the other endpoints and to estimate those that are to arrive as the outcome of the dossier evaluations and for the upcoming REACH revision. Results The total animal count as of December 2022 for these categories is about 2.9 million with an additional 1.3 million animals deriving from ongoing tests. The estimation is for other 0.6 to 3.2 million animals have been used for other endpoints. The latest REACH and CLP amendments will probably add up to 7 million animals. Discussion and Conclusion The total, 4.2 million, for just these three test categories exceeds the original EC forecast of 2.6 million for all REACH tests. This awareness should serve to increase awareness and support ECHA in its efforts to require new tests only when there is a clear benefit for the improvement of human and environment health. It also demonstrates the possibility to monitor animal use, which is important for measuring the impact of REACH in this area, and efforts by ECHA and the EC to increase use of alternative strategies.

O-1-2
Guidance on the use of biomarkers of effect in regulatory risk assessment of chemicals - integration of new approach methodologies
ABSTRACT #88

Lucian Farcal[1], Sara Levorato[1], Georgia Bompola[1], Zainab Al Harraq[1], Cristina Croera[1]
[1]*European Food Safety Authority (EFSA), Parma, Italy*

The European Food Safety Authority (EFSA) has initiated a project towards developing a guidance on the use of biomarkers of effect in regulatory risk assessment of chemicals, implemented with the support of a working group of experts established under EFSA's Scientific Committee. The project is proposed in the context of the risk assessment processes aiming to support the risk assessors in integrating additional biological measurements, such as the biomarkers of effect that indicate an early biological response due to exposure to a chemical or mixture. Moreover, the aim is to achieve an internationally agreed guidance by establishing consultation and co-creation mechanisms with EU and international organisations, contributing also to the EU chemicals strategy for sustainability and its action on 'one substance - one assessment' [1]. The goal of the project is to work towards guidance on the use of biomarkers of effect to derive Reference Points (RPs) for establishing Health Based Guidance Values (HBGV) or deriving Margins of Exposure (MoE). The implementation started with a feasibility study, including the definition and description of

biomarkers of effect applicable in this context and the establishment of the overall scope of the guidance. This also includes mapping of activities, initiatives and approaches relevant in this context. Among several challenges that need to be addressed, one refers to the integration of new approach methodologies (NAMs) for e.g. characterisation and validation of biomarkers of effect, understanding more regarding their variability, sensitivity and specificity or integration of knowledge via computational tools. The use of existing approaches and framework such as the adverse outcome pathways (AOPs) is also investigated. Finally, the feasibility study should recommend and identify a way forward in developing the guidance, jointly with other EU and international organisations.

O-1-3
EFSA's ongoing and planned activities in the application of NAMs for the risk assessment of chemicals
ABSTRACT #87

Maria Chiara Astuto[1], Irene Cattaneo[1], Djien Liem[1], Claudia Roncancio-Peña[1]
[1]*European Food Safety Authority (EFSA)*

The European Food Safety Authority (EFSA) considers New Approaches Methodologies (NAMs) strategic in the context of its remit. While responding to 3Rs principles, NAMs are considered a powerful tool to address data gaps in risk assessment and accelerate the transition towards a Next Generation Risk Assessment (NGRA). Over the last few years, EFSA has launched several projects aimed at promoting the implementation of NAMs in the risk assessment of chemicals to contribute to the safety of the EU food and feed chain. Several proof-of-concept case studies covering different regulatory frameworks and toxicological endpoints were launched with the ultimate goal of incorporating newly generated NAM-based data in EFSA's risk assessments through the design of Integrated Assessment and Testing Strategies (IATA). A roadmap for action on NAMs was recently published and is considered as a starting point for future priority setting. For example, a new EFSA Project called NAMs4NANO initiated to focus on the design of case studies in the area of nanomaterials and development of methodologies to promote the qualification of NAMs and its integration in EFSA risk assessment. All these activities will set the basis for the development of future guidelines to support risk assessors in the practical integration of NAM-based data within EFSA's risk assessments.

O-1-4
ACCELERATING VALIDATION THROUGH IN VITRO METHOD AUTOMATION AND HIGH THROUGHPUT TESTING
ABSTRACT #354

Taina Palosaari[1], Donatella Carpi[2], Aurora Fassi[2], Roman Liska[2], Peter Macko[2], Julia Malinowska[2], Emilio Mendoza[2], Stavroula Sampani[2], Maurice Whelan[2]
[1] *European Commission, Joint Research Centre (JRC), Ispra, Italy*
[2]*European Commission, Joint Research Centre (JRC), Ispra, Italy*

The development of international test guidelines that facilitate the mutual acceptance of data between OECD countries requires rigorous validation of newly proposed test methods. In this context, validation is the process assessing the reliability and relevance of a method intended for a particular purpose, for example to generate information on a toxicological effect of regulatory concern. Assessing the relevance of in vitro methods during a validation study is a challenging task since there are numerous aspects that need to be properly characterised, including sensitivity and specificity of detecting the effect of interest, concentration-response relationship, and applicability domain with respect to the properties of chemicals that can be reliably tested. In addition, the relative and collective relevance of a set of methods within a battery needs to be evaluated for the design of Integrated Approaches to Testing and Assessment. All this requires significant amounts of data that are usually generated by testing the methods under validation using libraries of reference chemicals. Historically, these data have been generated manually in either one or more laboratories, which usually requires considerable time and resources.

Method automation and high-throughput testing enables the generation of large reference datasets of high quality in a shorter time. However, to successfully automate an in vitro method, it is necessary to appropriately transform a manual protocol into an automated one, and to verify its implementation. Here we present considerations and practical steps involved in automating in vitro methods on high-throughput screening platforms to support validation. We illustrate this by discussing studies undertaken using the automated platforms of the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM).

O-1-5
VALIDATING NAMS ; EXPERIENCE GAINED ON THE PEPPER PLATFORM
ABSTRACT #68

Philippe HUBERT[1], Andrea RIVERO ARZE[1], Pauline LELANDAIS[1], Elise GRIGNARD[1]
[1]*PEPPER - Paris (FR)*

Pepper is a public-private platform dedicated to the validation of methods for the identification of endocrine disrupters. It is a French non profit association whose activities started in 2020. It is funded not on a case by case basis but for a global systematic work on validation, both organizing and funding it. Experience gained in two areas are illustrated : How to convince partners to support validation; Where are difficulties and cost issues in the validation process ? The challenge was to convince that validation is a “common good” for all those players, and that funding should therefore be pooled. Under the impetus of a “national strategy for endocrine disruptors”, French ministeries and industries have adopted this approach, and the Pepper funding, of about 3 M€ per year is almost evenly shared. A specific governance that was necessary to convince the partners, including new partners from Dutch (and soon from German and Belgium) ministries. Nine methods were selected and two other ones are to be added in October 2023. They are are Non Animal Methods, Five of them are on the OECD Work plan and a sixth one under submission. Challenges for NAMs are the Readiness of method, substance selection … and of course the various shortages and technical difficulties. An other is the transferability phasis. Preliminary exchanges with developer to identify problems can alleviate the burden. The various levels of support to laboratories are shown according to method complexity, and other factors (e.g. labor intensive, consumable intensive …). The cost of the manpower for the organization is compared to the costs for laboratory supports. The experience gained shows that validation costs can be lower than they are at present, but this implies a great improvement in the readiness of methods, hopefully through more pressure in the research funding.

Tuesday, 4 June 2024
09:00 - 10:30

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

Chairs: *Christian Pellevoisin (Urbilateria & Vitroscreen, France) & Arno Gutleb (Invitrolize & Luxembourg Institute of Science and Technology LIST, Luxembourg, Luxembourg)*

O-2a-1
Evaluation of in vitro and in silico new alternative methods for the identification of potential endocrine effects
ABSTRACT #147

Daniela Lange[1], Abdulkarim Najjar[1], Jaqueline Meinhardt[1], Mareike Boettcher[1], Nadine Mueller[1], Johanna Ebmeyer[1], Andreas Schepky[1], Anke Wilm[1]
[1]*Beiersdorf AG*

An evaluation of the potential of a chemical to cause endocrine disruption (ED) is an important aspect of a safety evaluation since the endocrine system is responsible for the production and regulation of hormones, which are crucial for the normal development, growth, and functioning of the body. Endocrine disrupting chemicals can interfere with normal hormone signaling, leading to a wide range of adverse health effects, including reproductive and developmental abnormalities, cancer, obesity, and neurological disorders (WHO, 2012). We have evaluated a suite of in silico prediction models and in vitro assays reflecting

estrogenic, androgenic and steroidogenic effects for their ability to identify the ED properties of ten test chemicals (tamoxifen, 4-tert-octylphenol, mestanolone, daidzein, benzyl butyl phthalate, mono-benzyl phthalate, 2-(4-(diethylamino)-2-hydroxybenzoyl)benzoic acid; 2-[4- (dibutylamino)-2-hydroxybenzoyl]benzoic acid, isoeugenol and terephthalic acid). In vitro methods included receptor binding, CALUX transactivation, H295R steroidogenesis and aromatase activity inhibition assays. In silico prediction models were evaluated for their ability to detect binding, agonism and antagonism of the estrogen receptor and androgen receptor, and aromatase inhibition. These included Derek, Vega, Case Ultra, Danish (Q)SAR, ADMETLab, Opera, ADMET Predictor and ProToxII. In silico final calls were mostly in agreement with the in vitro assays, and predicted ER and AR effects well. This study highlights the importance of combining in silico and in vitro assays to evaluate EAS effects. While in vitro assays provide mechanistic information, in silico assessments provide guidance at an early stage of risk assessment for screening or for in vitro testing. Nevertheless, other mechanisms and exposure should be considered when making a conclusion with respect to ED effects.

O-2a-2
A 3D model applicable to reproductive toxicology
ABSTRACT #220

Francesco Carriero[1], Francesca Rescigno[1], Christian Pellevoisin[2], Marisa Meloni[1]
[1]*VitroScreen, Milan, Italy*
[2]*Urbilateria, Tours, France*

In the field of reproductive biology and its clinical applications, the study of pathologies affecting the reproductive system, and fertility and pregnancy, has reached an increasing importance. For this reason, the interest in developing predictive and versatile models is high(1). Because the endometrium, the tissue that lines the uterus, plays an important role in the menstrual cycle and pregnancy we developed a 3D model of this epithelial tissue. The VitroScreen ORA® Endometrium is a 3D scaffold free spheroids composed of endometrial fibroblasts (T-HESC cells) and epithelial endometrial cells (HEC-1-A) that mimics the physiological tissue architecture in terms of compartment organization and cells interactions. It expresses specific endometrial morphological biomarkers (vimentin, CK7, ITGb1, CDH and ki67) and secretes characteristic cytokines (LIF, VEGF, IL-1 β, IFN-γ). Due to its high biological relevance, the model responds to sexual hormones stimulation as estradiol and progesterone at different doses mirroring the phenotypical changes of tissue during different phases of menstrual cycle. The endometrium is a key organ in many adverse outcome pathways developed to study endocrine disruptors(2). Therefore, the morphological and physiological characteristics of this 3D model of the endometrium make it an oustanding platform for the in vitro study of compounds with a potential impact on reproductive biology.

O-2a-3
Human based brain barrier models for studying thyroid hormone transport
ABSTRACT #198

Kim Heikamp[1], Timo Hamers[1], Ellen Hessel[2], Majorie van Duursen[1]
[1]*Amsterdam Institute for Life and Environment (A-LIFE), Vrije Universiteit, Amsterdam, The Netherlands.*
[2]*Centre for Health Protection (GZB), National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands.*

Thyroid hormone (TH) plays a key role during pregnancy and is involved in many important processes during brain development. In order for TH to reach the fetal brain it has to pass the blood-brain-barrier (BBB) or the blood-cerebrospinal fluid barrier (BCSFB). TH transport across both barriers is mediated via differentially expressed TH transmembrane transporters (THTMTs), as well as the TH distributor protein (THDB) transthyretin. The transport of TH across brain barriers can be disrupted by a wide range of substances via different mechanisms. To study possible molecular initiating events for TH transport inhibition and subsequent key events of changes in the TH molecular pathways, it is essential that models are utilized that closely

mimic the in vivo situation. An overview is given of a broad range of in vitro and ex vivo BBB and BCSFB models. From least to most complex, these include mono-cultures, co-cultures, spheroids, organoids, barrier-on-a-chip, and explant cultures. Furthermore, different assays are highlighted that can be employed in these models to study TH transport. All in vitro models come with advantages and disadvantages, and a choice for which one to utilise must be made based on the aim of the study. Use of a combination of barrier model systems to answer one question might be necessary. The more complex models offer the opportunity to study TH transport in human based systems that closely represent the in vivo (developmental) BBB and BCSFB. This could lead to new insights into the distribution of THTMTs and the role of THDBs at the brain barriers and how chemical exposure might effect this. Next to this, regulatory testing to assess possible substance toxicity involves in vivo tests. In the future, a combination of in vitro models could possibly replace animal testing, or at least reduce the need for in vivo models.

O-2a-4
The identification of thyroid hormone-disrupting chemicals from complex environmental mixtures using pull-down assay coupled with non-target analysis
ABSTRACT #63

Petra Mikušová[1], Zuzana Toušová[1], Luděk Sehnal[2], Kateřina Grabicová[3], Roman Grabic[3], Klára Hilscherová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany*
[3]*South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Vodňany, Czech Republic*

As thousands of chemicals of anthropogenic or natural origin occur worldwide, the environment faces a huge problem with contamination by highly complex mixtures, which cause adverse effects in biota including human. Exposure mixtures can contain micropollutants causing endocrine disruption through diverse modes of action. Potential to disrupt thyroid hormone regulation by binding of compounds from mixtures to human transthyretin (hTTR), transporter protein of thyroid hormones, has been detected, but causative chemicals remain largely unknown. Current methods for the identification of bioactive chemicals like high-performance liquid chromatography with high-resolution mass spectrometry (HPLC-HRMS) for target and non-target analyses combined with bioassays and fractionation have several limitations like insufficient reduction of sample complexity. That is why a pull-down assay, a novel highly specific protein-ligand interaction-based approach, was developed for the identification of active compounds in complex mixtures. Human transthyretin (hTTR) protein was engineered, expressed, purified and used in pull-down assay for active compounds' separation and identification from several environmental mixtures. Complex samples as well as the eluted ligands were subjected to TTR bioassay and non-target screening workflow based on differential analysis of HPLC-HRMS data for different pull-down assay eluates. Water samples from several sites like including treated wastewater from small streams in the Czech Republic (Europe), and surface water from an urban area in Malawi (Africa), were used to identify known and novel hTTR ligands. Identified compounds were confirmed and quantified by the targeted analysis of the original samples. Retained activity in pull-down eluates was measured in the TTR bioassay, and the effects and potencies of identified ligands were determined. The contribution of identified ligands to the overall effect was calculated. The presented method is a promising tool for the effective identification of bioactive compounds from complex mixtures. The project was funded from the Czech Science Foundation (GACR) under grant agreement GX20- 04676X.

O-2a-5
Novel in vitro assay for thyroperoxidase inhibition – comparison of different models and detection methods
ABSTRACT #98

Runze Liu[1], Jiří Novák[1], Jan Kuta[1], Klára Hilscherová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*

Interference with thyroid hormone (TH) regulation can result in health issues in both children and adults, including neurodevelopmental and thyroid disorders and cardiovascular risks. Thyroid hormone disruptors (TDs) can disrupt the TH system, affecting TH synthesis, transport, actions on target tissues, or metabolism. A key molecular initiating event (MIE) within the adverse outcome pathway (AOP) network in TH synthesis is the inhibition of thyroperoxidase (TPO). The study aims to develop, characterize, and optimize an in vitro assay for the efficient assessment of TPO peroxidation inhibition. We conducted an in-depth characterization of existing in vitro human and rat cell-based models (e.g. Nthy-ori 3-1, FRTL5) and rat microsomes together with a newly developed human TPO-transfected cell model. Selected models were coupled with previously used high-throughput peroxidase activity assays (Luminol and Amplex UltraRed (AUR) assays), along with a novel sensitive and selective method using HPLC-ICP-MS detection [1]. The suitability of these methods for assessing TPO inhibition was determined using a set of 21 priority model chemicals relevant to human exposure. The Luminol assay was highly non-specific, detecting peroxidation activity even in cell lines with very low or undetectable TPO expression (Nthy-ori 3-1 and HEK293T). On the other hand, the AUR assay was more specific and exhibited mostly lower IC50 levels than the Luminol assay, while the HPLC-ICP-MS assay was shown to be the most sensitive but with lower throughput. In summary, the AUR assay proved to be the most suitable high-throughput method for assessing TPO inhibition. The successful creation of a human TPO-transfected cell line allows for high-throughput screening, avoiding the need for ex vivo material. This innovation provides valuable insights into the impact of thyroid hormone-disrupting chemicals on human molecular targets. The project received funding from the EU H2020 research and innovation program under the ERGO project (grant agreement No. 825753).

Session 2b: Challenges in cosmetics safety

Chairs: *Bambou Tan (L'Oréal, Paris, France) & Erin Hill (ICCS, New York, USA)*

O-2b-1
Improved Confidence of Quantitative Sensitizing Potency Assessment for Point of Departure Using GARDskin Dose-Response
ABSTRACT #35

Robin Gradin[1], Andy Forreryd[1], Ulrika Mattson[1], Johan Andersson[1], Frédéric Amaral[2], Fleur Tourneix[2], Nathalie Alépée[2], Henrik Johansson[1]
[1]*SenzaGen AB*
[2]*L'Oreal Advanced Research*

Identification of skin sensitization hazard and potency characterization are central aspects of risk assessment of chemicals. Current legislation advocates a transition from hazard assessment using in vivo methods to UN GHS potency subclassification and quantitative risk assessment by use of New Approach Methodologies (NAM:s) as well as Defined Approaches (DA). However, the ability of NAM assays to quantitatively estimate sensitizing potency and thereby establish a point of departure (POD) for next-generation risk assessment (NGRA) strategies is currently lacking. To this end, the GARDskin Dose-Response (DR) method, adapted from the OECD TG 442E method GARDskin, was recently introduced. The GARDskin DR method evaluates test chemicals in a titrated range of concentrations, in order to investigate the dose-response relationship between the output from the GARDskin prediction algorithm (Decision Values; DV:s) and test chemical concentration. The combined information can be used to derive a quantitative estimation of sensitizing

potency, defined as the cDV0-value, i.e, the least required dose required to elicit a positive response by the prediction model. The current work focuses on optimizing the ability of GARDskin DR to derive a quantitative POD based on conversion to a composite Potency Value (PV; µg/cm2). A total of 25 chemicals were used to construct predictive regression models fitted to reference PV:s. Results show that the updated models produced more accurate potency predictions compared with models fitted with, and aiming to predict, only LLNA EC3 and NOEL, respectively. Mean fold-change errors ranged between 2.8 and 3.2, with predicted POD:s being within or close to the range of the variation of the historical in vivo data. Lastly, uncertainty in predictions was reduced, as estimated by a minimum 2-fold reduction of 95%-confidence intervals. In conclusion, such improved ability to predict POD:s may be used as a starting point to determine safe use levels of chemicals.

O-2b-2
Deriving a continous point of departure for skin sensitization assessment using a bayesian network model
ABSTRACT #46

Fleur Tourneix[1], Leopold Carron[1], Lionel Jouffe[2], NATHALIE ALEPEE[1]
[1]*L'Oreal R&I*
[2]*Bayesia SAS*

Regulations of cosmetic ingredients and products have been the most advanced in embracing new approach methodological (NAM) solutions. Consequently, cosmetic industry has assumed a forerunner role in the development and implementation of animal-free defined approaches (DA) to assess skin sensitisation potential characterisation of ingredients. Among them, we have developed a DA based on a Bayesian network, referred as the SkinSens-BN to predict skin sensitisation hazard, UN GHS categories and to derive a potency prediction of being a non, weak, moderate, or extreme/strong sensitizer. The Bayesian network was constructed against reference Local Lymph Node Assay for a total of 297 substances. The accuracy of prediction was 61% for the four potency categories, increasing to 67% for UN GHS categories and to 85% for hazard classes. To derive a continuous Point of Departure (PoD), the SkinSens-BN probability distribution for the four potency classes was associated with fixed weights. The selection of a PoD by picking only the most likely class was too restrictive. The derived PoD for substances in the weak, moderate, or strong/extreme sensitisers show a PoD capable to fall in neighboured class. The PoD for substances classified as non-sensitisers did not overlap with any others. Among the derived PoD, 77% (228/297) were similar or more conservative than the EC3 LLNA. To exemplify this PoD approach, 2-methyl-undecanal was considered as moderate by picking the most likely class. As this potency class corresponds to an EC3 □ 2 to 10%, a worst-case approach would have resulted in a PoD of 2%. Applying the approach proposed here resulted in a continuous PoD estimate of 7.86% almost four times higher compared to the PoD assigned by considering the most likely potency class only. The predictive PoD may, using appropriate assessment factors, be used as a starting point to determine safe use levels of chemicals.

O-2b-3
A novel 3d Nrf2-are reporter epidermis model for skin sensitization testing
ABSTRACT #195

Johanna Ebmeyer[1], Katrin Brandmair[1], Denise Dising[2], Doris Finkelmeier[2], Andreas Schepky[1], Jochen Kühnl[1], Anke Burger-Kentischer[2]
[1]*Beiersdorf AG, Hamburg, Germany*
[2]*Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Cell and Tissue Technologies, Stuttgart, Germany*

Skin sensitization assessment has shifted towards the application of New Approach Methodologies. Several in vitro assays covering key events 1-3 of the AOP for skin sensitization are accepted for regulatory use. [1, 2, 3]. Most in vitro tests are based on submersed cell culture conditions that limit applicability domain and biological functionality. Culture conditions are rarely suitable for lipophilic or precipitating compounds. Additionally, 2D cell cultures lack important biological features such as skin barrier

functionality. Consequently, test results for challenging compounds and mixtures lack confidence. Here, we present a new 3D reporter epidermis model that shows promise to broaden the applicability domain for skin sensitization assessment by enabling topical application and by partially implementing skin barrier functionality. Our 3D epidermis models were developed by stable transfection of the immortalized keratinocyte cell line Ker-CT with an antioxidant response element (ARE)-containing promotor regulating the expression of a Secreted Embryonic Alkaline Phosphatase (SEAP)-reporter. ARE elements are regulated by the Keap1-Nrf2-ARE signaling pathway, being the fundament of skin sensitization assays described in OECD Test guideline 442D [2]. Our novel KerCT-Nrf2 reporter cell line showed typical keratinocyte morphology and proliferation rate in 2D culture. Air-liquid interface cultivation formed stratified 3D epidermis models that demonstrated in vivo-like morphology and skin barrier functionality. A set of reference chemicals was used to evaluate the cell line's capability to identify skin sensitizers in 2D and 3D cultures. Compared to LLNA and human benchmark data, our results imply a promising predictive capacity with high intra- and interlaboratory reproducibility. All experiments applied culture conditions free of animal-derived materials warranting ethical testing and avoiding serum batch-dependent variability. We conclude that our novel 3D epidermis model may broaden the applicability domain by emulating in vivo-like exposure of the epidermis and show promise for coping with challenges in the skin sensitization assessment of mixtures.

Tuesday, 4 June 2024
11:00 - 13:00

Session 3a: Human Cells as New Approach Methodologies for Immunotoxicity Testing

Chairs: *Victor J. Johnson (PhD, Burleson Research Technologies, Inc., Morrisville, NC, USA) & Emanuela Corsini (PhD, Università degli Studi di Milano, Milan Italy)*

O-3a-1
Building a comprehensive toolbox for Immunotoxicology safety assessment using human whole blood.
ABSTRACT #253

Victor Johnson[1]
[1]*Burleson Research Technologies, Inc.*

The immune system is charged with maintaining overall health as well as a critical role in maintenance of homeostasis in most organ systems of the body. Therefore, the immune system is highly complex and dynamic and built to excel in an environment of continually shifting challenges from intrinsic and extrinsic forces. Given the pinnacle role for the immune system in homeostasis and health, the field of Immunotoxicology has been charged with protecting the immune system from toxicity. Traditional approaches for immunotoxicity testing have relied on animal models to study the effects of xenobiotics on complex multi-cellular functions. However, there is immense pressure to adopt the 3R principle of Replacement, Reduction, and Refinement of animal use in toxicity testing. Considering the ever-expanding ban on animal testing for cosmetic ingredients, the field of Immunotoxicology has done an exemplary job of developing and validating non-animal alternative testing methods for skin sensitization. Successful recapitulation of the complex process of skin sensitization using in silico and in vitro technologies provides promise for adopting NAMs for other complex immune functions. It is critical for a comprehensive safety assessment to investigate the impact of xenobiotics on innate, humoral, and cell-mediated immunity that demand complex interactions of many immune cell types. The goal of this talk is to present evidence for development of in vitro techniques capable of assessing immunotoxicity to all major arms of the immune system. Human whole blood has been used to develop antigen-driven functional immune responses including Natural Killer activity, T-cell activation, antibody production, and cytokine profiling. Together with comprehensive immunophenotyping for identification of cell populations and cytotoxicity, this human whole blood model provides promise as a comprehensive toolbox for in vitro hazard identification for xenobiotics affecting the immune system. Proof of

performance will be illustrated using known immunosuppressive compounds including polycyclic aromatic compounds.

O-3a-2
Using in vitro human immune system models to understand the immunotoxicity risks associated with PFAS exposure.
ABSTRACT #254

Emanuela Corsini[1]
[1]*Università degli Studi di Milano*

Per- and polyfluoroalkyl substances or perfluorinated alkylated substances (PFAS) have been used extensively in commercial/industrial applications for the last 70 years. In vivo available data mainly highlights an immunosuppressive effect resulting in a reduction of the antibody response to vaccination and reduced resistance to infection, demonstrated both in experimental animals and humans. Starting from this evidence and to get more insights on the mode of action, an in vitro human immune system approach has been developed to assess both T cell-independent and T cell-dependent antibody responses. A successful primary antibody response in vitro specific to keyhole limpet hemocyanin (KLH) was elicited, as well total immunoglobulin release by polyclonally activated B cells. Antigen specific and polyclonal antibody responses showed clearly decreased responses following in vitro exposure to perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS). In addition, the use of primary human peripheral blood mononuclear cells allowed for identification of differences according to gender and type of PFAS tested, demonstrating the value of this in vitro human immune system model. Results obtained using our in vitro human immune system model following treatment with PFOA and PFOS, perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) will be presented.

O-3a-3
A Path Forward: Current and Future Perspectives of Alternatives to Developmental Immunotoxicity Testing.
ABSTRACT #255

Fenna Sillé[1], Costanza Rovida[2]
[1]*Johns Hopkins University, Bloomberg School of Public Health & Center for Alternatives to Animal Testing*
[2]*CAAT-Europe*

The development of non-animal New Approach Methods (NAMs) has been widely acknowledged as a critical need for toxicity testing. Although critical windows of developmental immunotoxicity (DIT) have been defined, DIT testing is intimately linked to the use of whole animal studies with inherent limitations in translatability to humans. Sensitive in vitro assays are hampered by the complex nature of effects and partially missing information on interrelationships in the human system during the developmental period. Clinical information on the effects of drugs and other exposures on the developing immune system are scarcely available. Unfortunately, the status of in vitro and other alternative assays for DIT screening is in its infancy. The “International Working Group on Alternatives to Developmental Immunotoxicity Testing” is identifying and addressing critical knowledge gaps in the field of alternative DIT. First, the need for alternative DIT testing strategies will be discussed from applied and regulatory end-user perspectives. Second, an updated and refined network of key molecular and biological events in developmental immunology that are important to assess during DIT testing will be presented. Current efforts to translate scientific advances into adverse outcome pathways (AOP) that can inform regulatory hazard or risk assessment will be discussed. Examples of existing alternative strategies that are useful for DIT testing will be provided. Areas in need of development of alternative DIT models and tests will be highlighted through the introduction of a novel framework to encourage the refinement and development of new alternative test methods suitable for screening of (large numbers of) DIT compounds. Finally, a future outlook on how ground-breaking innovations and state-of-the art technologies can benefit the switch to alternatives to DIT will be

presented. The ultimate goal is to develop a methodology for alternative DIT screening and develop test guidelines that can be incorporated in OECD guidance documents.

O-3a-4
Human cord blood derived CD34+ hematopoietic stem cells as an in vitro model for investigating developmental immunotoxicity.
ABSTRACT #256

Norbert Kaminski[1]
[1]*Michigan State University*

The large array of different cell types that constitute the immune system arise from CD34+ hematopoietic stem cells (HSCs). The cell fate decisions involved in the development of these various immune cell lineages is facilitated by the transition of HSCs to multipotent progenitors, followed by the appearance of common myeloid or common lymphoid progenitors ultimately resulting in cell lineage specification. Historically, xenobiotics that interfere with immune cell development were identified using rodent models, primarily mice. However, it is also well established that although there are many similarities between the mouse and human immune system, critical differences also exist. The objective of this presentation is to discuss a novel in vitro model system using human cord blood derived CD34+ HSCs that recapitulates critical events allowing for the cellular development and lineage commitment for most of the major immune cell types, as confirmed by scRNAseq and flow cytometry. Moreover, an aryl hydrocarbon receptor (AHR) ligand will be used as a case study to demonstrate how perturbations of the events involved in lineage commitment can markedly and selectively skew cell type specification. With the current emphasis on establishment of new approach methodologies for toxicity evaluations, this model system focused on developmental immunotoxicology provides numerous advantages over murine based models, the most significant being the utilization of human primary HSCs, which eliminates the need for extrapolation across animal species.

O-3a-5
In vitro assessment of the sensitizing potential of bisphenol A substitutes
ABSTRACT #146

Mélanie Mourot-Bousquenaud[1], Isabelle Langonné[1], Maurane Buchheit[1], Samuel Muller[1], Amélie Coiscaud[1], Julianne Mathiot[1], Sandrine Jacquenet[1], Fabrice Battais[1]
[1]*French Research and Safety Institute for the Prevention of Occupational Accidents and Diseases*

Background and Objectives: Bisphenol (BP-) A is a chemical substance used in Europe to produce polycarbonate plastics and epoxy resin or as color developer in thermal paper. Due to its toxic properties for human health, BPA was banned in feeding bottles in 2011 and its presence in thermal paper was restricted by European regulations in January 2020. Therefore, substitute chemicals with poorly described immune toxicological properties are gradually replacing BPA. The objective of this study was to assess the allergenic sensitizing potential of 27 substitutes to BPA used in the industry. Material and Methods: The expression of two costimulatory molecules: CD80 and CD86; and six cytokines: interleukine-6 (IL-6), tumor necrosis factor (TNF), C-C motif chemokine ligand (CCL-) 2, CCL3, CCL4 and CCL5, were analysed by flow cytometry in mouse bone-marrow derived dendritic cells (BMDCs) exposed to the chemicals at sub-cytotoxic concentrations. Results: All substances except one induced overexpression of at least one receptor on BMDCs and were thus identified as having sensitizing potential. Based on the BMDC model, those substances were classified as extreme (1 out of 27), strong (20 out of 27) and moderate (5 out of 27) sensitizers. BPA was classified as a moderate sensitizer and BPF was the only substitute classified as a non-sensitizer. The more potent BPA substitutes induced more than 2-fold secretion of CCL3, CCL4 and/or CCL5 by dendritic cells. Discussion and Conclusion: Most of the BPA substitutes tested in this study have a sensitizing potential with the BMDC model; 24 of them being more potent than BPA itself. Only BPE, BPF and 2,4-BPS appeared to be weaker sensitizers than BPA. Chemical-induced allergies are major occupational diseases and

caution should therefore be exercised when handling such chemicals at workplace.

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

Chairs: *Benoît Maisonneuve (NETRI, Lyon, France) & Erika Ferrari (BiomimX, Milano, Italy)*

O-3b-1
Evaluating the potential of novel physiologic-like liver-on-chip models for in vitro hepatotoxicity screening
ABSTRACT #72

Erika Ferrari[1,2], Mattia Ballerini[2]
[1]*BiomimX srl*
[2]*Politecnico di Milano*

Background and Objectives: Accurately reconstructing the 3D architecture of the natural liver is crucial for developing effective in vitro hepatic models that can be integrated into the drug development pipeline (DDP) [1]. LoC platforms capable of recapitulating pivotal features of liver physiology are expected to increase the robustness of compounds screening for hepatotoxicity earlier in the DDP, as more than 30% of drug recalls are due to drug-induced liver injury (DILI) [2]. We recently developed a 3D liver-on-chip (LoC) platform able to mimic the in vivo space of Disse between hepatocytes and endothelial cells. The in vitro space of Disse improved liver-specific functionality (e.g., albumin and CYP3A4 production) compared to direct contact of hepatocytes with the vasculature [3]. We here further improved our device establishing a novel physiologic-like 3D LoC model amenable for hepatotoxicity testing. Material and Methods: We evaluated the effect of APAP, Bosentan and Troglitazone on models generated by HepG2, HepaRG and primary human hepatocytes (PHH) 3D constructs, co-cultured with hepatic stellate cells (e.g., LX-2) embedded in an extracellular matrix (ECM) layer mimicking Disse space and human umbilical vein endothelial cells (HUVECs) lining the vascular channels. Results: The effect of these drugs on liver viability and functionality was assessed through live/dead immunofluorescence (IF), albumin and enzymatic assays, as well as IF staining and RT-PCR of peculiar hepatic biomarkers. Changes on endothelial barrier permeability were also monitored by 4kDa FITC-Dextran diffusion tests. Discussion and Conclusion: The developed 3D LoC models can be thus used as screening tools in the pre-clinical phases of the DDP, as well as to study the behaviour of the immune components flowing in the vasculature in phato-physiological conditions.

O-3b-2
A Pilot Study to Evaluate the Kinetics, Metabolism and Toxicity of a Fragrance Material (Coumarin) in an In Vitro Three-Organ Integrated Microphysiological System (MPS) Model
ABSTRACT #102

Kaushal Joshi[1], James M. McKim[2], Olive Chon[1], Anne Marie Api[1]
[1]*Research Institute for Fragrance Materials, Mahwah, NJ*
[2]*LifeNet Health, Life Sciences, Kalamazoo, MI*

The Research Institute for Fragrance Materials (RIFM) ensures the safe use of fragrance materials, while proactively investigating alternatives to animal testing. New toxicological methods are needed, particularly for endpoints that have been dependent on animal testing, like repeated dose toxicity. The human multiple organ platform provides a means to accomplish these objectives. In this project, coumarin was evaluated in a three-organ circuit (intestine, liver, and kidney). Human EpiIntestinal (Mattek Corp.) was used for the intestine, human primary hepatocytes (LifeNet Health) were used for the liver, and renal HK-2 cell line was used for the kidney. Communication between organs was through a simulated blood flow system that incorporates a semipermeable membrane inside each organ compartment. This allows test chemicals to move by osmotic diffusion into and out of the simulated blood and organ compartments. Flow was accomplished with a precision micro syringe pump (5 µL/min), and the simulated blood consisted of buffered saline (pH 7.4) with human serum albumin. Coumarin was applied (100 µL) to the apical surface of the intestine chamber, yielding a final

dose of 100 µg. Samples (50 µL) were collected from each compartment at different timepoints from 0 to 72hrs. Kinetic concentration versus time curves showed rapid intestinal absorption and distribution to liver and kidney. The primary metabolite, 7-OH coumarin, was detected in the liver, reaching a maximum after 1.5 hrs. After 72hrs, cell viability measured by lactate dehydrogenase (LDH) release was approximately 90% in all organs. In comparison, the liver viability measured by the MTT assay was about 50%. These findings indicate a direct action of coumarin on mitochondria, consistent with in vivo reports. The kinetic, metabolism, and toxicity data provide a means of parameterizing the human in vitro MPS platform for building quantitative models designed to improve next generation risk assessment (NGRA).

O-3b-3
Cytotoxic effects induced by individual and combined exposure to sterigmatocystin, ochratoxin A, and patulin on sh-sy5y spheroids
ABSTRACT #76

Veronica Zingales[1234], Elisa Cimetta[34], Maria-Jose Ruiz[12]
[1]*Research Group in Alternative Methods for Determining Toxics Effects and Risk Assessment of Contaminants and Mixtures (RiskTox)*
[2]*Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia*
[3]*Department of Industrial Engineering (DII), University of Padova*
[4]*Fondazione Istituto di Ricerca Pediatrica Cittá Della Speranza (IRP), Lab BIAMET, Padova*

Mycotoxins are toxic natural food contaminants produced as secondary metabolites by filamentous fungi. Various evidence point that humans are exposed to more than one mycotoxin at once through diet. However, relatively few studies have addressed the interactions between concomitantly occurring mycotoxins and, no studies examining the putative effects of multi-mycotoxins exposure on a 3D cell culture system have yet been reported. This study investigates the cytotoxic effects induced by individual and combined exposure to the mycotoxins sterigmatocystin (STE), ochratoxin A (OTA) and patulin (PAT) on human neuroblastoma SH-SY5Y spheroids by ATP assay after 72h of exposure. The concentration ranges used were 50-1.56 µM for STE, 25-0.78 µM for OTA and 5-0.16 µM for PAT. The combination ratios used for the mixtures were 1:2 (OTA:STE), 1:10 (PAT:STE), 1:5 (PAT:OTA) and 1:5:10 (PAT:OTA:STE). The individual exposure resulted in IC50 values equal to 18.52 µM, 7.74 µM and 7.13 µM for STE, OTA and PAT, respectively. For STE and OTA, no differences were obtained between the binary and tertiary combinations and the mycotoxins taken individually. On the other hand, a higher cell viability reduction was induced by the mixture STE+PAT, OTA+PAT and STE+OTA+PAT compared to PAT alone. The type of interaction that occurred when STE, OTA and PAT were in binary and tertiary combination was described by the median-effect/combination index (CI)-isobologram equation. Antagonistic and additive effects were observed. In particular, the binary combinations OTA+ PAT and STE+PAT showed an additive effect, while STE+OTA was antagonistic. In the tertiary combination, an antagonistic effect was detected at low fractions affected (fa), tending toward an additive effect at high fa. This work is the first step towards a realistic risk assessment scenario for these mycotoxins.
Acknowledgments: Spanish Ministry of Science and Innovation (PID2020-11587RB-100); Generalitat Valenciana post-doctoral grant (Ref.CIAPOS/2021/228); ERC Starting Grant MICRONEX UERI17_01.

O-3b-4
Novel strategy to assess the neurotoxicity of organic solvents such as glycol ethers: Combining in vitro and in silico methods with human controlled exposure experiments
ABSTRACT #189

Nancy B. Hopf[12], Laura Suter-Dick[32], Jörg Huwyler[42], Myriam Borgatta[12], Lucie Hegg[12], David Pamies[52], Hélène Paschoud[12], Ramya Deepthi Puligilla[42], Elena Reale[12], Sophie Werner[342], Marie-Gabrielle Zurich[52]
[1]*Center for Primary Care and Public Health (Unisanté), University of Lausanne, Epalinges-Lausanne, Switzerland*

[2]*Swiss Centre for Applied Human Toxicology (SCAHT), Basel, Switzerland*
[3]*School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland*
[4]*Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland*
[5]*Department of Biomedical Sciences, University of Lausanne, Lausanne, Switzerland*

Exposure to chemicals may contribute to the development of neurological diseases, however chemicals are not required to be systematically tested for their neurotoxic potency. The absence of systematic testing may partially be explained by the current OECD test guidelines relying on animal experiments that are expensive, as well as scientifically and ethically debatable. This study aims to provide a strategy to rank solvents according to their neurotoxicity, using the glycol ethers as a case study. The proposed strategy focuses on a complex 3D in vitro brain model (BrainSpheres) derived from human induced pluripotent stem cells, as well as in vivo, in vitro and in silico models for blood-brain barrier (BBB) and in vitro models for liver metabolism. Data are integrated in a toxicokinetic (TK) model. Internal concentrations predicted with this TK model are validated with results from in vivo human controlled exposure experiments. Results show that the cytotoxicity of propylene glycol ethers (PGEs) in BrainSpheres, BBB and liver models, as well as in Zebrafish larvae is correlated to their carbon chain length. One-week repeated exposure of BrainSpheres to PGEs decreases synaptic and astrocytic markers at subcytotoxic concentrations. Metabolites seem more neurotoxic than their parent compounds, and interestingly, not only liver but also brain cells are able to metabolize the parent compounds. In Zebrafish larvae, acute PGEs exposure decreases BBB integrity and impairs behavioral patterns. Finally, the PGEs brain concentrations predicted for workers exposed at the occupational exposure limit are in the magnitude order of the PGE concentrations found neurotoxic in vitro. Altogether, these results suggest that PGEs are potentially neurotoxic for the human brain. The TK model will be used in reverse dosimetry to predict air concentrations deemed safe for the human brain, in order to contribute to the protection of workers and the general population exposed to PGEs.

O-3b-5
A sensitive and robust human liver microphysiological system for assessing drug-induced liver injury ABSTRACT #113

Ovidiu Novac[1], Emily Richardson[2], Tomasz Kostrzewski[2]
[1]*CN Bio Innovations*
[2]*CN Bio Innovations*

Drug-induced liver injury (DILI) remains the most common cause for acute liver failure in the USA and Europe and is a leading cause of attrition of compounds in drug development. As an alternative to classical 2D cell cultures, which have significant limitations in assessing DILI, we developed a human liver microphysiological system (MPS) comprised of human primary liver hepatocyte parenchymal and nonparenchymal cells, cultured in 3D microtissues on an engineered scaffold under perfusion up to two weeks. The methodology has been qualified with a broad set of thirteen severely and mildly hepatotoxic test articles aligning with the IQ Consortium MPS affiliate recommended list of small molecule drugs for predicting DILI [1]. Robustness of the model was calculated for three main soluble biomarkers that are measured in the cell culture media at Day 4 to assess the liver microtissues quality control. Excellent intra-study coefficient of variance (CV) was calculated for LDH, urea and albumin (below 15%) and inter-study CV falls below 20% (N = 360 wells/liver microtissues) proving a sensitive and robust in vitro liver model for predicting DILI. At a threshold of 50x Margin of Safety (MOS, ×Cmax) our liver MPS in vitro model showed superior sensitivity and specificity over classic 2D primary hepatocytes cultures and even some standard non-MPS 3D models in detecting DILI matching clinical data (with sensitivity 100%, accuracy 85%, and precision 100%) highlighting the clinical translatability of our liver MPS model. Due to the human relevance and predictivity of the system if the liver MPS had been utilised in early drug development the hepatotoxicity of

pharmaceutical compounds would have been identified at a much higher rate, helping to de-risk drug attrition in clinic. Subsequently, mechanistic insights of the toxicity may be investigated and a better alternative designed, saving cost in both drug development and human lives.

O-3b-6
Oral absorption and intestinal first-pass metabolism of pesticides using an in vitro human gut-model
ABSTRACT #323

Eileen Hallscheidt[1], Marc Lamshöft[1], Maria Hahn[1]
[1]*Bayer AG Crop Science*

Investigating human safety of pesticides for regulatory purposes is currently driving from in vivo animal studies to new approach methodologies (NAMs) supporting the 3R approach. Within the framework of this approach, an advanced human gut-model was implemented to investigate the exposure to humans after oral absorption and intestinal first-pass metabolism of the radiolabeled fungicide trifloxystrobin. Human intestinal Caco2 and HT29-MTX cell lines were co-cultivated on collagen I coated transwell membranes to form physiologically relevant barrier models. On day 21, cells were exposed to 10 µM trifloxystrobin, atenolol and diclofenac, the latter serving as permeability references. Sampling of apical and basolateral specimens was performed in a kinetic approach. Specimens were quantitatively analyzed by high resolution mass spectrometry to determine permeability coefficients and identify respective metabolites. Barrier integrity was monitored by transepithelial electrical resistance measurement. Viability, differentiation and endogenous metabolic functionality of the cells was confirmed by apical determination of lactate dehydrogenase, alkaline phosphatase and glucose consumption, respectively. Results revealed in vitro permeability coefficients predicting medium to low (10 min, 30 min and 2 hours) and low (4- and 24-hours) in vivo oral absorption in humans. However, Caco2/HT29-MTX cells metabolized trifloxystrobin to the known phase-I hydrolyzed carboxylic acid metabolite. In apical and basolateral specimens, a 4- to 6-fold increase was observed compared to cell-free controls. Overall, these results show low absorption and high metabolic capacity of the cells towards trifloxystrobin indicating low oral bioavailability. Additionally, it is assumed that the metabolite crosses the intestinal barrier via the transcellular route based on its intracellular and basolateral abundance. The human gut-model provides the opportunity to investigate human absorption and first-pass metabolism of orally exposed pesticides. Predicting the exposition of pesticides towards downstream organs is crucial for human risk assessment. Moreover, the envisioned transfer into organ-on-a-chip technologies provide the basis of future organ interactions.

Tuesday, 4 June 2024
14:00 - 16:00
Session 4a: Computational toxicology – in silico modelling, read-across, artificial intelligence

Chairs: *Thomas Hartung (Johns Hopkins Bloomberg School of Public Health and the Whiting School of Engineering, Baltimore, USA) & Emily Reinke (Inotiv, Research Triangle Park, NC, United States)*

O-4a-1
Case Studies for the SARA-ICE Defined Approach for Skin Sensitization – Application to Different Chemistries
ABSTRACT #55

Emily Reinke[1], Judy Strickland[1], Dori Germolec[2], Jim Truax[1], Joe Reynolds[3], Georgia Reynolds[3], Nicole Gilmour[3], David Allen[1], Gavin Maxwell[3], Nicole Kleinstreuer[2]
[1]*Inotiv*
[2]*NIH/NIEHS/DTT/NICEATM*
[3]*SEAC Unilever*

The Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) defined approach (DA) for skin sensitization is a Bayesian statistical model that estimates ED01, a human-relevant metric of sensitizer potency that represents the dose with a 1% chance of inducing skin sensitization. The model

accounts for variability of input data, which are largely from in vitro and in chemico new approach methodologies (NAMs), and explicitly quantifies uncertainty. SARA-ICE is currently under evaluation by the Organisation for Economic Co-operation and Development for inclusion in Guideline No. 497: Defined Approaches on Skin Sensitisation. It is also being evaluated by the U.S. Environmental Protection Agency Office of Pollution Prevention and Toxics for predicting a point-of-departure for risk assessment. As part of the SARA-ICE evaluation by both organizations, several case studies for different chemical classes have been conducted. These case studies highlight the model's utility for predicting hazard and potency categorization and providing a human-relevant point-of-departure for risk assessment. This presentation will share results from several ongoing case studies that apply SARA-ICE to different chemical and product use classes, including chemicals used in preservatives (e.g. isothiazolinones), fragrances (e.g. geraniol), agrochemicals, and personal care products. We will present comparisons to other DAs and existing human and mouse data, where available. Our results indicate that SARA-ICE accurately predicts skin sensitization hazard and potency relative to currently utilized methods. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

O-4a-2
Metabolic Mechanisms of Action modelling to sustain safety evaluation of cosmetic ingredients
ABSTRACT #236

Fresnais Louison[12], Perin Olivier[2], Riu Anne[2], Grall Romain[2], Ott Alban[2], Fromenty Bernard[3], Frainay Clement[4], Jourdan Fabien[4], Poupin Nathalie[4]

[1]*Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France*
[2]*L'Oréal Research and Innovation, Aulnay-sous-bois, France*
[3]*INSERM, Univ Rennes, INRAE, Institut NUMECAN (Nutrition Metabolisms and Cancer) UMR_A 1341, UMR_S 1241, F-35000 Rennes*
[4]*Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France*

The animal testing ban for safety evaluation of cosmetic ingredients still addresses challenges. Developing New Approach Methodologies (NAMs) for systemic toxicity evaluation and to better understand xenobiotic metabolic Mechanisms of Action (mMoA). in silico and in vitro tools, bringing both mechanistic insight and identification of biological analogs, are developed to conduct Next generation Read Across (NGRA). Some of these approaches are based on transcriptomic data. They describe a biological behavior captured at a specific dose, time and in a defined cellular context. Despite being more and more used, these are known to have some flaws that need improvements: indirect representation of metabolism, genes considered independent and post-translational modifications ignored. To overcome these shortcomings, new systems biology approaches based on Genome Scale Metabolic Networks (GSMN) have emerged (1). One way to exploit those GSMN is to build condition-specific metabolic networks. To do this we developed a dedicated workflow to integrate transcriptomics data into GSMN, highlight significantly activated reactions (DAR) and finally extract specific subnetworks of closely located DARs. Metabolic network extraction is presented here to address two kinds of questions related to NGRA: toxicity classification and MoAs weight of evidence. Extraction of mMoA-specific subnetworks of DARs from the whole GSMN paves the way to classification for safety assessment purposes. Knowledge of the flux of metabolites is also of great interest. Thus, integration of experimental metabolomic data into GSMN As today in silico read across strategies are mostly structure-based approaches, this workflow represents a promising tool for systemic toxicity evaluation of the metabolic impact of defined structure molecules, as well as for the evaluation of complex mixtures such as natural ingredients generating strong interest for cosmetic industries.

O-4a-3
New Approach Methods for Regulatory Risk Assessment of Azo Dyes in Textiles
ABSTRACT #124

Prachi Pradeep[1], Panagiotis Karamertzanis[2], Suna Nicolai[1], Andreas Luch[1], Ralph Pirow[1]
[1]*Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany*
[2]*Computational Assessment and Alternative Methods, European Chemicals Agency (ECHA), Helsinki, Finland*

Azo dyes are the most commonly used synthetic coloring agents in textile industry. Azo dyes, by reductive cleavage of the azo bond, can biotransform into primary aromatic amines (pAAs), some of which are known to be genotoxic. Systematic evidence mapping for available data on human health hazards of market-relevant azo dyes (Keshava et al. 2023) and studies to identify cleavage products and toxicity data for 470 non-regulated azo dyes (Brüschweiler et al. 2014, 2017) revealed a significant toxicity data gap leading to regulatory challenges in azo dye risk assessment. In the absence of experimental data, New Approach Methodologies (NAMs) such as read-across and quantitative structure-activity relationship models (QSARs) are often used for data-gap filling. This study aims to utilize experimental genotoxicity data received under REACH to develop predictive models for assessing the genotoxic potential of azo dyes lacking reliable experimental information. Firstly, azo dyes were grouped into structure-based clusters using the unsupervised K-means algorithm. Resultant clusters and associated data were analysed to assess if structurally similar clusters had similar genotoxicity outcome data. Secondly, experimental in-vitro genotoxicity data, fingerprints and chemical descriptors were used to develop QSAR models. Finally, confidence scores were assigned to model predictions based on performance across previously derived clusters. This study utilized over 4500 azo dyes registered on the US EPAs CompTox Chemicals Dashboard. Experimental REACH genotoxicity data (Klimisch score of 1 or 2) was available for 276 azo dyes. QSAR models developed using these data predict genotoxicity outcomes with an external balanced accuracy of 72% and a confidence score in the range of 39-82%. This study demonstrates a promising approach to using in-silico NAMs for azo dye risk assessment. Additionally, NAMs tailored to azo dye chemistry can help utilize REACH data more efficiently for data-gap filling with respect to regulatory risk assessment of non-regulated azo dyes.

O-4a-4
AnthroDrugs-Tox: Personalized Toxicology through Population Genetics
ABSTRACT #94

Simon Perera del Rosario[12], Laureano E. Carpio[1], Eva Serrano-Candelas[1], Jorge García Calleja[2], Laura Vilà-Valls[2], Elena Bosch[2], Francesc Calafell[2], Rafael Gozalbes Botella[1]
[1]*ProtoQSAR SL*
[2]*Departament de Ciències Experimentals i de la Salut, Institut de Biologia Evolutiva (UPF-CSIC), Universitat Pompeu Fabra*

Personalized medicine is one of the elements of the P4 medicine of the future, and has already shown its ability to improve patients' lives in an individualized way (e.g. Soverini 2018, Gambardella 2020). However, a series of limitations hinder its widespread implementation in the immediate or short term, which has led to the development of different methods of population stratification (McGuire 2020, O'Hanlon Cohrt 2021, Litman 2019). One of these approaches is stratification based on the biological ancestry of patients, which has seen significant progress in recent years thanks to developments in DNA and ancient DNA analysis. It has proven to be a successful strategy throughout the value chain of drug design and development, both in diagnosis and therapeutic efficacy as well as in pharmacology and toxicology, being specially advanced in the pharmacokinetics and pharmacodynamics (PKPD) area. Stemming from our team's previous work in personalized medicine and drug development (e.g. Font-Porterias 2021, Bhat-Ambure 2023), in this work, we propose the optimization of current drugs by leveraging the current human population genetics knowledge. We analyse drug-related genes (DRGs) specifically in the toxicology area, thus

allowing for newer, optimized, safer-by-design drugs for all human populations. This contributes to avoiding the current pitfalls of a drug development process specifically focused on people of European descent). In particular, we analyse a dataset of 59 Tox-DRGs (drug toxicity-related genes) collated from the literature, describing their variability in populations, and searching for potential signatures of natural adaptive selection in specific continental regions (Africa, East Asia, South Asia, Europe, and America). For selected genes, we analyse how the population variability can affect the toxicity experienced by individuals when taking the drugs involved (e.g. affected adverse outcome pathways (AOPs) and molecular initiating events (MIEs) or key events (KEs), and whether drugs can be improved in this respect.

O-4a-5
Integration of multi-domain data and interpretable in-silico models for computational toxicology
ABSTRACT #230

Vijay Gombar[1], Alex Sedykh[1], Adrian Green[1], Jason Phillips[1], Austin Ross[1], Kristine Witt[2], Warren Casey[2], Ruchir Shah[1]
[1]*Sciome LLC, Research Triangle Park, NC, United States*
[2]*National Institute of Environmental Health Sciences (NIEHS), Division of Translational Toxicology (DTT), Research Triangle Park, NC, United States*

QSAR in silico models and read-across approaches are critical to transitioning away from animal-based toxicity measurements. Essential for the success of both these approaches are high-quality experimental in vivo and in vitro data. OrbiTox, an interactive web-based tool, houses cleaned and harmonized experimental data: E.g., bacterial mutagenicity (~7000 compounds); rodent, and human carcinogenicity (~5000 compounds); Tox21 bioassay (~8000 compounds); cardiotoxicity (~9000 compounds) from over 400 ToxCast assays; and acute rat oral LD50 (~7800 chemicals). These data are represented as over 400000 connections across multiple domains, namely ~900000 substances, ~22000 annotated human gene targets, ~2000 biological pathways, and ~200 test organisms. Besides being rich in data content, OrbiTox contains validated, QSAR models for predicting outcomes in multiple assays covering a variety of toxicity endpoints, e.g., 13 models for Ames test outcome in OECD-recommended Salmonella and E. coli strains, 80 models for Tox21 assays at 10uM and 100 uM concentration thresholds, 10 models to predict potential cardiotoxicity failure modes, etc. Over 500 QSAR models use our extensively benchmarked collection of chemist-interpretable molecular substructures, Saagar*, as descriptors and are highly accurate (AUROC 0.77-0.86) and provide chemistry-backed reasoning for a prediction. Like OrbiTox, our Windows-based software, SCiP also contains these QSAR models. Both OrbiTox and SCiP help visualize substructures leading to a prediction while showing as support chemistry-aware structural analogues. SCiP allows computation of descriptor vectors using built-in Saagar substructures and user-added substructures covering proprietary chemistry to build QSAR models using a variety of modeling methods. SCiP can automatically extract combination of substructures to visualize SAR trends for designing safer chemicals. The duo of intuitive web application OrbiTox and stand-alone Windows software SCiP, thus, makes an excellent predictive toxicology tool set with large collection of multidomain data intelligently connected and QSAR models providing chemistry-backed reasoning to propose potential mechanistic understanding and chemical design.

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

Chairs: *Helena Kandarova (CEM SAS, Bratislava, Slovakia) & Eugene Choi (OECD, Paris, France)*

O-4b-1
Adoption of OECD TG 439 and 431 for the assessment of skin irritation and corrosion properties of 2D nanomaterials
ABSTRACT #84

Michela Carlin[1], Silvio Sosa[1], Ester Vazquez[2],

Maurizio Prato[3], Aurelia Tubaro[1], Marco Pelin[1]
[1]*University of Trieste, Department of Life Sciences*
[2]*University of Castilla-La Mancha, Department of Organic Chemistry,*
[3]*University of Trieste, Department of Chemical and Pharmaceutical Sciences*

The increasing use of bidimensional (2D) nanomaterials in many technological applications needs a careful evaluation of their impact on human health. Skin contact is one of the most relevant exposure routes to these materials and can be associated with direct adverse effects.1 Therefore, this study is focused on the assessment of skin irritation and corrosive potential of selected 2D nanomaterials: i) graphene-based materials (GBMs), such as few-layer graphene, graphene oxide, reduced graphene oxide and graphene nanoplatelets; ii) hexagonal boron nitride (hBN); iii) molybdenum disulfide (MoS2). To obtain robust and reliable toxicological data through standard methods for safety testing not implying the use of animals, skin irritation and corrosion potential were assessed following the Organization for Economic Co-operation and Development (OECD) Test Guidelines (TGs) 439 and 431, respectively, using an in vitro advanced 3D model of epidermis (SkinEthic™ Reconstructed human Epidermis; RhE). Even though not validated for nanomaterials, both OECD TGs resulted suitable for testing powdered 2D materials, and showed that none of the tested materials is potentially irritant or corrosive. Only GBMs prepared with irritant surfactants, not subjected to washing procedures, reduced RhE viability at levels lower than those predicting skin irritation (≤50%). To implement the data obtained by the OECD TG 439, considering irritation as a pro-inflammatory reaction, the release of selected inflammatory mediators (IL-1α, -1β, -6, -7, -8, -18, -33, TNF-α, PGE2 and RANTES) from treated RhE was quantified. Hierarchical clustering analysis on the amounts of pro-inflammatory mediators released by RhE exposed to each 2D nanomaterial, as compared to those of negative (untreated RhE) and positive (RhE exposed to sodium dodecyl sulfate) controls, supported the lack of irritation properties. In conclusion, by adopting the appropriate OECD TGs on an advanced 3D epidermis model, the selected 2D nanomaterials appear to be devoid of skin irritation and corrosion potential.

O-4b-2
Eye damage reversibility in an in vitro model of bovine cornea to replace the Draize test completely
ABSTRACT #235

Martina Daniela Benedetti[12], Mariela Lenze[12], Julieta Roco[12], Romina Martinez[3], Maria Laura Gutierrez[12]
[1]*CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina)*
[2]*Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Argentina*
[3]*Hospital Dr Julio Méndez*

One of the requirements for the registration of substances such as agrochemicals is to provide evidence about their potential eye damage. The Draize test performed in rabbits allows the products to be classified into four categories, considering both the severity of the lesions produced in the animal's eye as well as its healing time. The available alternative methods to this live animal test do not allow documenting the damage reversibility, nor the time necessary for such reversibility to occur, as required by the UN GHS classifications. Our proposal is to complement the in vitro model that uses the bovine cornea as a substrate to predict whether a substance is irritating or non-irritating (BCOP), with a strategy that allows predicting if the observed irritation is reversible and the time it takes to revert. This is necessary to finally replace the Draize test completely. Limbal stem cells are known to play an important repairing role in corneal injury; therefore we isolated these cells from bovine cornea and used them to evaluate the cell sensitivity to reference products. A wound healing assay was also performed to study whether these products differentially affect the replication and migration capacity of the cells. Furthermore, a tissue explant and an organotypic cornea culture model were implemented to study if the chemical exposure alters cell's replication, migration and overall wound healing differentially. In conclusion, a combination of the approaches used have been

proven effective to detect the four categories of GHS reference products.

O-4b-3
The interleukin 18 is an in vitro biomarker for the prediction of photosensitization in reconstructed human epidermis: the development of PhotoSENSIL-18 method for the safety assessment of raw materials and finished products
ABSTRACT #143

Rodrigo Azevedo Loiola[1], Richard Nguyen[2], Mouctard Barry[1], Christophe Dini[1], Pierre-Jacques Ferret[2], Eric Andres[1]
[1]*Oroxcell*
[2]*Pierre Fabre Laboratories*

Background and objectives: The photosensitization is an adverse effect induced by a substance that becomes a sensitizer upon exposure to ultra-violet (UV) light. Although the photosensitization remains a major toxicological endpoint for the safety assessment, there is no validated in vitro method available for its evaluation so far. We have previously developed a method (SENSIL-18) using the interleukin 18 (IL- 18) production by reconstructed human epidermis (RHE) as a specific biomarker for in vitro sensitization assessment[1, 2]. Herein, we discuss our most recent study [3] demonstrating the efficacy of a new in vitro assay using IL-18 to predict the photosensitization in the RHE model (PhotoSENSIL-18). Methods: EpiCS™ RHE were incubated with a set of known sensitizing / phototoxic / photosensitizing substances and exposed to ultra-violet (UV) irradiation. Then, the cell viability was analyzed by MTT assay, while the IL-18 secretion was quantified by ELISA. This protocol was used to test 16 substances and the induction of IL-18 production (UV+/UV- ratio) was calculated. Results: We found that a cut-off of 1.5 induction (UV+/UV- ratio) is the most predictive model, being capable of identifying true positive photosensitizers (8 of 9) with a good prediction (sensitivity of 89%) in comparison with in vivo data. This approach provides complementary information regarding the toxicity and sensitization potential of tested substances and it can be integrated with other assays (e.g. hCLAT and DPRA) for an accurate prediction. Discussion and conclusion: Our data suggests that the PhotoSENSIL-18 is a valuable in vitro method for identification of photosensitizing substances, allowing the administration of higher concentrations and test lipophilic compounds. In a nutshell, our studies have highlighted that the SENSIL-18 and PhotoSENSIL-18 are promising approaches to evaluate the skin sensitization induced by raw material and finished products, as well as complex mixtures and medical devices.

O-4b-4
Toxicity of polylactic acid and polyethylene terephthalate nanoplastics, aged in environmental conditions, on human intestinal cells
ABSTRACT #89

Vérane Bard[1], Maeva Boulée[1], Aliro Villacorta[2], Thierry Douki[1], Sylvie Motellier[3], Alba Hernández[2], Ricard Marcos[2], Marie Carrière[1]
[1]*Univ. Grenoble Alpes, CEA, CNRS, IRIG, SyMMES-CIBEST, F-38000 Grenoble, France*
[2]*Univ. Autònoma Barcelona, Dept. Genet & Microbiol, Group Mutagenesis, Campus Bellaterra, 08193 Cerdanyola del Vallès, Spain*
[3]*Univ. Grenoble Alpes, Lab Measure Securing & Environm, LITEN, DTNM, STDC, CEA, 17 Av Martyrs, 38000 Grenoble, France*

Plastic production has exponentially increased over the past 50 years, leading to plastic accumulation in the environment. In the oceans and soils, plastics are subjected to physicochemical and biological stresses responsible for plastic fragmentation into smaller fragments called micro and nanoparticles (MNPLs) that may show a higher adsorption rate for pollutants than pristine particles. Hence, they are more likely to transport at their surface metals, additives or pathogens present in the environment. We are exposed daily to plastic fragments mostly via the consumption of seafood and water. In this context, our objective was to evaluate, in realistic environmental conditions, the biological effects of polyethylene terephthalate (PET) MNPLs, derived from plastic water bottles, and of commercial polylactic acid (PLA) MNPLs, a biosourced and biodegradable

plastic. These MNPLs were used both in their pristine state and after artificial ageing in a Q-SUN test chamber. The biological effects of PET and PLA were evaluated on human intestinal epithelial cell lines representative of healthy individuals and of people suffering from inflammatory bowel disease. To this, co-cultures of wild-type Caco-2 or Caco2-LV-Nod21007fs cells, which is one of the most frequents mutation in Crohn's disease patients, and HT29-MTX were used. These cells were exposed to 120, 200 and 450 nm PLA or to 200 nm PET particles. Particles were characterized by TEM and DLS and their degradation upon ageing was investigated via HPLC-MS/MS. Cytotoxicity, DNA damage, and intracellular ROS levels were assessed after 24 h of exposure to realistic concentration of nanoparticles. The results of the study do not show any toxicity of PET and PLA MNPLs on these cell models. These results are consistent with the literature, suggesting that the toxicity of MNPLs may rather lay in their role of pollutant carrier than in intrinsic hazard, but further work is needed to confirm this hypothesis.

Wednesday, 5 June 2024
09:00 - 10:30

Session 5a: PARC session – Partnership for the Assessment of Risks from Chemicals

Chairs: *Denis Sarigiannis (Aristotle University of Thessaloniki, Greece) & Philip Marx-Stoelting (German Federal Institute for Risk Assessment, Berlin, Germany)*

O-5a-1
Towards the development of safe and sustainable by design chemicals, materials and products
ABSTRACT #378

Dimosthenis Sarigiannis[1234], Spyros Karakitsios[124]
[1]*National Hellenic Research Foundation*
[2]*Aristotle University of Thessaloniki*
[3]*University School of Advanced Studies IUSS*
[4]*ENVE.X*

Background and objectives: Within PARC, a toolbox is currently under development, aiming at supporting the operationalization of SSbD. It is designed to integrate tools for safety and sustainability assessment originating from different policy areas and strategies, as well as new tools developed inside and outside PARC. Material and Methods: The PARC SSbD toolbox comprises interoperable models building on the EC SSbD framework, covering both the requirements for (a) de novo developed chemicals and materials and (b) existing ones. The toolbox builds technically on innovative and beyond-the-state-of-the-art methods for hazard, exposure (both occupational and for the general population), human health risk assessment, and sustainability assessment covering all aspects of sustainability, environmental, social and economic. Regarding safety by design, the source-to-impacts continuum is followed, aiming to integrate functionally environmental releases, environmental concentrations, multi-pathway and multi-route exposure, internal dose assessment, early biological effects, adverse outcome pathways, risk assessment and health impact assessment. Our development method adopts concurrent engineering, and integrates NGRA methods that can accelerate the innovation process of developing newer and safer chemicals. Results: The toolbox is empowered with quantitative structure-activity relationship models for property prediction supported by a machine learning-based search engine that explores the relevant chemical space for alternative molecules that would eventually minimize their environmental and human health hazards associated. Results of a first case study to assess toolbox applicability on bisphenol A and two substitutes (BPAP and isosorbide) are given herein. Discussion and Conclusion: The experience obtained from the development of the toolbox to date highlights the most important user requirements and how they need to be integrated into the subsequent development stages. Enhancement and operationalisation of NAMs including in silico methods embodying the rapid developments in artificial intelligence, multi-omics and computational systems toxicology are key aspects of operational tools facilitating the SSbD transition of the chemical industry.

O-5a-2
Hazard Assessment in PARC
ABSTRACT #359

Kiara Aiello-Holden[1], Celia Garcia Arenas[1], Thalia de Castelbajac[2], Gilles Riviere[2]
[1]*BfR*
[2]*ANSES*

Current approaches for the assessment of environmental and human health hazards due to exposure to chemical substances have served their purpose well. However, the assessment systems in place for different chemical regulations are faced with numerous challenges, ranging from a growing number of chemicals and mixture aspects to changes in the types of chemicals and materials produced. This has triggered awareness of the need for a paradigm shift, notably appearing in the EU Chemicals Strategy for Sustainability (CSS), requiring new concepts for chemical risk assessment. As a result, new approach methods (NAM) and next-generation risk assessment (NGRA) are commonly regarded as the way forward. However, incorporating new scientific insights and innovative approaches into hazard assessment in such a way that regulatory needs are adequately met has appeared to be challenging. The European Partnership for the Assessment of Risks from Chemicals (PARC) has been designed to address various challenges associated with innovating chemical risk assessment. Its overall goal is to consolidate and strengthen the European research and innovation capacity for chemical risk assessment to protect human health and the environment. With around 100 participating organisations from all over Europe, including three European agencies, and a total budget of approximately 100 million euros, the PARC work package (WP) on hazard assessment is one of the largest projects of its kind. It has a duration of seven years and is coordinated by ANSES, the French Agency for Food, Environmental and Occupational Health & Safety and BfR, the German Federal Institute for Risk Assessment. The WP hazard assessment focusses on closing data gaps for substances where data are lacking, development of new approach methods as well as increasing their regulatory readiness.

O-5a-3
NGRAroute and PARCopedia
ABSTRACT #373

Sebastian Schmeisser[1], Isabella Apruzzese[1], Magnus Løfstedt[2], Sónia Namorado[3], Rita Paiva Pessoa[3], Maria Uhl[4], Matthias Herzler[1]
[1]*German Federal Institute for Risk Assessment (BfR), Berlin, Germany*
[2]*European Environment Agency (EEA), Copenhagen, Denmark*
[3]*Instituto Nacional de Saúde (INSA), Lisbon, Portugal*
[4]*Environment Agency Austria, Vienna, Austria*

Within PARC Work Package 2 (“A common science-policy agenda”), Task 2.2 (“Knowledge management and uptake into policy”) is responsible for two major overarching activities directed towards expediting the regulatory uptake of New Approach Methods (NAMs) and Next-Generation Risk Assessment (NGRA): The activity NGRAroute, kicked off in October 2022, aims to provide a concrete and applicable roadmap proposal for implementing NGRA as the default approach to chemical risk assessment in EU chemicals legislation by 2025. In July 2023, the European Commission announced their plan to prepare a roadmap for phasing out animal testing in chemical safety assessments. A jointly organised roadmap workshop was held in Brussels in December 2023 and in the meantime, both activities have joined forces along with the European Partnership for Alternatives to Animal Testing (EPAA) and an NGO initiative to create a “roundtable” supporting the Commission’s roadmap. Recent developments including guiding principles for a future NGRA framework will be reported in this presentation. The transition to NGRA as the default chemical risk assessment approach requires broad participation and consensus from a large and diverse chemical risk assessment community. To overcome knowledge gaps as well as communication barriers and to create a lively community for jointly solving the complex challenges lying ahead, PARC Task 2.2 has created the online platform PARCopedia (https://parcopedia.eu) which was launched in November 2023. Although created by PARC, PARCopedia is open to everyone involved professionally in working on chemicals safety for human health and the environment, explicitly aiming to connect PARC with other

initiatives and stakeholders in the field, in Europe and worldwide. In this presentation, a short overview of the main features of the platform is given, along with examples of how users may benefit from it in their daily work.

O-5a-4
How can PARC contribute to promoting the regulatory uptake of New Approach Methodologies?
ABSTRACT #355

Dimitra Nikolopoulou[1], Mirjam Luijten[2]
[1]*Benaki Phytopathological Institute (BPI), Greece*
[2]*National Institute for Health and Environment (RIVM), The Netherlands*

The European Partnership for the Assessment of Risks from Chemicals (PARC) [1] aims to protect human health and the environment through development and implementation of Next Generation Chemical Risk Assessment (NGRA) approaches that support transition to a circular economy and relevant policy-related strategies [2]. In the context of PARC, the concept of NGRA refers to the use of data from New Approach Methodologies (NAMs) for chemical Risk Assessment (RA). NAMs include, but are not limited to, tiered combinations of in silico tools, in vitro systems, organ models, omics approaches, physiologically-based toxicokinetic modelling and complex exposure models [3]. Work package 6 (WP6) of PARC, entitled 'Innovation in regulatory risk assessment', contributes to this goal by strengthening the scientific basis of NGRA approaches, by reviewing current practices used in regulatory risk assessment and by developing and implementing the best scientific achievements in the risk assessment processes. WP6 is developing Integrated Approaches to Testing and Assessment (IATAs) for selected human health effects, using Adverse Outcome Pathway (AOP) networks and relevant NAMs to measure individual events. Databases and inventories of available NAMs are being compiled. A wide range of case studies is being performed to identify specific challenges and needs related to the use of NAMs for regulatory purposes both for human health and environmental risk assessment. Barriers that hamper the regulatory uptake of NAMs are being identified and addressed where appropriate. Overall, PARC will contribute in multiple ways to advancing the regulatory uptake of NAMs, through interconnection of different scientific domains, consideration of current EU legislation and sharing common visions and roadmaps towards achieving the 'one substance, one assessment' approach.

Session 5b: Knowledge sharing and education

Chairs: *Bambou Tan (L'Oréal, Paris, France) & Erin Hill (ICCS, New York, USA)*

O-5b-1
Storytelling skills for a more effective scientific communication
ABSTRACT #127

Aviva Vetter[1], Bambou Xuezhu TAN[2], Pascale Mora[2]
[1]*Humane Society International*
[2]*L'Oréal R&I*

Effective communication is fundamental in the scientific research[1]. It is crucial for knowledge sharing within the scientific community, it is also significant in bridging the gap between scientific details and the general public's comprehension. However, many researchers are not trained to present their findings in an engaging manner. They often lack the refined tools and techniques to effectively communicate their insights. Our presentation aims to providing researchers with a comprehensive guide on the essentials of effective scientific communication. Among the essential communication tools available, storytelling emerges as a particularly effective technique. Why is storytelling so effective? It humanizes the content. It transforms abstract data into narratives, fosters emotional connections, and crafts compelling hooks that captivate an audience. Such techniques elevate a presentation from a transfer of information to an impactful exchange of knowledge and understanding. By integrating storytelling elements into scientific presentations, researchers could disseminate their work for a broader, more empathetic, and

highly engaged reception to not only amplifies the reach of their findings but also fosters a deeper, more meaningful connection with audiences.

O-5b-2
Skills for Next-Generation Safety Scientists in Cosmetics: Bridging Academic Expertise to Industry Relevance
ABSTRACT #130

Véronique Poulsen[1], Eric Antignac[1], Bambou Xuezhu Tan[1], Pascale Mora[1], Beta Montemayer[2], Sébastien LUEZ[1], Matthew Burbank[1], Stéphane Dhalluin[1], Nicolas Fabre[1]
[1]*L'Oréal R&I*
[2]*Cosmetics Alliance Canada*

During the last decade, the safety evaluation of cosmetic ingredients has been submitted to tremendous changes, moving from animal-based approaches to NAMs, leading to the NGRA. This paradigm shift requires that safety scientists adeptly integrate traditional expertise with NAMs. Today's industries are on the lookout for versatile safety scientists who can create innovative safety evaluation methods and master in vitro and in silico models, who can adapt fluidly to a constantly evolving professional landscape. As they transition from academia to industry, it's crucial for researchers to understand how the industry perceives safety scientists and what skills they value most This review dives deep into the key skills next-generation safety scientists need from industry perspective. The essential skills are divided into three main categories: Digital Proficiency, Human Safety Skills, and Environmental Safety Skills. Digital Skills: Rigorous data analysis capabilities, with a focus on advanced statistical methodologies and probabilistic risk assessment. Human Safety Skills: In vitro and in silico ADME (Absorption, Distribution, Metabolism, and Excretion): Allows to predicting/define how a chemical is processed within an organism. PBPK (Physiologically Based Pharmacokinetic): This model allows to predict/estimate systemic exposure to a given chemical. Mechanistic Toxicology: Aimed to identify molecular processes that lead to toxicity. Computational Toxicology: Leveraging algorithms to formulate toxicity predictions. Environmental Safety Skills: TKTD (Toxicokinetics-Toxicodynamics) Modelling: processes that lead to toxicity at the level of organisms over time . AOP (Adverse Outcome Pathways): Decoding the sequences that culminate in ecotoxicological detriments. In-vitro Ecotoxicology Models: Recognizing the strengths and limitations of in vitro test models for environmental toxicity. By refining and/or improving these skills, safety scientists can enhance their professional standing and pave the way for significant advancements in safety science. Merging academic knowledge with industry demands prepares them to make meaningful contributions for a more ethical and sustainable approach to safety evaluations.

O-5b-3
International Collaboration for Cosmetics Safety (ICCS): Accelerating Global Adoption of Animal-Free Safety Science for Cosmetic Product and Ingredient Safety Assessment
ABSTRACT #2

Erin Hill[1], Amelie Ott[2], Torben Koenig[2], Arianna Guisti[2]
[1]*International Collaboration on Cosmetics Safety (ICCS)*
[2]*ICCS*

The International Collaboration on Cosmetics Safety (ICCS) was launched in 2023 to advance the adoption of animal free science for the human health and environmental safety assessment of cosmetics, personal care products, and their ingredients through investment in research, education, and regulatory engagement. While animal tests on cosmetic products and ingredients are increasingly banned, many regions still require animal tests and do not yet accept non-animal, new approach methodologies (NAM) data for all hazard endpoints or classes. ICCS plans to work with stakeholders, globally to understand regulatory needs and address them through advancing the development, evaluation and use of non-animal, Next Generation Risk Assessment (NGRA) frameworks for human health and environment. To do so, ICCS has brought together experts from over 35 cosmetics product and ingredient manufacturers, animal protection organizations

and industry trade associations to work with regulators and the broader scientific community towards achieving three objectives: ensuring safety through animal-free science; advancing regulatory acceptance; and delivering education and training. This presentation will share a summary of progress, ongoing priorities and planned activities across all ICCS objectives

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O-5b-4
New non-invasive, label-free monitoring approach for 2D and 3D cell culture
ABSTRACT #131

Anna Martina Jötten[1], Philipp Paulitschke[12]
[1]*Ludwig-Maximilians-University Munich*
[2]*PHIO scientific Gmbh, Munich*

Two major issues of cell-based toxicological and drug response assays are the lack of the temporal component of endpoint assays, and the strong dependency of reproducibility and significance on the quality and condition of the cells used. Thus there is a tremendous need to provide insight into the usually inaccessible processes inside the incubator. We developed a novel lensfree imaging method exploiting the optical properties of the cell itself for imaging inside the incubator, which allows non-invasive, super compact, label-free, live-cell monitoring. By applying AI to determine key cell culture parameters such as confluence, proliferation, and cell motility [1], high-quality, automated, objective, and real-time data can be collected. Applying our lensfree microscopy (LM) method, we find that memory effects from heterogeneous cell culture conditions lead to an increase of variance during subsequent assays like e.g. omics-readouts [2] or other cell based assays, like wound healing assays, motility and proliferation assays significantly. Furthermore, our LM is also suitable for 3D applications and will enable quantification of organoid growth dynamics and interactions. Our approach dramatically increases control and processing speed. In the context of the reproducibility crisis, we hope to make a contribution in the direction of standardization of cell-based research in the future.

O-5b-5
Continuing education for next generation risk assessment
ABSTRACT #129

Catherine Willett[1], Bambou Xuezhu TAN[2], Pascale Mora[2], Beta Montemayer[3], Gladys Ouedraogo[2]
[1]*Humane Society International*
[2]*L'Oréal R&I*
[3]*Cosmetics Alliance Canada*

As the global move towards non-animal cosmetic safety assessment gains traction, the need to equip toxicologists with contemporary knowledge on new approach methodologies (NAMs) and next generation risk assessment (NGRA) is becoming crucial. Many toxicologists are grounded in traditional animal testing methods, despite the increasing global restrictions on such approaches. The shift toward non animal safety assessment (human relevant safety science) necessitates continuous education to stakeholder from various sectors – regulators, testing companies, ingredient suppliers, or consumer goods companies. Given the evolving dynamics, this review aims at providing a comprehensive existing continuing education module to help safety scientists get the adequate training on the new generation risk assessment which is exposure-led, hypothesis-driven risk assessment approach that integrates existing knowledge with in silico, in chemico, and in vitro approaches. One of them is AFSA MasterClass, which is a free online course. The AFSA Collaboration has developed this Master Class to build confidence and global capacity in the use of animal-free data in safety decision-making. These state-of-the-art scientific tools support the goal of safe and sustainable products by design, and inform robust regulatory decisions, maintaining a high standard of consumer protection, and assisting compliance with animal testing restrictions. This, along with other notable resources like Episkin Academy Training, ESTIV courses, ASCTT training, IIVS Training, PETA International Science Consortium webinars, PCRM's New Approach Methodology (NAM) Use for Regulatory Application (NURA) and AlterTox webinars, provides the educational resources to accompany the paradigm shift towards human-relevant, animal-free safety science. Such

educational modules will foster wider acceptance and adoption, laying the foundation for informed regulatory safety decisions without animal testing.

Wednesday, 5 June 2024
11:00 - 13:00

Session 6a: Toxicokinetics and in vitro – in vivo extrapolation

Chairs: *Jochem Louisse (European Food Safety Authority EFSA, Parma, Italy) & Andreas Schepky (Beiersdorf AG, Hamburg, Germany)*

O-6a-1
Relevance of Toxicokinetics and ADME methods in Quantifying Exposure for Next generation Risk Assessment
ABSTRACT #1

Andreas Schepky[1], Abdulkarim Najjar[2], Daniela Lange[3], Sophia Krause[2]
[1]*Beiersdorf AG, Global toxicology*
[2]*Beiersdorf AG, Digital toxicology*
[3]*Beiersdorf AG, Dermal toxicology*

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 within NGRA, an estimate of the internal exposure should be provided. That requires implementations of a variety of ADME tools (in vitro parameterization and in silico simulations) to convert human external exposure from multiple routes (dermal) to concentration-time profiles within blood and specific organs. In this presentation, the progress towards modern ADME tools will be shown (incl. biokinetics) and it will be demonstrated how its application impacts highly on successful NGRA of chemicals, e.g. salicylates. Additionally, the roles of ADME, in vitro and in silico simulations, on internal Threshold of Toxicological Concern (iTTC) will be discussed. Furthermore, the possible impact of Lab-on Chip on toxicokinetics (e.g. skin-liver-thyroid) and its possible benefit on NGRA will be briefly discussed.

O-6a-2
Toxicokinetic assessment of perfluorooctanoic acid and perfluorooctanesulfonic acid in a 2-dimensional in vitro liver model
ABSTRACT #26

Daniela Brenner[1], Pierre-André Billat[2], Céline Brochot[2], Lucie Bláhová[1], Eliška Sychrová[1], Pavel Babica[1], Iva Sovadinová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Czech Republic*
[2]*Institut National de l'Environnement Industriel et des Risques, France*

The assessment of hazards and risks associated with chemical compounds is crucial for the preservation of a healthy environment, safeguarding the well-being of both humans and wildlife. In recent years, a significant shift towards replacing, reducing, and refining animal testing has emerged, advocating for the optimization and utilization of in vitro toxicity models as a promising alternative [1]. While in vitro toxicity models appear straightforward and convenient, they demand meticulous consideration to ensure their reliability and reproducibility, aligning with the ultimate goal of achieving robust in vitro-in vivo extrapolation. One prevalent weakness of many in vitro studies is the reliance on nominal concentrations rather than actual concentrations within the intracellular environment. The intricate interplay of chemicals within the in vitro system, including factors such as compound sorption to plastic equipment, interactions with serum proteins or lipids, potential evaporation, or bio(transformation), has been often neglected in hazard and risk assessments. Nevertheless, these chemical behaviours can exert a significant impact on the intracellular concentration of the studied chemical during exposure time [2]. The present study aims at determining the total, free and intracellular concentration of two selected perfluorinated compounds, namely perfluorooctanoic acid (PFOA) and perfluorooctaniesulfonic acid (PFOS), in a 2-dimensional (2D) HepG2 cell in vitro model. A multifaceted approach, combining various experimental techniques

and in silico modeling, is employed to unravel the kinetic behaviour of these perfluorinated compounds. The comprehensive assessments will be interlinked and compared to provide improved in vitro-in vivo extrapolation of perfluorinated compounds. Acknowledgments: This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement no 825712 - OBERON project (https://oberon-4eu.com), that is part of the European EURION cluster (http://eurion-cluster.eu/).

O-6a-3
Next-generation risk assessment for diterpene glycosides
ABSTRACT #48

Elena Reale[1], Styliani Fragki[2], Luis David Jimenez Franco[2], Stephan Schaller[2], Danilo Basili[1], Walburga Seefelder[1], Gina Montoya Parra[1], Alicia Paini[2]
[1]*Nestlé Research*
[2]*esqLABS GmbH*

Next-generation risk assessment (NGRA) is an innovative approach that supports animal-free safety decision-making for regulatory purposes. It combines exposure, in vitro, and in silico methodologies to assess the potential risks of chemical substances. NGRA relies on physiologically based kinetic (PBK) models to estimate internal chemical exposure by simulating the absorption, distribution, metabolism, and excretion (ADME) of chemicals within living organisms. To parameterize PBK models, physiological, biochemical, and physicochemical data are necessary. To assess the accuracy of PBK models, their predictions are typically compared to in vivo kinetic data. However, finding chemical-specific input parameters and in vivo data in the literature can be challenging. To address the challenges associated with data scarcity, this study used a methodological approach for data-poor chemicals, following the guidance document provided by the OECD PBK modeling guidance document. The approach was demonstrated using chemicals from the diterpene glycoside family present in food ingredients, and a user-friendly and open-source software for PBK modeling, PK-sim®. Physiological data from PK-Sim® and biochemical and physicochemical values from in silico predictors like OPERA, ADMET Predictor, and SwissADME were utilized. A sensitivity analysis identified parameters that required further verification through in vitro experiments. The PBK models for rodents and humans were evaluated using available published in vivo data for chemicals with structural and functional similarity. The PBK model predictions were then used for quantitative in vitro-to-in vivo extrapolation (qIVIVE) of in vitro toxicity data to predict safe levels for human consumption. In summary, this study presents a successful case study for NGRA, utilizing an open-source PBK modeling software, the parameterization of PBK models using in silico and in vitro data, and read-across for model evaluation. The integration of NGRA into regulatory decision-making processes has the potential to enhance safety evaluations while reducing the reliance on animal testing.

O-6a-4
A workflow for true dose considerations of in vitro test systems which are used as part of next-generation risk assessment
ABSTRACT #169

Beate Nicol[1], Evita Vandenbossche-Goddard[1], Charlotte Thorpe[1], Richard Newman[2], Hiral Patel[2], Dawn Yates[2]
[1]*Unilever Safety and Environmental Assurance Centre*
[2]*Charles River Laboratories*

With the increasing use of in vitro tests for decision making as part of Next Generation Risk Assessment (NGRA) the need to be able to confidently relate in vitro doses to in vivo exposures has become more prominent. We have previously described frameworks for a systemic toxicity NGRA (1) and we now present 40 case study chemicals to illustrate how in vitro dose considerations can be applied to characterise the true available in vitro dose and build confidence in the understanding of the biologically effective dose in in vitro test systems for the determination e.g. point of departures (PoDs) used in the NGRA. Furthermore, we have proposed a workflow that can assess whether the nominal test concentration can be considered a

conservative dose metric for use in NGRA. The workflow examines the implications of volatility, stability, hydrophobicity, binding to plastic and serum, solubility, and the potential use of in silico models for these parameters. For most of the case study chemicals we were able to build confidence that the use of nominal concentrations in a NGRA context would result in conservative decision making. However, for several chemicals the potential for underestimation of the risk in vivo based on in vitro effect concentrations was identified due to potential uncontrollable losses of chemicals from the test system. For these cases we propose follow up actions.

O-6a-5
High-throughput PBK modelling providing insights into ADME/TK in systemic toxicity
ABSTRACT #45

René Geci[12], Lars Küpfer[2], Alicia Paini[1], Stephan Schaller[1]
[1]*esqLABS GmbH, Saterland, Germany*
[2]*University Hospital RWTH Aachen, Aachen, Germany*

This abstract is part of the session proposal: "Adversity with new approach methodologies: use of complex and integrated systems for systemic toxicity" Absorption, distribution, metabolism and excretion (ADME) processes play an essential role in the risk assessment of chemicals, as these can undergo significant changes in location, concentration and structure within the body. Therefore, ADME processes ultimately dictate how chemicals interact with biological systems and their consequent toxicity. However, for the large variety of environmental chemicals we are exposed to, information about these processes is not available, nor is it possible to assess them. Here, we will introduce the ADME concepts, as well as a new high-throughput PBK (HT-PBK) modelling approach, as a potential solution to this problem. This new HT-PBK approach is built on the open-source PBK modelling software PK-Sim and will support the evaluation of a broader diversity of chemicals for which current methods of ADME characterisation are lacking. By this, it can help to guide chemical risk assessment and prioritisation. We will outline the HT-PBK model application and its relevance for systemic toxicity predictions, as well as the potential for future integrations with toxic effect models like quantitative adverse outcome pathways. The HT-PBK model, parameterised solely using in silico and in vitro data, is specifically tailored towards the needs of Next-Generation Risk Assessment and will support the reduction and replacement of animal testing.

O-6a-6
Towards a quantitative interpretation of in vitro DNT data using PBK-modelling based QIVIVE
ABSTRACT #306

Jochem Louisse[1], Iris Mangas[1], Alicia Paini[1], Magdalini Sachana[2], Andrea Terron[1]
[1]*European Food Safety Authority (EFSA)*
[2]*Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD)*

In June 2023, the OECD published the Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity In Vitro Testing Battery (DNT IVB), marking a major endeavour from extensive efforts by the European Food Safety Authority, the US Environmental Protection Agency, and academic groups to standardise a battery of 17 in vitro assays for DNT assessment. The OECD document aims to provide guidance on evaluating and interpretating results from the DNT IVB to identify substances with DNT potential. However, it does not address the use of such data for human health risk assessment, necessitating the development of specific criteria. To allow for a quantitative interpretation of the DNT IVB data and its integration in the chemical hazard and risk assessment process, the application of physiologically based kinetic (PBK) modelling is considered essential. PBK modelling facilitates quantitative in vitro to in vivo extrapolation (QIVIVE), translating in vitro bioactive concentrations into equivalent external (e.g., oral) exposure levels. While examples of QIVIVE exist in the scientific literature, no harmonized approach nor guidance is available. Also, QIVIVE is generally not applied in regulatory hazard and risk

assessment. Currently, a document is being developed with considerations for conducting QIVIVE of DNT IVB data, which will further support the regulatory use of the DNT-IVB. This presentation will summarize considerations for QIVIVE of DNT-IVB data in the regulatory risk assessment of pesticide active substances in the EU.

Session 6b: In vitro systems to assess respiratory toxicity

Chairs: *Arno Gutleb (Invitrolize & Luxembourg Institute of Science and Technology LIST, Luxembourg, Luxembourg) & Robert Bedford (Labcorp Early Development Laboratories Ltd., Harrogate, United Kingdom)*

O-6b-1
An In Vitro Model to Mimic Immune and Vascular Responses Following Airway Inflammation in Humans
ABSTRACT #9

Robert Bedford[1]
[1]*Labcorp Early Development Laboratories Limited*

Despite increasing use of in vitro models that closely resemble in vivo human biology, their application in modelling the downstream effects of airway inflammation, such as immune cell differentiation and atherosclerosis, are at an early stage. In this study, we used various assays to examine the inflammatory response induced in the organotypic bronchial MucilAirTM model and the alveolar epithelial A549 cell line. Following exposure to three products known to induce varying levels of toxicity, reduced barrier integrity and viability were observed in MucilAirTM tissues following exposure to each product. Similar changes in viability were also observed in A549 cells. Furthermore, both cell types that had been exposed to whole cigarette smoke were able to induce downstream phenotypic changes in THP-1 monocytes. Finally, adhesion of treated THP-1 monocytes to endothelial cells, a known marker of atherosclerosis, was confirmed under flow using the BioFluxTM microfluidic system. In contrast to the inflammatory response observed following airway exposure to whole cigarette smoke, exposure to next-generation delivery product aerosol did not induce this response. Cytokine, histological and RNA analysis highlighted increased biomarkers linked to inflammatory and immune cell differentiation pathways following exposure to whole cigarette smoke, including GM-CSF, IL-1β, cleaved caspase-3 and cytochrome P450 enzymes. As a result of similar observations in situ during inflammatory lung disorders such as chronic obstructive pulmonary disorder, we propose that our exposure platform could act as a representative model for studying such events in vitro. Furthermore, this model could be used to test the inflammatory or anti-inflammatory potential of inhaled compounds delivered to the lung.

O-6b-2
Responses insided by AEROSIL® R 504 using ALISENS®
ABSTRACT #111

Sabina Burla[1], Tobias B. Schuster[2], Arno C. Gutleb[1]
[1]*invitrolize sarl, Belvaux, Luxembourg*
[2]*Evonik Operations GmbH, Hanau-Wolfgang, Germany*

Synthetic amorphous silica (SAS) represents a wide category of man-made nanomaterials (NMs) which are utilized by a plethora of industries for many applications, such as consumer products, cosmetics, food, medical applications, and pharmaceuticals. The surface of the NM is often modified to render new physico-chemical properties, alterations that can change the safety profile of the SAS. As inhalation is one of the main routes of exposure to NMs, the respiratory health hazard assessment of SAS NMs is indispensable. The objective of this study was to assess the in vitro bioactivity of AEROSIL® R 504, a SAS NM treated with (3-Aminopropyl)triethoxysilane, chemical which may induce skin sensitization, and Hexamethyldisilazane, using ALIsens®, a three-dimensional (3D) alveolar model for respiratory sensitization. ALIsens® is built on semipermeable membranes using three human cell lines: alveolar epithelial (A549), endothelial cells (EA.hy926) and monocytes (THP-1). A stock suspension of the investigated SAS NM

was prepared by ultrasonication in bovine serum albumin aqueous solution. The working suspensions and controls were administered to the ALIsens® model at the air-liquid-interface (ALI) using the Vitrocell® Cloud-6 exposure device and in submerged conditions. 24h post-exposure cell viability was evaluated using the resazurin assay, cell surface markers relevant for respiratory sensitization were measured by flow cytometry, and secretion of cytokines and one chemokine was quantified by Luminex technology. The tested SAS NM did not decrease the cell viability of ALIsens® following 24h exposure in any of the exposure conditions. None of the established markers for respiratory sensitization (CD54, CD86, TSLPr, GM-CSF, MCP-1, MIP-3α) showed statistically significant differences at the low and the high tested doses, respectively concentrations following exposure at the ALI and in submerged conditions. We concluded that AEROSIL® R 504 did not show any indication of having a respiratory sensitization hazard based on the results obtained using the ALIsens® model.

O-6b-3
Inflammatory and carcinogenic potential of mineral fibres assessed through a physiologically relevant 3d in vitro model of human alveolar tissue
ABSTRACT #212

Vanessa Almonti[12], Michela Licciardiello[3], Serena Mirata[12], Mario Passalacqua[4], Anna Maria Bassi[42], Stefania Vernazza[42], Gianluca Ciardelli[3], Chiara Tonda Turo[32], Sonia Scarfì[12]
[1]*Dept. of Earth, Environment and Life Sciences, University of Genova, Itay*
[2]*Inter-University Centre for the Promotion of the 3Rs Principles in Teaching & Research, Pisa, Italy*
[3]*Department of Mechanical and Aerospace Engineering, Polytechnic University of Turin, Italy*
[4]*Dept. Experimental Medicine, University of Genova, Italy*

The severe health problems derived from the prolonged inhalation of particulate matter and mineral fibres, such as pulmonary fibrosis, asbestosis, silicosis, lung cancer and pleural mesothelioma, to cite a few, are a well-known issue for several classes of exposed workers even in the modern society [1]. Furthermore, given the considerable number of chemicals and new materials potentially producing respirable particulates continuously placed on the market, the setup of new high throughput approaches to evaluate the effects of multiple inhaled substances, could overcome many issues related to the massive use of expensive, poorly relatable, and ethically concerning animal models. In this context, a very good alternative would be the development of physiologically relevant 3D in vitro models of the human alveolar tissue for the prediction of the toxicity/carcinogenicity of inhaled substances/particulates. We propose the setup of two 3D models of the human pulmonary tissue in vitro, either mimicking the thinnest, either the thickest part of the alveoli, implemented by the addition of the human immune component to be able to fully recapitulate in vitro the inflammatory process caused by inhalation of hazardous agents. The thinner model is produced by the stratification of human alveolar and endothelial cells separated by an electrospun PCL-Gel membrane mimicking the alveolar basement membrane, while the thicker model also contains the collagen-embedded human fibroblast component stratified between the alveolar epithelium and the basement membrane. The THP-1 derived human macrophages are then added to the two 3D systems and the toxicity/carcinogenicity of chrysotile and crocidolite mineral fibers with asbestiform behavior is then assessed by comparison with a non toxic wollastonite mineral. The evaluation of cytototoxicity, genotoxicity and inflammation is then performed with the attempt to identify specific markers useful to develop standardized tests to assess the human pulmonary toxicity of hazardous agents.

O-6b-4
Development of an in vitro model of Dry Nose
ABSTRACT #202

Xiao-Yann Huang[1], Jimmy Vernaz[1], Song Huang[1], Samuel Constant[1]
[1]*Epithelix*

When the nasal cavities do not contain enough moisture, dry nose symptoms can manifest such as pain and swelling, nosebleeds, and even airway infections. Nasal dryness can often degrade the quality of life, therefore, novel topical treatments formulated as nasal sprays are needed to treat the severe cases.(1) To test simultaneously efficacy and toxicity effect of novel formulations, we developed an in vitro "Dry Nose" model based on a fully differentiated human nasal epithelium cultured at the air-liquid interface. Epithelia (MucilAir™-Pool) were reconstituted with a mixture of primary human nasal epithelial cells isolated from 14 different heathy donors. Dry air was applied onto the apical surface of the epithelia at a speed of 6 L/min for several exposure time (from 1 to 10 min). To measure the effect of dry air on nasal epithelial cells, the following End-points were evaluated: (i) tissue integrity (TEER); (ii) cytotoxicity (LDH); (iii) ciliopathic effect (Cilia Beating Frequency, active area and mucociliary clearance); (iv) pro-inflammation (IL-8 release). Proof-of-Conception for treatment as well as prevention of Dry Nose symptoms was provided by application of saline solution (0.9% NaCl). Data suggest that the optimal condition to simulate Dry Nose were 5 minutes of exposure to dry air (6 L/min), which induced a decrease of cilia beating frequency. Kinetics of CBF and Active Area could be restored after application of saline solution. The saline solution also prevented the cytotoxic effect of dry air measured by LDH assay. As expected, addition of saline solution abrogated the induced IL-8 secretion by dry air. This set of data suggest the human nasal epithelia is a versatile and convenient tool for assessing the efficacy and toxicity of moisturizing formulations designed to treat dry nose.

O-6b-5
Toxicity of mixtures: ethylbenzene and xylene have competitive effects in human lung cells
ABSTRACT #223

Sylvain Billet[1], Nour Jaber[1], Fabrice Cazier[1], Fabrice Bray[2], Christian Rolando[2], Dorothée Dewaële[1], Claude Emond[3]
[1]*University of the Littoral Opale Coast, France*
[2]*CNRS, France*
[3]*University of Montreal, Canada*

Background and Objectives: Very few experimental toxicology studies have characterised the mechanisms of action of m-xylene and ethylbenzene, a fortiori of the binary mixture, even though co-exposure exists, particularly in the workplace. The aim of this study was to investigate the acute toxicity of ethylbenzene and m xylene alone and in a binary mixture on human bronchial epithelial cells exposed at the air-liquid interface (ALI). Materials and Methods: The cells were exposed to VOCs alone and in a mixture at their Occupational Exposure Levels (8h and 15min) for 1 hour, followed by 5, 23 and 47 hours of incubation in order to characterise the kinetics of the following toxic effects: cytotoxicity, biotransformation of xenobiotics, antioxidant defence system, inflammatory response and apoptosis. Results: Biological responses to exposure to ethylbenzene and m-xylene are specific, whether alone or in a binary mixture. Ethylbenzene does not appear to be metabolised in BEAS-2B cells because it inhibits gene expression of the xenobiotic metabolising enzymes (XME) studied. It does not induce antioxidant defence systems or apoptosis. However, a slight inflammatory response was observed after exposure to OEL-15min. m-xylene is metabolised in BEAS-2B cells and upregulates the antioxidant defence system, as well as markers of inflammation and apoptosis. Discussion and Conclusion: With regard to co-exposure to the binary mixture, an inhibition phenomenon was observed, resulting in the inhibition of the toxic action mechanisms studied. These results have provided new information on the toxicity of ethylbenzene and m-xylene. They also show the importance of conducting exposures at ALI to mixtures of toxicants, as the responses observed cannot necessarily be predicted by conventional hypotheses such as additivity. These results may contribute to a better understanding of the effects of these compounds on human health.

O-6b-6
The Sabyna in vitro hazard testing strategy for adverse effects upon inhalation of nanomaterials: 'safe and sustainable by design' made practical

ABSTRACT #302

Nienke Ruijter[1], Ana Candalija Iserte[2], Virginia Cazzagon[2], Socorro Vázquez-Campos[2], Alberto Katsumiti[3], Isabel Rodríguez-Llopis[3], Itziar Polanco[3], Mari Venäläinen[4], Hanna Pulli[4], Julia Catalán[4], Matthew Boyles[56], Morgan Lofty[5], Jessica Marshall[5], Marie Carriere[7], Ralph Vanhauten[8], Lion Traas[8], Flemming R. Cassee[19], Hedwig Braakhuis[110]
[1]*National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*
[2]*LEITAT Technological Center, Barcelona, Spain*
[3]*GAIKER Technology Centre, Basque Research and Technology Alliance (BRTA), Zamudio, Spain*
[4]*Finnish Institute of Occupational Health (FIOH), Helsinki, Finland*
[5]*Institute of Occupational Medicine (IOM), Edinburgh, United Kingdom*
[6]*Edinburgh Napier University, Edinburgh, United Kingdom*
[7]*Univ. Grenoble Alpes, CEA, CNRS, SyMMES, Grenoble, France*
[8]*ThinkWorks B.V., Delft, The Netherlands*
[9]*Institute of risk assessment sciences (IRAS), Utrecht University, Utrecht, the Netherlands*
[10]*TNO Risk Analysis for Products in Development, Utrecht, the Netherlands*

The European Commission has set ambitious goals in its Green Deal and Chemicals Strategy for Sustainability (CSS) by aiming for a toxic-free society in 2050. Safe and sustainable by design (SSbD) is a key approach towards achieving these goals, and goes beyond regulatory requirements. In many cases, SSbD for nanomaterials (NMs) can be complicated as hazard data are scarce, and the datasets that can inform on classification, labeling, and packaging (CLP) are incomplete or nonexistent. There is a great need for clear guidance for manufacturers and innovators on what hazard testing is required and how to make accurate interpretation to ensure their NMs are SSbD, and correct SSbD decision-making is achieved. The SAbyNA online guidance platform is an integrative and interactive web-based guidance service that gives support towards the development of NMs and nano-enabled products (NEPs) that are SSbD throughout the entire life cycle. The platform integrates data on hazard, exposure, functionality, life cycle, and costs, into an SSbD advise at an early stage of product development. We present the SAbyNA hazard testing strategy of NMs for the inhalation exposure route. It facilitates filling hazard data gaps by guiding the user to perform simple and cost-effective in vitro assays in a tiered approach – ranging from measuring key physico-chemical properties to measuring cellular toxicity. The strategy aligns with the different stages of data availability within the NM development process, and is based on published adverse outcome pathways, relevant markers for toxicity, and previously established strategies. The goal of the SAbyNA hazard testing strategy is to estimate potential hazards of NMs and NEPs at the early stages of innovation, and to guide the user in making SSbD decisions. The SAbyNA project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 862419.

Wednesday, 5 June 2024
14:00 - 15:00

Session 7a: Bio-engineering, stem cells and disease models

Chairs: *Yasunari Kanda (National Institute of Health Sciences, Kawasaki, Japan) & Anna Maria Bassi (Universita degli Studi di Genova, Genova, Italy)*

O-7a-1
Differentiation of retinal organoids from human iPSC for modelling neurodegenerative diseases
ABSTRACT #237

Stefania Vernazza[12], Anna Maria Bassi[12], Serena Mirata[12], Sonia Scarfì[32], Sara Tirendi[12]
[1]*Department of Experimental Medicine (DIMES), University of Genoa, Italy*
[2]*Inter-University Center for the Promotion of the 3Rs Principles in Teaching & Research (Centro 3R), Italy*
[3]*Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Italy*

Optic nerve damage and progressive slow death of retinal ganglion cells (RGC) are the

hallmarks of glaucomas, a group of neurodegenerative diseases. The RGCs are the first retinal neurons generated during development from multipotent retinal progenitor cells. Recently, we set up an in vitro 3D-innovative human-based model (IVOM) of human trabecular cells on a millifluidic-platform, to define several key events of the onset of eye damage [1,2]. Since the underlying mechanisms of neurodegeneration in glaucoma remain mostly unknow, our goal has been to implement IVOM by adding retinal organoids (ROs), derived from human pluripotent stem cells (h-IPSCs), to investigate the early steps involved in the neurodegenerative cascade. Human iPSCs (Cell Applications Inc.), grown in Essential-8-FLEX medium, were submitted to ROs generation by culturing in BiN2 medium and then in Pro-B27 medium, according to Slembrouck-Brec et al [3]. ROs were maintained until 180 days. The retinogenesis was verified over time, by analysing the gene levels of several specific markers, such as NOGGIN, DKK1, LHX2, PAX6, and BRN3a. The RGCs were separated from ROs, by magnetically labelling with MACS MicroBeads and positively selected for CD90/THY-1. BRN3a protein levels, as marker of retinal organization, was measured in the selected cells, after 1 week culture. Differentiating h-IPSCs showed to express typical retinal marker genes, including NOGGIN, DKK1, LHX2, PAX6, and BRN3a, and displayed the ability to differentiate into ROs. Moreover, the RGC-positively selected for CD90/THY-1 on the 28th day showed a marked expression of BRN3a protein level. These preliminary data suggests a positive selection for THY-1 (CD90) and that obtained RGCs express several specific retinal markers. Further analysis is ongoing on other RGCs markers, that may be represent useful targets to better identify the key events of neurodegeneration outcome and to test strategies for neurodegeneration prevention. Project funded by Italian Ministry of Health, 2021

O-7a-2
Prediction of developmental neurotoxicity using an integrated in silico and in vitro approach

ABSTRACT #276

Yasunari Kanda[1], Yukuto Yasuhiko[1], Koichi Yoshinari[2]
[1]*National Institute of Health Sciences (NIHS)*
[2]*University of Shizuoka*

In silico methods have been widely used to predict and evaluate the toxicity of chemicals. However, it is still challenging to predict chemicals with complex toxicity mechanisms, such as neurotoxicity and developmental neurotoxicity. In the present study, we analyzed in vivo developmental neurotoxicity datasets from literature information (e.g., Neurotoxicol Teratol 52: 25-35, 2015) and tried to perform an in silico approach using molecular descriptors representing the physicochemical properties of chemicals. We found that the accuracy of developmental neurotoxicity assessment using molecular descriptors can be improved by setting a threshold for the distance between substances used to define neighbors, and by setting the number of neighbors to an appropriate value. Although there are chemical categories that correlate well with in vivo data sets, some categories showed discrepancies between in silico and in vivo. To improve the predictability, we performed in vitro studies using human iPSC-derived neurons, which neural network activities were recorded by a multi-electrode array (MEA) system. Some false-negative compounds by in silico method inhibited the network activities in human iPSC-derived neurons, suggesting that in vitro data are necessary to support the in silico analysis. Thus, the integrated approach using both in silico and in vitro would improve the prediction of developmental neurotoxicity in vivo.

O-7a-3
Genetic predisposition for anti-mash drug response: a population-based in vitro study

ABSTRACT #141

Alexandra Gatzios[1], Matthias Rombaut[2], Joery De Kock[2], Dinja De Win[1], Anja Heymans[1], Vera Rogiers[1], Robim M. Rodrigues[1], Joost Boeckmans[1], Tamara Vanhaecke[1]
[1]*In Vitro Liver Disease Modelling team, department of In Vitro Toxicology and Dermato-cosmetology (IVTD), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090*

Brussels, Belgium
[2]*Liver Therapy & Evolution team, department of In Vitro Toxicology and Dermato-cosmetology (IVTD), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*

Over the past decade, metabolic dysfunction-associated steatohepatitis (MASH) has become a major cause of morbidity and mortality. With a global prevalence estimated at 5% and no approved drug available, its socio-economic burden is booming. Our group recently showed that genetic predisposition may not only impact the pathogenesis of MASH, but also the response to certain treatments. Considering this pharmacogenetic background, we aimed to investigate the impact of a polygenic risk score for hepatic fat content (PRS-HFC) on the potential anti-steatotic effects of anti-MASH drug candidates (e.g., Aramchol, Nidufexor) using an in vitro population-based approach. The PRS-HFC was determined for 84 human skin-derived precursor (hSKP)-cell lines isolated from different donors, based on the presence of single nucleotide polymorphisms in four genes, namely patatin-like phospholipase domain-containing protein 3 rs738409 C>G, transmembrane 6 superfamily member 2 rs58542926 C>T, glucokinase regulator rs1260326 C>T and membrane bound O-acyltransferase domain-containing 7 rs641738 C>T. Using the PRS-HFC, donors were classified in three risk categories (RC1 low, RC2 intermediate, RC3 high risk) and five cell lines were selected from each category to be differentiated to hepatic progenitor-like cells (hSKP-HPC). The cultures were then exposed for 24 hours to MASH triggers and anti-MASH drug candidates. Neutral lipid load was quantified using flow cytometry and visualized using fluorescence microscopy. RC3 hSKP-HPCs exhibited a 3.5-fold higher lipid load in both control and MASH-triggered conditions compared to RC1 cultures, indicating that this system adequately represents the human in vivo population situation. In addition, the PRS-HFC positively correlated with the lipid-lowering effect of Aramchol (Pearson's r = 0.66, p = 0.0147), suggesting an underlying genetic effect that will be further examined. In conclusion, hSKP-HPC cultures can represent the genetic risk for hepatic fat accumulation and unveil a modifying effect of the PRS-HFC on the anti-steatotic effects of Aramchol, urging further mechanistic investigations.

Session 7b: Towards Liver models – developing session

Chairs: *Mathieu Vinken (VUB, Brussels, Belgium) & Amer Jamalpoor (Toxys, Leiden, The Netherlands)*

O-7b-1
Towards in silico modelling of qAOPs for liver steatosis
ABSTRACT #182

Anna Melina Steinbach[1], Viktoria Städele[1], Christain Tobias Willenbockel[1], Vikas Kumar[12], Tewes Tralau[1], Philip Marx-Stoelting[1]
[1] *German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str. 8-10, 10589, Berlin, Germany*
[2]*Environmental Engineering Laboratory, Departament d' Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007, Tarragona, Catalonia, Spain*

Background and Objectives: New Approach Methodologies can help in the context of hazard identification by allowing for effective, faster and less costly toxicological testing as well as supporting safety by design. Promising developments in this context are the increasing application of in silico tools and the ongoing transgression of Adverse Outcome Pathways (AOPs) towards quantifiable AOP (qAOP) networks. In line with these developments this project hence aims at the development of an qAOP for the prediction of liver steatosis. Materials and Methods: The focus for the development of the qAOP is the late key event relationship of triglyceride accumulation. Existing toxicological data from in vivo and in vitro studies were subjected to benchmark dose (BMD) analysis in order to verify data quality prior to further computation. Physiologically based toxicokinetic modelling (PBTK) was then used to calculate internal concentrations and the results subsequently compared to data from in vivo measurements. Further, internal concentrations for the in vitro exposure system were calculated by computational approaches. Results: BMD analysis of the data yielded good

precision factors. The reproducibility and performance of PBTK and in vitro exposure models was analysed. Discussion and Conclusion: Based on the precision factors of the BMD analysis the data used were suitable for the modelling, as they gave satisfactory dose-response curves. The models for the in vitro and in vivo simulations proved fit for the purpose of further modelling of QIVIVE.

O-7b-2
A framework for a chemically agnostic model for liver injury as a stepping stone to link in vitro outcomes to organ-level adverse outcomes
ABSTRACT #57

Heeseung Jo[1], Celine Brochot[1], James Stevens[2], Elias Zgheib[1]
[1]*Certara UK Limited, Simcyp , Sheffield, UK*
[2]*Leiden University, Leiden, Netherlands*

The RISK-HUNT3R consortium aims to develop reliable methods for chemical safety assessment based on non-animal methods through new approach methodologies (NAMs) combining in silico and in vitro methods. Adverse outcome pathways (AOPs) are a useful tool to democratize toxicology knowledge and establish a mechanistic framework leading from molecular initiating events (MIEs) to key events (KEs) to organ level adverse outcomes. Quantitative AOPs (qAOPs) have evolved to identify quantifiable measures of events and can facilitate development of quantitative systems toxicology models to help predict adverse outcomes with varying chemicals of differing pharmacokinetics. Currently available in silico models are not always applicable for safety assessments, particularly for organ level adverse outcomes and for ab initio compounds where little is known. As part of RISK-HUNT3R, we present a framework for a generalizable approach to predict adverse outcomes in liver by adapting a model by DeGracia et al. (2018) that describe a feedback relationship between a system's damage (D) and stress/adaptive response (S) induced by an external injury (I). This model presents the idea of a system's threshold in which regardless of the mechanism of "I", once "I" exceeds the system's threshold, the resulting "D" will overwhelm the "S" resulting in adverse outcomes. Conversely, if "I" is lower than the system's threshold, the system is robust enough to mitigate the "D" with a higher "S" response, preventing or minimizing adverse outcomes. We applied this concept to explore surgical liver injury models in rats through an in silico model guided by a qAOP for liver injury and using the surgical data to establish a chemically agnostic liver response model. This framework provides a basis for in vitro to in vivo extrapolation from in vitro measured chemical specific MIEs and KEs that establish the extent of "I" to predict late KEs and organ level adverse outcomes.

O-7b-3
Prioritisation of potential hepatotoxic co-formulants for chemical risk assessment using NAMs
ABSTRACT #42

Alkiviadis Stagkos-Georgiadis[1], Bright Baffour Duah[12], Denise Bloch[1]
[1]*Department of Pesticides Safety, German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str. 8-10, 10589 Berlin, Germany*
[2]*University of Potsdam, Institute of Nutritional Science, Department of Food Chemistry, Arthur-Scheunert-Alle 114-116, 14558 Nuthetal, Germany*

Plant protection products (PPPs) contain one or more active substances (AS) as well as a varying number of co-formulants. In particular, AS are responsible for a PPPs effectual functionality whereas co-formulants support the efficacy of ASs. While a comprehensive set of toxicity studies is required for the authorisation of AS, testing of PPPs is limited to acute and topological toxicity only. Co-formulants are not subject to any particular toxicological evaluation or authorisation as part of PPP Regulation (EC) No 1107/2009. Thus the potential contribution of co-formulants and their interaction with one another or the AS is not systematically assessed. Due to its relevance in toxicant excretion and high blood perfusion, the liver is one of the main sites of toxic action. Here, we propose an approach to identify potentially hepatotoxic co-formulants and prioritise PPPs for further testing using New Approach Methodologies (NAMs). In a first step

potentially hepatotoxic co-formulants were identified based on in silico screening using both rule-based and statistical models. A total of 502 co-formulants in currently authorised PPPs was subjected to model based scoring with scores ranging from zero (no alert) to two (alert from two independent models). In result, eleven co-formulants received a score of two and 132 a score of one. PPPs containing these co-formulants were prioritised for further testing if their percentage in the formulated PPP exceeded 0.1% (score of two) or 10% (score of one), respectively. In addition, potential kinetic interactions between hepatotoxic ASs and co-formulants were investigated using in silico tools. Comparative cytotoxicity testing of PPPs and AS is conducted for prioritised PPPs. Where the PPP is more toxic than the AS, further testing is required. Where a potential for kinetic interaction was predicted, active efflux transporter and CYP enzyme kinetics are investigated.

O-7b-4
A stem cell-based in vitro test battery to predict liver steatotic potential of diverse chemicals
ABSTRACT #66

Anouk Verhoeven[1], Alexandra Gatzios[1], Ramiro Jover[2], Robim M. Rodrigues[3], Julen Sanz-Serrano[3], Andrés Tabernilla[3], Joery De Kock[3], Mathieu Vinken[3], Tamara Vanhaecke[3]
[1] *Entity of In Vitro Toxicology and Dermato-Cosmetology, Department of Pharmaceutical and Pharmacological Sciences, Vrije Universiteit Brussel, Belgium*
[2]*Joint Research Unit in Experimental Hepatology, University of Valencia, Health Research Institute Hospital La Fe & CIBER of Hepatic and Digestive Diseases, Spain*
[3]*Entity of In Vitro Toxicology and Dermato-Cosmetology, Department of Pharmaceutical and Pharmacological Sciences, Vrije Universiteit Brussel, Belgium*

The identification of potential liver steatogenic chemicals without the use of animals remains challenging in modern toxicity testing. In this regard, new approach methodologies based on adverse outcome pathways (AOPs) may provide a solution to this ubiquitous issue1. As a case study in the ongoing ONTOX project, this work aims to establish a 2-tiered in vitro test battery, fully mechanistically anchored in the AOP network for chemical-induced liver steatosis. The first tier incorporates a transcriptional signature for chemical-induced liver steatosis and serves as an initial identifier. The second tier involves a series of in vitro assays at the translational and functional levels aiming at detecting and quantifying changes in molecular initiating events (MIEs) and key events (KEs) critical for the development of liver steatosis. To guide the selection of MIEs and KEs, the recently optimized and confidence-assessed AOP network for chemical-induced liver steatosis was utilized2. The in vitro test battery was established using a human postnatal skin precursor-derived hepatic cell model, which has previously demonstrated its capacity to detect liver steatotic effects of chemicals3. To evaluate the robustness of the in vitro test battery, six data-rich steatogenic reference chemicals from various application domains were examined. These included three pharmaceuticals (sodium valproate, tetracycline and amiodarone), two plasticizers (trimethyl phenyl phosphate and perfluorohexanesulfonic acid) and a pesticide (cyproconazole). Initial results showed intrahepatic lipid accumulation after daily exposure to a concentration range of each steatogenic chemical for a period of 72 hours. Modulations in the underlying MIEs and KEs (i.e., fatty acid uptake, de novo lipogenesis, mitochondrial beta-oxidation and triglyceride secretion) leading to lipid accumulation detected and quantified by the 2-tiered in vitro test battery for these six chemicals will be presented. This human-relevant hepatic model might be a promising tool for testing the steatotic potential of diverse chemical compounds.

Wednesday, 5 June 2024
16:30 - 18:30

Session 8a: Case studies for successful use and implementation of complex in vitro models

Chairs: *Andreas Stucki (PETA Science Consortium International e.V., Stuttgart,*

Germany) & Paul Carmichael (Unilever, UK and Wageningen University (WUR), Wageningen, The Netherlands)

O-8a-1
The INSPIRE Initiative: Assessing respiratory toxicity of surfactants to human cell-based in vitro systems by pipetting and aerosol exposure
ABSTRACT #148

Andreas O Stucki[1], Nuria Roldan[1], Monita Sharma[1], Sandra Verstraelen[2], An Jacobs[2], Karen Hollanders[2], Jo Van Laer[2], Sylvie Remy[2], Evelien Frijns[2], Amy J Clippinger[1]
[1]*PETA Science Consortium International e.V., Stuttgart, DE*
[2]*Flemish Institute for Technological Research (VITO), Health Department, Mol, BE*

Inhalation is a major route through which substances can cause toxic effects. The INSPIRE Initiative (IN vitro System to PredIct REspiratory toxicity) aims at building scientific confidence in non-animal methods to assess inhalation toxicity.[1] In this study, a human bronchial epithelial cell line (BEAS-2B) and a reconstructed human tissue model (MucilAir™) both grown at the air-liquid interface were used to predict the toxicity of two classes of chemicals. Building upon recently published data for the testing of silane vapors, [2] the same testing approach was used to assess surfactants exposed to cells as aerosols or liquids (pipetting). BEAS-2B cells and MucilAir™ were exposed to five different concentrations of the two surfactants (Triton X-100 and N-oleoylsarcosine). The surfactants were either aerosolized using a collision atomizer with a VITROCELL® 6/4 system or were directly pipetted (30µL) onto the apical side of the cells. Cellular effects were assessed ~24 hours (in BEAS-2B and MucilAir™) or seven days (in MucilAir™) after exposure. For both cell systems, these effects included cytotoxicity (lactate dehydrogenase release), cell viability (resazurin-based PrestoBlue® assay), inflammatory response (secretion of interleukins 6 and 8 (CXCL-8); MSD V-Plex Assay), and additionally for MucilAir™, transepithelial electrical resistance, cilia beat frequency and average active area (Sisson-Ammons Video Analysis), and histology. Preliminary results show concentration-dependent effects in both cell systems and for both exposure methods. Generally, BEAS-2B cells were more sensitive in comparison to MucilAir™. These observations are similar to previous studies testing silane vapors in these cell systems. [2] These results help to further increase scientific confidence in the use of in vitro testing approaches and show how they can be used to meet regulatory requirements. The study also helps to identify what cellular effects, exposure methods, and model systems may be most appropriate for use, depending on the purpose of testing.

O-8a-2
Validation and implementation of the toxtracker assay for mechanistic genotoxicity assessment
ABSTRACT #70

Giel Hendriks[1], Els Adriaens[2], Ashley Allemang[3], Jan van Benthem[4], Julie Clements[5], Gabrielle Cole[6], Maria Engel[7], Annie Hamel[8], Darren Kidd[5], Stephanie Kellum[9], David Kirkland[10], Tomomi Kiyota[6], Abby Myhre[9], Valerie Naëssens[11], Stefan Pfuhler[3], Marise Roy[8], Raja Settivari[9], Maik Schuler[7], Philippe Vanparys[12], Andreas Zeller[11]
[1]*Toxys, The Netherlands*
[2]*Adriaens Consulting, Belgium*
[3]*Procter&Gamble, United States*
[4]*RIVM, The Netherlands*
[5]*Labcorp, United Kingdom*
[6]*Genentech, United States*
[7]*Pfizer, United States*
[8]*Charles River Laboratories, Canada*
[9]*Corteva Agriscience, United States*
[10]*Kirkland Consulting, United Kingdom*
[11]*Roche, Switzerland*
[12]*Consultant Genetic Toxicology, Belgium*

ToxTracker is a mammalian cell reporter assay that accurately predicts the genotoxic properties of compounds. By evaluating induction of various reporter genes that play a key role in cellular pathways relevant for genetic toxicology, ToxTracker has the advantage of providing insight into chemical mode-of-action (MoA), thereby discriminating direct-acting genotoxicants from cytotoxic chemicals that induce DNA damage secondarily. To investigate how ToxTracker

may complement the standard battery of in vitro genotoxicity assays, a comprehensive interlaboratory validation trial was conducted. The goal of this prospective validation study was to explore the applicability of ToxTracker for regulatory applications, establish the transferability and reproducibility of the assay and to explore how it can be applied to improve the in vitro genotoxicity testing strategies. Additionally, reproducibility of the assay to predict genotoxic MoA was confirmed across participating laboratories and data were evaluated in terms of concordance with in vivo genotoxicity outcomes. In the validation trial, seven laboratories tested 64 genotoxic and non-genotoxic chemicals that together cover a broad spectrum of chemical spaces. The validation trial showed a good within-lab reproducibility (WLR) of 73-98%. The between-lab reproducibility (BLR) of ToxTracker was 83%. The interlaboratory validation confirmed the accuracy of ToxTracker to correctly predict in vivo genotoxicity of compounds with a sensitivity of 87% and a specificity of 90%. From this validation trial we concluded that ToxTracker is a robust in vitro assay for the accurate prediction of in vivo genotoxicity. With information on the MoA of chemicals that is provided by the assay, ToxTracker is a valuable addition to the battery of genotoxicity assays that is applied for regulatory applications. Considering its robust standalone accuracy, ToxTracker has potential to mature into one of the mammalian cell assay options as a genotoxicity test battery component.

O-8a-3
2D and 3D human endothelial cell models to assess vascular toxicity
ABSTRACT #132

Benoit Fischer[1], Johanna Alm[1], Francesca Moretti[1]
[1]*Novartis Pharma AG*

Vascular toxicity can be observed during pre-clinical and clinical development of novel compounds. Drug-induced endothelial dysfunction can manifest, for example, as increased adhesiveness and transmigration of leukocytes, increased endothelial barrier permeability and/or perturbation of angiogenesis. We set out to evaluate 2D and 3D human endothelial cell models for their ability to recapitulate drug-induced vascular toxicity. We used the Mimetas OrganoPlate system to generate 3D endothelial tubes subjected to gravity-driven perfusion and compared them to 2D static endothelial cell cultures. We observed that 2D static endothelial cell cultures are better suited to recapitulate vascular inflammation and leukocyte adhesion than 3D cultures. The 3D cultures allowed us to perform assessment of endothelial barrier integrity and recapitulate vascular leakage findings from the clinic. Furthermore, the 3D cultures allowed the establishment of angiogenic sprouting and the evaluation of compounds with both pro- and anti-angiogenic potential. In summary, we have established a combination of 2D and 3D cellular assays that allow the identification and characterization of various human vascular toxicities.

O-8a-4
Breaching the surface: in vitro Eye Hazard Assessment of surfactants based on an innovative Defined Approach
ABSTRACT #69

Els Adriaens[1], Takayuki Abo[2], Nathalie Alépée[3], Alessandra Cavarzan[4], Karsten R Mewes[5], Chelsea Odriscoll[6]
[1]*Adriaens Consulting bv*
[2]*Kao Corporation*
[3]*L'Oréal R&I*
[4]*Reckitt*
[5]*Henkel AG & Co KGaA*
[6]*Proctor & Gamble*

Currently, two defined approaches (DAs) for Serious Eye Damage and Eye Irritation for non-surfactant liquids were adopted by the OECD (TG 467, 2022). Recently, a new DA has been developed for chemicals having surfactant properties (Alépée et al., 2023) and is currently under OECD consideration. The DA for surfactants (DASF) is based on combination of Reconstructed human Cornea-like Epithelium (RhCE) test methods (OECD TG 492: EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the Short Time Exposure (STE, TG 491) method (5-min exposure to 0.5% test substance concentration). The DASF

is constructed as a tiered approach. The first tier can be the RhCE test method (Bottom-Up) or the modified STE test method (Top-Down). For example, in the Bottom-Up approach, an RhCE test method is used to distinguish No Cat. from classified substances. If the surfactant results in a positive call based on an RhCE method, the modified STE method is used to further classify the substance into Cat. 1 (positive call) or Cat. 2 (negative call). The DASF correctly identified 91.3% of Cat. 1 (N=23), 66.7% of Cat. 2 (N=9) and 76.0% of No Cat. (N=17) surfactants. Therefore, the minimum performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat. established by the OECD experts were met. The principle of the DASF was illustrated with two neat surfactants (No Cat.: Tween 80, Cat. 1: ethylhexyl acid phosphate ester) and several dilutions of one surfactant (cetylpyridinium bromide) covering the 3 UN GHS categories for eye hazard identification. For each surfactant, the prediction using variations of the DASF was concordant with in vivo classification. This case study illustrates an innovative approach for moving from animal testing to classifying new surfactants (neat or diluted) based on a DA within the IATA framework for the safety assessment of surfactants.

O-8a-5
Comparative Case Studies on NAMs: A Step Towards Enhancing Specific Target Organ Toxicity Analysis
ABSTRACT #165

Kristina Jochum[1], Andrea Miccoli[123], Helen Hammer[4], Albert Braeuning[2], Tewes Tralau[1], Oliver Poetz[45], Philip Marx-Stoelting[1]
[1]*German Federal Institute for Risk Assessment, Dept. Pesticides Safety, 10589 Berlin, Germany*
[2]*German Federal Institute for Risk Assessment, Dept. Food Safety, 10589 Berlin, Germany*
[3]*National Research Council, Institute for Marine Biological Resources and Biotechnology (IRBIM), 60125 Ancona, Italy*
[4]*SIGNATOPE GmbH, Markwiesenstr. 55, 72770 Reutlingen, Germany*
[5]*NMI Natural and Medical Sciences Institute at the University of Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany*

Background and Objectives: Risk assessment has traditionally relied on animal testing, but concerns exist about interspecies consistency, reproducibility, time, costs, and ethics. New Approach Methodologies (NAMs), like cell-based omics analysis, offer promise by understanding underlying mechanisms, rather than assessing organ pathology. However, NAMs face limitations, such as the absence of a whole organism system and limited toxicokinetic interactions. The question remains whether omics data can sufficiently predict target organ toxicity. Here, comparative studies between in vitro and in vivo results can help improve the acceptance of NAM-generated data. Materials and Methods: Six pesticide active substances with known in vivo hepato- and nephrotoxicity were tested on two cell lines, the liver cell line HepaRG and the kidney cell line RPTEC. Two non-cytotoxic concentrations were selected for targeted protein and transcriptomics analysis using multiplexed microsphere-based sandwich immunoassays and quantitative real-time PCR arrays, respectively. Results: Protein and mRNA from cultivated cells was successfully isolated and analyzed. A Weight of Evidence approach was established to identify relevant pathways from the data. Where possible, in vitro endpoints were connected to in vivo observations. Analyses of this data set from substances with a good in vivo database revealed various affected pathways, some of which could not be connected to in vivo observations. Discussion and Conclusion: The challenges associated with generating risk assessment conclusions from in vitro data and extrapolate to the in vivo situation can be addressed by comprehensive assessment frameworks incorporating kinetic considerations.

O-8a-6
New Approach Methodologies (NAMs) for use in Next Generation Risk Assessment (NGRA) for Systemic Safety: A pragmatic approach to 'validation' by establishing protectiveness and utility
ABSTRACT #22

Paul Carmichael[1]

[1]*Unilever*

There is an increasing recognition that new frameworks and approaches are required for establishing scientific confidence in the use of NAMs to achieve regulatory acceptance. In the context of NGRA, which is an exposure-led and hypothesis-driven approach for conducting safety assessments using non-animal NAMs, we need to establish that the 'toolboxes' are fit-for-purpose. This requires that the combination of NAMs employed are evaluated in the context of their use (risk assessment) and that they can give the correct 'call' that a given chemical and relevant exposure is safe or not. To address this, we recently proposed a NAM-based toolbox and workflow for conducting systemic safety assessments, together with an approach for evaluating how protective it is (Middleton et al., Tox Sci, 2022). The approach is based on the principle of benchmarking safety decisions made using the NAM toolbox against historical safety decisions. The toolbox includes physiologically based kinetic (PBK) models and a broad range of different in vitro assays, from which points of departure (PODs) are estimated. The feasibility of the evaluation strategy was initially tested using 24 benchmark exposure scenarios from 10 different chemicals (Middleton et al.) and has since been expanded to include 69 additional benchmark exposure scenarios from 38 additional chemicals. The results of the extended evaluation will be presented.

Session 8b: Complex chemical mixtures - how can NAMs contribute?

Chairs: *Anne-Marie Vinggaard (DTU, Denemark) & Beate Escher (Helmholtz Centre for Environmental Research in Leipzig, Germany)*

O-8b-1
Using NAMs for predicting adverse effects of chemical mixtures on male reproductive health
ABSTRACT #377

Anne Marie Vinggaard[1], Maria João Valente[1], Yanying Ma[1]

[1]*Technical University of Denmark*

We explored a novel methodology using new approach methodologies (NAMs) for mixture risk assessment of chemicals. As a case, we focused on adverse effects on human male reproductive health disorders caused by chemical mixtures with an antiandrogenic mode of action at realistic human exposure levels. Androgen insufficiency in the male fetus can lead to adverse effects on male reproductive health later in life. The major molecular initiating event causing androgen insufficiency is human androgen receptor (hAR) antagonism and therefore chemicals with potent hAR antagonistic properties can cause a range of male reproductive health disorders. Research has shown that a shortened anogenital distance (AGD) at birth predicts well male reproductive health disorders in animals and humans, and we have previously shown that by using NAMs (a combination of hAR data and physiologically-based kinetic modeling), the in vivo outcome on AGD in male pups can be predicted [1]. To address the question whether chemical mixtures at realistic human exposure levels may affect male reproductive health, we calculated risk quotients for each chemical based on hAR antagonism data combined with human biomonitoring levels. Our work identified 231 hAR antagonists and among these were 61 finally identified as having both reliable hazard and exposure data [2]. Risk quotients indicated that the major chemical mixture drivers were PCB 118, BBP, PFOS, DBP, and the UV filter benzophenone-3, together contributing 75% of the total effect. This viable way forward for mixture risk assessment has the advantages of including human data only and being more comprehensive also covering data-poor chemicals. However, the approach is subjected to uncertainties in terms of translating urine to blood levels. Still, the results indicate a concern for adverse effects on reproductive function in highly exposed boys, when considering additional exposure to data-poor chemicals and chemicals acting by other mechanisms of action.

O-8b-2
In vitro assays for quantification of adverse effects of chemical mixtures extracted from human serum
ABSTRACT #150

Beate Escher[12], Jean-Philippe Antignac[3], Marx Audebert[4], Peter Cenijn[5], Timo Hamers[5], Maria João Portugal Couto Valente[6], Laure Khoury[4], Maria König[1], Jungeun Lee[1], Yanying Ma[6], Maria Margalef Jornet[5], Solène Motteau[3], Kostja Renko[7], Martin Scholze[8], Andreas Treschow[6], Anne Marie Vinggaard[6], Marja Lamoree[5]
[1]*Department Cell Toxicology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany*
[2]*Eberhard Karls University Tübingen, Germany*
[3]*Oniris, France*
[4]*Preditox, France*
[5]*Free University Amsterdam*
[6]*Denmark Technical University*
[7]*Federal Institute for Risk Assessment Germany*
[8]*Brunel University*

In vitro bioassays have a long tradition for hazard assessment of chemicals as well as for water quality monitoring. Here we apply these tools for the first time to mixtures extracted from human serum. We developed a test battery of 22 in vitro bioassays that broadly covered developmental neurotoxicity, thyroid hormone system disruption, reproductive toxicity, genotoxicity and adaptive stress responses. Putative adverse outcome pathways synthesized from diverse literature sources served as a starting point for the selection of the bioassays. Serum samples pooled from diverse population groups in Australia and Europe were extracted with solid-phase extraction yielding broad-spectrum extracts of chemicals of a medium hydrophobicity range including neutral and charged organic chemicals. 50-60 % of the bioassays responded to the dosed serum extracts. All groups of mode of action were affected with the exception of genotoxicity. High specificity was observed for disruption on thyroid hormone system and neurotoxicity. Through a global profiling approach (suspect screening), 25 endogenous chemicals and environmental pollutants were identified with high confidence and were quantified, among many more qualitatively confirmed. Mixture modelling using detected concentrations and bioassay data for single chemicals demonstrated that little of the effect could be explained by the quantified chemicals. Endogenous compounds also contributed to mixture effects and future work will need to establish a baseline of effects caused by endogenous compounds and differentiate those from the effects triggered by environmental pollutants. Bioassays are a promising tool to identify bioactive chemical mixtures in human biomonitoring.

O-8b-3
Combined effects of pesticide product ingredients in zebrafish and mixture effect predictions at the whole transcriptome scale
ABSTRACT #103

Wibke Busch[1], Paul Michaelis[1], Gianina Jakobs[1], Andreas Schüttler[1]
[1]*UFZ*

Humans and environmental organisms are constantly exposed to complex mixtures of chemicals. Extending our knowledge about the combined effects of chemicals is thus essential for assessing the potential consequences of these exposures. Comprehensive molecular readouts as retrieved by omics techniques are advancing our understanding of the diversity of effects upon chemical exposure. However, omics profiles induced by chemical exposures have rarely been systematically considered in mixture contexts. In this study, we aimed to investigate the predictability of chemical mixture effects on the whole-transcriptome scale. We predicted and measured the toxicogenomic effects of a synthetic mixture on zebrafish embryos. The mixture contained the compounds diuron, diclofenac, and naproxen. To predict concentration- and time-resolved whole-transcriptome responses to the mixture exposure, we adopted the mixture concept of concentration addition. Predictions were based on the transcriptome profiles obtained for the individual mixture components in a previous study. Finally, concentration- and time-resolved mixture exposures and subsequent toxicogenomic measurements were performed and the results were compared with the predictions. This comparison of the predictions

with the observations showed that the concept of concentration addition provided reasonable estimates for the effects induced by the mixture exposure on the whole transcriptome. Although nonadditive effects were observed only occasionally, combined, that is, multicomponent-driven, effects were found for mixture components with anticipated similar, as well as dissimilar, modes of action. Overall, this study demonstrates that using a concentration- and time-resolved approach, the occurrence and size of combined effects of chemicals may be predicted at the whole-transcriptome scale. This allows for improving effect assessment of mixture exposures on the molecular scale that might not only be of relevance in terms of risk assessment but also for pharmacological applications. The study is published in EHP: https://doi.org/10.1289/EHP7773.

O-8b-4
Unraveling the impact of a real-life organochlorine mixture on male reproductive health: an AOP-driven approach combined with lipidomics
ABSTRACT #18

Ishita Virmani[1], Eliška Sychrová[1], Eliška Řehůřková[1], Darshak Gadara[1], Veronika Vidová[1], Zdeněk Spáčil[1], Jiří Novák[1], Iva Sovadinová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*

Exposure to chemical cocktails, especially mixtures containing organochlorines, raises substantial concerns for male reproductive health. Organochlorine mixtures are notably prevalent in regions such as Greenland and are associated with male reproductive toxicity. While individual chemical responses are extensively documented, the consequences of mixture exposure have been overlooked. Our study aims to bridge this knowledge gap by delving into the intricate mechanisms that underlie the reproductive toxicity of a real-life organochlorine mixture. We employed an adverse outcome pathway (AOP)-driven approach combined with lipidomics to resolve this critical issue. Our comprehensive approach began with an assessment of the toxicity profile of the mixture through transfected reporter gene assays targeting various receptors. These assays revealed potent dioxin-like and antiandrogenic activities of the mixture, accompanied by its subtle estrogenic effect. Furthermore, we investigated the direct impacts of the mixture on testicular cells, specifically Leydig TM3 and Sertoli TM4 cells. Our mixture of interest induced oxidative stress and cytotoxic effects on both cell types. Notably, the key mechanism behind this toxicity lay in the disruption of lipid homeostasis, resulting in lipid droplet accumulation and alterations in lipid species. Interestingly, our investigation uncovered that this lipid imbalance was not triggered via the androgen or aryl hydrocarbon receptor or peroxisome proliferator-activated receptor gamma, highlighting the need to identify the initiator at the molecular level. This lipotoxicity detrimentally affected the functionality of these testicular cell types, impacting hormone production in Leydig cells and the barrier functionality of Sertoli cells. Given the alarming prevalence of environmental exposure to such cocktails, our work sheds light on the importance of addressing this critical issue, particularly in the context of male reproductive health. Furthermore, it underscores the significance of chemically-induced lipid disruption as a potential contributor to male reproductive disorders. Acknowledgment: Research is supported by the Czech Science Foundation project no. GA22-30004S.

O-8b-5
Endocrine disruptive potencies of air and dust samples from different indoor environments
ABSTRACT #145

Jiří Novák[1], F. Vidal[2], L. Melymuk[2], J.W. Martin[3], A. Sunyer[3], K. Hilscherová[2]
[1]*RECETOX, Masaryk University, Czechia*
[2]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[3]*Department of Environmental Science, Science for Life Laboratory, Stockholm University*

The fact that the human population spends most of their life in the indoor environment makes the complex mixtures of chemicals that tend to cumulate there very relevant for human exposure and potential impact on health. Many

of the chemicals have been identified as endocrine disruptors [1]. In order to characterize the endocrine disruptive potential of the indoor chemical mixtures, a battery of in vitro bioassays was employed for a set of indoor and outdoor air samples and indoor settled dust samples obtained from different indoor environments. The samples were assessed for the effects associated with endocrine system disruption, such as estrogenicity, anti-androgenicity, and AhR-mediated toxicity, using human-based reporter gene assays. For most of the samples, several endocrine-disrupting potentials were detected. The obtained potentials were compared with the potencies modeled by the iceberg modeling based on data from chemical analyses. Due to either a lack of toxicity data for the detected chemicals or because the chemical analyses did not cover the main drivers of the effects, the explicability was relatively low for most of the effects. The gaps in identifying indoor pollutants responsible for the assessed endocrine-disruptive potencies and the pervasive presence of ED potentials within indoor environments raise concern regarding the possible health risks associated with the indoor environment for the human population. This project received funding from the European Commission Horizon Europe, HADEA, research and innovation programme under grant agreement Project No 101057499 - INQUIRE.

Thursday, 6 June 2024
09:00 - 10:00

Session 9a: Developmental Toxicity and Developmental Neurotoxicity (DNT)

Chairs: *Yasunari Kanda (National Institute of Health Sciences, Kawasaki, Japan) & Amer Jamalpoor (Toxys, Leiden, The Netherlands)*

O-9a-1
High-Throughput Screening of FDA-Approved Compounds on Human Brain Organoids for Safety Assessment
ABSTRACT #174

Robin Pronk[12], Petra Szeszula[1], Hanna Axelsson[3], Brinton Seashore-Ludlow[3], Anna-Lena Gustavsson[3], Mats Nilsson[2]
[1]*BrainZell*
[2]*Stockholm University, Department of Biochemistry and Biophysics*
[3]*Karolinska Institutet, Chemical Biology Consortium Sweden*

Introduction: Accurate assessment of brain toxicity and function is paramount to reduce risks in clinical trials and improve the success rate in treating disease. The abysmal success rate for neurological compounds is 6% and underscores the need for more effective methods. Advancements in technology, such as hiPSC derived brain organoids, provide a promising tool that mimics critical features of the developing human brain (1). This allows for testing compounds in a more physiologically relevant model system. Methods: In this study, we present a high-throughput screening (HTS) approach synergizing with existing HTS workflows. We conducted screenings on 15,000 brain organoids using 58 FDA-approved compounds and known toxic substances. This was accomplished with minimal resource utilisation, using automated liquid handling and microscopy, accomplishing a high degree of similarity between the organoids. Results: Our results revealed comprehensive dose response profiles of all tested compounds. The validity of our assay was confirmed by identification of the highly toxic effects of Rotenone, a potent inhibitor of mitochondrial complex I, and known to induce a Parkinsonian phenotype in rats. In addition to toxicity assessment, we simultaneously measured spontaneous and potassium chloride-induced calcium activity. This dual assessment provided a comprehensive view of the compounds, their safety, and their impact on neural activity. Conclusion: The data presented in this study exemplifies the potential of human brain organoids as a powerful tool for assessing compound toxicity in a more physiologically relevant model system. This approach holds promise for improving the safety assessment of new drug candidates in the early discovery and preclinical stages of drug development, potentially reducing the risk of failures in downstream clinical trials. The use of human brain organoids for high-throughput screening offers a valuable bridge between in vitro and in vivo assessments, with implications for

enhancing the safety and success rates of drug development.

O-9a-2
Evaluating the Reprotracker assay as a NAM for developmental and reproductive toxicity testing
ABSTRACT #101

Jade Houghton[1], Magdalena Sawicka[1], Luke Flatt[2], Iris Muller[1], Alistair Middleton[1], Marleen Feliksik[2], Giel Hendriks[2], Amer Jamalpoor[2], Mark Liddell[1], Sophie Malcomber[1], Claire Peart[1], Katy Wilson[1]
[1]*Safety and Environmental Assurance Centre, Unilever, Colworth Science Park, Sharnbrook, Bedfordshire, UK*
[2]*Toxys B.V., De Limes 7, 2342 DH Oegstgeest, Netherlands*

Encouraged by the successful application of New Approach Methodologies (NAMs) in an exposure-driven Next Generation Risk Assessment (NGRA) approach for systemic toxicity (Middleton et al., 2022), we created a developmental and reproductive toxicity (DART) framework that includes additional in vitro assays covering specific DART-related biology (Rajagopal et al. 2022). One of the DART-specific assays included in the framework was ReproTracker® (Toxys). ReproTracker® is a human stem cell-based assay that rapidly and reliably identifies developmental toxicity hazards of chemicals (Jamalpoor et al., 2022). The assay captures changes in the key cellular events of stem cell differentiation into cardiomyocytes, hepatocytes and neural rosettes and upon exposure to the chemical of interest in a dose dependent manner. We have multiple on-going activities to evaluate and build confidence in including the use of ReproTracker® as a NAM for NGRA. First, we have assessed the utility of ReproTracker® as a NAM for NGRA by testing ~40 chemicals in the NGRA-adapted version of the ReproTracker® assay. We have compared calculated Point of Departure (PODs) to internal exposure estimates (relevant to specific known human exposure scenarios) to assess the ability of the assay to detect exposure relevant teratogenicity. We have then conducted a transferability study of ReproTracker® across two independent laboratories. After an initial stage of training and proficiency testing a study of 10 blinded chemicals were selected and tested in both laboratories. Identical data analysis pipelines will be applied to both result sets and compared for evaluations of inter-lab reproducibility. Lastly, we have performed high throughput transcriptomics on samples throughout the differentiation process to understand the biological coverage of ReproTracker® and to map transcriptional changes to biological functions and pathways.

O-9a-3
Advancing the use of New Approach Methodologies for assessing teratogenicity: A tiered approach
ABSTRACT #51

Matthew Burbank[1], Florian Gautier[1], Nicky Hewitt[2], Audrey Noel-Voisin[1], Nazanin Golbamaki[1], Sébastien Grégoire[1], Anne Riu[1], Ann Detroyer[1], Typhaine Bringel[1], Laurent Guillet-Revol[1], Léopold Carron[1], Emilie Le Mevel[1], Noémie De Croze[1], Gladys Ouédraogo[1]
[1]*L'Oréal Research & Innovation, Aulnay-sous-Bois, France*
[2]*Cosmetics Europe, Brussels, Belgium*

New approach methodologies (NAMs) to assess the developmental and reproductive toxicity (DART) potential of substances have been developed in recent years where there have been advances as a result of international collaborative programs. Research into this endpoint is a priority, with the overall objective of developing and evaluating NAMs in an Integrated tested Strategy (ITS) for the evaluation of this endpoint for new ingredients. In this work we present the performance of three NAMs, alone and in combination, for their ability to assess the teratogenic potential (from the developing embryo to birth) of a panel of chemicals. DART decision tree model, the devTox assay and the Zebrafish Embryotoxicity Test (ZET) were used. The starting training set of 85 test chemicals comprised 46 which were classified as non-teratogenic and 39 which were classified as teratogenic, based on information provided in the open literature and public databases. All 85 chemicals were tested in the in silico DART decision tree model,

whereas 82 and 61 were tested in the devTox assay and the zebrafish assays, respectively. Fifty-three chemicals were tested in all three assays and when results were combined and based on a "2 out of 3 rule", the sensitivity and specificity were 96.0% and 71.4%, respectively. The specificity of the devTox assay for a sub-set of 43 chemicals was increased from 26.1% to 82.6% by incorporating human plasma concentrations into the assay interpretation. When all 85 chemicals were assessed in a decision tree approach, there was an excellent predictivity and assay robustness of 90%. In conclusion, all three models exhibited a good sensitivity and specificity, especially when outcomes from all three were combined or used in "2 out of 3" or a tiered decision tree approach. The latter is an interesting predictive approach for evaluating the teratogenic potential of new chemicals.

O-9a-4
Benchmarking in silico methods for identifying chemicals of developmental neurotoxicity concern
ABSTRACT #144

Chia-Chi Wang[1], Shan-Shan Wang[2], Chun-Wei Tung[2]
[1]*Department and Graduate Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei 10617, Taiwan*
[2]*Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 35053, Taiwan*

Background and Objectives Developmental neurotoxicity is an important but probably the least tested toxicity endpoint for both drug development and chemical risk assessment. Since the developing nervous systems are more vulnerable to toxic agents, drugs used in pregnancy, breastfeeding and pediatrics should be carefully assessed for their potential developmental neurotoxicity. However, experimental assessment of developmental neurotoxicity is both labor- and resource-intensive. This study assessed the performance of in silico methods for identifying chemicals of developmental neurotoxicity concern. Material and Methods A total of 170 chemicals consisting of 129 developmental neurotoxic and 41 non-neurotoxic compounds obtained from CompTox Chemicals Dashboard were utilized for evaluating prediction models. Several developmental toxicity-relevant models including seven quantitative structure-activity relationship (QSAR) models, one structural alert-based model and two scores of gene and disease obtained from the in silico toxicogenomics ChemDIS platform were evaluated. A weight-of-evidence (WoE) model optimized for a developmental and reproductive toxicity dataset was also assessed using the 170 chemicals. Results The chemical coverage of individual QSAR and structural alert methods is quite low (<50%). In contrast, a high coverage of 98.6% was obtained for the two toxicogenomics-derived scores. Generally, the toxicogenomics-derived scores tend to predict chemicals as toxicants with high sensitivity but low specificity, while QSAR and structural alert models tend to generate non-toxicant predictions with high specificity but low sensitivity. The WoE model integrating all available in silico evidence performs best with high coverage and precision of 100% and 81.3% and moderate sensitivity and specificity of 65.7% and 61.5%, respectively. Discussion and Conclusion The WoE method integrating toxicogenomics, QSAR and structural alert methods achieved a good performance for predicting developmental neurotoxicants compared to individual methods. The WoE model can be further optimized for developmental neurotoxicity.

Session 9b: Regulatory considerations for complex NAMs

Chairs: *Anne Gourmelon (OECD, Paris, France) & Nathalie Alepee (L'Oreal, Paris, France)*

O-9b-1
Quantifying the uncertainty of the new approach methodology U-SENS for skin sensitization assessment in a regulatory context
ABSTRACT #47

NATHALIE ALEPEE[1], Fleur Tourneix[1], Laurent Nardelli[1], Leopold Carron[1]

[1]*L'Oreal R&I*

Skin sensitisation is a widely prevalent human health concern affecting the consumers of chemical products. Many Defined Approaches (DA) combining New Approach Methodologies (NAM) were developed to identify chemicals with the potential to induce skin sensitisation. The first decision on whether each information element can be used is dictated by the limitations of the NAM (e.g. for the U-SENS method) as found in the test guidelines (OECD TG442E). Adding that the NAM results may be subject to variation and these variations increase the uncertainty especially when close to the classification cut-off, i.e. in the borderline range (BR), the aim of our project was to define areas where lower confidence in the U-SENS may exist. The U-SENS™ method quantifies the change in the expression of a cell surface marker associated with the process of activation of dendritic cells by sensitizers (stimulation index CD86). Computing the log_10 of the CD86 corrected values, the analysis was based on a total of 2741 data encompassing dose concentrations and multiple runs. The BR was determined for 4 laboratories based on the log pooled median absolute deviations. The determined BR mean of all laboratories was 133%-169% and the median was 134%-168%. A flowchart of the U-SENS prediction model, including the BR has been then established to facilitate its implementation by end-users. Illustrations of the different scenario with some chemicals i) cell viability<70% at low concentration (eg. CASRN#50-00-0), (ii) CD86 induction>169% (eg. CASRN#100-06-1), iii) CD86 induction ≤133% (eg. CASRN#108-90-7), iv) 133%<CD86<150% (eg. CASRN#637-07-0), and v) 150%<CD86<169% (eg. CASRN#369-36-8) are presented. Application of the U-SENS BR were therefore applied to the “2o3” DA determining the substitution of the h-CLAT by the U-SENS. Overall, the implementation of BR acknowledges uncertainties of the U-SENS and is therefore considered in regulatory context to be considered in OECD Guideline No. 497.

O-9b-2
The future of risk assessment at OECD
ABSTRACT #109

Patience Browne[1]
[1]*OECD*

The Chemical Safety Programme at OECD was established over 50 years ago with the aim of harmonising approaches for evaluating chemicals and sharing best practices and work of regulatory risk assessment. The Programme has evolved with modern toxicology and there are now ~35 in vitro methods included in OECD Test Guidelines, the OECD QSAR Toolbox includes freely available in silico predictive tools, and the Adverse Outcome Pathway Knowledgebase (AOP-KB) is a proof-of-concept framework for how in vitro methods can be used to predict in vivo outcomes. In addition to OECD Test Guidelines, a variety of initiatives were developed to help implement modern toxicological methods in regulatory decision making. OECD publishes harmonized data reporting templates and standards which contribute to structured databases and facilitate sharing chemical safety data globally. The Integrated Approaches to Testing and Assessment (IATA) Case Studies Project is beginning its 10th year and publishes examples demonstrating how innovative methods can be combined to assess chemical safety, in addition to general and topic-specific guidance associated with IATAs. Increasingly, OECD projects extend across multiple areas in the Chemical Safety Programme to provide examples of how technology and innovation can be included in Guideline methods covered under the agreement on Mutual Acceptance of Data (MAD) as well as standards for assessing the performance, reporting, and review of approaches that are not (yet) included in Test Guidelines but are nonetheless associated with a high level of confidence which regulators may choose to accept. The changing regulatory landscape and increased interest in using non-animal approaches to assess chemical safety lead to consideration of these methods in frameworks based on traditional toxicology data, as well as frameworks that that weigh the value of shorter time to decisions based on less data but remain protective of humans and the environment.

O-9b-3
NAMs in chemical mixture risk assessment
ABSTRACT #175

Philip Marx-Stoelting[1], Denise Bloch[1], Tewes Tralau[1]
[1]*German Federal Institute for Risk Assessment*

Data driven approaches for risk assessment of combined exposure to multiple chemicals are successfully applied by regulators to ensure a high level of food safety. For pesticide active substances cumulative assessment groups (CAGs) were built based on existing comprehensive toxicity data. Cumulative risk assessment (CRA) was performed for these CAGs considering wide-ranging exposure information also for sensitive parts of the population based on conservative assumptions. So far, no elevated risk has been detected for the CAGs of the thyroid and the nervous system as a result of combined exposure to multiple pesticide residues. For other groups of compounds with less comprehensive data packages like contaminants, structure based grouping and relative potency factors are used for CRA. Additionally whole mixture approaches, taking into account exposome data and new approach methods (NAM) for mechanism and adverse outcome pathway driven hazard identification are discussed. Their implementation would facilitate CRA across regulatory domains. As CAGs focus on toxicodynamic aspects, we recently proposed the complimentary introduction of common kinetic groups (CKG) and to evaluate the potential of compounds to interact at the level of xenobiotic metabolizing enzymes or transporters in vitro. A roadmap for action on risk assessment of combined exposure to multiple chemicals (RACEMiC) was prepared. Within a number of European projects such as EuroMix or more recently PARC steps toward the development and implementation of NAMs have been undertaken.

O-9b-4
Survey results to identify nam use in agrochemical regulation
ABSTRACT #17

Raechel Puglisi[1], Sandrine Deglin[1], Gina Hilton[2], Andrew Nguyen[2]
[1]*Health and Environmental Sciences Institute*
[2]*PETA Science Consortium International*

Although the existing agrochemical safety evaluation paradigm, based on classical toxicology methods, is well established, it is unlikely to meet the emerging challenges of a developing and ever-expanding sustainable agriculture. The scientific methods underpinning chemical toxicity testing are advancing at a remarkable pace, and the ability to leverage modern tools to assess the safety of crop protection products has dramatically increased. Despite the opportunities they offer, the potential presented by these new tools is far from being realized. It has now become critical to consider how to incorporate the technological and scientific advancements, leverage existing knowledge, and prioritize the needs for toxicity testing to better inform human health and environmental risk assessment decisions for plant protection products while improving the decision-making process. The Health and Environmental Sciences Institute (HESI) is currently leading a project called, Transforming the Evaluation of Agrochemicals, whose mission is to revamp the safety evaluation paradigm for plant protection products. As part of this project, they have surveyed members of the agrochemical industry to identify which new approach methodologies (NAMs) are used in product research and development, and/or included in regulatory submissions in various countries, with what frequency. The results from this survey offer a real-time assessment of NAM use in regulatory decisions and help to identify endpoints that need further development to be fit for safety assessment. This presentation will provide an overview of this survey and highlight areas of the agrochemical safety evaluation where the use of NAMs could be initiated or expanded.

Thursday, 6 June 2024
10:00 - 11:00

Session 10a: In vitro methods for safety testing of

biopharmaceuticals/biotherapies/vaccines

Chairs: *Yasunari Kanda (National Institute of Health Sciences, Kawasaki, Japan) & Benoît Maisonneuve (NETRI, Lyon, France)*

O-10a-1
In vitro prediction of drug-induced liver injury using toxprofiler
ABSTRACT #21

Bas ter Braak[1], Liesanne Loonstra-Wolters[1], Kim Elbertse[1], Eva de Tombe[1], Giel Hendriks[1], Amer Jamalpoor[1]
[1]*Toxys B.V., De limes 7, 2342 DH, Oegstgeest, The Netherlands*

Drug-induced liver injury (DILI) is a leading cause of clinical trial failure, and it accounts for 30% of market withdrawals. Poor predictivity of the current pre-clinical safety assessment strategies contribute to these high drug attrition rates. To improve the current safety evaluation of pharmaceuticals, there is an increasing emphasis on implementation of new approach methodologies (NAMs) that could shed light on the underlying mechanisms of toxicity. In this study we applied the imaging-based reporter assay ToxProfiler on a set of 60 drugs with known FDA DILI liabilities to gain insight into their biological activity and toxicological mode-of-action. The ToxProfiler assay consists of a panel of seven GFP reporter cell lines that are specifically activated upon induction of oxidative stress, ER stress, cell cycle stress, ion stress, protein stress, autophagy and inflammation. Using live cell confocal imaging of these seven reporters, an extensive toxicological profile of compounds was obtained. Hierarchical clustering of the compounds based on their ToxProfiler profile and calculation of the Point of Departure (PoD) concentrations for the different toxicological responses was used for grouping and potency ranking. ToxProfiler-derived PoDs and human cMAX information were used for an exposure corrected margin of safety approach to assess the ToxProfiler assay performance for the classification of DILI. Induction of oxidative stress, ER stress and autophagy in ToxProfiler were the most important predictors for DILI. Overall, ToxProfiler correctly predicted DILI with an accuracy of 83%. Together, quantitative dose-response modelling in ToxProfiler allowed for identification of primary mode-of-actions of compounds. Moreover, the ToxProfiler clustering and potency ranking aligned well with the relative risk of these compounds to induce DILI in humans.

O-10a-2
Pragmatic Approach to inculcate New Approach Methodologies (NAMs) in Vaccine Safety Assessment
ABSTRACT #217

Rajamuthu SRINIVASAN[1], Patrick Syntin[1], Paul DESERT[2]
[1]*Toxicologist, Nonclinical Safety, Sanofi*
[2]*Head, Nonclincal Safety, Sanofi*

New Approach Methodologies (NAMs) are being developed by sponsors and applied in the safety assessment following the harmonized mindset on 3R principles among the scientific community and ban on animal testing in a few domains like cosmetics (Westmoreland, 2022). With respect to drug development including the vaccines, the CBER and EMA offered several platforms and forums for the sponsors to develop NAMs and to integrate them in the drug development which will lead to an overall evidence generation on the reliability of human-tissue derived in vitro data while reducing the animal usage. The momentum is gaining towards reduction or replacement of animal testing through proper scientifically valid NAMs for development of biologicals. A positive outcome of the introduction of NAMs in drug development is the acceleration of clinical development of vaccines. It is well recognized by both the scientific community and regulatory authorities on a transition from current in vivo approach to human tissue-derived in vitro methodologies to increase the predictivity of potential safety issues (Schmeisser, 2023) It also has intrinsic challenges in terms of availability of functionally equivalent in vitro models, standardization and regulatory acceptance. We propose here a pragmatic framework towards integrated approach to inculcate NAMs in vaccine nonclinical safety assessment by developing a clear context-of-use for regulatory acceptance to support the

clinical development of vaccines.

O-10a-3
Evaluation of 3D human intestinal organoids as a platform for EV-A71 antiviral drug discovery
ABSTRACT #71

Fatma Masmoudi[1], Nanci Santos-Ferreira[2], Peter I Racz[1], Dasja Pajkrt[3], Katja C Walthers[4], Jeroen DeGroot[1], Maria L.H. Vlaming[1], Joana Rocha-Pereira[2], Ludovico Buti[1]

[1]*Charles River Laboratories, 2333 CR Leiden, The Netherlands*
[2]*Laboratory of Virology and Chemotherapy, KU Leuven-Department of Microbiology, Immunology and Transplantation, Rega Institute, 3000 Leuven, Belgium*
[3]*OrganoVIR Labs, Department of Medical Microbiology, Amsterdam UMC Location Academic Medical Center, Amsterdam Institute for Infection and Immunity, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands*
[4]*OrganoVIR Labs, Pediatric Infectious Diseases, Emma Children's Hospital, Amsterdam UMC Location University of Amsterdam, 1105 AZ Amsterdam, The Netherlands*

Enteroviruses are a leading cause of upper respiratory tract, gastrointestinal and neurological infections. Management of enterovirus-related diseases has been hindered by the lack of specific antiviral treatment. The pre-clinical and clinical development of such antivirals has been challenging, calling for novel model systems and strategies to identify suitable pre-clinical candidates. Organoids represent a new and outstanding opportunity to test antiviral agents in a more physiologically relevant system. However, dedicated studies addressing the validation and direct comparison of organoids versus commonly used cell lines as well to clinical data are still lacking. This study described the use of human small intestinal organoids (HIOs) as a model to study antiviral treatment against human enterovirus 71 (EV-A71) infection and compare this model to EV-A71-infected RD cells. Reference antiviral compounds that were previously tested in clinical trials were used such as enviroxime, rupintrivir and 2'-C-methylcytidine (2'CMC) to assess their effects on cell viability, virus-induced cytopathic effect (CPE), and viral RNA yield in EV-A71-infected HIOs and cell line. All compounds showed almost similar toxicity between HIOs and RD cells, except for 2'CMC showed toxicity in HIOs but not in RD cells. Enviroxime showed antiviral activity in HIOs while the determined EC50 in RD cells was 10-fold lower. Rupintrivir showed a strong antiviral activity in both HIOs and RD cells, resulting in full protection from EV-A71-induced CPE at the lowest concentration tested. 2'CMC only partially reduced HIOs from virus-induced CPE, while there was a complete protection from virus-induced CPE in RD cells. These results indicate a difference in the activity of the tested compounds between the two models, with HIOs being more sensitive to the infection, drug treatment and giving results that more closely align with patient's data. In conclusion, the outcome reveals the value added by using organoid model in virus and antiviral studies.

O-10a-4
A human ex vivo model for assessing the immunotoxicity risk of engineered nanomaterials
ABSTRACT #299

Josephine Blersch[1], Kurkowsky Birgit[1], Anja Meyer-Berhorn[1], Grabowska Agnieszka K.[2], Eva Feidt[2], Wera Roth[1], Dominik Stappert[1], Armin Kübelbeck[2], Philip Denner[1], Eugenio Fava[3]

[1]*Deutsches Zentrum für Neurodegenerative Erkrankungen e.V. (DZNE), CRFS-LAT, Bonn, Germany*
[2]*Ruprecht Karl University of Heidelberg, IPMB - Institute of Pharmacy and Molecular Biotechnology, SILVACX, Heidelberg, Germany*
[3]*Deutsches Zentrum für Neurodegenerative Erkrankungen e.V. (DZNE), CRFS, Bonn, Germany*

The unique physicochemical properties of engineered nanomaterials (ENM) have led to widespread use in various industries, increasing human exposure. However, there is a lack of ENM-tailored robust and screenable tools for assessing the risks associated to ENM

[1]. The inflammatory response is a critical aspect of ENM safety, affecting organs as lung, skin, and bowel. Upon exposure to ENM, the innate immune system triggers an inflammatory response, with secretion of inflammatory cytokines identified as key event in nanotoxicology's Adverse Outcome Pathways (AOPs) [2]. This work proposes a fully automated and miniaturized high-throughput cell-based assay using human peripheral blood mononuclear cells to assess the cytotoxicity and innate immunity effects of ENM. Exemplary results from a small library of SiO2-ENM is shown as SiO2 ENM are known to trigger NLRP3 inflammasome [3]. Data from this fully automated and miniaturized assay demonstrates outstanding performance parameters (Z'-score >0.5), making it suitable for large-scale screening campaigns in industrial settings. Immunotoxicity is collected in dose-response format. At different cellular states, multiparametric readouts for cytotoxicity (e.g., cell viability), and innate immunity (e.g., IL-1ß secretion) are conducted in a combinatorial method, avoiding e.g., bias by endotoxin contamination. For selected ENM, inhibitor panels (for e.g., inflammasome, caspase-I, cathepsin B, TLR-4) are used to understand mechanistic details in the immunotoxicity profile. In summary, integration of the high-dimensional data collected through this method, allows (i) holistic safety assessment of immunotoxicity effects caused by ENM, classification of safe and toxic ENM phenotypes, and (ii) deconvolution the mode of action of the ENM effect on PBMCs. The next step is an extensive validation to use this human tissue like system as new approach methodology (NAM) for next generation risk assessment (NGRA). As added value the data obtained can be used to troubleshoot ENM or for a safe-by-design approach in product development.

Thursday, 6 June 2024
11:30 - 12:45

Session 11a: In vitro methods for safety assessment of medical devices

Chairs: *Helena Kandarova (CEM SAS, Bratislava, Slovakia) & Christian Pellevoisin (Urbilateria & Vitroscreen, France)*

O-11a-1
Challenges in regulatory acceptance of NAMs for medical devices
ABSTRACT #100

Christian Pellevoisin[1], Marisa Meloni[2]
[1]*Urbilateria, Tours, France*
[2]*VitroScreen, Milan, Italy*

The publication of ISO 10993-23 in 2021 was a breakthrough for the replacement of animal testing by in vitro methods for assessing the irritation of medical devices (MD). Although not regulatory texts, ISO standards provide a presumption of conformity with regulatory requirements when assessing biocompatibility. Standardization is therefore an important step, but not always sufficient. Indeed, in some countries, recognition of these standards depends on national regulatory authorities. In the United States, for example, the FDA only partially recognizes this standard and continues to require tests on rabbits. Furthermore, part 23 also covers special irritation tests for MD in contact with mucosal membranes: ocular, oral, vaginal, etc. Qualification study conducted by ISO in 2017 did not cover these tests and animals are still the reference despite availability of in vitro methods with 3D models of these mucosal tissues. Existing in vitro OECD guidelines are important for developing ISO standards. However, OECD validations are conducted with pure chemicals, and their applicability to MD needs to be demonstrated. For skin sensitization, ISO TS11796 published in 2023 specify the procedure for qualification of OECD methods from guidelines 442C, D and E. The aim is to provide a clear framework in terms of data to be generated and acceptance criteria for the qualification of candidate methods to accelerate their regulatory acceptance. Qualification of these in vitro methods will eventually lead to the revision of ISO 10993-10 for skin sensitization. However, methods not included by ISO standards do not mean that they cannot be used. But, the complexity lies in the uncertainty of acceptance by the national authority that will review the biocompatibility dossier. This is therefore a case-by-case approach where data may or may not be accepted, depending on the type of MD, it's history of use and the scientific soundness

of the justification.

O-11a-2
Regulatory approval of medical devices according to MDR using in vitro data from GARDskin Medical Device for skin sensitization assessment
ABSTRACT #86

Andy Forreryd[1], Anna Chérouvrier Hansson[1], Tim Lindberg[1], lisa Theorin[1], Rose-Marie Jenvert[2], Monica Grekula[2], Anneli Johansson[3]
[1]*SenzaGen AB*
[2]*Veranex Sweden Ab*
[3]*Duearity AB*

Skin sensitizers in medical device extracts are conventionally assessed in vivo, primarily using the Guinea Pig Maximization Test and the Buehler Occluded Patch Test. However, there is a shift in the medical device toxicology field towards an increased use of in vitro methods for the evaluation of the biological safety of medical devices. Recently, in vitro methods for the endpoints skin irritation and skin sensitization have been included in the ISO 10993 standard, what makes it possible to perform this testing in vitro. The GARDskin assay is one of the in vitro methods for assessment of skin sensitization described in ISO 10993-10 and is the first OECD TG 442 method that has been adapted to work with oil, the non-polar extraction vehicle often used in in vivo studies for testing medical devices. Here we share an example of how in vitro testing results, including results from the GARDskin Medical Device assay, were submitted to obtain CE-marking according to the European Medical Device Regulation 2017/745 (MDR) for Tinearity® G1, an innovative tinnitus treatment medical device. Tinearity® G1 was classified as a non-sensitizer in both polar and non-polar extracts in the GARDskin Medical Device assay. This result was used together with in vitro cytotoxicity and in vitro skin irritation results as weight of evidence together with review of chemical data in the risk assessment and biological evaluation of the medical device.

O-11a-3
Developement of a macrophage-based in vitro assay for alternative prediction of foreign body reaction to implantable medical devices
ABSTRACT #49

Tom Meseberg[12], Susanne Kurz[1], Juliane Spohn[1]
[1]*Fraunhofer Institute for Ceramic Technologies and Systems IKTS Material Diagnostics, Dresden, Germany.*
[2]*Institute of Clinical Immunology, Faculty of Medicine, University of Leipzig, Leipzig, Germany.*

Foreign body reaction (FBR), which leads to chronic inflammation and fibrotic encapsulation of implants, poses a challenge in the development of implantable medical devices. Immunomodulatory coatings and materials have been extensively researched to overcome FBR and its consequences. Currently, in vivo animal studies according to ISO 10993-6:2016 are the gold standard for preclinical safety testing of medical devices and the prediction of the FBR, while immunotoxicology testing considerations of ISO/TS 10993-20:2006 are still in state of technical specification. However, ethical concerns about animal studies as well as the lack of specific and standardized in vitro alternatives for the FBR prediction necessitate the consideration of new approaches. Due to the central role of macrophages in the outcome of FBR, these cells are highly studied and represent the key in the development of promising in vitro test strategies. In this project, we propose a standardized in vitro testing system for immunomodulatory materials and coatings. Our approach is based on the integration of a THP-1 macrophage-based model into a novel cell culture interface (»ClicKit-Well«) that allows quantitative comparisons between materials. The main objective of this study is to define key events related to macrophages in the FBR, establish suitable in vitro test scenarios, and specify relevant analytical markers for characterizing the FBR based on an Adverse Outcome Pathway. This concept finds application not only in early-stage assessment of improved implant coatings and materials for various implantable medical devices but has the potential to contribute to the development of next-generation biocompatibility evaluation

standards derived from hypothesis-driven risk assessment approaches.

O-11a-4
Reproductive Toxicity of vaginal lubricants: effects on oocyte maturation, fertilization and sperm viability
ABSTRACT #91

Patrícia Gomes Ruivo[1234], Carlos Gaspar[134], Ana Sofia Oliveira[13], Joana Rolo[13], Sandra Duarte Fonseca Dias[135], Ana Palmeira-de-Oliveira[134], Denise Vaz Oliani[2367], José Martinez-de-Oliveira[31], António Hélio Oliani[2367], Rita Palmeira-de-Oliveira[134]
[1]*CICS-UBI- Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[2]*CHUCB - Centro Hospitalar Universitário Cova da Beira, EPE, Reproductive Medicine Unit, Covilhã, Portugal*
[3]*FCS-UBI- School of Health Sciences, University of Beira Interior*
[4]*Labfit-HPRD - Health Products Research and Development Lda*
[5]*Escola Superior Agrária do Instituto Politécnico de Castelo Branco, Portugal*
[6]*Reproductive and Fetal Medicine Institute of São José do Rio Preto, Brazil*
[7]*School of Medicine, University of São José do Rio Preto, Brazil*

Background and Objectives: Vaginal lubricants are commonly used, including couples that are trying-to-conceive (TTC), to improve comfort during intercourse, overcoming vaginal dryness. The lack of regulation to clearly state their impact in gametes, fertilization or embryo development led the Food and Drug Administration (FDA-USA) to create a new product code (PEB) for these medical devices that aims to avoid lubricants' negative impact on conception for TTC couples. This study aims to evaluate the effects of vaginal lubricants for personal use. Material and Methods: nine marketed lubricants (one with PEB code) were tested regarding key life/reproductive processes that have major impact in fertility and ability to conceive – sperm viability, oocyte maturation and fertilization - using in vitro alternative methods (bovine model for oocyte maturation/fertilization tests; WHO-Guidelines for sperm viability). An assessment of pH and osmolality was performed too. Results: oocyte maturation/fertilization tests both demonstrate that at 1% (v/v), five lubricants showed a significant decrease on percentage of matured/fertilized oocytes and for the other four, two showed a dose-dependent effect at higher concentration (10%v/v). For sperm viability, at 10%(v/v), four have significative effects on motility and lubricant penetration ability, six on sperm morphology, two in sperm vitality and one for DNA fragmentation. Two lubricants showed a dose-dependent effect at lower concentrations (5% and 1%v/v). Testing pH and osmolality, six lubricants are hyperosmolal, one hypoosmolal and only two within the range proposed by WHO advisory note. Discussion and Conclusion: Overall, PEB coded lubricant is the only one that didn't present any significant deleterious effect. This work demonstrate that vaginal lubricants can have a potential negative impact and those who pass through this wider test battery ensure greater safety for TTC couples. Further studies should still be made in order to develop the most effective way to regulate and assure a safety use.

Session 11b: Cardiotoxicity and cardiac efficacy models

Chairs: *Marketa Dvořáková (NIPH Prague, Praha, Czech Republic) & Andreas Stucki (PETA Science Consortium International e.V., Stuttgart, Germany)*

O-11b-1
A weight of evidence approach for assessing cardiotoxicity in a regulatory context integrating New Approach Methodologies (NAMs)
ABSTRACT #20

Georgia Papadimitriou[1], Nektaria Eirini Kompi[1], Nikolaos Avgeros[1], Christina Tsitsimpikou[2], Konstantinos Tsarouhas[3], Dimitrios Kouretas[4], George E. N. Kass[5], Nikolaos Georgiadis[6]
[1]*Department of Biochemistry and Biotechnology, University of Thessaly, Greece*
[2]*Directorate of Energy, Industrial & Chemical Products, General Chemical State Laboratory*

of Greece
[3]*Department of Cardiology, University Hospital of Larissa, Greece*
[4]*Department of Biochemistry and Biotechnology, University of Thessaly*
[5]*European Food Safety Authority, Parma, Italy*
[6]*European Chemicals Agency, Helsinki, Finland*

Background: Human health risks and hazards from chemical substances are well regulated internationally. However, cardiotoxicity, is not defined as a stand-alone hazard and therefore there are no defined criteria for classification of substances as cardiotoxic. In fact, in the last 10 years cardiotoxicity is very rarely recognised on a regulatory level at least in Europe. Identifying and regulating substances, which cause cardiovascular adverse effects would undoubtedly strengthen the national health systems. Methods: To overcome the aforementioned gap, a weight of evidence approach is proposed for identifying regulatory criteria using New Approach Methodologies (NAMs) and data from existing animal studies endorsing them into the legislation in order to classify substances as cardiotoxic. The approach consists of (i) assembling the evidence into lines of evidence of similar type (i.e. cardiac animal models from known cardiotoxicants, Adverse Outcome Pathways (AOPs), In silico data, Omics, Read-Across, QSARs, biochemical and echocardiographic indices and histopathological data) (ii) weighing the evidence using well established scientific methods and (iii) integrating the evidence using statistical analysis and meta-analysis for each line of scientific evidence. Results: Preliminary in depth review analysis indicate a clear distinction from normal values to echocardiographic indices (i.e. left ventricle (LV) fractional shortening (FS) and (LV) ejection fraction (EF)), biochemical indices (e.g. Creatine Kinase-Myocardial Band isoenzyme (CK-MB) and cardiac tissue glutathione (GSH)) and histopathological findings. These findings together with the establishment of NAMs are encouraging and indicate that there is room for targeted research to this end, and that these specific approaches such as AOPs, QSARs, Read-Across, Omics and others should be further investigated in order to be developed to regulatory criteria; Conclusions: Further research should be conducted both from the scientific and regulatory community aiming to clearly define the cardiotoxicity hazard caused by chemicals and develop a full set of scientific criteria based on NAMs.

O-11b-2
SmartHeart - Novel cardiac micro tissues to answer the evolving challenges of drug discovery
ABSTRACT #211

Patricia Davidson[1], Magali Seguret[2], Stijn Robben[1], Charlène Jouve[3], Celine Pereira[3], Cyril Cerveau[1], Maël Le Berre[1], Jean-Sébastien Hulot[2], Rita S. Rodrigues Ribeiro[1]
[1]*4Dcell*
[2]*Université de Paris Cité, PARCC, INSERM*
[3]*PARCC, INSERM*

Clinical trials often fail the efficacy assessment phase, with a success rate of only 21% for cardiovascular drugs. Furthermore, cardiotoxicity is the 3rd leading cause for withdrawal of drug candidates during clinical phase. These concerning statistics emphasize the prevalence and financial toll of ineffective drugs emerging from preclinical animal studies. Despite all the advances made in cardiac bioengineering there are still issues related with physiological relevance, cost and throughput. To tackle this, we developed a novel 3D cardiac model, the SmartHeart (SH), which not only guides the self-assembly and maturation of ring shaped cardiac tissues but also enables precise measurements of several readouts as contraction force and physiological beating parameters in-situ. The technology relies on standard 96-well-plates which are coated with a structured hydrogel consisting of an array of conical-shaped microwells each surrounding a central pillar. Less than 24 hours after cell seeding, a tissue composed of iPSC-derived cardiomyocytes and fibroblasts exhibited rhythmic contractions in this assay. The contractility force, beating rate, and beating amplitude of the tissues were quantified by monitoring the contraction of a centrally positioned pillar with adjustable stiffness. The tissues were also subjected to classic cardiotropic drugs (Verapamil, Isoproterenol, Dofetilide), and their responses were consistent with existing literature. The hydrogel's optical transparency allows compatibility with high-

resolution image-based techniques. This includes the use of fluorescent calcium-sensitive dyes, revealing the intensity increase during contractions. Likewise, cellular spatial organization and intracellular morphology, e.g. cardiomyocyte cytoskeletal fiber elongation and striation, can be visualized using immunofluorescence. The SH assay accelerates the formation and maturation of functional cardiac tissues. Contraction stress increased during the initial week, reaching a plateau on day 7 and maintaining stability thereafter. Thus, this assay efficiently provides several relevant redouts in a single platform, meeting HTS and HCS requirements for drug efficacy and safety assessments.

O-11b-3
Green chemistry, red flags: ensuring cardiovascular safety by performing multiparametric analysis of phytochemicals using hIPSC-CMs-MEA assay.
ABSTRACT #56

Laura-Sophie Frommelt[123], Katarina Mackova[12], Chiara Volani[14], Christoph Voutsinas[5], Claudia Altomare[6], Lucio Barile[6], Marzia De Bortoli[1], Giada Cattelan[17], Johannes Oberzaucher[5], Peter P Pramstaller[1], Serena Zacchigna[2], Alessandra Rossini[1]
[1]*Institute for Biomedicine, Eurac Research, Bolzano, Italy*
[2]*Cardiovascular Biology Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy*
[3]*Department of Life Sciences, University of Trieste, Trieste, Italy*
[4]*The Cell Physiology MiLab, Department of Biosciences, Università degli Studi di Milano, Milano, Italy*
[5]*Department for Health and Assistive Technologies IARA, Fachhochschule Kärnten, Klagenfurt, Austria*
[6]*Cardiovascular Theranostics, Istituto Cardiocentro Ticino, Ente Ospedaliero Cantonale, Lugano, Switzerland*
[7]*Faculty of science and technology, Free University of Bolzano, Bolzano (BZ) Italy*

Background and Objectives: Cardiotoxicity represents a significant challenge in drug development, with adverse cardiac events potentially causing late-stage project terminations or drug withdrawals. Traditional animal-based assays often fall short in predicting human cardiac safety reliably (1). Recent advances, such as using human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in combination with multi-electrode arrays (MEA), provide a more physiologically relevant and sensitive approach to evaluate drug-induced cardiotoxicity at an early stage (2). Here, we assess the effectiveness of the hiPSC-CMs-MEA assay in detecting both acute and chronic electrophysiological, cardiotoxic, and proarrhythmic effects caused by Prestwick's phytochemicals. Material and Methods: Commercial hiPSC-CMs (Ncardia) were seeded in 24-well MEA plates and exposed to a single dose (1µM) of Prestwick's phytochemicals, which includes 320 well-known and novel plant-based compounds. MEA recordings were taken at multiple time points: 30min, 2hrs (acute phase), 24hrs and 48hrs (chronic phase). Various electrophysiological, contractility and viability parameters were measured using the Maestro Edge MEA system (Axion BioSystems). The Toxicological Prioritization Index application was then employed to rank the screened compounds for overall cardiotoxicity (2). Results: The hiPSC-CMs MEA assay effectively identified a range of phytochemical-induced cardiotoxicities, including rhythmic abnormalities (112 compounds), alterations in field potential duration (56 compounds), as well as changes in contractility (29 compounds) and viability parameters (12 compounds). Remarkably, well-known pro-arrhythmic drugs such as quinidine and E-4031, whether included in the library or used as positive controls, consistently achieved high scores in their respective categories, underscoring the assay's accuracy. Discussion and Conclusion: This study highlights the hiPSC-CM-MEA assay's capacity to precisely detect cardiotoxic effects in preclinical studies, encompassing both established cardioactive drugs and novel compounds. The assay is ideally suited for initial high-throughput screening, allowing us to pinpoint the most intriguing compounds for an in-depth analysis utilizing the 3D engineered heart tissue model.

O-11b-4
Chronic Assessment of hERG Ion Channel Trafficking on Repolarisation and Cytotoxicity in iPSC Cardiomyocytes
ABSTRACT #327

Niall Macquaide[12], Taylor Watters[1], Shahrum Ghasemi[1], Lewis Henderson[1], Mark Bryant[1], Godfrey Smith[13]
[1]*Clyde Biosciences Ltd*
[2]*Glasgow Caledonian University*
[3]*University of Glasgow*

Background and Objectives The effects of drugs on iPSC-derived cardiomyocytes (iPSC -CMs) after short-term (<1hr) exposure are well-known 1,2. However, long-term (>4 hr) exposure can also cause toxic actions, such as hERG trafficking problems. These are difficult to detect with in vivo tests, which are costly, complicated and may vary by species. In this study we aim to evaluate an iPSC-CM assay to study the toxic actions of hERG trafficking over a longer period using serum-free solutions. Material and Methods We measured the following functional cellular assays over 72 hours, with 24hr intervals: (i) electrophysiology using optical voltage measurements (ii) contractile kinetics and amplitude using a camera-based algorithm (iii) iPSC -CM monolayer integrity based on assessment of wide field images and (iv) extracellular lactate dehydrogenase (LDH) measurements that assess plasmalemma integrity. We used iCell2 (FujiFilm-CDI) iPSC -CMs, which we plated on a 96well plate and incubated in a serum free media. We performed automated image and signal analysis using a proprietary analysis platform (CellOPTIQ®- Clyde Biosciences). We tested 6 concentrations of Pentamidine and Arsenic Trioxide (ATO) which are both known to affect hERG trafficking and assessed chronic toxicity, electrophysiological, metabolic dysfunction. Results Pentamidine showed obvious prolongation of APD90 in the clinical range (1-3uM) after 72hrs and in 24 hrs at (3-10uM). ATO induced APD prolongation at 1-3uM but shortening at 10uM indicating mixed ion channel trafficking or metabolic effects. Discussion and Conclusion IPSC-CMs can be used to study the medium-long term actions of drugs on the trafficking of hERG and other ion channels with additional toxicity and contractility measurements to allow mechanistic interpretation and prediction of in vivo data. This represents a highly specific system for chronic assessment of human cardiovascular toxicity risk such as hERG trafficking.

Tuesday, 4 June 2024
16:30 - 17:30

Session ESR1: Early Stage Researcher Session 1

Chairs: *Marketa Dvořáková (NIPH Prague, Praha, Czech Republic) & Mohamed F. Abdallah (Ghent University, Ghent, Belgium)*

O-ESR1-1
A new lung-on-chip inhalation platform for toxicity and therapy assessment
ABSTRACT #116

Arunima Sengupta[12], Nuria Roldan[3], Janick Stucki[3], Tobias Krebs[4], Nina Hobi[3], Olivier T. Guenat[1]
[1]*ARTORG Organs-on-Chip Technologies, University of Bern, Switzerland*
[2]*Alexis Technologies, Bern, Switzerland*
[3]*AlveoliX AG, Swiss Organs-on-Chip Innovation, Switzerland*
[4]*Vitrocell Systems GmbH, Germany*

Background: Exposure to toxic inhalants accelerate the development of chronic lung conditions like COPD (emphysema) and fibrosis. Innovative new-approach-methodologies that use human-derived cells and tissue have the potential to overcome the limitations of animal studies in clinical translation. Materials and Methods: We have employed here the new Cloud α AX12 [1] platform which integrates the cutting-edge AXlung-on-chip technology (AX12) [2] with a cloud-based exposure system (Cloud α) to mimic realistic inhalation exposure in the distal lung. A triple co-culture system was established on-chip with immortalized human alveolar epithelial cells (AXiAECs) [3], primary and human-derived macrophages and lung endothelial cells. Results: Exposure to nanoparticles (TiO2 and ZnO) and a toxic aerosolized chemical (PHMG) resulted in

significant cytotoxic effects, including barrier breakdown, increased secretion of reactive oxygen species and elevated gene expression of pro-inflammatory cytokines. Nebulized corticosteroid, fluticasone propionate, could effectively alleviate the inflammation symptoms induced by aerosolized PHMG in the cells-on-chip. Conclusion: The Cloud α AX12 platform thereby offers reproducible conditions and ease of use for inhaled medicine development and hazard assessment as an alternative to animal models in inhalation toxicity and drug efficacy testing, particularly in pre-clinical and precision medicine studies.

O-ESR1-2
Utilizing human induced pluripotent stem cells to enhance in vitro leydig cell models for reproductive health research
ABSTRACT #25

Eliška Řehůřková[1], Eliška Sychrová[1], Jan Raška[2], Iva Sovadinová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

Leydig cells, a crucial component of the testes, play a vital role in sex hormone production (steroidogenesis), driving male phenotype and secondary sexual differentiation. Imbalances in sex hormone levels, especially during the early stages of life, can lead to adverse health consequences later on. Growing concerns have arisen regarding the potential impact of environmental pollutants on male reproductive health via targeting hormone production. However, currently available in vitro steroidogenic models, predominantly based on rodent cancerous cell lines cultured as monolayers, primarily mimic the adult Leydig cell type and do not express the complete range of steroidogenic enzymes. Human induced pluripotent stem cells (hiPSCs)-based models successfully recapitulating Leydig cell development and functionality in vitro could be cutting-edge technologies for reproductive biomedical research and regulatory applications. Yet, these models remain relatively unexplored, with limited availability and utilization. This study aims to provide a comprehensive overview of the current landscape of available in vitro steroidogenic models, highlighting their respective advantages and limitations, especially concerning their relevance to the human context and widespread application. Additionally, we will introduce an innovative approach for developing 2D hiPSCs-based models of Leydig cells that accurately recapitulate developmental stage-specific characteristics and functionality. We will conduct a comparative analysis encompassing morphology, functionality, steroidogenic potential, and human relevance in comparison to commonly used mice Leydig TM3 cells stimulated with a cocktail of several factors enhancing their steroidogenic capacity. The ultimate objective is to construct a sophisticated testicular organoid, building upon the foundation of the established 2D hiPSCs-based model. The collective findings from this research are set to make significant contributions to the development of innovative non-animal testing models and methods for regulatory applications, specifically tailored to assess the impact of endocrine disruptors on male reproductive health. Furthermore, the model's development holds promise for broader applications in biomedicine and pharmacy.

O-ESR1-3
Male reproductive toxicity of real-life PFAS mixtures: an AOP-based investigation
ABSTRACT #36

Jana Navrátilová[1], Eliška Sychrová[1], Jiří Kalina[1], Petr Šenk[1], Michal Ješeta[2], Iva Sovadinová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine Masaryk University and University Hospital Brno, Brno, Czech Republic*

Per- and polyfluoroalkyl substances (PFAS) represent a diverse category of organic compounds with extensive applications, ranging from industrial uses to everyday products and fire-suppressing foams. However, their widespread use is counterbalanced by

their persistence in the environment and the associated health risks. Our research focused on assessing the potential male reproductive toxicity of three real-life PFAS mixtures containing seven PFAS. These mixtures mirror the PFAS compositions and concentrations detected in the seminal plasma of 47 firefighters, a population at risk due to occupational exposure. The mixtures represent a "median mixture" and two extreme compositions, with a focus on PFOS or PFOA. Using the Adverse Outcome Pathway (AOP) framework, we investigated how these PFAS mixtures directly affect the functionality of critical somatic testicular cell types, Leydig and Sertoli cells, with a primary focus on male reproductive health. Our study targeted specific molecular initiating/key events (MIE/KE), including interactions with human receptors responsible for androgens, glucocorticoids, estrogens, thyroid hormones, and peroxisome proliferator-activated receptors. These receptors are pivotal MIEs/KEs within AOPs related to Leydig and Sertoli cell functions and, consequently, male reproductive health. We then explored potential subsequent KEs such as cell proliferation, viability, lipid accumulation, steroidogenic pathways, hormone production and the integrity of the blood-testis barrier. These KEs were identified based on recommendations from established AOPs or scientific literature. Our investigations offered insights into the cellular processes influenced by PFAS exposure, confirming proposed AOPs. Our results indicate that the reproductive toxicity of PFAS mixtures may depend on their particular compositions. This study enhances our understanding of the reproductive health risks linked to real-life occupational exposure to PFAS and their potential impacts on male fertility. Acknowledgement: Research is supported by the Marie Skłodowska-Curie grant No. 101003355 and Czech Science Foundation project No. GA22-30004S.

O-ESR1-4
Evaluation of physiological repeated exposure of aluminium in a 3D intestinal tissue model
ABSTRACT #78

Giulia De Negri Atanasio[1], Giorgia Allaria[1], Lorenzo Dondero[1], Francesca Rispo[1], Francesca Tardanico[1], Erica Lertora[1], Katia Cortese[2], Sara Ferrando[1], Francesco Soggia[3], Jan Markus[4], Silvia Letasiova[4], Tommaso Filippini[5], Federica Robino[6], Matteo Zanotti-Russo[6], Elena Grasselli[17]
[1]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa, 16132 Genoa, Italy*
[2]*Cellular Electron Microscopy Laboratory, Department of Experimental Medicine (DIMES), Human Anatomy, University of Genoa, 16132 Genoa, Italy*
[3]*Department of Chemistry and Industrial Chemistry (DCCI), University of Genoa, Via Dodecaneso 31, 16146 Genoa, Italy*
[4]*MatTek In Vitro Life Science Laboratories, 82105 Bratislava, Slovakia*
[5]*CREAGEN—Environmental, Genetic and Nutritional Epidemiology Research Center, Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy*
[6]*Angel Consulting Via San Senatore 14, 20122 Milano, Italy*
[7]*Centro 3R, Interuniversity Centre for the Promotion of 3Rs Principles in Teaching and Research, Italy*

Background and Objectives One of the most prevalent elements in the crust of the Earth is aluminium (Al3+)*. The free cation of Al3+ is very physiologically reactive and might disrupt metabolism, perhaps causing gastrointestinal problems and neurotoxicity. Since soil contains a significant quantity of Al3+, the amount that plants absorb depends on how much of it is present there. Humans, indeed, consume Al3+ every day through their food and drink. The total weekly exposure to Al3+ was calculated to be 1 mg/kg body weight. Material and methods This study aimed to assess the impact of repeated exposure to varying concentrations of Al3+ on a 3D intestinal tissue model. Over a 12-day period, different Al3+ concentrations (5, 20, and 50 ppm) were applied on epiIntestinal 3D models (MatTek). The study employed multiple methods to evaluate the effects, including TEER measurements to assess tissue viability, ICP-AES analysis to quantify Al3+ trespass, histological assessment for tissue architecture, evaluation of tissue barrier integrity, transmission electron microscopy (TEM)

analysis of microvilli structure, and mRNA expression analysis (MT1A, MT2A, OCLN, CAT) to investigate oxidative processes at the end of the 12-day exposure period. Results Toxicity was not observed across all tested Al3+ concentrations, as indicated by TEER measurements that showed no significant differences between the treatments and the negative control. These findings were further corroborated through histological examination of the tissues. Discussion and Conclusion Overall, the results of this study suggest that the repeated exposure to Al3+ in the tested concentrations did not lead to significant detrimental effects on the 3D intestinal tissue model. These findings contribute to our understanding of the safety of Al3+ exposure within the context of the gastrointestinal system. Further research may be needed to explore any potential long-term effects and to understand the possible implications for human health.

Tuesday, 4 June 2024
17:30 - 19:30

Session ESR2: Early Stage Researcher Session 2

Chairs: *Marketa Dvořáková (NIPH Prague, Praha, Czech Republic) & Mohamed F. Abdallah (Ghent University, Ghent, Belgium)*

O-ESR2-1
A novel way to measure cell viability with electrochemical sensing for in vitro skin testing
ABSTRACT #67

Ignacio Risueño[12], Sandra Navarro[1], Mahmoud Amouzadeh Tabrizi[3], Pablo Acedo[3], Diego Velasco[124]
[1]*Department of Bioengineering, Universidad Carlos III de Madrid (UC3M), Madrid, Spain*
[2]*Fundación Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain*
[3]*Department of Electronic Technology, Universidad Carlos III de Madrid (UC3M), Madrid, Spain*
[4]*Instituto De Investigación Sanitaria Gregorio Marañón, Madrid, Spain*

During the last few years, extended efforts have been made to assess the toxicity of chemicals for skin applications. Cell viability is a fundamental parameter in biological research for preclinical testing. The use of MTT dye, as explained in the OCDE Test Guideline 439 [1], details the use of this tetrazolium salt as a valid measurement for testing skin irritation in reconstructed human epidermis. In this guideline, it is stated that this method could present several interference problems due to the nature of the test chemical. One of these measurement issues could be due to optical interference, leading to misestimate viability data. Furthermore, the use of MTS tetrazolium salt, which facilitates the viability test and shortens its time, is not still contemplated in these guidelines. In this work, we present a novel electrochemical analysis approach to measure cell viability based on MTS reduction. The procedure utilizes an electrochemical sensor that allows the measurement of the occurred reduction in the sample when applied to a cell culture, expressed as changes in the current intensity at specific voltages. Experiments using keratinocyte monolayers at different seeding confluences have shown a proper linear correlation between the number of cells and the intensity currents obtained from the sensor (R^2=0.9716). The correlation between these intensities and the colourimetric results obtained with the traditional method also presents a linear tendency (R^2=0.9758). Additionally, this method has shown promising results when estimating the damaging capacity of different chemicals such as isopropanol, potassium hydroxide or sulfuric acid, to reconstructed skin equivalents, following the OCDE guidelines, highlighting the potential of this approach as a reliable and rapid method for assessing cell viability.

O-ESR2-2
Analysis of vascular disruptors in zebrafish embryos - an endpoint of developmental toxicity
ABSTRACT #104

Julia Nöth[1], Paul Michaelis[1], Stefan Scholz[1]
[1]*UFZ*

Inhibition of angiogenesis is an important mode of action for the teratogenic effect of chemicals and drugs. There is a gap in the availability of simple, experimental screening models for the detection of angiogenesis inhibition. The zebrafish embryo represents an alternative test system that offers the complexity of developmental differentiation of an entire organism while allowing for small-scale and high throughput screening. Here we present a novel automated imaging-based method to detect inhibition of angiogenesis in early life stage zebrafish. Video subtraction was used to identify the location and number of functional intersegmental vessels according to the detection of moving blood cells. By exposing embryos to multiple tyrosine kinase inhibitors including SU4312, SU5416, Sorafenib, or PTK787, we confirmed that this method can detect concentration-dependent inhibition of angiogenesis. The new test method showed higher sensitivity, i.e. lower effect concentrations, relative to a fluorescent reporter gene strain (Tg(KDR:EGFP)) exposed to the same tyrosine kinase inhibitors. Additionally, we performed time-resolved transcriptome analyses to identify exposure windows that are sensitive and specific for the detection of angiogenesis disruption and could show, that exposures starting at 24 hpf are suitable to detect this specific teratogenic endpoint.

O-ESR2-3
In vitro biocompatibility assessment of engineered living materials: a high throughput strategy
ABSTRACT #14

Krupansh Desai[12], Shrikrishnan Sankaran[1], Aránzazu del Campo[12]
[1]*INM - Leibniz Institute for New Materials, Campus D2 2, Saarbrücken 66123, Germany*
[2]*Chemistry Department, Saarland University, Saarbrücken 66123, Germany*

Engineered living materials (ELMs) are advanced materials comprising engineered cells entrapped in either self-produced or polymer-based biocompatible matrices. Particularly, ELMs containing drug-eluting bacteria have the potential to revolutionize drug delivery. Their ability to produce drugs in situ eliminates costly post-production steps such as purification. Plus, bacteria can produce drugs on demand, allowing treatments with more complex drug regimes. Until now, most research has focused on the fabrication of these ELM systems and proof-of-concept devices for a variety of biomedical applications [1]. However, in vitro biocompatibility validation of these devices to the host has been widely overlooked. Here, for the first time, we present a 96-well plate-based method to screen ELMs to determine their biocompatibility potential in vitro. With this approach, we were able to encapsulate different strains of engineered bacteria in a model hydrogel (polyvinyl alcohol (PVA)-based) with a core/shell architecture. We showed the proliferation of bacteria in 3D confinement at different timepoints using alamarBlue assay and compared it with their growth in suspension. We could also track the morphology of mCherry-expressing bacteria using fluorescence microscopy. In addition, we studied the toxicity potential of our ELMs to fibroblasts and monocytes. This was quantified by lactate dehydrogenase and alamarBlue assays using culture supernatants of the ELMs. We further investigated the likelihood that our ELMs trigger inflammation. This was achieved by tracking cytokines such as interleukin-6 on monocytes. Our results suggest that the ELMs were cytocompatible and did not trigger strong immune responses. We also demonstrated the versatility of our approach by examining three PVA-based ELM systems embedding either gram-positive bacteria (Corynebacterium glutamicum or Lactiplantibacillus plantarum) or gram-negative bacteria (Escherichia coli). In summary, our work illustrates an easy-to-follow and replicable high-content workflow for determining the in vitro biocompatibility of ELMs, which is crucial for clinical translation.

O-ESR2-4
Successful generation of three-dimensional human precision-cut lung slices for pulmonary fibrosis modeling
ABSTRACT #118

Laurie Perdigon[1], Manon Barthe[1], Jean-Paul Thénot[1], Franck Chiappini[2], Agnes Choppin[2], Hanan Osman-Ponchet[1]
[1]*PKDERM Laboratories, 45 Boulevard Marcel Pagnol, 06130 Grasse – France*

[2]*OTR3, 4 rue Française, 75001 Paris – France*

Occurrence of diffuse alveolar damage is a real storm in the course of pulmonary diseases leading to an acute and/or chronic inflammatory processes with poorly understood etiologies and cellular mechanisms. To address this gap in knowledge, we developed a model of lung fibrosis using human precision-cut lung slices (hPCLS). hPCLS preserve the native 3D structure of the lung and contain all lung cell types, making them an ideal in vitro model for studying lung diseases and testing potential safety, toxicity, and efficacy of promising therapies. We generated hPCLS of 8 mm diameter and 300 µm thickness from surgical lung resections from nine patients using a vibrating microtome. We treated hPCLS with a profibrotic cocktail (PFC) for 5 days to induce fibrogenesis, and added nintedanib, an anti-fibrotic drug, to PFC-treated PCLS for 3 days to inhibit fibrogenesis. We used different methods to study the properties of PCLS, including metabolic activity, cell viability, actin staining, and mRNA expression of fibrotic markers. We obtained hPCLS from 6 of 9 lung resections, with an average of 120 hPCLS per lung (range: 48-190). hPCLS maintained metabolic activity, viability, and actin cytoskeleton for up to 14 days in culture. Treatment with PFC increased mRNA expression of fibrotic markers COL1A1 and FN1 by more than 4-fold, and nintedanib reduced PFC-induced expression of COL1A1 and FN1 by more than 60%, confirming its anti-fibrotic properties. We successfully generated large numbers and repeatable hPCLS that remained viable and structurally intact for up to 14 days in culture conditions. We also used these hPCLS to model pulmonary fibrosis, creating a model that can be used to evaluate new treatments. Generating hPCLS is a challenging task that requires skill and expertise. Therefore, our results demonstrate that hPCLS are a feasible and promising platform for studying lung diseases and developing new therapies.

O-ESR2-5
Toxic effects of metal oxide nanoparticles on healthy and psoriatic-like human epidermal keratinocytes
ABSTRACT #33

Liyi Tan[1], Magdiel Inggrid Setyawati[1], Kee Woei Ng[12]
[1]*School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Ave, 639798, Singapore*
[2]*Nanyang Environment and Water Research Institute, 639798, Singapore*

The use of metal oxide nanoparticles (NPs) in skincare products has drastically increased human skin exposure to these NPs, raising safety concerns. Although the ability of these NPs to penetrate the healthy skin barrier is minimal, in vitro and in vivo studies have shown that metal oxide NPs can induce toxicity in keratinocytes and fibroblasts through direct contact, even at low skin penetration levels. Safety guidelines have been established to restrict NP use in cosmetic products to 25 wt%. Regardless, there is a lack of knowledge on the effects of NPs on common skin disorders such as psoriasis, where barrier impairments and underlying inflammation could increase NP penetration and exacerbate nanotoxicity. In this study, we examined whether psoriatic-like human keratinocytes (Pso-HKs) would exhibit exacerbated toxic responses to metal oxide NPs compared to healthy HKs. ZnO NPs, in comparison to TiO2 and SiO2 NPs, gave rise to the most prominent toxic effects in both cell types by causing a significant decrease in cell viability and upregulation of oxidative stress, consistent with the literature. Exposure to ZnO NPs also activated the inflammasome pathway, with no observable difference between phenotypes. Interestingly, specific to ZnO NP treated Pso-HKs, trappin-2/pre-elafin, a serine protease inhibitor, was non-canonically cleaved in whole cell lysates, suggesting an increase in protease activity. This was coupled with a significant decrease in trappin-2 and non-consistent elevation of elafin levels in the supernatant. These results indicate an imbalance in protease and inhibitor activity. As proteases play a crucial role in desquamation of the epidermis, the drop in trappin-2 levels may serve to intensify serine protease activity and aggravate psoriatic symptoms. Future work focussing on the relationship between ZnO NPs and protease activity would be valuable to fully understand the differential impact of ZnO NPs on healthy and psoriatic keratinocytes.

O-ESR2-6
Development Of Protocol For Evaluation Of Safety Of Medical Devices Intended For Oral Cavity
ABSTRACT #366

Peter Pôbiš[1], Tatiana Milasová[1], Helena Kanďárová[12]
[1]*Center of Experimental Medicine, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia*
[2]*Faculty of Chemical and Food Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovakia*

Medical Devices (MDs) play an important role in a wide spectrum of medical interventions, ranging from simple treatment of wounds to intricate surgical interventions. Their use extends beyond medical facilities, increasingly finding applications in home settings. MDs are being used to cope with degenerative diseases, treat traumatic injuries, or in personal and dental care. With up to 24,000 types[1] recognized, ensuring their safety is an important task addressed by the defined biological evaluation outlined in ISO 10993. With the progress in NAMs, ISO 10993 is progressively integrating NAMs, such as in vitro testing, as exemplified by the successful validation of ISO 10993-23 for sub-cutaneous irritation testing[2,3]. Building on this, we developed an in vitro protocol for testing MDs intended for use in the oral cavity. This protocol utilizes a 3D reconstructed human tissue model that mimics the soft tissues of the oral cavity. In pilot studies, EpiOcular, a non-keratinized model closely resembling the oral cavity's soft tissues was utilized. Various products were tested and categorized into three groups: (I) materials intended for surgical application, (II) sore-throat pills, and (III) oral care products. The tissue responses to the tested materials matched the expected responses, although providing slightly higher sensitivity towards some types of adhesive products. In a further step, we incorporated the EpiOral model to better mimic the target tissues. EpiOral tissues were exposed to the same materials as EpiOcular tissues, and we recorded a nearly identical response. Establishing an in vitro protocol based on reconstructed 3D tissues aims to enable the early detection of medical devices (MDs) that pose health risks, ensuring patient safety. Once validated, this protocol has the potential to replace animal testing, which is still used in the preclinical phases of MD safety assessment. Acknowledgment: The research has been supported by grants Vega 20/0153/20, DS-FR-19-0048, and APVV-19-0591.

Thursday, 6 June 2024
10:00 - 11:00

Session ESR3: Early Stage Researcher Session 3

Chairs: *Andreas Stucki (PETA Science Consortium International e.V., Stuttgart, Germany) & Bambou Tan (L'Oréal, Paris, France)*

O-ESR3-1
Physiological Maps and their Curation Guidelines: paving the way for mechanistic NGRA
ABSTRACT #226

Luiz Ladeira[1], Alessio Gamba[1], Liesbet Geris[123], Bernard Staumont[1]
[1]*Biomechanics Research Unit, GIGA In Silico Medicine, University of Liège*
[2]*Skeletal Biology and Engineering Research Center, KU Leuven*
[3]*Biomechanics Section, Department of Mechanical Engineering, KU Leuven*

Physiological maps (PM) are comprehensive and machine-readable graphical representations of the intricate mechanisms governing organ function. As they represent undisturbed physiology, they are ideal tools to study chemical-induced perturbations, focusing on detailed mechanisms and modes-of-action. Developed within the ONTOX project (Vinken et al., 2021), PMs allow the integration of biological knowledge available in the scientific literature. They have great potential in in silico toxicology, New Approach Methodologies, and digital twin technologies. By integrating data from various sources, these maps can be used to benchmark adverse outcome pathways, identify therapeutic targets, and visualize omics data, facilitating the potential development of personalized medicine and NAMs for next-generation risk assessment (NGRA). In

ONTOX, organ-specific PMs are currently being developed: bile secretion & lipid metabolism (liver), nephron physiology (kidney), neural tube closure (updated version of Heusinkveld et al., 2021) & cognitive function development (brain). To unlock the full potential of PMs, they should adhere to the FAIR principles of Findability, Accessibility, Interoperability, and Reusability. The ONTOX's PMs Curation Guidelines (PMCG) aim to improve the curation efforts and documentation of PMs, assuring quality management in all development phases. The PMs require a collaborative effort from domain experts and biocurators, oriented by specific planning documents, PMCG, and detailed metadata, ensuring compliance with the FAIR principles, facilitating their use by other projects, and guaranteeing interoperability among platforms. The modular structure simplifies the review process, increasing engagement in curation tasks, and facilitates complex data visualization for stakeholders. The architecture provides a framework for integrating data as it becomes available, ensuring that the maps remain up-to-date and accurate. Ultimately, the PMCG resulted in an organized and FAIR-aligned structure. It is expected to benefit the scientific community by improving data management, accessibility, and interoperability, facilitating the integration of different physiological knowledge, and paving the way for a more human-relevant NGRA.

O-ESR3-2
Ontology-based AI-driven innovative approach using DNT NAMs for NGRA
ABSTRACT #218

Eliska Kuchovska[1], Kristina Bartmann[1], Luiz Carlos Maia Ladeira[2], Arif Donmez[1], Lynn-Christin Saborowski[1], Nicolai Gorts[1], Denis Polozij[1], Farina Bendt[3], Mats Schade[1], Georgea Raad[1], Raphaëlle Lesage[4], Alessio Gamba[2], Bernard Staumont[2], Malene Lislien[5], Oddvar Myhre[5], Hubert Dirven[5], Liesbet Geris[246], Ellen Fritsche[173]
[1]*IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany*
[2]*GIGA In Silico Medicine, University of Liège, Belgium*
[3]*DNTOX Gmbh, Düsseldorf, Germany*
[4]*Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*
[5]*Department of Chemical Toxicology, Norwegian Institute of Public Health, Oslo, Norway*
[6]*Biomechanics Section, KU Leuven, Belgium*
[7]*Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany*

The current regulatory guidelines for assessing developmental neurotoxicity (DNT) are inadequate for hazard assessment of the vast array of chemicals in our environment. Additionally, our understanding of human brain development often relies on animal-derived data, which may not accurately represent the human context. Therefore, there is a critical need for more reliable and efficient human-centered new approach methodologies (NAMs), ideally combining in vitro and in silico methods: a strategy applied in the European H2020 ONTOX project [1]. To construct the DNT ontology, a framework for data integration, we first shaped its pillars. The first pillar consists of a comprehensive physiological map of the developing human brain, serving as a foundational knowledge base that provides insights into the biological underpinnings and physiological mechanisms crucial for healthy brain development. The second pillar is a meticulously curated adverse outcome pathway (AOP) network which describes events leading to the adverse outcome of decreased cognitive function and impaired learning and memory. Subsequently, a tailored human in vitro battery was selected and characterized regarding its biological applicability domain to determine its relevance to human physiology and neurodevelopmental disorders. The DNT in vitro NAMs, as well as the physiological map, also serve to derive new AOPs to complete the current AOP network. Furthermore, the DNT ontology and assay characterization contribute to bolstering the regulatory acceptance of in vitro NAMs by reducing their uncertainty. Ultimately, the final iteration of this ontology-based approach, which combines in silico and in vitro NAMs with exposure assessment, will predict the effects of chemicals on the developing human brain without the need for animal testing and advance human risk assessment in line with the principles of next generation risk assessment (NGRA).

O-ESR3-3
Artificial intelligence (AI)-driven morphological assessment of zebrafish embryo for developmental toxicity chemical screening
ABSTRACT #229

Arpit Tandon[1], Brian Howard[1], Adrian Green[1], B. Alex Merrick[2], Keith Shockley[2], Helen Cunny[2], Kristen Ryan[2], Jui-Hua Hsieh[2], Ruchir Shah[1]
[1]*Sciome LLC, Research Triangle Park, NC, United States*
[2]*National Institute of Environmental Health Sciences (NIEHS), Division of Translational Toxicology (DTT), Research Triangle Park, NC, United States*

Evaluating toxicity using the zebrafish embryo developmental toxicity bioassay requires assessing larval morphological changes by experienced screeners using imaging data. The process is time-consuming and prone to subjectivity. However, deep learning models trained using labeled image data can help classify subtle changes in images more efficiently and objectively. We are developing a deep-learning model using labeled image data from Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) to classify over 20 different types of larval morphological changes collected from embryos exposed to various test substances for 5 days. We developed a customized multi-view convolutional neural network (MVCNN) using EfficientNet architecture which uses the distinct dorsal and lateral views of each embryo. For almost all the malformations (8 out of 10), which were annotated by direct visual inspection, our classification models had F1 scores of more than 0.90. For most of the rest of the malformations (8 out of 10) that were annotated according to the presence of statistical outliers of automatically measured features, the model's performance had an F1 score of more than 0.78. Additionally, we are developing a deep-learning segmentation model to identify and quantify larval organ structures: eye, bladder, notochord, yolk, otolith, otic vesicle, brain, pigment, and fish body. We are building segmentation models using UNet++ architecture with encoders from the EfficientNet family. The segmentation models for most organs (6 out of 9) exhibit an Intersection over Union (IoU) score of more than 0.85 on a held-out validation dataset. For two organs, otolith and pigment, the model's IoU score was a bit lower, but still more than 0.75. Together, these models facilitate rapid review of larval morphological changes in images obtained from zebrafish. Such models can provide more efficient and standardized output than manual visual assessment of zebrafish images alone, thereby enhancing data reliability.

O-ESR3-4
A Next Generation Risk Assessment case study for the hair dye HC Yellow 13 focused on liver steatosis
ABSTRACT #160

Sara Sepehri[1], Dinja De Win[1], Anja Heymans[1], Robim Marcelino Rodrigues[1], Tamara Vanhaecke[1]
[1]*Vrije Universiteit Brussels*

Next Generation Risk Assessment (NGRA) deploys New Approach Methodologies (NAMs) to obtain dose-response data, bypassing the need for in vivo animal tests. This modern risk assessment approach holds particular importance for the EU cosmetics industry, where animal testing has been prohibited since March 2013 (1). However, a significant challenge in NGRA is the lack of animal-free models for evaluating repeated dose systemic toxicity effects. In this case study, we exclusively rely on a combined in silico-in vitro approach to assess the safety of the prevalent fluorinated hair dye HC Yellow n° 13 (HCY 13) with a focus on liver steatosis as the adverse outcome. First, the internal liver concentration (Cliver) of HCY 13 has been estimated by employing a Physiologically-based Kinetic (PBK) model, assuming a typical dermal hair dye exposure scenario under non-oxidative conditions to a maximum of 2.5% HCY 13 and a dermal absorption per treatment of 1.82 mg (SCCS/1322/10)(2). Subsequently, four different in silico Quantitative Structure-Activity Relationship (QSAR) tools were used that collectively indicated structural alerts in HCY 13 for systemic liver toxicity. These in silico findings were followed up by in vitro testing of HCY 13 for steatogenic properties using human skin stem cell-derived hepatocyte-like cells (hSKP-HPC). Guided by the Adverse Outcome Pathway (AOP) for liver steatosis, we analyzed

the expression of 10 marker genes associated with lipid metabolism, as well as intracellular triglyceride accumulation, after 72 hours of daily repeated exposure to a range of non-cytotoxic concentrations (3). To establish the in vitro point of departure (PODNAM) for the different steatogenic biomarkers, Benchmark concentration (BMD) modeling was conducted. Finally, the Bioactivity-Exposure Ratio (BER) was calculated as the lowest PODNAM/PBK Cliver, with a BER>1 indicating low risk. Overall, our case study underscores the value of using NAMs to make informed safety assessments.

Tuesday, 4 June 2024
16:30 - 17:30

Session Industry: Industry session – In vitro inhalation toxicity and future trends for Fiber Safety assessment

Chairs: *Arno Gutleb (Invitrolize & Luxembourg Institute of Science and Technology LIST, Luxembourg, Luxembourg) & Samuel Constant (Epithelix, Plan-les-Ouates, Switzerland)*

O-Industry-1
Toxicology of synthetic vitreous fibers (svfs): Historical overview and outlook for next generation new approach methods (NAMs)
ABSTRACT #171

Amy Madl[1]
[1]*Valeo Sciences*

Background and Objectives: In vivo toxicology of synthetic vitreous fibers (SVFs) has been extensive over the last three decades, and has contributed to a well-established understanding that fiber dimension, durability/dissolution, and biopersistence are critical factors for risk of fibrogenesis and carcinogenesis. In the modern era to reduce, refine, and replace animals in toxicology research, the application of in vitro test methods to measure fiber durability or dissolution and predict in vivo biopersistences is paramount for hazard evaluation and designing SVFs for safe use. Material and Methods: This presentation will (1) provide a historical toxicological overview of the animal and in vitro toxicology studies SVFs, (2) discuss key aspects of the historical research, which contributed to the understanding that long durable fibers pose a risk of fibrogenic and tumorigenic responses and not short fibers or long soluble fibers, (3) summarize available in vivo and in vitro test methods for biodurability and biopersistence and thresholds for solubility and lung clearance which are not associated with fibrosis or tumors, and (4) offer recommendations for hazard and toxicological testing of SVFs in the context of the next generation of new approach methods (NAMs). Results: SVFs (fiber lengths >20 µm) with in vitro fiber dissolution rates greater than 100 100 ng/cm2/hr (glass fibers in pH 7 and stone fibers in pH 4.5) and in vivo fiber clearance half-life less than 40 or 50 days were not associated with fibrosis or tumors. Long biodurable and biopersistent fibers exceeding these fiber dissolution and clearance thresholds may pose a risk of fibrosis and cancer. Discussion and Conclusion: In vitro fiber dissolution assays provide a promising avenue to predict in vivo fiber biopersistence, hazard, and health risk.

O-Industry-2
The problem of lung overload in animal studies
ABSTRACT #158

Annette Bitsch[1]
[1]*Fraunhofer Institute for Toxicology and Experimental Medicine*

• Background and Objectives Although the term "lung overload" has a long history, it has recently gained renewed importance due to regulatory decisions and ongoing discussions about human relevance. Several more recent workshops addressed this topic. Lung overload due to physical overload of macrophages leading to loss of motility and impaired elimination of fibrous material was described more than 30 years ago (Morrow, 1988). Key factors for lung exposure to fibres (MMWFs) are dose metrics, geometric features and biopersistence. This will be discussed in the context of toxicological mechanisms and the use of alternative approaches to support toxicological assessments. • Material and

Methods The knowledge about lung overload effects in vivo is compared to data from studies specifically addressing alternative test methods and discussed against the background of regulatory requirements. The focus is on inhalation toxicity but instillation is considered as well. The quality of fibre characterization and information about dose metrics is crucial. • Results Integration of new approaches (in vitro testing and kinetic modelling) will be an important step towards next generation risk assessment in the field of fibre toxicology. So far only a limited number of in vitro studies is available in lung-relevant in vitro systems. The derivation of thresholds for a safe working place is still relying on in vivo studies. However, tools such as adverse effect pathways have already been developed for the assessment of carbon nanofibres. A similar approach could help to overcome the discussions about overload effects and their relevance for humans and reveal the toxicologically relevant properties of fibres. • Discussion and Conclusion One way to incorporate alternative methods in the toxicological evaluation of MMWF and use them to differentiate fibres according to their toxicological properties could be in combining mechanistic in vitro studies with data-driven approaches.

O-Industry-3
Advanced immunocompetent in vitro primary human lung models for point-of-contact toxicity evaluation of Man-Made Vitreous Fibres (MMVF)
ABSTRACT #125

Samuel Constant[1]
[1]*Epithelix*

The main function of the human airway epithelium is to generate sterile atmosphere for the alveolar region where the gas exchange occurs. As first line of defence against airborne pathogens or xenobiotics, the airway epithelium acts not only as key physical barrier endowed with mucociliary clearance and innate host defence mechanisms, but also as an important immunoregulator through production of key messengers and physical interactions with immune cells especially macrophages.(1) We will describe the development and characterization, as well as the use of fully primary human cell based co-culture models made of nasal, tracheal, bronchial, small-airways and alveolar epithelia (MucilAir™, SmallAir™ and AlveolAir™) and alveolar macrophages.(2) Impact of Man-Made Vitreous Fibres (MMVF) on these advanced immunocompetent ALI models will be discussed via evaluation of: (i) local tolerance; (ii) pro-inflammation; (iii) macrophage activity; (iv) ciliopathic effect.

Tuesday, 4 June 2024
17:30 - 19:30

Session PRVS: Peer Review for Validation Studies – building confidence and transparency into a new validation paradigm

Chairs: *Amanda Ulrey (IIVS, Gaithersburg, USA) & João Barroso (JRC, Ispra, Italy)*

O-PRVS-1
The Art of Validation and Peer Review: evolving practices while ensuring scientific confidence
ABSTRACT #157

João Barroso[1]
[1]*European Commission - Joint Research Centre*

Method validation and peer review are indispensable components in the regulatory assessment of chemical hazards and safety. The principles of validation in a regulatory context were first established in the 1990s and gained international recognition with the adoption of OECD Guidance Document No. 34 (GD34) in 2005 [1]. GD34 also specifies that all test methods/approaches should undergo a formal and independent peer review at the end of validation, and prior to submission to regulatory authorities for acceptance. Even if the principles of validation and peer review remain relevant today and the process described in GD34 was successful in pioneering the regulatory acceptance of non-animal methods, an evolution of practices is needed to embrace emerging technologies

(e.g. high throughput, artificial intelligence), the increasing complexity of the information measured (e.g. 'omics), and the need for data integration to address complex endpoints [2]. Moreover, as more validation studies are sponsored and managed by method developers, ensuring data integrity, transparency and independent review of the study is becoming paramount. Raw data and well-documented information supporting the method/approach's fitness for purpose, human biological relevance, and technical characterisation should be accessible for review. Developers should be able to fund but not directly manage an independent review of their methods/approaches. It is also important to guarantee that members of peer review panels have no conflicts of interest in order to guarantee the independence of a review. This talk will give an historical perspective on validation and peer review and will provide insights into potential future directions to keep pace with scientific progress whilst ensuring scientific confidence and the protection of human and environmental health. Future directions in the regulatory context will involve adapting to technological advancements, fostering international collaboration, enhancing data transparency, embracing computational tools, and promoting inclusivity in the validation and peer review processes.

O-PRVS-2
Ensuring Transparency and Rigor in the Peer Review of New Approach Methods for Skin Sensitization Assessment
ABSTRACT #13

G. Frank Gerberick[1]
[1]*GF3 Consultancy, LLC*

Conducting a robust peer review of validation studies is imperative to obtain regulatory acceptance and facilitate the progression toward the development of an OECD Test Guideline. In the current scientific landscape, test developers are seeking innovative approaches to streamline the peer review process, enabling the comprehensive evaluation of methods for their suitability in regulatory contexts. In this particular case study, a test developer sought to identify individuals with expertise in the domain of skin sensitization research, and who were well-versed in the evaluation of New Approach Methods (NAMs). Importantly, it was agreed with the test developer that the panel foster an environment of autonomy and transparency throughout the review process. A fundamental aspect of this collaborative effort was the commitment to providing regular updates to both the test developer and the relevant OECD National Coordinator, who would ultimately receive the comprehensive validation report produced by the expert panel. Ensuring transparency at every stage, each panel member disclosed their Declaration of Interest and Confidential Disclosure agreements, accessible on a publicly available server. Additionally, for panel members receiving financial compensation, their Letters of Engagement were made publicly available. Comprehensive meeting minutes were made readily available to maintain transparency throughout the review process. Upon the formal establishment of the panel and the identification of co-chairs, the panel crafted Evaluation Criteria and embarked on the rigorous peer review process, setting the stage for the continued advancement of innovative approaches in skin sensitization assessment.

O-PRVS-3
Peer Review for Validation Studies - building confidence and transparency into a new validation paradigm- The test developer perspective
ABSTRACT #8

Hervé Groux[1]
[1]*ImmunoSearch Labs, Grasse, France*

To achieve regulatory acceptance and advance the development of an OCDE test guideline for the SENS-IS assay, we established an ad-hoc peer review committee. This presentation aims to offer insights from the test developer's perspective on the comprehensive steps undertaken to ensure a transparent and impartial review process, guaranteeing the submission documents' completeness and accuracy. The development and initial validation of the assay took over a decade. During this period, we blazed trails in various

areas: we introduced the inaugural genomic assay, proposed the first-ever patented assay, pioneered multiple gene expression targets, and provided both hazard and potency data. Given the innovative scope of these undertakings, articulating the extensive gene expression data persuasively posed significant challenges. Our initial step involved a detailed revision of the ECVAM Test Submission Template (TST), accounting for ten years of changes. During this revision, the documents were scrutinized to standardize content, eliminate superfluous explanations, and integrate fresh data. Subsequent to this, an independent and seasoned laboratory rigorously audited the gene expression files. This ensured the precision of the assay's calculations and the consistency of reportings across all associated documents. Following the audit's confirmation, both the data and the updated TST were submitted to the peer review committee for an independent assessment. At the request of the committee, we engaged in meetings chaired by co-leaders to address questions and elucidate various aspects of the assay and the assembled data.

O-PRVS-4
Comprehensive reporting of validation studies starts at inception and ends with peer review
ABSTRACT #85

Sebastian Hoffmann[1]
[1]*seh consulting + services*

Prospective validation remains a long and demanding process - from inception to final peer reviews it takes years, not rarely covering more than a decade. The enthusiasm and eagerness at the beginning of a validation usually fade when first issues and delays are encountered. Problem-solving skills and patience are needed to successfully navigate the first experimental phases to reach the stage of producing the decisive evidence on the reliability and relevance of a test method. Once all data needed for progressing to peer review are available, the next major task is the appropriate documentation of the validation study. Especially for lengthy studies, this can become a challenge, for example as not all decisions and documentation can be traced. This presentation will discuss how to ensure that a validation study can be comprehensively and transparently reported. To be able to readily compile all relevant information and to allow for an efficient peer review process, without delays due to unclear, inconsistent and incomplete documentation, reporting starts at the planning stage. Proper documentation of responsibilities, meetings, study plans, decisions etc. is key to retrace them years later. Guidance and reporting templates are available to help understand the information required for peer review. If accessible, validation expertise and documentation of successfully peer reviewed validation studies can also be of great help. During the conduct of the study, activities related to transferability and revisions of plans and protocols should be completely documented. In addition, a validation-ready data pipeline for the generation of the data informing reliability and relevance should be set-up to transparently record the flow from raw data to data analysis to reported results. The resulting documentation package will not only maximise the change of a smooth peer review but will provide a comprehensible repository for a potential regulatory acceptance process.

O-PRVS-5
Assessing data integrity: a deep dive into data review in method validation
ABSTRACT #10

Amanda Ulrey[1]
[1]*Institute for In Vitro Sciences, Inc*

In the realm of scientific method validation, data review plays a pivotal role in establishing the credibility and reliability of experimental outcomes. This presentation aims to dissect the data review process within the context of a scientific method validation packet. Key components of the data review process used to assess the accuracy, completeness, and integrity of the submitted data are discussed. The test submitter's data is traced, from its capture as raw data on the laboratory equipment, through its presentation in a validation packet. Tools to help in this process, like data flow diagrams, features within data analysis software, audit trails, and metadata are covered. Real-world case studies are presented, offering insights into common

challenges encountered during the data review phase and the strategies employed to address them, where possible. Emphasizing the importance of data governance strategies, especially in the context of multi-laboratory endeavors, the presentation underscores their role in safeguarding the integrity of both present and future laboratory and validation data. These concepts, though derived from Good Laboratory Practices and Good In Vitro Method Practices, extend their relevance to any scientific work environment. Solid data integrity is necessary in assessing a method's validity and a robust data review process is essential to support data integrity.

O-PRVS-6
Peer Review for Validation Studies - building confidence and transparency into a new validation paradigm
ABSTRACT #90

Emily Reinke[1], Sebastian Hoffmann[2], Joao Barroso[3], Amanda Ulrey[4], Hervé Groux[5], Frank Gerberick[6]
[1]*Inotiv*
[2]*seh consulting + services*
[3]*European Commission, Joint Research Centre*
[4]*IIVS, Inc*
[5]*ImmunoSearch*
[6]*GF3 Consultancy, LLC*

Conducting a peer review of a validation study is a key step in gaining regulatory acceptance and moving forward toward development of an OECD test guideline. Historically, these peer review panels have been convened under the purview of the various validation organizations, e.g. ECVAM, NICEATM, or JaCVAM, following a comprehensive, multi-laboratory validation study, typically also managed by the same organizations. However, OECD has called for external support for validation studies, potentially even suggesting that test method developers fund validation studies and subsequent peer review. This introduces a plethora of questions and concerns about how the validation and peer review can be conducted in a sufficiently transparent manner, without concern of conflicts of interest that may bias the outcome. In this workshop, we will use a recently conducted peer review panel that was supported by the test method developer as a case study. We will discuss the steps taken by the peer review panel to ensure independence from the test method developer to foster a transparent and unbiased process, confirmation that data and supporting documentation submission packets were complete and correct, perspectives from the test method developer, and all of the lessons learned throughout the process. The session will conclude with a discussion to allow the audience to provide feedback on what was done and share ideas/suggestions on further improvements to the process. Joao Barroso – History of the validation and peer review process at JRC Frank Gerberick – Ensuring Transparency and Rigor in the Peer Review of New Approach Methods for Skin Sensitization Assessment Sebastian Hoffmann - Comprehensive reporting of validation studies starts at inception And ends with peer review Amanda Ulrey – Assessing Data Integrity Hervé Groux – Perspectives from a Test Method Developer Emily Reinke – Lessons Learned – Peer Review Panels Outside a Validation Oversight Committee

POSTER PRESENTATIONS

ALL POSTER PRESENTATIONS

BIO-ENGINEERING, STEM CELLS AND DISEASE MODELS

P1
Hepatic programming of human skin-derived precursor cells into functional hepatocyte-like cells for in vitro liver research
ABSTRACT #138

Marth Stinckens[1], Matthias Rombaut[2], Alexandra Gatzios[1], Robim Marcelino Rodrigues[1], Tamara Vanhaecke[1], Joery De Kock[2]
[1]*In Vitro Liver Disease Modelling Team, In Vitro Toxicology and Dermato-Cosmetology (IVTD) Research Group, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090, Brussels, Belgium*
[2]*Liver Therapy & Evolution Team, In Vitro Toxicology and Dermato-Cosmetology (IVTD) Research Group, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090, Brussels, Belgium*

Primary human hepatocytes (PHHs) are the current gold standard for in vitro liver research, but their use is hindered by scarce availability and dedifferentiation in culture. Alternatively, human skin-derived precursors (hSKPs) differentiated towards hepatic progenitor-like cells (hSKP-HPCs) serve as a valuable in vitro model for studying drug-induced liver steatosis and metabolic dysfunction-associated steatohepatitis. However, the hepatic phenotype of hSKP-HPCs is immature compared to PHH. Therefore, this study aims to improve the hepatic maturity of these cells by overexpressing hepatogenic programming factors. Hepatogenic programming of undifferentiated hSKPs into hepatocyte-like cells (hSKP-HEPs) was achieved by co-overexpression of three liver-enriched transcription factors (LETFs), namely FOXA3, HNF1α and HNF4α using an all-in-one hepatic programming cassette. The induction of these LETFs was achieved using a CRE/LoxP-based flip excision system. After switching on the hepatic programming cassette in hSKPs, the previously established 24-day hepatic differentiation protocol was used. At day 24, the transcriptomic profile of the obtained hSKP-HEPs was evaluated using microarray and compared to the unswitched hSKP-HPCs (control) and paediatric PHHs. Results were confirmed using RT-qPCR. Microarray analysis showed significant higher levels of multiple hepatic markers in switched hSKP-HEPs when compared to unswitched hSKP-HPCs, including serum markers such as ALB (655-fold), SERPINA1 (88-fold),… and biotransformation markers such as CYP2C9 (20-fold), UGT1A family (107-fold), ABCC2 (11-fold),… The gene expression changes of several of these markers were also verified and confirmed by RT-qPCR i.e. ALB (2246-fold), SERPINA1 (561-fold), CYP2C9 (682-fold), UGT1A9 (4679-fold) and ABCC2 (306-fold). An improved hepatic phenotype was successfully achieved after overexpression of the hepatic programming cassette in hSKPs. Although further characterization at protein and functional levels is still required, our findings provide promising prospects for generating hSKP-HEPs with a superior hepatic phenotype compared to hSKP-HPCs, which would present as a valuable new tool for in vitro liver toxicity studies and disease modelling.

P2
3D bioprinting of human skin and squamous cell tumors as advanced models for precision medicine
ABSTRACT #204

Stefano Lorenzetti[1], Milena Mikhail[1], Laura Di Benedetto[1], Valeria Bartolocci[2], Rosanna Monetta[2], Sofia Raniolo[3], Isabella De Angelis[1], Elena Dellambra[2], Cristina Maria Failla[2], Sara Maria Giannitelli[3]
[1]*ISS - Istituto Superiore di Sanità*
[2]*IDI-IRCCS*
[3]*University Campus Biomedico*

Background and objectives. The BIOSQIN project (https://www.biosqin.org/) intends to develop, validate and disseminate the use, among companies in the pharmaceutical and biomedical sector of a 3D model of human skin and squamous cell carcinoma (cSCC) obtained through bioprinting (3D bioprinting). These models, highly relevant from a physiological

point of view, will be aimed at the identification and characterization of new tumor-specific drugs and the evaluation of their efficacy or toxicity. Materials and methods. As in vitro model systems, two cell lines SCC-12 and SCC-13, identified and phenotypically characterized by Rheinwald and Beckett in 1981, have been used. Both cell lines derive from human squamous cell carcinomas (cSCC) of the facial epidermis: SCC-12 (https://www.cellosaurus.org/CVCL_4026) is from a 60-year-old male who had previously received a kidney transplant and had been treated with immunosuppressive drugs for the previous 7 years, whereas SCC-13 (https://www.cellosaurus.org/CVCL_4029) is from a 56-year-old female subject who had received a series of radiation therapy treatments for the tumor prior to surgical removal. Both cell lines have been treated with inhibitory molecules of the epidermal growth factor (EGF)- or brain-derived neurotrophic factor (BDNF)-underlying pathways, namely PD-0325901, MK-2206 and ANA-12. Results. Data on cell viability/cytotoxicity, apoptosis, necrosis, migration and epitelial.to-mesenchymal transition (EMT) in classical bidimensional cell culture will be presented. Furthermore, data obtained on 3D bioprinted GelMA-based skin scaffolds will be presented considering as main outputs cell viability/cytotoxicity and migration and epitelial.to-mesenchymal transition (EMT).

P3
3D human stem-cell-derived neurons to complement the in vitro neurotoxicity testing for Methylglyoxal
ABSTRACT #257

Uliana De Simone[1], Francesca Caloni[2], Laura Villani[3], Luciana Alessandra Russo[3], Teresa Coccini[1]
[1]*Istituti Clinici Scientifici Maugeri IRCCS SpA-SB, Laboratory of Clinical and Experimental Toxicology, Toxicology Unit, Pavia, Italy*
[2]*Università degli Studi di Milano (ESP)*
[3]*Istituti Clinici Scientifici Maugeri IRCCS, Pathology Unit, Pavia, Italy*

Methylglyoxal (MGO), the most potent glycating agent in humans, mainly generated as a by-product of glycolysis, is associated with age-related diseases including neurodegenerative disorders. In our study, 3D human based stem-cell-derived neuronal spheroids, an in vitro advanced model, was set up in order to evaluate adverse effects after short-term (5 to 48h) exposure to different MGO concentrations (5-500 µM), through different endpoints. MGO, from 5-10 µM, reduced cell growth proliferation and viability, and, from 100 µM, diminished the spheroid compactness, apparently without affecting spheroid size. A loss of the neuronal markers MAP-2 and NSE from 10-50 µM was also observed. The 3D cell culture model of neurons, was useful to study cytochemical alterations, occurring at morphological level and density degree of spheroid, and modifications of cell-cell and cell-ECM interactions as revealed by areas with decreases of cell-matrix components such as collagen and E-cadherin. Effects were induced from 50 µM MGO and more evident at the higher concentrations (300-500 µM). The findings demonstrated that our 3D human based model of spheroids from primary neuron-like cells can be suitable to complement the in vitro neurotoxicity testing strategy for MGO.

P4
IN VITRO 2D AND 3D KIDNEY MODELS TO STUDY THE NEPHROTOXIC RESPONSE OF URANIUM EXPOSURE
ABSTRACT #268

Marie frerejacques[1], Victoria Powell[1], Sebastien Giraud[2], Annabelle Manoury[1], Clémence Finet[1], Céline Bouvier-Capely[1], Clara Steichen[23], Thierry Hauet[234], Yann GUEGUEN[1]
[1]*Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE, SESANE, Fontenay-aux-Roses, F-92262 France.*
[2]*Inserm U1313 – IRMETIST (Ischémie Reperfusion, Métabolisme et Inflammation Stérile en Transplantation), Poitiers, F-860000, France*
[3]*Université de Poitiers, Faculté de médecine et de pharmacie, Poitiers, F-86000, France*
[4]*CHU de Poitiers, service de biochimie, Poitiers, F-86000, France*

As a heavy metal and alpha emitter, uranium presents chemical and radiological toxicity risks, accumulating preferentially in the

kidneys, specifically in proximal convoluted tubules. The biological mechanisms and pathways impacted by uranium exposure can be formulated into an adverse outcome pathway (AOP) (https://aopwiki.org/aops/447), allowing us to identify the different steps and gaps between an initiating event to the kidney toxicity. Using in vitro models of renal proximal tubule epithelial cells (hRPTEC TERT1) or induced kidney organoids (iKO), this study aims to reinforce and contribute to the development of the AOP of kidney toxicity. After identifying the U(VI) concentrations that induce adverse outcomes (apoptosis or necrosis), key events linked to oxidative stress, apoptosis, survival, inflammation, and renal damage are studied at the gene and protein levels. Apoptosis (Casp 3/7, Bax/Bcl2, cytochrome C) is significantly induced starting from exposure to 300 µM for hRPTEC and from 500µM for iKO, and necrosis (LDH) from 500 µM for both models. A time-dependent rise in ROS production and an antioxidant response -increased HO-1, NQO-1, GCLC- are observed in hRPTEC cells. Whereas SOD2, NQO-1 are augmented in iKO but GCLC, CYP2E1 and CAT are diminished in iKO. Uranium concentrations >300µM induced a marked inflammatory response in both models, with increased levels of TNFα, IL-6 and IL-18 in hRPTEC cells and only TNFα in iKO. The study of renal damage markers revealed a slight increase in KIM-1 at 500 µM and a decreased of Collagen IV in both models and an increased level of Calbindin in iKO. In conclusion, this work provides an insight into the key events in the AOP of uranium-induced renal failure featuring a human phenotype of proximal tubule epithelial cells and kidney organoid model, while considering the dose-dependent effect.

P5
DEVELOPMENT OF A NOVEL RIBOSWITCH-BASED CELLULAR MODEL OF TDP-43 PROTEINOPATHY ABSTRACT #304

Vito D'Alessandro[12], Eleonora De Vitis[12], Alessia Foscarini[2], Velia La Pesa[3], Angelo Quattrini[3], Alessandro Romano[3], Sarah Mizielinska[4], Francesca Gervaso[2], Alessandro Polini[2], Giuseppe Gigli[12]

[1]*Dipartimento di Matematica e Fisica E. De Giorgi, Università del Salento, Lecce, Italy*
[2]*Institute of Nanotechnology, CNR Nanotec, Lecce, Italy*
[3]*Division of Neuroscience, Institute of Experimental Neurology, IRCCS San Raffaele, Milan, Italy*
[4]*UK Dementia Research Institute at King's College London, London, UK*

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of motor neurons leading to paralysis and death(1). The underlying causes of ALS remain elusive, but the accumulation of insoluble aggregates of TDP-43 has been proposed to play a critical role(2). The development of effective treatments for ALS requires establishing convenient and reliable in vitro models that faithfully recapitulate the pathological features of the disease. In this context, a number of mammalian neuronal cell types have been used to overexpress the different forms of TDP-43, to investigate the neurotoxicity of its aggregation within the cells. In particular, inducible gene expression systems are preferred over stable expression systems in several applications, including the study of the toxic effects of specific proteins involved in various disorders. Most of these systems use more than one plasmid or large plasmids and exogenous-inducing molecules with potential off-target effects, which can negatively affect their reliability as disease models. Herein, we combine a novel approach that exploits the potentialities of a riboswitch sequence as a post-transcriptional regulator that is sensitive to small, non-toxic and endogenous molecules(3) with the need to regulate TDP-43 overexpression in a controlled manner while minimizing unwanted cellular perturbations. This study addresses the pressing need for improved ALS models and lays the groundwork for more advanced in vitro systems with enhanced translational relevance. By providing a platform for studying TDP-43 proteinopathy in a controlled and easy-to-use manner, our research offers promise for advancing our understanding of ALS-related proteinopathies and facilitating the development of urgently needed therapeutic strategies.

P6
Next -Generation Full-Thickness Human Skin Models Produced using 3D Electrospun Scaffolds and Animal-Component-Free Culture Media
ABSTRACT #312

Patrick Hayden[1], Rayan Hodroj[1], Jayashree Chakravarty[1], Lisa Fitzgerald[1], Jake Pierson[1], Elizabeth Labbe[1], Jacob Le Do[1], Kyle Golden[1], Nathaniel Long[1], Matthew Phaneuf[1]
[1]*BioSurfaces, Inc.*

Background and Objective: In vitro full-thickness skin models commonly utilize animal-derived collagen as a main structural element of the stromal matrix. These constructs suffer from stability and contraction issues, resulting in short lifespan and poor reproducibility. Additionally, culture media utilized to produce these models commonly contain animal-derived components including fetal bovine serum (FBS) and bovine pituitary extract (BPE). To address these shortcomings, we developed full-thickness skin models using electrospun scaffolds as structural components of the stromal constructs, together with FBS/BPE-free culture media. Methods: Polyester (Bio-Spun™-PET) electrospun scaffolds consist of randomly oriented fibers with diameters similar to native collagen fibers. These scaffolds were attached to Transwell® inserts in place of the typical microporous membranes. The scaffolds were seeded with human dermal fibroblasts and cultured under submerged conditions in FBS/BPE-free medium. Human epidermal keratinocytes were then seeded onto the stromal components. The constructs were cultured at the air-liquid interface (ALI) in FBS/BPE-free medium to produce fully developed full-thickness epidermal tissues. Results: Histochemical and immunohistochemical staining revealed fibroblasts and freshly synthesized extracellular matrix throughout the dermal scaffold. H&E sections also showed well-developed stratified epidermis by Day 10 following ALI culture, consisting of basal, spinous, granular and stratum corneum components. A viable epidermal layer with stable thickness was maintained out to at least Day 36 after ALI, providing an extended window of useful experimentation time. Barrier development (TEER) was evident by Day 8 following ALI culture and persisted to Day 36. Conclusions: Next-generation in vitro full-thickness human skin models were produced using animal collagen-free 3D electrospun scaffolds and FBS/BPE-free culture media. The fully human skin models provide long-term stability and do not suffer from contraction and stromal degradation issues. These next-generation full-thickness human skin models offer promise for completely animal-product-free testing of cosmetics and chemicals, screening of new pharmaceuticals and more human-relevant disease modeling.

CARDIOTOXICITY AND CARDIAC EFFICACY MODELS

P7
Assessment of the proarrhythmic effects of repurposed antimalarials for COVID-19 treatment using a comprehensive in vitro proarrhythmia assay (CiPA)
ABSTRACT #281

Seung Hyun Yoon[1], Ki-Suk Kim[1]
[1]*Korea Institute of Toxicology*

Backgroud and Objectives: Due to the outbreak of the SARS-CoV-2 virus, drug repurposing and Emergency Use Authorization have been proposed to treat the coronavirus disease 2019 (COVID-19) during the pandemic. While the efficiency of the drugs has been discussed, it was identified that certain compounds, such as chloroquine and hydroxychloroquine, cause QT interval prolongation and potential cardiotoxic effects. Drug-induced cardiotoxicity and QT prolongation may lead to life-threatening arrhythmias such as torsades de pointes (TdP), a potentially fatal arrhythmic symptom. Here, we evaluated the risk of repurposed pyronaridine or artesunate-mediated cardiac arrhythmias alone and in combination for COVID-19 treatment through in vitro and in silico investigations using the CiPA initiative. Material and Methods: The potential effects of each drug or in combinations on cardiac action potential (AP) and ion channels were explored using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and Chinese hamster ovary (CHO) cells transiently expressing cardiac ion channels (Nav1.5,

Cav1.2, and hERG). We also performed in silico computer simulation using the optimized O'Hara-Rudy human ventricular myocyte model (ORd model) to classify TdP risk. Results: Artesunate and its active metabolite dihydroartemisinin (DHA) were classified as having low TdP risk. Artesunate had no significant impact on hiPSC-CMs' action potential even at 100 times the maximum concentration (Cmax), while DHA only modestly prolonged action potential duration at 90% (APD90) at 100 times Cmax (10.16%). Pyronaridine and the combination therapy were classified as having intermediate TdP risk when tested considering Cmax, but when considering unbound concentrations, the TdP risk was low. Discussion and Conclusion: Pyronaridine, artesunate, and their combination are anticipated to have a low risk of TdP occurrence. The outcomes of these were consistent with clinical results. Therefore, the tool of CiPA Initiative may be suitable for regulatory use and provide novel insights for evaluating drug-induced cardiotoxicity.

P8
INTERPLAY BETWEEN CARDIOMYOCYTES AND CARDIAC FIBROBLASTS IN CARDIOTOXICITY CAUSED BY ANTHRACYCLINES, INVOLVEMENT OF THE ANGIOTENSIN-SIGNALLING PATHWAY
ABSTRACT #310

Ray Alsuhaibani[12], Gavin Richardson[3], Jason Gill[1]
[1]*Transitional and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, United Kingdom*
[2]*Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Kingdom of Saudi Arabia*
[3]*Biosciences Institute, Newcastle University, United Kingdom*

Background and Objectives: Delayed cardiotoxicity is a major clinical issue with anthracyclines and cancer treatment, with the effectiveness of this therapeutics limited by life-threatening heart failure (Hahn et al., 2014). In recent studies, drugs interfering with angiotensin-signalling system have shown promise in the reduction of anthracycline-induced cardiotoxicity (AIC) in the clinic (Cardinale et al., 2006). Unfortunately, the mechanisms underpinning mitigation of AIC by these drugs remains unclear. In this study, we aimed to identify the molecular mechanism of anthracycline induced cardiotoxicity and evaluate the role of angiotensin II signalling in different types of human cardiac cell lines. Method and Result: We have previously shown both angiotensin II stimulation and exposure to sub-therapeutic concentrations of the anthracycline doxorubicin induce cellular hypertrophy in human cardiomyocyte cells in vitro models, an effect associated with a significant upregulation of expression of the angiotensin receptor (AT1R). In contrast, our recent studies have demonstrated that no such morphological changes are observed in primary human cardiac fibroblasts (HCF) evaluated in real-time by xCELLigence cell analysis and fluorescently using green actin tracking staining. However, despite no observable structural change to HCF, exposure to doxorubicin did cause a time and concentration-dependent increase in AT1R expression determined by quantitative real-time PCR. This suggests a potential interplay between these two cell types of the myocardium in AIC, involving crosstalk of the angiotensin-signalling pathway. From a therapeutic perspective, the hypertrophic response of cardiomyocytes was mitigated by pre-exposure to the angiotensin-receptor blocking drug telmisartan, offering an explanation for the cardioprotective effects of blocking angiotensin-signalling in AIC. In addition, AC10 sensitivity to doxorubicin was mitigated by knockdown of AT1R using small interfering RNA. Conclusion: Together these findings support an involvement for angiotensin signalling in drug-induced hypertrophy and subsequent cardiotoxicity, with scope for interaction of this pathway for mitigation of chronic cardiotoxicity in the clinic.

P9
Qualification of a 3D beating heart-on-chip platform for drug-induced cardiac contractility alterations analyses
ABSTRACT #315

Caterina Pernici[1], Marco Mondini[1], Eva Vermersch[2], Ambroise Garry[2], Roberta

Visone[1], Marco Rasponi[3], Paola Occhetta[13]
[1]*BiomimX Srl*
[2]*Global Investigative Toxicology Group Sanofi*
[3]*Politecnico di Milano*

Detecting cardiotoxicity during early stages of drug development process still represents a critical step. Hence, the development of more representative in vitro models able to generate human-relevant data holds great potential. Here we present a human functional 3D cardiac model, named uHeart[1], developed within a beating Organ-on-Chip platform for the quantitative assessment of drug-induced changes in cardiac microtissues contractility. uHeart platform features uBeat® patented technology, an actuating mechanism providing a physiological uniaxial cyclic strain (i.e., 10% stretching, 1 Hz)[2]. Human induced pluripotent stem cell derived cardiomyocytes (h-iPSC-CMs, iCell2) and human cardiac fibroblasts (h-CFs), 75%-25% ratio, were embedded in fibrin hydrogel (125*106 cells/ml) and cultured for up to 7 days. Upon achievement of functional microtissues synchronously and spontaneously beating, 20 compounds affecting different variables (e.g., Calcium homeostasis, Sodium channel inhibition, sarcomere) were selected to qualify the model. Drug-induced alterations were evaluated at incremental doses by analysing contractility parameters form video analysis by means of MuscleMotion (e.g., beating period-BP, contraction time-CT, relaxation time-RT, contraction amplitude-CA, contraction velocity-CV). DMSO was used as vehicle and as negative control. Human cardiac microtissues showed synchronous beating after 6 days of culture. Drug screening campaign evidenced that ORM 10-962 (3 µM, Ca2+ inhibitor) and Levosimendan (3, 30 µM, enhancer of myocytic calcium sensitivity) increased the CA, showing a positive inotropic effect, while Ryanodine (0.3, 3, 30 µM, RyR inhibitor) and Mavacamten (0.3 µM, cardiac myosin inhibitor) reduced the CA, thus eliciting a negative inotropic effect. Ivabradine (0.03, 0.3, 3 µM, If inhibitor) and Mexiletine (10, 100 µM, Na+ channel inhibitor) prolonged the BP, showing a negative chronotropic effect. DMSO (1 µM) did not statistically alter myocardial contraction. uHeart human 3D cardiac in-vitro model was demonstrated able to predict compound specific chronotropic and inotropic effects, thus resulting suitable to detect drug-induced changes in cardiac microtissues contractile behaviour.

CASE STUDIES FOR SUCCESSFUL USE AND IMPLEMENTATION OF COMPLEX IN VITRO MODELS

P10
Decision making in Next Generation Risk Assessment (NGRA) for Skin Sensitisation: How Useful Read-Across Analogue Data Can Be
ABSTRACT #81

Nathalie Alépée[1], Aynur Aptula[2], Dagmar Bury[1], Els DeConinck[3], Johanna Ebmeyer[4], Francoise Gautier[1], Nicola Gilmour[2], Sebastian Hoffmann[5], Petra Kern[3], Jochen Kühnl[4], Cathy Lester[6], Masaaki Miyazawa[7], Kanako Nakayama[7], Hayato Nishida[8], Erwin van Vliet[9], Gang Yan Yan[6]
[1]*L'Oreal R&I, France*
[2]*Unilever, United Kingdom*
[3]*Procter & Gamble Services NV/SA, Belgium*
[4]*Beiersdorf AG, Germany*
[5]*seh consulting + services, Germany*
[6]*The Procter & Gamble Company, USA*
[7]*Kao, Japan*
[8]*Shiseido Global Innovation Center, Japan*
[9]*Innovitox Consulting & Services, The Netherlands*

Next generation risk assessment (NGRA) frameworks have been increasingly utilized in safety assessments. Advancements have been made in the evaluation of skin sensitization hazard and potency by using new approach methodologies (NAM) and Defined Approaches (DA) for decision-making. However, the derivation of a point of departure (PoD) remains a challenge for chemicals for which NAM data and/or DA outputs are associated with uncertainty. To address this limitation, information from read-across can be applied and will be demonstrated here. The present work outlines a case study exploring risk assessment outcomes for two cosmetic consumer exposure scenarios. Anisylalcohol (AA) was selected as ingredient, because of available NAM data and data on metabolism and bioavailability. Its chemical structure was suitable for identifying read-across analogue(s)

with skin sensitization data. Suitable structural analogues were identified using a variety of approaches considering not only structural similarity, but also biological and toxicological features, reactivity, metabolism and expert knowledge. Five analogues were selected by experts, all of which had some historical human or animal data, or some NAM information. The aligned set of analogues was used in combination with NAM data and input from several DA to derive a PoD. As observed in previous case studies, the outputs of DAs differed considerably, which may undermine the confidence in potency predictions for PoD setting. However, some of the DA outputs were consistent with PoDs of the selected analogues which increased confidence in the predicted value. For DAs providing a hazard prediction or a GHS potency category only, the analogues data were utilized in a weight-of-evidence approach to derive a PoD. This case study illustrated how data from analogues either as stand alone or in combination with NAM/ DA information can support PoD derivation. Read-across information can be critical in an NGRA for decision making to overcome high uncertainty.

P11
TOPICAL PROBIOTIC FORMULATION PROMOTES RAPID HEALING IN DOG KERATINOCYTE CELLS: A PROMISING APPROACH FOR WOUND MANAGEMENT
ABSTRACT #117

Manon Barthe[1], Lionel Gillot[2], Laurie Perdigon[1], Aline Jacobs[2], Gregory Schoonbroodt[2], Paul Mauhin[2], Emna Bouhajja[2], Hanan Osman-Ponchet[1]
[1]*PKDERM Laboratories, 45 Boulevard Marcel Pagnol, 06130 Grasse – France*
[2]*Probiotic Group Luxembourg S.A., Research and Development Department, 9944Weiswampach, Luxembourg*

Background and Objectives. The use of probiotics has gained increasing attention as a means of promoting wound healing while decreasing microbial resistance to disinfectants and antibiotics. This study investigated the potential of a non-medicated topical cocktail of probiotic bacteria (CPB) to promote wound healing in dogs using an in vitro scratch assay. Materials and Methods. Canine Progenitor Epidermal Keratinocytes (CPEK) were exposed to three different concentrations of a prototype product containing CPB (PPP), non-formulated CPB, and the vehicle. The viability of CPB and CPEK cells was first evaluated in the co-culture model. Then, cell migration and wound closure were analyzed over time using the Omni Cytosmart automated image analysis platform. Each treatment condition was performed in duplicate and each experiment was repeated twice. Results. The CPB required a minimum concentration of 75 CFU/mL for better viability with CPEK. While the CPEK preserved 100% of their viability when PPP was diluted to up to 75,000 CFU/mL. At higher concentrations, the viability of CPEK was reduced by the concomitant effect of the non-formulated CPB and the vehicle. The formulated and non-formulated CPB and the vehicle induced a dose-dependent increase in cell migration compared to the control. Importantly, at the concentration of 750,000 CFU/mL, the PPP showed a 20% increase in wound closure. indicating a synergistic effect between the CPB and the vehicle. Conclusion. Wound healing is a complex biological process that entails interactions amongst diverse cell types with various events such as hemostasis, inflammation, proliferation, and remodeling. Our investigation focused on evaluating the effects of the probiotic cocktail on CPEK cell migration. Our findings suggest the potential beneficial effects of the probiotic-based topical cocktail (PPP) on wound healing and provide a foundation for future research in this area.

P12
Beneficial effect of hydrolysed marine collagen on physiology of skin models
ABSTRACT #128

Lorenzo Dondero[1], Giulia De Negri Atanasio[1], Erica Lertora[1], Giorgia Allaria[1], Francesca Rispo[1], Francesca Tardanico[1], Federica Robino[2], Elisabetta Perata[2], Raffaella Boggia[3], Federica Turrini[3], Damien Dupin[4], Juliana De Souza Nunes[4], Natividad Diaz[4], Matteo Zanotti-Russo[2], Sara Ferrando[1], Elena Grasselli[15]
[1]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa,*

16132 Genoa, Italy
[2]*ANGEL CONSULTING S.a.s. , Italy*
[3]*Department of Pharmacy (DIFAR), University of Genoa, 16132 Genoa, Italy*
[4]*CIDETEC, Basque Research and Technology Alliance (BRTA), Parque Científico y Tecnológico de Gipuzkoa, Donostia-San Sebastián, Spain*
[5]*Interuniversity Center for the Promotion of 3R Principles in Teaching and Research (Centro 3R), Italy*

Background and objectives Collagen is the most abundant protein in the dermis of the human skin. This is continuously deteriorated by different factors accelerating skin ageing. This work falls within the EcoeFISHent project; the goal is to evaluate the beneficial effect on 3D skin models treated with hydrolysed marine collagen. Material and methods Hydrolysed collagen was characterized through hydroxyproline assay and UV spectroscopy. 3D skin models were used as model for this work; three different percentages (0.05%; 0.06%, 0.1% w/v) of collagen were solubilized in a phosphate-buffered saline solution and used as active ingredient within a cosmetic product. EpiDerm FT were treated with 25 µL of each treatment topically; each tissue was then cultured at 5% CO2, 37 °C for 24 hours. At the end of the experiment tissues were frozen or fixed for mRNA expression (AQP3, COL1A1, COL3A1, ELN) and histological analysis to highlight extracellular matrix components. Results The presence of a 0.06% marine collagen solution leads to a higher expression of both COL3A1 and AQP3 genes. Moreover, when examining COL1A1 gene expression, a notable increase is reported with the introduction of a 0.1% marine collagen solution. Additionally, a 0.05% collagen solution has been found to elevate the expression of the ELN gene. Moreover, the cosmetic product with 0.1% marine collagen exhibits heightened expression levels of COL3A1 and COL1A1 genes. In contrast, cosmetic product with 0.05% marine collagen boosts the expression of the ELN gene, while the cosmetic product incorporating 0.06% marine collagen demonstrates a substantial upregulation of the AQP3 gene. Discussion and conclusion Hydrolysed marine collagen, can increase gene expression on 3D skin models, enhancing anti-wrinkle effect, especially when used within a cosmetic product. This marks the beginning of the validation process for a human cosmetic product formulated with an active ingredient derived from marine fish side streams.

P13
NEW APPROACH METHODS – WHAT DETERMINES VALIDATED? A CASE STUDY USING THE IN VITRO BHAS42 CELL TRANSFORMATION ASSAY FOR CARCINOGENICITY PREDICTION ABSTRACT #176

Darren KIDD[1], Daniel BRAMHAM[1], Robel MEDHANE[1], Joanne LARNER[1]
[1]*Labcorp Early Development Laboratories Ltd, In Vitro Toxicology Group, Harrogate, UK*

The 3Rs were first described in 1959 (Russell and Burch) and within a decade, the in vitro Cell Transformation Assay (CTA) was first developed. Three protocols were developed aiming to replace the Rodent Carcinogenesis Bioassay (RCB). In the 2000s, an Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) was initiated, but limitations in each CTA protocol were noted. Further development ensued during the 2010s enabling publication of OECD Guidance Documents (GD) 214 (2015) and 231 (2016). NAMs span multiple purposes (Discovery, Screening, Hazard Identification, Risk Assessment), each requiring a level of "validation" (Parish et al., 2020) / being "Fit-For-Purpose" (van der Zalm et al, 2022) forcing a review of OECD GD 34 (validation of test methods). The in vitro Bhas42 CTA has recently been included in the ICH S1B(R1) Addendum under a new approach for carcinogenicity testing. Carcinogenicity is a multi-stage process requiring "initiation" and "promotion". The Bhas42 CTA uses a cell line already "initiated" (a by-product from v-Ha-ras gene transfection) meaning a single Promotion Transformation Assay (preceded by an Initial Cell Growth assay for cytotoxicity) is required for the assessment of carcinogenicity potential (OECD GD 231). To cover all purposes, we performed an in-house validation to Good Laboratory Practice (GLP). To increase robustness, we incorporated triplicate Cell Growth Assays and triplicate Transformation Assays due to the small chemical list in OECD

Guidance Document 231. Inclusion of the CTA in ICH S1B(R1) plus the methods being reassessed by OECD for test guideline adoption demonstrates significant regulatory-level interest in this NAM – a trend also seen with skin sensitization (OECD TG 497) and eye damage/irritation (OECD TG 467) in recent years. Acceptance (through confidence in the data) is the goal for all NAMs but confidence (on the scale of suitability / fit-for-purpose / validity) is not a single point.

P14
A BARRIER-ON-CHIP PLATFORM FOR CLINICALLY RELEVENAT SAFETY AND TOXICITY ASSESSMENT: A CASE STUDY OVERVIEW
ABSTRACT #216

Janick Stucki[1], Giulia Raggi[1], Lea Lara de Maddalena[1], Laurène Froment[1], Nicole Albrecher[1], Andreas Hugi[1], Léa Todeschini[1], Nina Hobi[1]
[1]*AlveoliX AG, Swiss Organs-on-Chip Innovation, Bern, Switzerland*

Novel drugs are developed for first-in-human studies or approved drugs are repurposed for new therapies numerously every year. These drugs require safety and efficacy testing for their specific purpose and application route. Traditionally, those tests are done on animal models. However, animal testing is time and cost-consuming, while animal to human translation holds many challenges and leads to a high attrition rate. Therefore, alternatives to animal models, which are reliable, cost-effective, and clinically relevant are needed. The AXBarrier-on-chip system allows the creation of multicellular biomodels suited to assess preclinical parameters such as, tissue integrity, target identification, pharmacodynamics, vascular leak, and MoA. Here we describe the lung and gut biomodel that are qualified for pre-clinical decision-making and IND submissions. Our distal lung models are composed of well-characterized primary alveolar epithelial cells or immortalized alveolar cell lines (AXiAEC). In a primary cell derived fibrosis model, the potency of Nintedanib was shown (gene and protein level). Further, on a primary cell based immune-competent system the safety concern of Proleukin® was confirmed (increased inflammation, immune cell recurrent, barrier disruption). In a third case for the distal lung, the damaging effects of environmental molecules and toxins (CdCl2 and LPS) were shown on a cell-line derived co-culture model (immune cell activation, vascular leak, increase toxicity). To simulate the human gut, a co-culture model (Caco-2/HT29 cells) with peristaltic movement considering the day/night cycle was engineered. The protective effect of the approved drug Azithromycin was shown in terms of barrier integrity (TER, permeability) and cytokine release (IL-8) after a proinflammatory cocktail was used to model chronic inflammatory bowel disease. Models developed with the AXBarrier-on-chip system show translational capability and potential to decrease attrition rate and assist in decision-making within the drug development pipeline finally helping to bring safer drugs faster on the market.

P15
BREAKING BARRIERS: IMPEDANCE-BASED ASSESSMENT OF CELLULAR FUNCTIONALITY IN COPD MIMICS
ABSTRACT #248

Thanh Huyen Phan[1], Huaikai Shi[23], Christopher E. Denes[4], Alexander J. Cole[56], Eline Geervliet[7], Yiwei Wang[28], Yuen Yee Cheng[39], Daniel Hesselson[56], Susan H. Roelofs[7], Graham Gregory Neely[45], Jun-Hyeog Jang[10], Wojciech Chrzanowski[1]
[1]*The University of Sydney, Sydney Nano Institute, Faculty of Medicine and Health, Sydney School of Pharmacy, Pharmacy and Bank Building A15, Camperdown, NSW 2006, Australia.*
[2]*Burns Research and Reconstructive Surgery, ANZAC Research Institute, Concord Hospital, University of Sydney, Sydney, Australia.*
[3]*Asbestos Disease Research Institute, Concord Hospital, Sydney, Australia.*
[4]*The Dr. John and Anne Chong Lab for Functional Genomics, Charles Perkins Centre and School of Life & Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia.*
[5]*Centenary Institute, The University of Sydney, Sydney, NSW 2006, Australia.y Institute, The University of Sydney, Sydney, NSW 2006,*

Australia.
[6]*Faculty of Medicine and Health, The University of Sydney, Sydney, NSW 2006, Australia.*
[7]*Locsense B.V., Locsense B.V., Enschede, The Netherlands.*
[8]*Jiangsu Provincial Engineering Research Centre of TCM External Medication Development and Application, Nanjing University of Chinese Medicine, Nanjing, China.*
[9]*Institute for Biomedical Materials and Devices (IBMD), School of Mathematical and Physical Sciences, Faculty of Science, University of Technology Sydney, Broadway, NSW 2007, Australia.*
[10]*Department of Biochemistry, College of Medicine, Inha University, Incheon 400-712, South Korea.*

Background and purpose: Animal testing is under pressure due to ethical concerns and poor predictability in translation to clinical trials [1]. Especially in lung pathophysiology, animal lungs differ substantially from those in humans [2]. Therefore, developing in vitro alternatives to animal models is one of the priorities in biotechnology. In order to correctly mimic a healthy and or diseased cell, platform functionality testing is necessary. In lung mimics, assessment of barrier functionality is essential [3]. Here we developed a non-invasive, accurate barrier function test using transepithelial electrical resistance (TEER) and impedance measurements. Methods: A complex healthy and chronic obstructive pulmonary disease (COPD) in vitro platform was developed. To determine whether the lung models are reproducible and reliable, we developed a quality control (QC) strategy, which included real-time TEER-based cellular barrier function. Results: We developed reproducible functional healthy and COPD in vitro platforms which were distinguished by different barrier functions. The healthy model is characterized by continuous tight junctions and physiological, differentiated cellular barrier function, while the disease model emulates human COPD disease by dysfunctional cellular barrier function. TEER and impedance spectroscopy successfully analyzed these characteristic differences. Conclusions: Our model can be utilized to test safety, efficacy, and superiority of new therapeutics as well as to test toxicity and injury induced by inhaled pollution or pathogens. TEER/impedance measurement are highly accurate, non-invasive measurement techniques for in vitro cellular barrier function and cellular development testing, which is highly necessary in the discovery of functional respiratory mimics. [1] Van Norman, G, 2019, doi: 10.1016/j.jacbts.2020.03.010 [2] Spits H, 2019, doi: 10.1038/s41587-019-0269-x [3] Chen H, 2014, doi: 10.1371/journal.pone.0101925

P16
A MULTIMODEL WORKFLOW TO TACKLE THE TOXICITY OF FOODBORNE COMPOUNDS AT INTESTINAL LEVEL
ABSTRACT #314

Janice Bergen[123], Francesco Crudo[1], Doris Marko[1], Franz Berthiller[4], Giorgia Del Favero[12]
[1]*Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria*
[2]*Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, Vienna, Austria*
[3]*Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Vienna, Austria*
[4]*Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU), Austria*

Modulation of the plasma membrane leads to modification of cell biophysical properties [1]. Hunting for molecular mechanisms of toxicity that can stem from these events, we started to investigate whether foodborne compounds can exert their activity through this route. Implementing the colon cancer cell line HCT116, the immortalized non-transformed intestinal epithelial cell line HCEC-1CT, and the Caco2/HT29-MTX-E12 human intestinal co-culture, we started to explore effects of food constituents and contaminants on cell motility and mechanotransduction. As test substance the mycotoxin fumonisin B1 (FB1, 10-100 µM) was selected, hence it affects sphingolipid metabolism, and it can contaminate various food commodities [2]. FB1 was compared to the ubiquitous dietary lipid - palmitic acid (PA, 25-100 µM). Aligning with the putative mechanism

of action of FB1, targeted mass spectrometry analysis revealed an increase in the sphinganine to sphingosine ratio. This was measurable also in combination with PA and particularly in the HCT116 cells. In line, membrane fluidity adapted to the presence of the FB1 and PA. When cells were cultivated in the microfluidic system, morphological adaption to shear stress (area spread) significantly changed after exposure to the tested compounds. When expanding the complexity of the model to differentiated Caco2/HT29-MTX-E12 cells, FB1 and PA maintained the capacity to modulate the cell membrane and induced significant changes in the thickness of the cell monolayer. In sum, harmonizing multiple models we could describe molecular mechanisms of toxicity that largely "escape" the evaluation in 2D static monocultures and promise to be relevant for a predictive toxicity profiling of the intestinal compartment.

P17
TAILORMADE SOLUTIONS FOR CHALLENGES IN TESTING SKIN IRRITATION WITH MEDICAL DEVICES USING IN VITRO 3D SKIN MODELS ABSTRACT #326

Ramona Gillere[1], Ulrike Theisen[1], Constantin Brinkmann[1], Helge Gehrke[1]
[1]*Eurofins BioPharma Product Testing Munich GmbH*

Medical devices are used daily, either inside the body or on the body's surface, consequently the safety requirements for medical devices are high. The high number of medical devices and increasing safety standards require a great number of laboratory tests. In vitro tests provide fast results at high through-put without the ethical burdens of using laboratory animals. Animal-free in vitro 3D skin models are routinely used to test liquid and solid substances for skin irritation. Guidelines for using model tissues to test medical device for skin irritation by extraction have been established. However, medical devices come in a great variety of types, shapes, and materials, which can be difficult to extract by standard procedures. Medical devices that are applied to the skin, such as films and patches, can be particularly challenging to extract. Their light material tends to float upon the extraction medium, resulting in only the underside or part of the medical device being extracted. They also often possess adhesive surfaces designed to stick to other surfaces in the presence of water, thus potentially making parts of the medical device inaccessible for extraction. Here we present in two case studies, how tailormade approaches for difficult to extract medical devices can be performed and thus, testing facilitated. Firstly, we will discuss the development of novel strategies (extraction vessel selection, weights, additional control) for the immersive extraction of a floating and sticky-surface medical device. Secondly, we will discuss the advantages of applying a non-sticking body-surface medical device directly to the in vitro 3D skin model, thereby circumventing the extraction step and better mimicking the conditions during usage. These examples and their novel processing methods extend the applicability of 3D in vitro skin models to difficult-to-extract medical devices, reducing the need for animal testing.

P204
EX VIVO EVALUATION OF THE OPHTHALMIC BIODISTRIBUTION OF THE PESTICIDES TEBUCONAZOLE AND PYRACLOSTROBIN ABSTRACT #363

Asley Thalia Medeiros Souza1[1], Leila Bastos Leal[1], Davi Pereira de Santana[1], Laís Matias da Silva[1], Juliana Falcato Vecina[2]
[1]*Federal University of Pernambuco, Pharmaceutical and Cosmetics Development Center (NUDFAC)*
[2]*Mérieux NutriSciences*

The exposure of rural workers to pesticides has been widely reported (FILIPPI et al., 2021). It is noteworthy that the ocular route must be the object of attention because, after substances come into contact with the eyes, ophthalmic permeation can occur through the cornea and conjunctiva/sclera (KANG-MIELER et al., 2014). The objective of this work was to evaluate the ophthalmic biodistribution of Tebuconazole (TBZ) and Pyraclostrobin (PIRA), using bovine and porcine eyes. Dilutions of Rival 200 EC® (Tebuconazole 200g/L) and Comet 250® (Pyraclostrobin 250g/L) were prepared, obtaining for TBZ: 0.2

and 0.4 mg/mL and for PIRA: 0.3 and 0 .5 mg/mL. 30 and 50 µL were applied to porcine and bovine eyes respectively and the concentrated products were also evaluated, and the system was kept in an incubator at 32 ± 1°C and 65% ± 3. At 15, 30, 45, 60, 90 and Aqueous humor was collected at 120 minutes and at time intervals of 15 and 120 minutes the cornea, sclera, iris, vitreous humor and choroid were collected. When applying the lower concentration dilution for TBZ and PIRA, there was a decrease in permeation within one hour, probably due to its elimination from the aqueous humor to the other ocular structures. When the highest dilution concentrations were applied, the opposite was observed, an increase in the total amount permeated, until the end of the study. Regarding biodistribution, for both pesticides tested, in both bovine and porcine eyes, a greater amount of product was found in the cornea, followed by the iris, vitreous humor, choroid and retina. The application of higher concentrations of TBZ and PIRA also resulted in greater retention in all internal structures of the eyes evaluated.

P210
Hepatic 3D cell models for assessment of genotoxic effects induced by BPA, BPC, BPAP and their complex mixtures
ABSTRACT #370

Martina Štampar[1], Tim Ravnjak[12], Alja Štern[1], Bojana Žegura[12]
[1]*Department for genetic toxicology and cancer biology, National Institute of Biology, Slovenia;*
[2]*Jozef Stefan International Postgraduate School, Slovenia*

There is a significant need for reliable research models, techniques and approaches to better assess the safety of newly developed chemicals. In this study we tested bisphenol A (BPA), which has become a major concern due to its harmful effects on human health prompting the increasing use of alternative bisphenols such as BPC and BPAP among many others. Despite the increasing use of alternatives, not much is known about their toxicity especially information on chronic exposure and combined effects is lacking. We investigated the adverse toxic effects of BPA and its analogues BPC and BPAP with emphasis on their (cyto)genotoxic activities after short and prolonged exposure in an in vitro hepatic 3D cell model developed from HepG2 cells. 3-days old spheroids were exposed to BPA, BPC, BPAP and their binary mixtures BPA/BPC and BPA/BPAP for 24 and 96h and then evaluated for cell viability (ATP), DNA damage (comet assay) combined with toxicogenomic analyses. The results showed that all tested compounds did not affect cell viability after 24 and 96 hours of exposure. All BPs and binary mixtures induced DNA single-strand break formation at ≥ 20 µM (24 h) and 4 µM (96 h) for single compounds and ≥ 10 µM (24 h) and 1 µM (96 h) for binary mixtures. The results showed concentration and time-dependent effects, highlighting the problem of long-term and repeated exposure of humans and animals to bisphenols and mixtures, where delayed effects may occur. At mRNA level, bisphenols and binary mixtures deregulated expression of genes encoding phase I (CYP1A1, CYP1A2) and II (UGT1A1, UGT1A2 and NAT2) enzymes and DNA damage-responsive genes (P53, GADD45α, CDKN1A). In conclusion, the study suggests that not all analogues are safer alternatives to BPA. It highlights the importance of studying not only the effects of individual BPA compounds, but also combined exposures.

P214
DEVELOPMENT OF IN-VITRO 3D OSTEOARTHRITIS MODEL FOR DRUG SCREEING
ABSTRACT #375

Eun-Ji Gwak[1], Chaeyeon Lee[1], Hyuna Lee[1], Suhhee Yeom[1], Su-Hyon Lee[1], Jungsun Lee[1]
[1]*R&D Institute, Biosolution Co.,Ltd., Seoul, Republic of Korea*

The prevalence rate of osteoarthritis (OA) is considerably rising up all over the world due to the increase in the population aging and obesity. For these reasons, there is a heightened interest in joint healthy, leading to the development of various related nutritional supplements. Especially, the evaluation of their functionality and efficacy is crucial during the development process. Recently, many studies have been conducted to develop in-vitro models that mimic physiological characteristics

to research this assessment easily. One of them, the in-vitro two-dimensional (2D) OA model, was developed by treating cultured chondrocytes with OA-specific inflammatory cytokines. This model has the advantage: simple, inexpensive, however 2D cultured chondrocytes in-vitro easily turn into dedifferentiation states and cause phenotypic changes. Therefore, it has different physiological conditions in-vivo. In this study, we developed in-vitro three-dimensions (3D) of cartilage and OA using chondrocytes to overcome these limitations. To reconstruct an in-vitro 3D cartilage model, human chondrocytes were expanded up to passage 8. The cells were then cultured in three dimensions on a transwell to induce chondrogenesis. To establish an in-vitro OA 3D model, we treated the 3D cartilage model with IL-1β and TNF-α for 72 hours. The reconstructed 3D cartilage model in-vitro consisted of cells and an extracellular matrix (ECM) similar to the articular cartilage tissue in-vivo. Induced OA model, showed not only ECM degradation, but also increases in the expression of catabolic factors. In the in-vitro OA 3D model treated with therapeutic agents, the recovery of the ECM was observed, and the expression of catabolic factors decreased. This suggested that these in-vitro models will be useful for OA research, such as drug screening.
Key words: 3D model, 3D Osteoarthritis Model, In vitro, Drug screening

Representative tests include the Bovine Corneal Opacity and Permeability (BCOP) Test, which use by-products from abattoirs, and cell-based tests such as the Short Time Exposure (STE) in vitro method, Reconstructed Human Cornea-like Epithelium (RhCE) Test and Vitrigel-Eye Irritancy Test (EIT). These tests were developed to determine the eye irritation potential of chemicals, so there has not been much research on predicting eye irritation of cosmetic products. In this study, we used the Vitrigel-eye irritation test and the STE test, a cell-based test method that does not use by-products from abattoirs, to predict the eye irritation of wash-off products. When evaluated at the concentration specified in the test method, the irritation level was high due to the characteristics of the product type, making it difficult to compare the irritation levels. Therefore, by changing the concentration conditions defined in the test method, we were able to determine the test concentration that being suited the characteristics of the surfactant-containing product type and identify the irritation level between the products. When comparing the results of the two other test methods, we found that the irritation level of the products was similarly predicted. The results of this study show the potential of using both test methods to assess the eye irritation level of products. With more product testing results and additional test methods, we may be able to more accurately predict product irritation levels.

CHALLENGES IN COSMETICS SAFETY

P18
Predicting eye irritation for wash-off products using in vitro testing methods
ABSTRACT #279

Minseok Choi[1], Go Un Kim[1]
[1]*Safety and Microbiology Lab, R&I Center, AMOREPACIFIC, Yongin, Republic of Korea*

In 2013, animal testing of cosmetic products and ingredients was banned in the EU, and in 2017, animal testing was banned in South Korea. As a result, various alternative animal testing methods have been developed and are actively used in the cosmetics industry. Alternative animal testing methods for eye irritation are one of the most studied areas.

P19
Safety Assessments Of Cosmetic Ingredients Exclusively Based On Data From New Approach Methodologies
ABSTRACT #284

Thorsten Diedam[1], Martina Hermann[1], Simone Hoffmann-Doerr[1], Detlef Keller[1]
[1]*Henkel AG & Co. KGaA, Düsseldorf, Germany*

Both the EU ban on animal testing for cosmetic products and ingredients and the increasing social rejection of animal testing require an intensive search for alternative methods for testing and safety assessment of substances. In this work, using hair dyes as an example, it was shown that a safety assessment of cosmetic ingredients after dermal exposure is

basically possible based exclusively on data from New Approach Methodologies (NAMs), i.e. without in vivo test data. Exposure and in vitro dermal absorption data of hair colorants from the assessments of the Scientific Committee on Consumer Safety (SCCS) were used to predict realistic systemic exposure. By using in vitro-in vivo/high-throughput toxicokinetics extrapolation data [1], plasma concentrations could be calculated for the final assessment. In the subsequent safety assessment of the hair dyes, the respective most sensitive in vitro bioactivity assays from US EPA's ToxCast program [2], [3] conservatively served as the starting point for calculating the Margin of Safety (MoS). Based on the calculated MoS, 18 of the 28 hair dyes were identified as safe for specific use as hair dyes. This corresponds to a specificity of 64% compared to the assessments according to SCCS guideline. In addition, using this methodology, a lower MoS was calculated for 26 of the 28 hair dyes (93%). This suggests that this evaluation approach could be more conservative than the conventional evaluation involving in vivo data, even without further uncertainty factors. The proposed methodology still has limitations. There is a lack of sensitivity data, the significance of a positive in vitro result for a specific Adverse Outcome Pathway is often poorly understood and the question of additional assessment factors has not yet been conclusively discussed. Nevertheless, it could be shown that this method represents a promising possibility for cosmetic ingredients assessments exclusively based on data from NAMs.

P20
Read-across supported by new approach methodologies for assessing repeated-dose systemic toxicity : a case study with octisalate
ABSTRACT #294

Ann Detroyer[1], Gladys Ouedraogo[1], Audrey Noel-Voisin[1], Anne Riu[1], Françoise Gautier[1], Sebastien Gregoire[1], Nazanin Golbamaki[1], Anne Moustie[1], Romain Grall[1], Matthew Burbank[1]
[1]*L'Oreal R&I, France*

Read-across is accepted as an approach for assessing systemic toxicity for cosmetic ingredients. A point of departure (POD) can be derived from data-rich analogues. When analogues are data-poor, one possibility, recently described by C. Alexander-White et al., is to fill data gaps with new approach methodologies (NAM's) covering toxicokinetic and toxicodynamic properties. Their 3-tier workflow was applied here in the hypothetical case there is a gap in systemic toxicity data for octisalate used at 5% as sunscreen. In tier-0, analogues were searched by chemical structure and narrowed down based on repeated-dose legacy in vivo data and in silico similarity (i.e. alerts relevant to systemic toxicity, physico-chemical and metabolism simulations). By adding existing absorption, metabolic and ToxCast data, homosalate and cyclohexylsalicylate were retained as the closest analogues with alerts on hepato- and reproductive toxicity. Using the POD of homosalate (LOAEL) or cyclohexylsalicylate (NOAEL) a margin of safety (MOS) of 95 and 857 respectively were calculated considering a 1.4% cutaneous absorption for octisalate. As the MOS using homosalate is < 100, refinement is needed. In tier-1, ADME properties were measured, but also transcriptomics in MCF7 and HepG2 cells, in vitro pharmacology profiling and cell stress panel in HepG2. For tier-2, Endocrine bioactivity was evaluated by transactivation assay with/without metabolic activation and with/without co-culture with reconstructed skin. Overall, similarity with homosalate and cyclohexylsalicylate was confirmed. Next, the ADME properties were incorporated into a human PBK model calculating plasma concentrations (Cmax) for octisalate. Plasma concentrations in rat for homosalate were also calculated using a PBK model with in silico/in vitro input data. A margin of internal exposure (MoIE) was calculated (= Cmax rat/Cmax consumers) which is higher than 25, the threshold protective of human health. Overall, NAMs could be used to fill data gaps in read-across. Case study funded by Cosmetics Europe.

P21
CHEMICAL CHARACTERIZATION, VIABILITY, AND OXIDATIVE DAMAGE PREVENTION IN MACROPHAGES WITH Ocimum gratissimum OIL NANOEMULSION
ABSTRACT #336

Julia Salles Gava[1], Hildegardo Seibert França[23], Iana Soares Pessoa[4], Tamires Cruz dos Santos[4], Marcio Fronza[14]
[1]*Pharmaceutical Sciences Graduate Program, Vila Velha University, Vila Velha, ES, Brazil*
[2]*Federal Institute of Espírito Santo, Vila Velha, ES, Brazil*
[3]*Multiuser Biomolecular Analysis Laboratory, Federal University of Espírito Santo Vitória, ES, Brazil*
[4]*Postgraduate Program in Plant Biotechnology, Vila Velha University, Vila Velha, ES, Brazil*

Background: Essential oils, such as Ocimum gratissimum, are targeted by the pharmaceutical industry due to their antioxidant, anti-inflammatory and antifungal properties. However, its high volatility and low water solubility make it difficult to incorporate into stable formulations. Nanoemulsions effectively encapsulate essential oils, ensuring the delivery of bioactive compounds and increasing bioactivity, preserving their stability and effectiveness. However, studies demonstrate that high concentrations of surfactants in some formulations can be toxic, requiring in vitro toxicity tests to assess their safety. Thus, the present research proposes the development of a nanoemulsion formulation containing Ocimum gratissimum essential oil (NEO), and evaluate the viability and oxidative damage prevention in macrophages. Methods: NEO were produced using low energy techniques and was dissolved in distilled water, at a concentration of 10 mg/mL, while the organic phase consisted of EO and Tween 80 (surfactant). Dynamic light scattering analysis (DLS) was employed to determine the nanoemulsion particle size and the Zeta potential value. The viability and its protective effects against oxidative damage by the developed nanoemulsion was assessed by the colorimetric MTT [3-(4,5-dimethylthiazol-2yl)-2,5diphenyltetrazoliumbromide] assay using macrophages RAW 264.7 cells. Results: The formulation had an average diameter of 94.47 nm ± 11 nm. The results revealed that, after 24 hours of cellular exposure to the nanoemulsion at concentrations of 1.000 to 1µg/mL no cytotoxic effects were observed in macrophage cell line. Furthermore, in the same range of concentrations , the nanoemulsion demonstrated a protective effect against oxidative damage induced by hydrogen peroxide (H2O2) at a concentration of 500 µM in RAW 264.7 macrophages, specifically at a concentration of 1.000 µg/mL. The results showed that the nanoemulsion was effective in protecting cells against cytotoxic effects and oxidative damage, standing out as a promising biotechnological formulation for the development of cosmetic products, due to its ability to modulate oxidative processes.

P22
NEXT GENERATION RISK ASSESSMENT (NGRA) FOR SKIN SENSITISATION: A CASE STUDY INCORPORATING READ-ACROSS
ABSTRACT #345

Petra Kern[1], Nathalie Alépée[2], Nora Aptula[3], Dagmar Bury[4], Els DeConinck[5], Johanna Ebmeyer[6], Francoise Gautier[4], Nicola Gilmour[3], Sebastian Hoffmann[7], Jochen Kühnl[6], Cathy Lester[8], Masaaki Miyazawa[9], Kanako Nakayama[9], Hayato Nishida[10], Erwin van Vliet[11], Gang Yan[8]
[1]*Procter & Gamble Services NV/SA, Strombeek-Bever*
[2]*L'Oréal, Aulnaysous-Bois, France*
[3]*Unilever, Bedford, United Kingdom*
[4]*L'Oréal, Clichy, France*
[5]*Procter & Gamble Services NV/SA*
[6]*Beiersdorf AG, Hamburg, Germany*
[7]*seh consulting + services, Paderborn, Germany*
[8]*The Procter & Gamble Company, Mason, OH*
[9]*Kao, Tochigi, Japan*
[10]*Shiseido Global Innovation Center, Takashima, Japan*
[11]*Innovitox Consulting & Services*

Next generation risk assessment (NGRA) frameworks is being introduced in safety assessments of cosmetic consumer products due to the animal testing ban. Substantial advancements have been made in the evaluation of skin sensitization potency by

using new approach methodologies (NAM) and Defined Approaches (DA) for decision-making within a NGRA. However, the derivation of a point of departure (PoD) remains challenging for cases with data limitations and excessive uncertainty. A hypothetical case study is presented on how such limitations can be addressed with read across analogue information, separately or in combination with NAM data. Anisylalcohol (AA) was selected as case study ingredient for exploring the NGRA for a cosmetic consumer exposure scenario, for which NAM data and data on metabolism and bioavailability were available. Its chemical structure was suitable for investigating and identifying read-across analogue(s). Structural analogues were identified using several approaches considering structural similarity, biological/toxicological features, reactivity, metabolism and expert knowledge. Three analogues with some historical human, animal data, or NAM information were selected: Anisaldehyde, Anisyl acetate, and Benzylalcohol. The analogues were used in combination with NAM data and input from several DA to derive a PoD. The outputs of DAs differed considerably, potentially undermining the confidence in potency predictions for PoD setting. However, some of the DA outputs were consistent with PoDs of the selected analogues which increased confidence in the predicted value for AA. For DAs providing a hazard prediction or a GHS potency category only, the analogues data were in a weight-of-evidence approach for PoD derivation. This case study illustrated how data from read-across analogues either as stand alone or in combination with NAM/ DA information can support PoD derivation. Read-across information can be critical in an NGRA for decision making when NAM data and DA outputs are associated with limitations and high uncertainty.

COMPUTATIONAL TOXICOLOGY – IN SILICO MODELLING, READ-ACROSS, ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

P23

A STRUCTURE-BASED VIEW ON THYROID HORMONE HOMEOSTASIS

ABSTRACT #28

Julia Kandler[1]
[1]*University of Vienna*

Neurodevelopmental disorders in children have been reported to become more prevalent in recent years. Pre- and postnatal exposure to environmental chemicals is considered to play a significant role in the pathogenesis. Hence, an urgent need for test methods for uncovering such substances exists. To allow a better understanding and prediction of adverse events without using animal experiments, the adverse outcome pathway (AOP) concept has been established. In this, a molecular initiating event (MIE) in which a stressor interacts with a biomolecule, leads to a series of key events, and results in an adverse outcome, such as the herein-discussed developmental neurotoxicity (DNT). Several reported MIEs leading to DNT are associated with thyroid hormone synthesis, transport, or metabolism. The objective of this study is to create structure-based models of MIEs leading to DNT through disruption of thyroid hormone homeostasis. These MIEs were collected and the data availability for in silico experiments was assessed. Initially, thyroid peroxidase (TPO) inhibition was modelled by the implementation of a virtual screening workflow and docking of a congeneric series of flavonoids. As the next target class, transporters from the solute carrier family, beginning with the sodium-iodide symporter (NIS), were approached. Since only inward open/occluded conformations of NIS are available, outward open homology models were created which were subsequently used for virtual screening of a library of compounds with experimentally determined inhibitory activity on NIS. The virtual screening workflows allowed a ranking of active vs inactive compounds. Furthermore, docking of congeneric compound series demonstrated a reasonable correlation with in vitro results. The correlations between the rankings allowed the establishment of a binding hypothesis for flavonoids in TPO. The created models can be used to elucidate the binding modes of additional compounds and can assist in the prediction of potential inhibitors of thyroid hormone synthesis.

P24
Enriched Systemic Fingerprints for Predicting Drug-Induced Cholestasis
ABSTRACT #39

Palle Helmke[1], Gerhard Ecker[1]
[1]*Department of Pharmaceutical Sciences, University of Vienna, Josef-Holaubek Platz 2, 1090 Vienna, Austria*

The rising importance of data science in Next-Generation Risk Assessment (NGRA) is driven by the increasing volume of data in life sciences. New Approach Methodologies (NAMs) are key, enabling researchers to detect complex patterns and predict toxicity endpoints, such as drug-induced liver injury (DILI). Cholestasis, a non-idiosyncratic (dose-dependent) subtype of DILI, involves liver bile flow impairment. Understanding these conditions is vital for identifying safer drug candidates. This study focuses on characterizing cholestasis by highlighting the role of compound-target and -pathway fingerprints in assessing drug candidates. However, public data on compound-target interactions are still extremely sparse, but could be enriched by respective target prediction tools. The retrieval of compound-interaction profiles was conducted for a cholestasis active/inactive dataset, consisting of 1754 compounds in an open-source KNIME workflow called “Path4Drug” [1]. Compound-target interactions were retrieved through 5 publicly available databases (ChEMBL, Drugbank, IUPHAR/BPS, PharmGKB, TTD) with annotated IC50 values and were enriched by the consensus of target prediction webservices HitPick and SEA. To connect a drug to a systemic effect, the targets were annotated to pathways using Reactome pathway analysis API service. In the following, binary matrices were created for the compound-target and -pathway interactions with 1 indicating an interaction and 0 representing no interaction. These matrices served as input for different machine learning algorithms. Moreover, a feature importance analysis was conducted for a Gradient Boosted Tree model with the combined descriptor set. For the target descriptor set, the highest Matthews Correlation Coefficient of 0.35 was reached with Undersampling in an XGBoost model. The feature importance analysis revealed the Bile Salt Export Pump (BSEP) as top ranked feature. Inhibition of BSEP is known to cause toxic accumulation of bile acids in the liver and is considered one of the primary reasons for cholestasis [2].

P25
Application of induced-fit docking for elucidation of a molecular initiating event at the human cytochrome b-c1 complex
ABSTRACT #75

Karin Grillberger[1], Gerhard F. Ecker[1]
[1]*University of Vienna*

In predictive toxicology, there is a paradigm shift towards applying new approach methods (NAMs) for chemical safety assessment. Amongst those NAMs, in vitro and in silico methods represent useful alternatives to animal testing. The in silico process starts with predicting binding to a respective target protein as the molecular initiating event (MIE), which leads to a cascade of key events, that result in a toxic adverse outcome, such as developmental neurotoxicity. In this study, we apply molecular docking as in silico tool to predict the binding of pesticides and insecticides. This aims to elucidate the crucial protein-ligand interactions that are responsible for triggering the MIE. In this study, the target protein is the mitochondrial cytochrome b-c1 complex. The focus lies on the Qo-binding site, also known as the quinol outer binding site. To generate a suitable docking protocol, we modified the human apo structure of the complex (PDB-ID: 5xte [1]) by rotating the amino acid Glu271 outward. In this way, the structure is more similar to a bovine co-crystallised complex (PDB-IDs: 1sqb [2]). We continued with an induced fit protocol [3], and applied the standard sampling protocol, which generated 80 docking poses for the 12 case study compounds. Subsequently, we used interaction fingerprint clustering to identify the most representative docking poses. These poses were rescored by using 3 different binding energy calculation methods. Finally, an aggregated ranking of different scoring functions identified 6 synthetic strobilurins, that are likely to bind at the mitochondrial complex 3. Furthermore, we could show that tentative uncoupling compounds had worse ranking and

scoring, which indicates that they would have a different MIE. To conclude, this study showed that molecular docking is useful for the elucidation of binding modes and to prioritise molecules that can be further tested in in vitro systems for further analysis.

P26
EVALUATION OF THE HEALING CAPACITY OF AN AMPHIBIAN SKIN PEPTIDE IN TWO-DIMENSIONAL CELLULAR MODELS AND ANALYSIS THROUGH AUTOMATIC SEGMENTATION
ABSTRACT #96

Erica Lertora[1], Lorenzo Dondero[1], Giulia De Negri Atanasio[1], Francesca Tardanico[1], Francesca Rispo[1], Giorgia Allaria[1], Vito Paolo Pastore[2], Alessandro Catenazzi[3], Matteo Zanotti-Russo[4], Elena Grasselli[15]
[1]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa, 16132, Genoa, Italy*
[2]*Department of Informatics, Bioengineering, Robotics and System Engineering (DIBRIS), University of Genoa, 16132, Genoa, Italy*
[3]*Department of Biological Sciences, Florida International University, Miami, FL, USA*
[4]*Angel Consulting, 20122 Milano, Italy*
[5]*Interuniversity Center for the Promotion of 3R Principles in Teaching and Research (Centro 3R), Italy*

Background and Objectives The skin has developed a complicated mechanism, called the Wound Healing (WH) response, to close breaks in its barrier thanks to growth factors and cytokines. Disruption in cellular processes associated with the wound can lead to the development of chronic, non-healing wounds along with healing dysfunction. Chytridiomycosis, a pandemic-infectious disease brought on by the chytrid fungus Batrachochytrium dendrobatidis (Bd), is primarily to blame for the abrupt global decline in amphibian populations. Bd causes ulcers and significantly reduces an individual's fitness, though survival rates vary amongst species. In addition to performing a wide range of physiological functions, the amphibian skin secretes antimicrobial peptides, involved in a variety of biological effects including accelerating the wound healing process and providing protection against UV radiation. Material and Methods Using an in vitro two-dimensional cellular model of WH, this work evaluates the WH effect of an amphibian skin peptide in both its inactive and active forms. Endothelial (HECV) and keratinocyte (HaCaT) cells are used, and IBIDI Culture-Insert 2 Well is employed to standardize the wound size. PHIO Cellwatcher M serves to automatically perform high-quality, objective image and data analysis based on cutting-edge AI, but it can also monitor migration behavior, speed, spatial and temporal distribution of cells. Results Given the evolutionary conservation of wound healing in tetrapods, it is likely that amphibians experience the same responses as those shown by human cells. Automatic Image Processing permits to extract additional valuable data from an image, reducing the time needed per analysis and improving accuracy and precision of area measurements. Discussion and Conclusion The WH capacity of the peptide has been evaluated using Image Processing drastically reducing the time needed to analyze the data. Moreover, the standardization of the wound width makes the process reproducible and reliable.

P27
Data-driven approaches to elucidate toxicological effects at different scales in zebrafish embryos
ABSTRACT #105

Wibke Busch[1], Paul Michaelis[1], Nils Klüver[1]
[1]*UFZ*

In today's society, hundreds of thousands of chemicals are on the market and for many of them, effects on environmental and human health are not completely known. New approaches are needed in toxicology to handle the huge number of novel compounds and speed up chemical risk assessment. One way forward is, to make use of the vast amount of already available data, and integrate and analyze them to gain insights on novel compounds. Here, we present two tools that aim to provide a way to generate data-driven hypotheses and facilitate the process of chemical risk assessment. The toxicogenomic fingerprint browser is an R-Shiny-based

application that displays the effects of chemical perturbation on a self-organizing map of the zebrafish embryo (ZFE) transcriptome. Most recent findings show that clusters and patterns occurring on the map due to visualizing chemical transcriptome perturbances on map can be associated with general chemical stress responses as well as with specific modes of action (MoA). On the way to interconnecting these patterns with morphological features indicative of adverse outcomes, we developed a database (INTOB), a tool to record and store ZFE toxicological observations on the organism scale. While INTOB is still being developed further, data for more than 300 chemicals have already been recorded and preliminary analyses show that MoA assignments due to morphological patterns are possible as well. Our approaches can be applied for read-across analyses in toxicological pharmacological screenings as well as for the generation of hypotheses about the MoAs of chemicals.

P28
ProtoPRED: a fast tool to predict physicochemical, (eco)toxicological and pharmacokinetic properties of chemicals with QSAR models
ABSTRACT #140

Salvador Moncho[1], Laureano E. Carpio[1], Addel Goya[1], Eva Serrano-Candelas[1], Rafael Gozalbes[1], Simon Perera del Rosario[1]
[1]*ProtoQSAR*

The development and optimization of chemicals requires the determination of their relevant properties such as their toxicity and environmental effects, or their pharmacokinetic properties in the case of drugs. Conventionally, the assessment of these parameters implies laboratory assays and animal experimentation, with high costs in terms of time and money. The economic and ethical impact of in vivo research boosted the study and application of New Approach Methodologies (NAMs) to replace or reduce the use of animals. Quantitative Structure-Activity Relationships (QSARs) are mathematical models relating the structure of molecules with a property, through the use of statistical tools and machine-learning algorithms. They can be used to predict properties and biological effects, quickly and at a very low cost in comparison to in vitro and in vivo experiments. Thus, QSAR models are widely applied in different sectors, such as cosmetics and pharmaceutics, to predict a broad spectrum of properties of scientific, industrial and regulatory interest. We have developed a computational platform, ProtoPRED (https://protopred.protoqsar.com), that allows the reliable, easy, fast and user-friendly prediction of a wide range of properties of chemical compounds. ProtoPRED only requires the input of the chemical structure (accepting several formats) and performs predictions in seconds, for one or several substances. The predictions come along with the QMRF and QPRF dossiers that are required to assess their quality and to use them for registration purposes. ProtoPRED includes more than 50 endpoints, grouped in different modules. There are two specific regulation-focused modules (ProtoICH and ProtoREACH) which include models that are linked to the ICH-M7 and REACH regulations, respectively. Other modules are based on the type of endpoints: physicochemical properties (ProtoPHYSCHEM), human toxicity (ProtoTOX), ecotoxicity and environmental fate (ProtoECO) and pharmacokinetics (ProtoADME). In addition, there is a module dedicated to predict the properties of nanomaterials (ProtoNANO).

P29
Bridging AI/ML Advancements with Risk Assessment Needs: A Journey Towards Effective Use and Regulatory Acceptance
ABSTRACT #167

Serhii Kolesnyk[12], Nicolas Roth[12], Angela Bearth[12], Martin Wilks[12]
[1]*Swiss Centre for Applied Human Toxicology, University of Basel, Switzerland*
[2]*Department of Pharmaceutical Sciences, University of Basel, Switzerland*

Toxicology, historically rooted in observational in vivo studies, is experiencing a paradigm shift towards mechanistic approaches embracing in vitro, in silico, advanced high-throughput and data-rich methods. While the rise of Artificial Intelligence (AI) and Machine Learning (ML) is reshaping numerous sectors, it is anticipated

that toxicology and chemical risk assessment (CRA) will benefit from these developments. Within the context of the Partnership for the Assessment of Risks from Chemicals, the present study evaluates the alignment of AI/ML advancements with the risk assessors' needs. Building on structured interviews and a survey with risk assessors, we performed a high-level exploration of the current status and integration of AI/ML into CRA, driven by the need to understand the barriers and drivers that might prevent or facilitate the use of these methods in the daily workflow. While the regulatory landscape often mandates classical in vivo studies, it also sets conditions for the implementation of alternative methods in CRA. Within this context AI/ML approaches present significant potential. The technological landscape maps out the increasing suite of AI/ML tools either applied or applicable for evidence management within CRA; data generation spanning hazard identification, characterization, and exposure assessment; and decision support systems enhanced by AI/ML for refined risk evaluations. The advent of Large Language Models has unlocked the potential to convert unstructured information into structured data primed for AI-based tools. However, applying such tools within CRA presents challenges (e.g., algorithm transparency, data ethics, replicability, trust) and calls for a better understanding of the underlying drivers for the effective uptake of AI/ML methods. In light of our study's findings, three critical paths emerge for successful AI/ML integration into CRA: collaboration between AI specialists and toxicologists; validation of AI/ML models; and education reforms ensuring CRA courses contain technological proficiency while technology courses - a stronger foundation in natural sciences.

P30
In silico profile of 14 microcystins (MCs): data regarding hepatoxicity and carcinogenicity to help prioritize MCs of concern
ABSTRACT #192

Enzo Moreira Silva[12], Cynthia Bomfim Pestana[1], Marize Marzo Solano[2], Philip Marx-Stoelting[2], Daniela Morais Leme[12]
[1]*Department of Genetics, Federal University of Parana, Curitiba, PR, Brazil*
[2]*German Federal Institute for Risk Assessment*

Microcystins (MCs) are cyanobacteria cyclic peptide toxins of seven amino acids. So far, many MCs have been found in the environment and as part of human diet and microcystin-leucine arginine (MC-LR) is the most common and investigated one, with lesser amounts of data available for the others. MC-LR has been classified as possibly carcinogenic to humans (Group 2B, IARC, 2010) based on a mechanism associated with enzymatic inhibition of protein phosphatases (PP) 1 and 2A. The PPs play an important role in apoptosis, mitosis, and DNA damage responses. Thirteen additional MCs have been analyzed and compared with MC-LR using in silico tools. Similarity using the OECD QSAR Toolbox (ver 4.4.1) based on Tanimoto coefficient and PubChem fingerprints was calculated to assess the similarities between MC-LR and all other molecules in the group. Physicochemical properties, ADME properties, and acute oral toxicity were collected when available in the literature. Predictions were conducted in QSAR software databases such as OECD QSAR Toolbox, VEGA, Toxtree and Swiss ADME. Results from similarity analysis yield a score ranging from 0.72 to 1. In vitro assays in the literature confirmed the ability to inhibit dephosphorylation of phosphorylase by PP1 and PP2A for MC-LR, MC-RR, and MC-LA, suggesting the same mechanism of action for these three MCs. Carcinogenicity alerts by ISS and IRFMN models were found for all analyzed molecules, as well as hepatotoxicity alerts using the IRFMN model. However, no genotoxicity alerts were detected, suggesting that possible carcinogenic abilities are related to non-genotoxic carcinogenic mechanisms. Although there is a gap on most MCs, in silico predictions suggest similar activity with MC-LR: enzymatic inhibition of PP1 and PP2 leading to hepatotoxicity and/or carcinogenicity. Possible additive effects and synergistic toxicity of these substances when present together in the environment or in the diet should also be further investigated.

P31
GENOITS: A WEB-BASED TOOL IMPLEMENTING A PREDICTIVE GENOTOXICITY INTEGRATED TESTING STRATEGY USING QSAR-BASED TOOLS.
ABSTRACT #197

José Luis Vallés Pardo[1], Donna S. MacMillan[2], Mar Crespo[3], Salvador Moncho[1], Addel A. Goya-Jorge[1], Eva Serrano-Candelas[1], Rafael Gozalbes[14]
[1]*ProtoQSAR*
[2]*Humane Society International*
[3]*Toxiconsultant S.L.*
[4]*MolDrug AI Systems SL*

To ensure the safety of chemicals and to assess their potential impact on human health and the environment, the EU's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation requires registrants to undertake a battery of in vitro and in vivo tests to fulfil Standard Information Requirements (SIR). These tests raise two main concerns, (1) the economic burden due to the resources needed to perform the complete list of assays, both in materials and time invested, and (2) the ethical considerations of using animals in the in vivo assays. For this reason, nowadays the regulatory agencies encourage registrants to minimize as much as possible the number of experiments performed on animals (3Rs). In this work, we have developed an exclusively computational Integrated Testing Strategy (ITS) to assess the genotoxicity of chemicals by using (QSAR) predictions. A complete battery of QSAR models for predicting gene mutation (in bacteria, in vitro and in vivo) and cytogenotoxicity (in vitro and in vivo) has been developed. This automated workflow is based on the schema proposed by REACH to assess the genotoxicity, and leads the user through a testing strategy, delivering a binary classification result (genotoxic/not genotoxic) as a final output. Additionally, the tool allows the users to work with their own data, filling the gaps with our in-house QSAR models, to obtain a regulation-ready prediction, which provides a significant benefit to the community using REACH and other regulatory guidelines as ICHM7 for assessing mutagenic impurities in pharmaceutical products. This workflow is implemented into the ProtoPRED prediction suite as the GenoITS module (https://protopred.protoqsar.com/).

P32
Improving understanding of toxicologically relevant molecular and cellular targets: The ICCS Higher Tier Evaluation Working Group
ABSTRACT #203

Matthew Burbank[1], Amaia Irizar[2], Predrag Kukic[3], Silja Voß[4], Torben Koenig[5], Gladys Ouedraogo[1], Catherine Mahony[6]
[1]*L'Oréal Research & Innovation, Aulnay-sous-Bois, France*
[2]*The International Fragrance Association (IFRA), Switzerland.*
[3]*Unilever, Colworth Science Park, Bedford, UK*
[4]*Beiersdorf AG, Hamburg, Germany*
[5]*International Collaboration on Cosmetics Safety (ICCS), NJ, USA.*
[6]*Procter & Gamble Technical Centers LTD, Reading, UK*

The higher tier evaluation (HTE) working group of the International Collaboration of Cosmetic Safety (ICCS) is aiming to support safety decision making, helping case study projects understand possible relationships between target hits and adverse outcomes and advising on methodologies that could be used for follow-up testing, to predict the likelihood of adversity. In order to deliver this support, the group seeks to better understand the landscape of targets that matter most for systemic toxicity, both the molecular initiating events as well the key events further downstream in adverse outcome pathways. As a starting point we have undertaken a review of mechanistic and key characteristics literature spanning carcinogenicity, cardiovascular, immune, developmental and reproductive, liver, lung, hematologic and kidney toxicity as well endocrine disruption and from this have sought to surmise the properties of substances that associate with these toxicities as well as the overlap between them and currently available testing approaches. As a next step, a proof of concept using systematic review and large language modelling is being explored to extract an understanding of key events, adverse outcomes, their molecular targets as well chemical stressors from a small number of

carefully chosen public data sources. These potential credible data sources that the HTE WG has already gathered as different possible starting points to build this ICCS project include MedDRA terms, current in vitro pharmacology panel, AOP wiki as well as MeSH ontology. Such endeavours will not only improve our epistemic understanding of the biological coverage but in turn will better inform us on possible gaps and actions that could be taken to fill them with the current toolbox.

P33
The nephron physiological map and associated disease ontologies: developing NAMs for chemical risk assessment in the kidney
ABSTRACT #227

Alessio Gamba[1], Luiz Ladeira[1], Devon A. Barnes[2], Manoe J. Janssen[2], Marc Teunis[3], Rosalinde Masereeuw[2], Liesbet Geris[14], Bernard Staumont[1]
[1]*Biomechanics Research Unit, GIGA In Silico Medicine, University of Liège, Belgium*
[2]*Utrecht Institute for Pharmaceutical Sciences, Div. Pharmacology, Utrecht University, The Netherlands*
[3]*Innovative Testing in Life Sciences & Chemistry, Utrecht University of Applied Sciences, The Netherlands*
[4]*Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

The nephron is a crucial functional unit in the urinary system and performs several vital functions, such as blood filtration to remove waste and maintain fluid and chemical balance in the body, as well as vitamin D activation. Because of its role, it is relevant to consider it carefully in chemical and drug safety assessment. In order to better understand the possible toxicities affecting the nephron, we have developed a systems biology approach based on physiological maps (PMs) and associated ontologies as part of the European ONTOX project [1]. PMs and ontologies offer a detailed and interactive view of cellular and molecular processes associated with specific functions, in order to study human toxicological mechanisms and improve risk assessment without animal testing. To build the PM, we developed a workflow inspired by the Disease Maps Project [2], that includes data extraction from literature and databases (e.g., Reactome, WikiPathways, KEGG) followed by a review process supervised by domain experts. The PM is assembled using the CellDesigner software, visualised on the MINERVA platform [3] and archived on the BioStudies database. The human nephron PM comprises genes, proteins, and metabolites, assembled in pathways involved in urine production and vitamin D metabolism. By integrating additional layers with complementary information (e.g. chemicals, phenotypes) to the PM, we obtained two associated multilayer ontologies to study crystallopathy and tubular necrosis. Being rich in biological mechanisms and related phenotypes, the PM and ontologies can serve as a basis for parallel or subsequent development of novel adverse outcome pathways. Moreover, they can be used for the development of dynamic models that can represent different cellular and tissue level responses to perturbations. In summary, PM and ontologies can offer the possibility to study human toxicities from an innovative perspective, qualitatively and quantitatively improving toxicological predictions.

P34
Ecotoxicological Evaluation of Bisphenol A and alternatives: A Comprehensive in silico Modelling Approach
ABSTRACT #232

Liadys Mora Lagares[1], Marjan Vračko[1]
[1]*National Institute of Chemistry*

Bisphenol A (BPA), a compound widely used in industrial applications, has raised concerns due to its environmental impact. As a key component in the manufacture of polycarbonate plastics and epoxy resins used in many consumer products [1], concerns about potential harm to human health and the environment are unavoidable [2]. This study seeks to address these concerns by evaluating a range of potential BPA alternatives, focusing on their ecotoxicological properties. The research examines 76 bisphenols, including BPA derivatives, using a variety of in silico ecotoxicological models, although it should be noted that these models were not developed

exclusively for this particular class of compounds. Consequently, interpretations should be made with caution. The results of this study highlight specific compounds of potential environmental concern and underscore the need to develop more specific models for BPA alternatives that will allow for more accurate and reliable assessment.

P35
Development of a generic physiologically based kinetic model to compare internal exposure to organophosphate pesticides in rats and humans
ABSTRACT #277

Thijs Moerenhout[1], Jiaqi Chen[1], Hans Bouwmeester[1], Ivonne M.C.M. Rietjens[1], Nynke I. Kramer[1]
[1]*Division of Toxicology, Wageningen University and Research*

Since their introduction in agriculture, the toxicity of organophosphate pesticides has been widely studied in animal models. However, next generation risk assessment (NGRA) intends to maximize the use of non-animal derived data for the hazard and risk assessment of pesticides, using new approach methodologies (NAMs), including in vitro and in silico methods. Therefore, without generating new animal data, a generic in silico physiologically based kinetic (PBK) model for oral organophosphate exposure in rats and humans was developed. This PBK model incorporates quantitative structure property relationships (QSPRs) and absorption, distribution, metabolism and excretion (ADME) properties from in vitro studies for chlorpyrifos, diazinon, fenitrothion, methyl-parathion, ethyl-parathion, chlorfenvinphos, dimethoate, profenofos and their metabolites, and was evaluated using experimental data from rat and human toxicokinetic studies available in literature. Simulated and observed available in vivo blood and plasma concentration-time, and urinary excretion profiles, as well as toxicokinetic parameters were compared. More than 35 exposures in over 20 in vivo studies were simulated. Results indicate that nearly all organophosphate pesticide exposures were well predicted, with 87% and 57% of the predictions for rat falling within a 5 and 2-fold difference from observed in vivo data, respectively. For humans these values were 91% and 43%. While parent compounds and nearly all metabolites were well predicted, metabolites of the di-methyl-organophosphates fenitrothion and methyl-parathion were predicted with a 5 to over 10-fold difference when compared to in vivo data, suggesting additional metabolic pathways for the oxon-metabolites of these pesticides need to be included in the model. It is concluded that the developed generic model adequately predicts blood and plasma concentrations after organophosphate pesticide exposure and may therefore be used in NAM-based safety evaluation of other organophosphates as well. This generic approach could also be applied for risk assessment of other pesticide and chemical classes.

P36
Mapping Physiology As A Basis For Building Disease Ontology Maps
ABSTRACT #324

Bernard Staumont[1], Luiz Ladeira[1], Alessio Gamba[1], Anouk Verhoeven[2], Julen Sanz Serrano[2], Eliška Kuchovská[3], Job Berkhout[4], Devon A. Barnes[5], Manoe J. Janssen[5], Marc Teunis[6], Thomas H. Luechtefeld[7], Thomas Hartung[8], Ramiro Jover[9], Tamara Vanhaecke[2], Ellen Fritsche[10], Harm J. Heusinkveld[4], Aldert Piersma[11], Rosalinde Masereeuw[5], Mathieu Vinken[2], Liesbet Geris[12]
[1]*Biomechanics Research Unit, GIGA In Silico Medicine, University of Liège, Liège, Belgium*
[2]*Department of In Vitro Toxicology and Dermato-cosmetology, Vrije Universiteit Brussel, Brussels, Belgium*
[3]*IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany*
[4]*Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*
[5]*Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands*
[6]*Innovative Testing in Life Sciences & Chemistry, University of Applied Sciences Utrecht, The Netherlands*
[7]*ToxTrack, Baltimore, MD, USA*
[8]*Center for Alternatives to Animal Testing*

(CAAT) - Europe, University of Konstanz, Germany; CAAT, Johns Hopkins University, Baltimore, MD, USA; Johns Hopkins University, Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, MD, USA
[9]*Departamento de Bioquímica y Biología Molecular, Universidad de Valencia, Valencia, Spain; Experimental Hepatology and Liver Transplant Unit, Health Research Institute La Fe, Valencia, Spain; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain*
[10]*IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany; Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany*
[11]*Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands*
[12]*Biomechanics Research Unit, GIGA In Silico Medicine, University of Liège, Liège, Belgium; Skeletal Biology and Engineering Research Center, Department of Development and Regeneration, KU Leuven, Leuven, Belgium; Biomechanics Section, Department of Mechanical Engineering, KU Leuven, Leuven, Belgium*

Physiological Maps (PM) can be defined as comprehensive graphical representations of biological processes and interactions using the standardized Systems Biology Graphical Notation (SBGN). Using the CellDesigner software and the MINERVA platform, PMs allow for describing physiological processes in a mechanistic and modularized manner, enabling the representation of complex interactions, and for integrating and organizing data coming from various sources. Inspired by the efforts of the Disease Maps community [1], organ-specific PMs are being developed as part of the H2020 ONTOX project [2]: bile secretion & lipid metabolism (liver), nephron physiology (kidney), neural tube closure & cognitive function development (developing brain). PMs can be exploited in many possible ways: (omics) data visualization, benchmarking and filling gaps in adverse outcomes pathways (AOPs), identifying new in vitro assays, exploring drug targets and developing in silico models. Importantly, in ONTOX, PMs represent a foundation for the development of (disease) ontology maps that aim to integrate and annotate pathological, toxicological, chemical and kinetic data in addition to physiology. As an example, ontology maps aim to include an SBGN representation of AOP networks that will be fully integrated with the PM (biological layer) and will be usable in other systems biology workflows. Ontology maps will be designed for 6 adverse outcomes (for which the organ-specific PM has been developed): cholestasis and steatosis (liver), tubular necrosis and crystallopathy (kidney), neural tube closure and cognitive function defects (developing brain). PMs and ontology maps are the result of a collaborative effort by domain experts and biocurators, using standardized guidelines for comprehensive annotation and documentation, ensuring compliance with the FAIR principles, including interoperability with different modeling tools and resources. Their development is expected to support and accelerate the generation of new approach methodologies for next-generation risk assessment, only possible through collaboration between the toxicology and systems biology communities.

P37
In silico prediction and real-life exposure to a chemical mixture of pesticides
ABSTRACT #343

Yana Kolianchuk[1], Inna Rashkivska[1], Mojmir Mach[1]
[1]*Centre of Experimental Medicine of the Slovak Academy of Sciences, Bratislava, Slovakia*

In recent years, a growing number of publications have highlighted the adverse effects of anthropogenic contaminants, even at doses considered safe for consumers. These contaminants are compounded by various physical, chemical, and social stressors associated with modern life. Around 5.2 billion units of plant protection products (PPPs) are globally used annually. The term "pesticide" encompasses a range of substances used to prevent or control harmful organisms, including insecticides, fungicides, herbicides, and others. While pesticide operators, farm workers, and

bystanders face the highest exposure, pesticide residues in food and water pose a potential risk to the general population. The following pesticides: boscalid, tebuconazole, acetamiprid, azoxystrobin, captan, chlorantraniliprole, and glyphosate, were found as residues in food samples according to the 2020 EU report on pesticide residues in food [1]. Individual chemical compounds underwent toxicological evaluation using the VEGA platform in silico. Various endpoints were assessed, including mutagenicity, developmental and reproductive toxicity, as well as activity related to estrogen, androgen, thyroid, glucocorticoid, and thyroperoxidase inhibition. The findings revealed that three compounds, namely boscalid, captan, and glyphosate, exhibited mutagenic/genotoxic potential. All compounds demonstrated potential developmental toxicity effects, with boscalid and tebuconazole showing high reliability. Additionally, two compounds, captan and tebuconazole, displayed positive alerts for androgen receptor-mediated effects, while one compound (captan) showed a positive alert for estrogen receptor relative binding affinity, albeit with low reliability. All compounds were negative for thyroid activity and glucocorticoid receptor affinity. The results indicate that some individual pesticides may not adversely affect certain toxic endpoints. However, considering that consumers are exposed to complex chemical mixtures, relying solely on this assumption may not accurately reflect real-life exposure scenarios and could result in an underestimation of safety concerns. Therefore, additional in vitro and/or in vivo studies are necessary to assess the toxic potential of this pesticide mixture comprehensively. This research was funded by the Grant Agency of the Ministry of Education and the Slovak Academy of Sciences (grant No. VEGA 2/0163/24).

P38
In Silico Predictions of Toxicological Effects and Real-Life Exposure Risks of Common Food Additives
ABSTRACT #344

Inna Rashkivska[1], Yana Kolianchuk[1], Mojmir Mach[1]

[1]*Centre of Experimental Medicine of the Slovak Academy of Sciences, Bratislava, Slovakia*

Over the past fifty years, the global food industry has experienced significant expansion and development, leading to a dramatic increase in the use of substances as food enhancement agents. These include additives, flavouring agents, and more. Consequently, individuals of all ages are routinely exposed to these compounds, not only through direct contact with food products but also via the food chain, water consumption, and other indirect environmental sources. Sodium benzoate, calcium diamine tetraacetate (EDTA), ethylparaben, butylparaben, bisphenol A, and aspartame food additives are found in everyday food products as residues, according to the 2020 EU report [1]. For those individual chemical compounds, in silico predictions were made using the VEGA platform. This comprehensive assessment covered various toxicological endpoints, including mutagenicity, developmental and reproductive toxicity, and activity related to estrogen, androgen, thyroid, glucocorticoid, and thyroperoxidase inhibition. The study findings indicate that two compounds, aspartame and bisphenol A, showed mutagenic/genotoxic potential with high reliability, while edetic acid exhibited similar potential but with low reliability. Additionally, all compounds displayed a high risk of developmental and reproductive toxicity. Furthermore, bisphenol A, butylparaben, and ethylparaben showed positive alerts for estrogen receptor relative binding affinity, and Bisphenol A also exhibited positive alerts for androgen receptor-mediated effects with high reliability. However, all compounds were found to be inactive for thyroid activity and glucocorticoid receptor affinity. Notably, in silico prediction for bisphenol A suggested a positive alert for thyroperoxidase inhibitory activity with moderate reliability. The findings suggest that certain individual chemicals may not pose adverse effects on specific toxic endpoints. However, since consumers encounter complex chemical mixtures of food additives, relying solely on this finding might not accurately represent real-life exposure scenarios and could lead to an underestimation of safety concerns. Therefore, additional studies are crucial to comprehensively evaluate the toxic

potential of this combination of food contaminants. This research was funded by the Grant Agency of the Ministry of Education and the Slovak Academy of Sciences (grant No. VEGA 2/0163/24).

P209
Prediction of bird reproduction study endpoints via toxicokinetic-toxicodynamic modelling of in ovo exposure data
ABSTRACT #369

David Heckmann[1], Thomas Martin[2], Barbara Bauer[2], Vanessa Baier[3], Alicia Paini[3], Stephan Schaller[3], Patrick Hubbard[4], Markus Ebeling[1], André Gergs[1]
[1]*Bayer AG, Crop Science Division, Monheim, Germany*
[2]*Rifcon GmbH, Goldbeckstraße 13, 69493 Hirschberg an der Bergstraße, Germany*
[3]*esqLABS GmbH, Am Sportplatz 7, 26683 Saterland, Germany*
[4]*Eurofins EAG Agroscience, LLC, Easton, MD, USA*

Environmental risk assessment (ERA) of pesticides requires the investigation of effects on the growth and reproduction of birds. Toxicokinetic-toxicodynamic (TKTD) models are promising tools to predict these effects mechanistically, thus enabling extrapolations relevant for ERA and the reduction of animal use. We used TKTD models to predict the effects of the fungicide and nematicide fluopyram on offspring in the northern bobwhite quail (Colinus virginianus). To do so, we parametrized a dynamic energy budget (DEB) TKTD model for the embryo stage using data from an egg injection study. We found that data on various endpoints, such as survival, growth, and yolk utilization, were needed to clearly distinguish between the modes of action used in the TKTD model. In order to extrapolate the results of the DEB-TKTD model to the offspring endpoints of a fluopyram quail reproduction study, we developed a physiologically-based toxicokinetic (PBK) model. This PBK model allowed us to describe the exposure of the quail embryos during the quail reproduction study. We then combined the standard DEB model of the bobwhite quail with the TKTD module calibrated to the egg injection studies and used it to predict effects on hatchling and 14-day chick weight based on the exposure predicted by the PBK model. Observed weight reductions, relative to controls, were accurately predicted, thus providing a case study for the combination of in ovo exposure and TKTD modelling in avian ERA.

IN VITRO AND IN SILICO METHODS FOR SAFETY ASSESSMENT OF MEDICAL DEVICES

P39
Exploring the acceptance of non-animal methods within the global regulatory landscape of medical devices
ABSTRACT #29

Charlotte Laupheimer[1], Arne Jaksch[1]
[1]*Department of Biocompatibility and Toxicology, JAKSCH LIFESCIENCE CONSULTING GmbH*

Current global regulations require medical devices to be exempt from unacceptable risks and operate safely according to their purpose. To test the safety of their products, medical device manufacturers are requested to conduct biological safety and toxicological risk assessments following the ISO 10993-x series [1]. Traditionally, the safety of medical devices has been assessed following a standardized battery of in vivo tests requiring many animals. However, the paradigm has changed since the introduction of New Approach Methodologies (NAMs). Currently, the number of alternatives being developed, validated and implemented keeps increasing periodically, enabling the reduction and replacement of in vivo tests. Tiered approaches using in vitro assays have been included in the ISO 10993-x standards to replace, e.g., traditional skin irritation, skin sensitization, and cytotoxicity tests. Moreover, the recently revised ISO 10993-17:2023 standard recommends in-depth literature reviews and leveraging existing data while conducting the hazard characterization of medical devices [2]. It also includes read-across approaches, in silico modeling, and the threshold of toxicological concern approach [2]. The validation of these NAMs sets a precedent

for reducing animals employed in traditional testing. While some progress has been accomplished, global regulatory acceptance remains limited. The present work evaluates the actual regulatory landscape, providing an overview of NAMs acceptance status in the biological safety and toxicological risk assessments of medical devices and their constituents. Tailor-made testing strategies developed and implemented with expert judgment are paramount to assessing medical device safety and making them acceptable to competent authorities. When done smartly and embracing NAMs, regulatory toxicologists can implement the 3Rs, significantly reduce the need for animal testing, and shorten the required assessment time of medical devices for global market submissions.

P40
New Approach Methods for Challenging Toxicological Risk Assessments of Medical Devices: A Case Study of Phenyl Propyl Carbonate
ABSTRACT #30

Charlotte Laupheimer[1], Shaheena Parween[1], Yana Kolianchuk[1], Arne Jaksch[1]
[1]*Department of Biocompatibility and Toxicology, JAKSCH LIFESCIENCE CONSULTING GmbH*

Medical devices are expected to be without unacceptable toxicological risks in clinical use. For regulatory consideration and to determine the risk to patient safety, manufacturers of medical devices should provide a toxicological risk assessment (TRA) of all device constituents according to ISO 10993-17:2023 [1]. The newly published standard introduces the application of new approach methodologies (NAMs), including in silico modeling and read-across approaches, and the application of the threshold of toxicological concern. These newly recognized tools allow the reduction of classical animal testing for irritation, systemic toxicity, genotoxicity, reproductive/developmental toxicity, and carcinogenicity, which is in accordance with 3R principles [1]. Here, we present a case study applying the new ISO 10993-17:2023 [1] methodology to phenyl propyl carbonate present on a long-term implant. A toxicological risk assessment was conducted for this data-poor substance, combining a literature review followed by an in silico analysis and read-across approach. Several in silico models (e.g., Toxtree, Lazar, Danish QSAR) were applied to address the following toxicological endpoints: toxicokinetic, irritation, sensitization, systemic effects, reproductive and development toxicity, genotoxicity, and carcinogenicity. In addition, a read-across approach was applied for further data-gap filing based on a representative analog substance. This tailored approach allowed us to successfully perform a toxicological risk assessment of a data-poor substance, waiving additional testing and thus reducing the number of in vivo tests. The case study highlights the applications of NAMs in filling data gaps concerning toxicological risk assessment of medical devices.

P41
IN VITRO EVALUATION OF ABSORPTION PROFILE AND EFFICACY OF LACTOFERRIN CONTAINED IN A NASAL SPRAY FORMULATION
ABSTRACT #133

Alessandra Zerboni[1], Sara Stoilova[1], Daniele Colombo[1], Michela Mori[1]
[1]*1MED SA, Via Campagna 13, CH-6982 Agno Switzerland*

Absorption tests on ingredients of substance-base medical devices are recommended for the classification under Rule 21 of the Medical Devices Regulation (EU) 2017/745 (MDR) [1], in fact absorption may influences both the efficacy and safety of a product. The medical device object of this study is a spray formulation intended to create a protective muco-adhesive film at the level of nasal mucosa. The aim of this analysis was to evaluate the absorption of lactoferrin, present as functional ingredient at the concentration of 1 mg/ml, and to evaluate the performance in terms of recovery and protection. The absorption test was performed, according to OECD 428 guidelines [2], on an in vitro 3D insert model of Human mucociliary Epithelium (AIR-606, MatTek), using Franz-diffusion cells under finite dose conditions, at 37°C, and stirring of the receptor fluid at 600 rpm. The fluid from receptor compartment was

sampled and analyzed at different time points: t0, 15 min, 1h, 2h, 4h, 6h and 24h. In order to test the performance of the device, inflammation inducing stimuli Lipopolysaccharide (LPS) (2.5 ug/ml) and Polyinosinic:polycytidylic acid (Poly I:C) (25 ug/ml) were added to medium of the basal cells to mimic bacterial and viral insults respectively. IL-6 and IL-8 in the basal medium were quantified as inflammation markers. The tissues were subjected to morphological evaluation by optical microscopy through Hematoxylin and Eosin and Alcian blue staining. The results demonstrate that the absorption of lactoferrin can be considered negligible. The test item resulted effective in preventing inflammation against Poly I:C. Tissues in contact with the lactoferrin formulation showed significant decrease in IL 8 and IL 6 concentrations as well as absence of alterations to mucous and Goblet cells versus the untreated tissue. The device showed recovery properties both against LPS and Poly I:C. This study was sponsored by: D-TAILS S.r.l.

P42
IN VITRO EVALUATION OF INNOVATIVE BIOMATERIALS FOR TISSUE ENGINEERING: WHEY PROTEIN ISOLATE AND CHITOSAN DERIVATES INTERACTION PRODUCTS ABSTRACT #134

Daiana Ianev[1], Barbara Vigani[1], Michela Mori[2], Caterina Valentino[1], Marco Ruggeri[1], Giuseppina Sandri[1], Silvia Rossi[1]
[1]*Department of Drug Science, University of Pavia, Via T. Taramelli 12, 27100, Pavia*
[2]*1MED SA, Via Campagna 13, CH-6982 Agno Switzerland*

Polysaccharides (PL) and proteins (PR) are among the most widely used biopolymers in the design and development of scaffolds for tissue regeneration [1]. The study of the interactions between these biopolymers could lead to the development of innovative biomaterials, capable of assuming different geometries and sizes. The interaction between PL and PR may lead to the formation of both covalent and non-covalent PL-PR complexes [2]. The present work aims to produce and characterize new sustainable and high-value biomaterials, consisting of complexes between whey protein isolate (WPI) and chitosan (CS) or trimethyl chitosan (TMC). The in-depth characterization of the biomaterials highlighted that the functional properties of PL-PR complexes strictly depend on the biopolymer properties (MW, charge density and chain conformation) as well as on the experimental set-up (pH and denaturation conditions). WPI denaturation before PL addition is responsible for the formation of soluble complexes, characterized by higher Deta/eta and lower absorbance values with respect to the values observed for coacervates, obtained as a result of WPI denaturation after PL addition. Moreover, rheological analysis highlighted that low MW CS was able to form stronger interactions with WPI than medium MW CS. ELS analysis suggested that the electrostatic interactions are the main driving forces for TMC:WPI complex formation, while the intrinsic tryptophan fluorescence spectroscopy highlighted the prevalence of hydrophobic interactions involved in the formation of CS:WPI complexes. In vitro studies were performed on Normal Human Dermal Fibroblasts (NHDF): MTT assay allowed to prove the biocompatibility of the new biomaterials obtained; while Alamar blue assay was performed after 7 days contact of the PL-PR complexes with NHDF and confirmed the proliferation enhancement properties of such samples. Further studies are on-going to investigate the use of such biomaterials for the design and development of dissolving microneedles intended to remodel hypertrophic scars or to treat keloids.

P43
IN VITRO IRRITATION OF MEDICAL DEVICES INTENDED FOR APPLICATION TO THE ORAL, VAGINAL OR RECTAL MUCOSA ABSTRACT #210

Barbara De Servi[1], Christian Pellevoisin[2], Marisa Meloni[3]
[1]*VitroScreen, Milan, Italy*
[2]*Urbilateria, Tours, France*
[3]*VitroScreen*

Local tolerance is a common pre-requisite before placing topical products on the market

and its assessment is increasingly based on in vitro methods (EMA/CHMP/SWP/2145/2000 Rev.1). Validated methods are available to classify chemicals and mixtures, but they are not applicable to assess the irritation potential of complex mixtures, such as topical formulations to be applied on skin and mucosal tissues. ISO 10993-23:2021 has validated the use of reconstructed human epidermis (RhE) to assess irritation potential of medical device (MD) extracts. However, for special irritation tests relevant for medical devices intended to be applied to a specific area, i.e. mucosal, eye, vaginal or rectal epithelia ISO standard states that "RhE models are not adapted and it is recommended to explore the use of other in vitro models with relevant cells or tissues if qualified for use with medical devices". To this end, we conducted a retrospective analysis of our internal data using human reconstructed tissues to assess tolerance of different formulation types (emulsions, sprays, gels, surfactant-based formulations, ovules) intended to be used on oral, vaginal or rectal mucosa. The experimental protocols were based on 1h exposure followed by product removal and 16h recovery on oral, vaginal or rectal human reconstructed mucosa. In accordance with ISO 10993, cell viability was used as an endpoint and determined on the basis of MTT reduction. Treated 3D tissues were compared to negative controls and SDS was used as positive control. Tested formulations were classified as irritant or non-irritant to oral, vaginal or rectal mucosa according to cell viability with a cut-off value of 50%. Secondary endpoints including tissue integrity, morphology and inflammatory response were used. This retrospective study confirms the relevance and predictivity of in vitro approaches using 3D human tissue models, as well as their flexibility and applicability to formulations regardless of their regulatory status.

P44
USE OF AN IN VITRO-TO-IN VIVO EXTRAPOLATION (IVIVE) APPROACH TO DERIVE COMPOUND-SPECIFIC TOLERABLE iINTAKE (TI) VALUES FOR PHTHALATE ESTERS
ABSTRACT #265

Anh Nguyen[1], Ronald Brown[1]
[1]*Risk Science Consortium, LLC*

Background and Objectives Toxicity data from in vivo studies are not available to derive Tolerable Intake (TI) values for many extractable and leachable (E&L) compounds released from medical devices and drug delivery systems. In the absence of in vivo toxicity data for these data-poor compounds, it may be possible to use chemical-specific activity concentration data from high throughput in vitro toxicity studies as input to toxicokinetic models to derive equivalent in vivo Point of Departure (PoD) for these compounds using an in vitro-to-in vivo extrapolation (IVIVE) approach (Chang et al., 2022). Materials and Methods This project investigated the ability of three toxicokinetic models (1C, solve_pbtk, solve_3comp) in the IVIVE module in the US National Toxicology Program (NTP) Integrated Chemical Environment (ICE) program to predict oral PoD values from high throughput in vitro toxicity data for one chemical class of E&L compounds, specifically, phthalate esters. Results and Discussion The results of this exercise show that two of the three models (solve_pbtk and solve_3comp) were able to successfully predict in vivo PoD values from the in vitro data for a number of phthalate esters; in most cases, the in vivo PoD values were overpredicted. Based on the ability of the models to provide conservative estimates of in vivo PoD values in a validation exercise, the models were then used to predict in vivo PoD values for phthalates lacking experimentally derived in vivo PoDs. Since some model-derived predictions of in vivo PoD were greater than their respective experimentally derived values, it is recommended to run all three models to perform the IVIVE procedure, then use the lowest model-derived PoD as the basis for a compound-specific TI value for each data-poor compound.

P45
Development of an in vitro test method for irritation of medical devices used in the oral cavity
ABSTRACT #287

Marek Puskar[1], Jennifer Molignano[2], Christian Pellevoisin[2], Silvia Letasiova[1], Mitchell

Klausner[2]
[1]*MatTek In Vitro Life Science Laboratories, Mlynske nivy 73, 821 05 Bratislava, Slovakia*
[2]*MatTek Corporation, 200 Homer Ave, MA 01721 Ashland, Massachusetts, United States of America*

The irritation of any medical device (MD) contacting oral tissues (gingival, buccal, lingual, etc) needs to be evaluated. The objective of this project is to develop and validate in vitro assay to assess the oral irritation of MDs. This assay is intended to replace historical in vivo assay performed on Syrian hamsters. The ISO 10993-23 standard requires that MDs be evaluated using an in vitro irritation test based on reconstructed human epidermis (RhE) prior to animal or human patch testing is performed. However, RhE models are not appropriate for MDs designed for use in oral cavity, therefore ISO recommends use of other in vitro models with relevant cells or tissues. The EpiOral tissue model consists of normal, human-derived oral epithelial cells cultured to form multilayered, highly differentiated model of the human buccal tissue (1). To assess the feasibility of an in vitro method, initial experiments tested solutions of irritant chemicals contained in MDs used in oral cavity. Increasing concentrations of ethanol, lactic acid, methyl methacrylate, sodium dodecyl sulfate, phosphoric acid, sodium hypochlorite, hydrogen peroxide, and chlorhexidine digluconate in NaCl or sesame oil were applied to the EpiOral model. The time required to reduce tissue viability by 50% (ET-50), was determined. The results showed a clear relationship between tissue viability and exposure time and between ET-50 and concentration of the irritant chemical. Compared to historical in vivo data, the in vitro method classified the samples containing an irritant at the expected concentration (2). In addition, the ET-50s allowed differentiation between strong and mild irritants. The data demonstrate that this in vitro assay has equivalent or superior performance to in vivo method.

P46
Identification of compounds with weak skin irritation potential using human 3D reconstructed epidermis model

ABSTRACT #296

Jan Markus[1], Jana Halajova[1], Lenka Hudecova[1], Christian Pellevoisin[2], Marek Puskar[1], Mitchell Klausner[2], Silvia Letasiova[1]
[1]*MatTek In Vitro Life Science Laboratories, Mlynske Nivy 73, 82105 Bratislava, Slovakia*
[2]*MatTek Corporation, 200 Homer Ave, Ashland, MA, USA*

Implementation of 3R guidelines into the practice in chemical, pharmaceutical and cosmetic industries led to marked reduction in the utilization of animals for safety testing. Besides further reduction of animal use, the introduction of relevant in vitro models led to reduction of the incidence of false positive results, and increased efficiency in terms of time and cost. Currently there are multiple tests for skin sensitization, skin corrosion, skin absorption and skin irritation accepted for regulatory purposes, but there is still need for refinement and further development. This work was focused on utilization of an in vitro reconstituted human skin model to identify substances with a weak irritation potential using in vitro methods for determining skin irritation according to OECD TG439 and ISO 10993-23:2021. Fifteen test articles (TAs) in 5 concentrations (0.1%, 0.5%, 1%, 5%, 10%) in non-polar and polar solvents were tested. If no effects were observed, TAs were also tested neat. The EC-50 was calculated, and the release of selected cytokines (IL-1α, IL-6, IL-8 and IL-18) were determined using ELISA. The test according to OECD TG 439 identified 3 TAs that decreased tissue viability below 50%, (heptanoic acid, SDS and sodium hypochlorite). Seven of the compounds only affected viability in neat form, but not at any dilution, remaining 5 TAs did not affect viability in any form. When using the skin irritation test for medical device extracts (ISO 10993-23:2021) we found that 3 compounds did not decrease viability at any condition and 2 compounds only in the undiluted form. All other compounds caused decreases in viability in at least one solvent and in the undiluted form. Observed cytokine release data confirmed the above results. These data provide an important first step in the development of the method capable of predicting weak irritants in vitro.

P47
Integrated Risk Assessment and Compliance Strategies for Nanomaterials in Medical Devices
ABSTRACT #334

Chiara Gazerro[1], Maria Labianca[1], Carla Landolfi[1]
[1]*ToxHub Srl*

Nanomaterials, characterized by their unique properties at the nanoscale, have been on the spotlight in the scientific world due to their promising applications across diverse fields. These materials, often engineered to exhibit specific functionalities, offer unprecedented opportunities across diverse domains such as medicine, electronics, and materials science. Nevertheless, a univocal method to assess their safety in different kind of products is still lacking. In fact, the novel properties of nanomaterials introduce complex challenges in assessing their safety and biocompatibility. We delved into the specificities of conducting risk assessments for nanomaterials, focussing on both established and novel in vitro techniques to evaluate biocompatibility, toxicity, and potential patient exposure scenarios. Our approach integrated a comprehensive review of MDR guidelines relevant to nanomaterials and a series of in vitro assessments designed to evaluate their safety and biocompatibility in medical devices. We highlighted the crucial role of in vitro methodologies in toxicological testing, with a particular focus on adequate assays, as the unique properties of nanomaterials pose significant challenges to the applicability of traditional in vitro assays. There are notable gaps in standardization and documentation, particularly in the characterization of nanomaterials and the assessment of long-term effects. As nanomaterials' fame rises, so does the need for clearer regulatory guidelines specifically addressing them, emphasizing the establishment of standardized testing methods and risk assessment frameworks. The evolving landscape of nanotechnology necessitates clear and comprehensive regulatory measures to address potential risks, fostering confidence in the responsible development and application of nanomaterials across various industries.

IN VITRO SYSTEMS TO ASSESS RESPIRATORY TOXICITY

P48
Dosing concepts in air-liquid interface (ALI) in vitro inhalation approaches.
ABSTRACT #27

Detlef Ritter[1], Tanja Hansen[1]
[1]*Fraunhofer ITEM, Hannover, Germany*

In vitro inhalation methods using air-liquid interface (ALI) cultures mimic the lung's biological barrier function and provide a reliable basis for assessing the effects of inhaled substances in vitro. Dose estimation is crucial for quantitative in vitro to in vivo extrapolation (QIVIVE) and enables the development of safety assessment strategies using alternative testing methods. The standard definition of "dose" is D = c x t x Q (dose D, concentration c, exposure time t, exposure volume flow Q). Traditional study designs involve keeping exposure time (t) and volume flow (Q) constant while varying the concentration of the airborne test substance. However, this approach is inflexible, time-consuming, and requires significant amounts of materials. This exploratory study aimed to evaluate whether varying exposure times and flows in in vitro inhalation experiments could also provide reliable doses. The hydrophilic gas formaldehyde (FA) was selected as test item and human lung epithelial A549 cells were exposed to FA by using the P.R.I.T.® ExpoCube® device. Cytotoxicity was assessed (WST-1) and a reference dose-response was established using constant exposure times (60 minutes) and volume flows (3 ml/min) while varying FA concentrations (1-100 ppm). Additional exposures included varying exposure times (1-120 min) and volume flows (1.5-25 ml/min), which were compared to the reference response based on the dose-metric "D". The results displayed an experimental window within specific limit values for exposure time and volume flow where the standard definition of "D" remains valid. This finding demonstrates the potential to improve efficiency and optimize experimental design in in vitro inhalation studies by considering variations in exposure times and flows. To expand these possibilities to a wide range of airborne test substances, additional types of substances, such as hydrophobic gases,

vapors, and different aerosols, need to be tested using similar approaches. This work was funded by NC3Rs [NC/C017102/1].

P49
The effects of nicotine base on oxidative stress and autophagy in the alveolar-capillary barrier in vitro show similarities with the pathophysiological features of COPD
ABSTRACT #136

Amelia-Naomi SABO[1], Emma FILAUDEAU[1], Guillaume BECKER[1], Laurent MONASSIER[1], Véronique KEMMEL[1]
[1]*Laboratoire de Pharmacologie et Toxicologie Neurocardiovasculaire, University of Strasbourg, France*

Background: Cigarette smoke is the main cause of chronic obstructive pulmonary disease (COPD), as it provides exogenous radical oxygen species (ROS) that upset the balance between oxidising and antioxidising molecules in the lungs. Nicotine is the main addictive substance in tobacco, but very little research has been done into its role in COPD. Nicotine is also present in electronic cigarettes (ECs), recent nicotine delivery devices. Our aim was to decipher the dose-related effects of nicotine on the alveolar-capillary barrier (ACB) in vitro. Methods: A validated cell co-culture model was exposed to a wide range of nicotine consistent with in vivo pulmonary levels. This cell model consisted of the NCI-H441 alveolar cell line and the HULEC-5a pulmonary endothelial cell line cultured on either side of a cell insert. We studied pulmonary mitochondrial ROS (MitoSOX® assay), cellular autophagy (LC3-II specific marker), barrier disruption (trans-epithelial/endothelial electrical resistance – TEER), cell viability (MTS and BrdU assays) and apoptosis (TUNEL assay) and necrosis (LDH assay) of the cells. Results: The results showed increased mitochondrial ROS production above 2.5 µM nicotine, partially reversed by the addition of a ROS scavenger, N-acetylcysteine (NAC). Autophagy was correlated with ROS production, with increased LC3-II expression and increased autophagosome puncta in the cells, partially reversed by NAC. The TUNEL assay showed a weak but significant increase in DNA fragmentation in NCI-H441 and HULEC-5a cells 24 hours after nicotine exposure, revealing induction of apoptosis. Nicotine concentrations in the millimolar range altered the integrity of the barrier, leading to a drop in TEER associated with reduced cell viability and necrosis. Discussion: The results reveal features of COPD and draw attention to ECs, which are therefore likely to trigger these deleterious processes. The mechanism by which nicotine induces ROS and the cellular pathways involved in autophagy have yet to be elucidated.

P50
ADVANCING AEROSOL NANOPARTICLE CYTOTOXICITY ASSESSMENT THROUGH A NOVEL "CELLS ON PARTICLES" IN VITRO MODEL
ABSTRACT #149

Edvinas Krugly[1], Edvardas Bagdonas[2], Jovilė Raudoniūtė[2], Preethi Ravikumar[1], Aistė Pupiūtė[1], Darius Čiužas[1], Tadas Prasauskas[1], Rūta Aldonytė[2], Dainius Martuzevičius[1]
[1]*Faculty of Chemical Technology, Kaunas University of Technology, Lithuania*
[2]*Centre for Innovative Medicine, Vilnius, Lithuania*

The potential health risks associated with aerosolized nanoparticles remain poorly understood. Traditional in vitro models for assessing aerosol particle toxicity often necessitate multiple steps of particle sampling, extraction, dilution, and dispersion prior to cellular exposure, which could potentially alter the inherent characteristics of the nanoparticles and, consequently, the accuracy of the toxicity assessment. In response to this challenge, we have innovatively conceived and developed a pioneering in vitro model termed "Cells on Particles". This model employs nanofibrous platforms fabricated from a variety of biocompatible polymers, serving a dual purpose: efficient collection of aerosol particles, and providing a substrate for subsequent cytotoxicity analysis using adherent cells, such as BEAS-2B. The biocompatibility of these platforms was established and further embarked on a thorough evaluation of the cytotoxic effects of engineered silver (Ag),

copper (Cu), and graphene oxide (GO) nanoparticles aerosolized and deposited on these platforms. A distinct dose-response relationship was registered, indicating the increased cellular sensitivity to lower nanoparticle concentrations during direct exposure compared to conventional in vitro models. Exploration of gene expression patterns revealed differential cellular responses to these nanoparticles, contributing invaluable insights into their potential health impacts. The "Cells on Particles" model significantly diminishes the pre-analysis processing steps, thereby facilitating a more realistic and accurate assessment of aerosol toxicity. This potentially accelerates the hazard assessment trajectory of airborne nanoparticles, thus bridging a crucial gap in our current understanding of nanoparticle-induced cytotoxicity and its broader implications on public health.

P51
Evaluation of the predictiveness of the acellular inhalation bioaccessibility test of ultrafine combustion particle-bound polycyclic aromatic hydrocarbons for assessing particulate matter lung toxicity
ABSTRACT #151

Albana Boutamba Mbina[1], Tiphaine Rogez-Florent[2], Christelle Monteil[2]
[1] *Univ Rouen Normandie, Université Caen Normandie, Normandie Univ, ABTE UR 4651, F-76000 Rouen, France*
[2]*Univ Rouen Normandie, Université Caen Normandie, Normandie Univ, ABTE UR 4651, F-76000 Rouen, France*

Airborne particulate matter (PM) remains of concern due to their sizes and chemical compositions adversely affecting health. PM comprises many chemical compounds, including polycyclic aromatic hydrocarbons (PAHs) resulting from incomplete combustion. Associated with PM, these PAHs can penetrate the respiratory tract after inhalation, be deposited in the pulmonary extracellular fluids, enter the cellular compartment, and induce effects, or even diffuse across the alveolar-capillary membranes and be absorbed. The amount of PAHs released into biological fluids can thus be available for absorption or induce local toxicity to respiratory cells. Therefore, it seems necessary to determine this so-called “bioaccessible” fraction and to assess its predictive value for health risk assessment. In this context, we have developed, using different simulated lung fluids (SLF) and by varying relevant parameters like the solid-liquid ratio and the type of SLF, an acellular in-vitro method to evaluate the inhalation bioaccessibility of PAHs associated with ultrafine particles (UFP). The tested SLF were the Gamble's Solution (GS), which composition is closed to the composition of the human pulmonary fluid and the Artificial Lysosomal Fluid representing the acidic intracellular environment of lung macrophages (1). The PM used are UFP rich in PAHs, without heavy metals, generated in a controlled manner using a soot generator (MiniCAST) set on the CAST3 operating condition (2). The relevance of the bioaccessibility measure as a predictive tool for PM toxicity will be assessed by in-vitro tests using a model of human bronchial epithelial cells, the BEAS-2B cells (3). The cellular response of BEAS-2B after exposure to bioaccessible fractions, PM suspensions as well as PM extracted PAHs organic fractions will be evaluated and compared considering certain biomarkers of effects such as cell viability and cytotoxicity, the induction of the xenobiotic metabolic response, the generation of an oxidative stress and the inflammatory response.

P52
Chemical analysis and cytotoxicity studies of different berry e-liquid flavors according to two exposure methods on an alveolar-capillary barrier cell model
ABSTRACT #154

Emma Filaudeau[1], Amelia-Naomi Sabo[12], Anita Lebert[1], Laurent Monassier[1], Véronique Kemmel[12]
[1]*Laboratoire de Pharmacologie et Toxicologie Neurocardiovasculaire, UR 7296, Faculté de Médecine de Maïeutique et des Métiers de la Santé, Centre de Recherche en Biomédecine de Strasbourg (CRBS), 67085, Strasbourg, France*
[2]*Laboratoire de Biochimie et Biologie*

Moléculaire, Hôpitaux Universitaires de Strasbourg, 67200, Strasbourg, France

Background: Studies about e-cigarettes toxicity are sparse, while they are largely used. Concerning e-liquids (EL), we previously have shown differences in term of toxicity depending on flavors and nicotine concentrations thanks to an alveolar-capillary barrier cell model. Since then, we have set up two exposure methods (EM) to study red fruit-flavored EL. Our aim was to identify specific compounds linked to oxidative stress (Ox) and cell death responses. Methods: Our coculture model is based on the use of NCI-H441 cell line as alveolar epithelial cells and EA.hy926 cell line as endothelial cells. Cells were treated with 4 nicotine-free EL flavored with red fruits in a 76/24 Propylene Glycol/Vegetable Glycerin ratio as vehicle for 24h. The 1st EM was the dilution of different EL (0.2-30%, V/V) and the 2nd EM was the trapping of the aerosol (10 to 240 puffs) in culture medium. Mitosox® assay was used to study the mitochondrial Ox, whereas, cell vitality and cell necrosis were explored by MTS and LDH assay, respectively. The LC-MS-TOF technique was used to discriminate compounds found in ELs and their trapped form. Results: For the 1st EM, we identified a decrease of the cell proliferation above 20% for 3 ELs coinciding with an increase in necrotic processes. For the 2nd EM, we did not find any clue about an effect on proliferation/necrotic processes up to 240 puffs. For Ox, Mitosox® assay showed several differences between the 4 EL tested: Blackberry flavored EL seemed to be the most toxic in term of Ox (from 0.2%) by dilution (1st EM) but not by trapping the aerosol (2nd EM). Furthermore, LC-MS-TOF analysis showed some composition differences between EL and trapped aerosol. Discussion: These results show interesting differences between flavored EL and their aerosolized form in term of toxicities but also in term of chemical composition.

P53
Assessing lung metabolic competency for in vitro inhalation toxicology applications: a single-cell RNA sequencing strategy
ABSTRACT #163

Nuria Roldan[1], Vivek S Patel[2], Joseph Hughes[2], Méry Marimoutou[2], Jose Alvarez[2], McKenzie Obermok[2], Carlos Rodriguez[2], Thomas Kallarakal[2], Andreas O Stucki[1], Amy J Clippinger[1], Holger P Behrsing[2]
[1]*PETA Science Consortium International e.V., Stuttgart, DE*
[2]*Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA*

The metabolic capacity of human lung cells influences local toxicity, molecule bioavailability, and downstream adverse effects, and various in vitro models used for the assessment of inhalation toxicity have been designed to mimic the metabolic situation in the human lung. With recent advancements in the cryopreservation of human precision-cut lung slices (hPCLS), it has become important to comprehensively characterize metabolic activity at the cellular level. In this study, fresh or cryopreserved hPCLS were characterized using single-cell RNA sequencing (scRNAseq) and compared to primary lung tissues retrieved from the tracheobronchial or the bronchiolo-alveolar region of the respiratory tract. To assess any effects related to time in culture, fresh hPCLS were acclimated for up to 72h and evaluated directly post-acclimation (day 0) and after 14 days of cultivation (day 14). To investigate any effects of cryopreservation, frozen hPCLS were thawed, acclimated, and also analyzed on day 0 and day 14 post-acclimation. Preliminary results show different metabolic signatures depending on the cell populations and lung regions, as expected in a human lung. General cell populations (epithelial, mesenchymal, endothelial, and immune cells) were present in both fresh and frozen hPCLS at day 0 and day 14, and were consistent with their original anatomical location. Taken together, the scRNAseq data demonstrate that fresh or frozen hPCLS reflect key characteristics of the human lung, increasing confidence in their use as a human-relevant test system. This sequencing approach can be applied to other lung models to, for example, characterize their metabolic capabilities and thereby assist end-users in selecting which model to use for in vitro inhalation toxicology assessments.

P54
Advanced immunocompetent in vitro primary human lung models for toxicity assessment and infectious disease research
ABSTRACT #184

Samuel Constant[1]
[1]*Epithelix*

The main function of the human airway epithelium is to generate sterile atmosphere for the alveolar region where the gas exchange occurs. As first line of defence against airborne pathogens or xenobiotics, the airway epithelium acts not only as key physical barrier endowed with mucociliary clearance and innate host defence mechanisms, but also as an important immunoregulator through production of key messengers and physical interactions with immune cells especially dendritic cells and macrophages and neutrophils. (1) We will describe the development and characterization, as well as the use of fully primary human cell based co-culture models made of nasal, tracheal, bronchial, small-airways and alveolar epithelia (MucilAir™, SmallAir™ and AlveolAir™) and dendritic or alveolar macrophages or neutrophils. Several applications of these advanced immunocompetent ALI models will be discussed: (i) inhalation toxicity assessment with highlight on OECD Case study 367; (ii) screening of antiviral drugs against SARS-CoV-2, rhinoviruses, influenza, RSV and flue; (iii) host-pathogen interactions in co-culture models of upper or lower airway Epithelium & Alveolar Macrophages using bacteria (pseudomonas aeruginosa, Streptococcus pneumonia and staphylococcus aureus) in context of new antibiotic development.

P55
TOXICITY OF REAL-WORLD SUBWAY EMISSIONS IN A MOBILE ALI EXPOSURE MODEL
ABSTRACT #186

Micol Introna[1], Ana T. Juárez-Facio[1], Sarah S. Steimer[1], Minghui Tu[2], Ulf Olofsson[2], Hanna L. Karlsson[3], N. V. Srikanth Vallabani[3], Karine Elihn[1]
[1]*Stockholm University*
[2]*KTH Royal Institute of Technology*
[3]*Institute of Environmental Medicine, Karolinska Institutet*

The subway is a highly efficient transportation mode widely used in cities due to its capacity and reduced use of urban space. Because of its location and the limited number of exits, the subway represents a partially isolated microenvironment in which particle matter (PM) can reach significantly higher levels than in the open outdoor urban environment. In Stockholm subway, the concentration of PM2.5 reached 5-10 times higher levels compared to busy streets (1,2). Nevertheless, the PM of the subway differs from the road traffic pollution because it is generated by the friction of the train wheels, brakes, and via sparking at electric cables. Consequently, its chemical composition mainly comprises metals: iron, manganese, copper, lead, and other elements (e.g., silicon, sulfur, and bromine) (3). The study aims to evaluate cell viability and inflammatory response (IL-8, IL-6, IL-1β, TNF-α) of human lung cells after direct exposure to fresh PM2.5 emitted at a subway platform. A mobile Air Liquid Interface (ALI) system was used to simulate particle inhalation and deposition in the lungs. The exposure was performed in the subway during working days in winter (closed ventilation) and summer (open ventilation) and cells were then transported back to the laboratory for incubation. In parallel, PM2.5 subway filters were collected to perform experiments at submerged condition at fixed concentrations. Cytokine release was reduced after exposing A549 at ALI condition to subway PM2.5 (low doses). At submerged conditions, cell viability of A549 and THP-1 decreased at high doses (150-200 µg/mL) and increased inflammatory markers.

P56
In situ exposure of human lung epithelial cells cultured in air-liquid interface to assess effect of air pollution of different influences
ABSTRACT #193

Suen Boulé[1], Fabrice Cazier[2], Dominique Courcot[1], Yann Landkocz[1]

[1]*Unité de Chimie Environnementale et Interactions sur le Vivant, UCEiV UR4492, Univ. Littoral Côte d'Opale (ULCO), Dunkerque, France.*
[2]*Centre Commun de Mesures, Univ. Littoral Côte d'Opale (ULCO), Dunkerque, France.*

Air pollution is a major public health issue. The World Health Organization has estimated in 2022 that 99% of the global population breath polluted air (WHO, 2023). Air pollution is a complex mixture of particulate matters (PM) and gaseous compounds like nitrogen oxides (NOx), ozone (O3) or volatile organic compounds (VOCs). The main route of entrance of air pollutants is the inhalation so the respiratory tract represents the first target of these toxicants. There is a lack of knowledge of the biological effects of air pollution on lungs. This project aims at assessing the effects of ambient air pollution on pulmonary cells. Human bronchial epithelial cells (Calu-3) and alveolar epithelial cells (hAELVi), cultured in air-liquid interface, have been exposed directly in the field to air pollution, using Vitrocell® system with isokinetic sampling device for cell exposure. In parallel, air quality was evaluated for pollutant concentrations (O3, NOx, VOCs and PM2.5) using real-time analyzers and particle samplers for chemical characterization. In order to study different pollution profiles, 4 sites were selected because of their major influence (urban, rural, industrial or road traffic). Different cellular parameters were evaluated: maintenance of barrier integrity by transepithelial electric resistance measurement and paracellular permeability, cell viability, xenobiotic metabolization and inflammatory response. We have therefore developed a system based on the chemical analysis of atmospheric pollutants and the biological response of lung cells in order to improve our understanding of the mechanism involved in respiratory toxicity. The first results show variations in the biological response between the different pollution profiles, which will be completed with further analysis. This work has been produced within the framework of the French Program "Territoire d'Innovation: Dunkerque, l'énergie creative", which has received funding from the "Grand Plan d'Investissement". The work also involved the health Services "Observatoire Local de Santé".

P57
Impact of airborne particulate matter from port, industrial and urban areas on epithelial-to-mesenchymal transition on lung cells.
ABSTRACT #206

Yann Landkocz[1], Margaux Cochard[1], Fréderic Ledoux[1]
[1]*Unité de Chimie Environnementale et Interactions sur le Vivant, UCEiV UR4492, Univ. Littoral Côte d'Opale (ULCO), Dunkerque, France*

The relationship between increase of environmental pollution, in particular by fine particulate matter (PM2.5) and the incidence of respiratory diseases such as asthma or lung cancer are currently proven. Moreover, particles emission sources have an impact on their chemical composition therefore inducing various toxicity responses. Epithelial to mesenchymal transition (EMT) is a fundamental process that underlies cancer progression and metastasis but little is known about the relation between exposure to PM2.5 and induction of EMT phenomenon The aim of this work is to chemically characterize PM2.5 from distinct origins and to study the toxicological potency of them on pulmonary cells in order to establish the link between specific particle constituents with specific cell responses, especially EMT. Thus, A549 epithelial cells were exposed to organic extract and water-soluble fraction of PM2.5 from the 4 influences (urban, industrial, road traffic, and port and maritime traffic emissions) and a toxicological study including the metabolization of xenobiotics, pro-inflammatory response or oxidative stress was carried out. Then, EMT was characterized through changes in cellular morphology and EMT markers expression such as E-cadherin, N-cadherin, vimentin, ZO-1 and transcription factors Snail, Slug and Twist. Our results were able to demonstrate that depending on the influence of PM2.5 particles, oxidative stress and inflammation responses and the activation of EMT mechanisms was more or less marked. To go further, an RNAseq analysis was carried out in order to compare pathways that could have led to the identified mechanisms specifically activated by source of PM2.5. In conclusion, our study provides a detailed examination of the EMT process in lung cells, revealing the molecular and cellular

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changes associated with exposure to PM2.5. This work is part of the ToxTEM project and was supported by ADEME (Convention no. 1962C0005). UCEIV UR4492 participates in the CPER ECRIN project.

P58
DEVELOPMENT OF A MOBILE ALI EXPOSURE SYSTEM FOR TOXICITY TESTING OF EMISSIONS FROM DIFFERENT TRANSPORTATION MODES
ABSTRACT #215

Ana Teresa Juárez Facio[1], Micol Introna[1], Sara S. Steimer[1], Karine Elihn[1]
[1]*Stockholm University*

Emissions from different transports cause several human health effects. An Air Liquid Interface (ALI) exposure system can examine the toxicity of airborne particles (Latvala et al., 2016), and be used to assess their effects on human health. So far, only a few studies have been performed using ALI systems in outdoor environments (Augustin et al., 2020; Gualtieri et al., 2018). We developed a mobile ALI system designed to facilitate the deposition of particles, especially nanoparticles, using an electrostatic field. It has controlled temperature and humidity and enables online characterization of the particle size distribution to estimate the particle exposure dose on cells. In outdoor locations, a concentrator can be used to increase the particle concentration and ease toxicity evaluation. Several parameters have been optimized for best cell survival (aerosol flow, temperature, humidity, incubation time). Our ALI system has already been used to evaluate particle emissions' toxicity in a road tunnel, subway station, tribology laboratory, chassis dyno, harbor, and outdoor background locations. This work was supported by the European Commission's Horizon 2020 research and innovation program nPETS (grant agreement 954377).

P59
An integrated human-in vitro approach to explore the role of miRNAs in the allergic asthma
ABSTRACT #238

Valentina Galbiati[1], Filippo Liviero[2], Gloria Melzi[1], Francesca Carlotta Passoni[1], Emanuela Corsini[1], Marina Marinovich[1]
[1]*Università degli Studi di Milano*
[2]*Azienda Ospedale - Università di Padova*

Asthma is a heterogeneous and chronic disease of the lower airways that affects around 300 millions people worldwide. The most common and well-studied form of asthma is the allergic type affecting both children and adults. The main pathological feature of allergic asthma is due to the complex interactions between immune cells and immunological mediators. Recent evidences reported alterations in miRNA expression in a variety of lung diseases, including allergic asthma. Several miRNAs have been associated with asthma and airways inflammation but target identification remain not yet determined for the majority of the studies. The specific aim of this study, entirely based on human cells, is to identify miRNA patterns and consequently which specific targets are involved in the interaction between the respiratory system and the immune system in the allergic asthma disease. For the in vitro approach the human lung epithelial cells, namely Calu-3, are exposed to 5 respiratory sensitizers (hexamethylen, methylene diphenyl, and toluene diisocyanate; ammonium hexachloroplatinate; trimellitic anhydride), 1 skin sensitizer (2,4-dinitrochlorobenzene) and 1 irritant (sodium dodecyl sulphate) through a liquid aerosols system (Cloud Alpha System – VITROCELL® Systems). A microRNA panels is than performed in control conditions and exposed conditions. miRNA expression will be then evaluated and compared with miRNAs expressed in human samples from healthy and asthma donors (collected in collaboration with Dott. Liviero, Occupational Unit of Padova University). The translational potential of the project is due to the use of a primary cell culture model as well as human samples. Furthermore, implementation of miRNA patterns could be useful as prevention tool (biomarkers for diagnosis) of allergic asthma.

P60
Effects of repeated exposure of human 3D bronchial tissue to fresh smoke and aerosol from heated tobacco products and electronic vapour products
ABSTRACT #259

Sarah Jean Pour[1], Roman Wieczorek[1], Edgar Trelles Sticken[1], Fiona Chapman[2], Liam Simms[2], Matthew Stevenson[2]
[1]*Reemtsma Cigarettenfabriken GmbH, an Imperial Brands PLC Company*
[2]*Imperial Brands PLC*

Background and Objectives: Combustible cigarette smoke consists of more than 6,000 chemicals and is a cause of serious diseases in adult smokers, including lung cancer, heart disease and emphysema. Public health experts worldwide have concluded that it is the toxicants in cigarette smoke, not the nicotine, which is the cause of smoking-related diseases. For this reason, many public health bodies have indicated that Next Generation Products (NGPs), like Heated Tobacco Products (HTPs) and E-Vapor Products (EVPs) have a role to play in tobacco harm reduction (THR). In the present study we compared the biological impact of EVP and HTP aerosol compared to combustible cigarette smoke using an in vitro 3D human reconstituted bronchial tissue model. Methods and Results: A smoking robot was used to expose MucilAirTM (Epithelix) tissue models to fresh whole aerosol/ smoke at the Air Liquid Interface (ALI) over a period of 28 days. Due to a strong cytotoxic effect, cigarette smoke was diluted (1/14 and 1/17) with fresh air leading to a lower nicotine delivery (up to 25-fold) than the EVP and HTP. The cigarette smoke caused significant, dose dependent toxicity in a variety of assessed endpoints (cilia function, barrier integrity, histology, and pro-inflammatory markers), whereas the EVP and HTP aerosols showed minimal effects. Discussion and Conclusion: Fresh aerosol from the tested EVP and HTP has a substantially reduced in vitro toxicity activity in human 3D bronchial cell models compared to cigarette smoke. These results support the scientific evidence that EVPs and HTPs represent a less harmful alternative to cigarettes. Both HTP and EVP offer promising THR potential when used by adult smokers as an alternative to combustible tobacco products.

P61
Assessment of a Heated Tobacco Product in the ToxTracker and ToxProfiler assays reveal marked reductions in biological activity compared to a combustible cigarette
ABSTRACT #262

Ourania Komini[1], Remco Derr[2], Inger Brandsma[2], Bas ter Braak[2], Liesanne Wolters[2], Edgar Trelles Sticken[3], Roman Wieczorek[3], Sarah Jean Pour[3], Fiona Chapman[1], Liam Simms[1], Matthew Stevenson[1]
[1]*Imperial Brands PLC*
[2]*Toxys BV*
[3]*Reemtsma Cigarettenfabriken GmbH*

Heated Tobacco Products (HTPs) are an emerging category of Next Generation nicotine delivery Products which heat a stick containing reconstituted tobacco to a temperature below that of combustion. Rapid and mechanistically insightful in vitro methods are required for the biological assessment of these products. Here we explored the ToxTracker and ToxProfiler assays for a comparative assessment of a HTP (Pulze and iD sticks) and a combustible cigarette (1R6F). The ToxTracker genotoxicity assay utilises 6 green fluorescent protein reporter cell lines measuring DNA damage, oxidative stress, p53 activation and protein damage; the ToxProfiler assay determines activation of seven specific cellular stress response pathways (oxidative stress, cell cycle stress, ER stress, autophagy, ion stress, protein stress, inflammation). Both assays were exposed to 1R6F smoke or HTP aerosol fractions, generated by bubbling smoke / aerosol through PBS (bPBS), forming stock solution concentrations of 1.8puffs/ml for 1R6F and 4puffs/ml for the HTP. The ToxTracker assay predicted that the 1R6F bPBS was genotoxic, inducing DNA damage, oxidative stress and p53 activation markers, whereas the HTP bPBS did not induce genotoxicity. The ToxProfiler assay detected that 1R6F bPBS induced oxidative stress and cell cycle stress; with effects appearing from 0.23% bPBS concentrations. In contrast, a higher concentration of 0.7% bPBS was required to induce oxidative stress for the HTP. The

reduced biological activity of HTP aerosol relative to combustible cigarette smoke, using bPBS extracts, in both the ToxTracker and ToxProfiler assays add to the growing evidence that these products have harm reduction potential.

P62
MULTIPLEX ENDPOINT FOR LONGITUDINAL CHARACTERIZATION OF OXIDATIVE STRESS USING IN VITRO 3D LUNG EPITHELIAL CULTURES
ABSTRACT #283

Jorge Pereira[1]
[1]*PMI R&D, Philip Morris Products S.A., Switzerland.*

Authors: Jorge Pereira, Laura Ortega Torres, Neda Haghayegh Jahromi, Solene Carrichon, Anna Goralczyk, Diego Marescotti Presenting Author: Jorge Pereira. jorge.pereira@pmi.com Affiliation: PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland. Oxidative stress is a common hallmark of toxicity induced by environmental agents and is also at the center of fundamental intrinsic cellular processes. It is regarded as a redox imbalance from excessive production of reactive oxygen species (ROS) that is not counterbalanced by antioxidant defenses, including glutathione. Oxidative stress represents an important and multifaceted biological research endpoint, although it is often challenging to quantify due to its complex and dynamic nature. Here, we characterize the response of an in vivo-mimicking 3D lung epithelial model to the well-described oxidative agent menadione. We adapted two commercially available assays from Promega (Madison, WI, USA): the ROS-Glo assay for the quantification of ROS and the GSH/GSSG-Glo assay to measure the reduced-to-oxidized glutathione ratio from 2D to 3D cell culture. Multiplexing of both assays using a single biological sample enabled longitudinal assessment of tissue responses based on the intensity of the stress. As two oxidative stress biomarkers are assessed simultaneously from the same biological sample (instead of three different samples), this new approach methodology saves tissues and provides a more holistic view of oxidative stress. Funding: Philip Morris International is the sole source of funding and sponsor of this research.

P63
Contemporary in vitro Toxicology Assessment of a Novel Herbal Heated Product (HHP) Compared to Cigarette Smoke
ABSTRACT #316

Emma Bishop[1], Fan Yu[1], Stela Bozhilova[1], Fabio Miazzi[1], Yihe Li[1], Lauren Smith[1], David Smart[1], Damien Breheny[1]
[1]*B.A.T. (Investments) Ltd.*

To support Tobacco Harm Reduction (THR) there have been many new emerging product categories coming to market, with the aim to give adult smokers alternative and potentially reduced harm alternatives to traditional cigarettes. Heated Tobacco Products (HTP) emissions show reductions in the number of chemical analytes present and in vitro toxicity, as compared to traditional cigarettes, due primarily to the absence of combustion. Here, we present the in vitro assessment of a tobacco-free herbal heated product (HHP), assessed against reference cigarette responses. Composition includes non-tobacco heated substrate, nicotine and flavours. Test products 1R6F reference cigarette and novel herbal heated product (HHP), variants were examined in two test matrices. Firstly, aqueous extracts (AqE) were produced (1) under HCI or HCIm regime as appropriate using Glo™ Hyper X2 device for the HHP variants. Test extracts were assessed in Real time Cell Analyser (RTCA) as a measure of cytotoxicity in H292 lung cells and ToxTracker™ screening assay for genotoxicity. Furthermore, whole aerosol experiments were also performed (2) with undiluted aerosol exposure using the Korber LM4E vaping robot and MucilAir™ organotypic lung cultures (Epithelix), measuring cytotoxicity via MTT, trans epithelial electrical resistance (TEER) and cilia beat frequency and active area. In all assays conducted, HHP variants showed reductions in cytotoxicity and genotoxicity compared to 1R6F research cigarette, showing their potential as a reduced harm product in comparison to traditional

cigarettes.

P64
EXPLORING THE POTENTIAL OF EPIGENOTOXICITY TO ASSESS RESPIRATORY EFFECTS OF NANOMATERIALS TOWARDS THEIR RISK ASSESSMENT AND REGULATION
ABSTRACT #317

Célia Ventura[12], Luís Vieira[12], Catarina C. Silva[12], Henriqueta Louro[12], Maria João Silva[12]
[1]*National Institute of Health Doutor Ricardo Jorge (INSA), Department of Human Genetics, Lisbon, Portugal*
[2]*Centre for Toxicogenomics and Human Health (ToxOmics), NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal*

Epigenomics addresses the regulation of gene expression through fine-tuned mechanisms involving histone tails modification, DNA methylation changes and expression of small non-coding RNAs (e.g, microRNAs). Besides mantaining its homeostasis, it also reprograms the genome and adapts its functions to comply with environmental stressors1. Epigenome disturbances can lead to stable changes in cells and organisms' phenotype, contributing to human diseases, e.g., immune, metabolic and oncological diseases. In contrast with the regulatory requirements to test for the potencial genotoxicity of new substances, e.g., pharmaceuticals or food additives, there are no regulatory requests for screening their potential epigenotoxicity. In this study, we investigated the epigenotoxicity of a nanocrystalline (CNC) cellulose in the respiratory system. The CNC was derived from Eucaliptus globulus kraft pulp and has potential for innovation in industry and biomedicine2. To identify the differentially expressed microRNAs (DEmiRNAs), BEAS-2B cells were exposed to CNC (3 µg/cm2, 24h) and microRNAs were extracted and sequenced on a NextSeq 550 (Illumina). DEmiRNAs were obtained using sRNAtoolbox, and only DEmiRNAs identified by two different methods were considered for further analysis. The results showed that CNC induced the over- or under-expression of 52 microRNAs. The bioinformatics analysis of the DEmiRNAs-enriched cellular pathways identified pathways associated to cell cycle, apoptosis, cell proliferation and differentiation (e.g., Hippo, p53, TGF-B, FoxO, PI3-Akt signaling pathways), metabolism (e.g., fatty acids, O-glycans) and cancer (e.g., transcriptional dysregulation in cancer, non-small cell lung cancer). The present epigenotoxicity results deemphasize the negative genotoxicity results (comet and micronucleus assays) produced using co-cultures of human alveolar cells/monocyte-derived macrophages exposed to the same CNC3. This study highlights the relevance of developing screening methodologies to identify epigenotoxic effects of new substances as a complement to their genotoxicity assessment, at early stages of their development/application.
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P65
FLAVOURS IN E-LIQUIDS – TOXICOLOGICAL INVESTIGATIONS ON RELEVANT IN VITRO LUNG MODELS
ABSTRACT #325

Leonie Gloger[1], Jan Heide[2], Sebastian Malke[3], Johannes Becker[1], Evelyn Kuhn[1], Anja Huber[1], Sven Ehlert[2], Selina Rinaldi[3], Elke Pieper[3], Mathilde N. Delaval[1], Ralf Zimmermann[12]
[1]*Munich, Germany*
[2]*Rostock, Germany*
[3]*Berlin, Germany*

Since 2004, when e-cigarettes (e-cigs) were launched as smoking cessation aids, they have gained popularity worldwide. However, exposure to e-cig aerosol has shown to cause adverse effects on the respiratory system [1]. Yet, the role of flavours in e-cig toxicity is unclear. Therefore, this pilot study aims at evaluating the potential effects of different e-liquid flavours by combining advanced exposure procedures, state-of-the-art aerosol generation methods and an in vitro lung model. Alveolar (A549) epithelial cells were exposed at the air-liquid interface to clean air or to aerosol from in-house e-liquids (base-solvent

consisting of 30% propylene glycol and 70% vegetable glycerine with or without vanillin, cinnamaldehyde, and eugenol) in the VITROCELL® Automated Exposure System using 20 puffs, 55 mL/puff, 3 s/puff every 30 s. The emissions were monitored on a puff-by-puff basis by a PI time-of-flight MS combined with single photon ionisation (SPI) and resonance enhanced multi photon ionisation (REMPI). A slight increase of lactate dehydrogenase (LDH) release, was observed in the exposure to base-solvent aerosol, accompanied by a decrease of metabolic activity in A549 cells. Xenobiotic enzyme cytochrome P-450 (CYP) 1A activity was decreased in A549 cells exposed to all e-cig flavour aerosols while all e-cig (base-solvent only and flavours) aerosols led to an inhibition of CYP 2B activity. Only eugenol flavoured aerosol led to a slight increase of cytokine IL-8 release by A549 cells. Additionally, base-solvent aerosol exposure induced a slight increase in MDA release. In conclusion, we observed only minor toxicological effects in alveolar epithelial cells exposed to e-cig flavoured aerosols compared to negative controls and base-solvent aerosol exposed cells. However, even after a very short exposure differential biological responses are induced by the e-cig flavoured aerosols, emphasizing the need to investigate the role of flavour toxicity caused by e-cig aerosols on human respiratory health.

P66
Coupling an atmospheric simulation chamber to an air/liquid interface cell exposure device to study the toxicity of prenol, a second-generation biofuel, and its ozonolysis products
ABSTRACT #329

Reem Al Mawla[12], Cécile Coeur[1], Nicolas Houzel[1], Antoine Truffier[2], Sylvain Billet[2]
[1]*Laboratoire de Physico-Chimie de l'Atmosphère (LPCA), Université du Littoral Côté d'Opale, Dunkerque, France*
[2]*Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV), Université du Littoral Côte d'Opale, Dunkerque, France*

Background and Objectives Because air pollution affects air quality, climate, and human health, the use of biofuels has expanded significantly. One identified candidate for second-generation biofuel is prenol (3-methyl-2-buten-1-ol)(Nascimento, 2022). This Volatile Organic Compound (VOC) is released into the atmosphere while it is transported, stored, or unburned in automobile engines (Belgiorno, 2018). Prenol reacts with atmospheric oxidants, which can lead to the formation of several gaseous oxidation products as well as Secondary Organic Aerosols (SOAs), that are potentially hazardous. The objective of this study was to assess, in vitro, the toxicologic effects of prenol and its ozonolysis products formed in the atmosphere. Material and Methods The ozone reaction of prenol was performed in the atmospheric simulation chamber CHARME (Dunkerque, France). A Proton Transfer Reaction Mass Spectrometer and a Scanning Mobility Particle Sizer were used to monitor the concentrations of VOCs and SOAs, respectively. To investigate the impact of prenol and its ozonolysis products on health, an air/liquid interface (ALI) cell exposure device (Vitrocell) was coupled to CHARME to expose human lung cells (Calu-3 cell line) for 1 hour to prenol alone, ozone alone or prenol + ozone. Results The major oxidation products detected in the gas phase were methylglyoxal, glycolaldehyde, and acetone. The formation of SOAs was also observed. In addition, no significant cytotoxicity following any of the exposures was detected. Comparing the cells exposed to prenol, ozone or both in combination with filtered air used as a control, RT-qPCR analyses revealed gene induction of proteins involved in xenobiotic metabolism (CYP2E1 and NQO1) and antioxidant defense (HMOX1, NOQ1, and NRF2). Additionally, a pro-inflammatory response was measured at the protein and gene levels. Discussion and Conclusion To the best of our knowledge, this study is the first one presenting the coupling of an ALI exposure device to an atmospheric simulation chamber.

P201
Use of 3D Airway Tissue Models Cultured from Rat, Primate, or Human Cells for Translational Inhalation Studies
ABSTRACT #356

Seyoum Ayehunie[1], Robert Jackson[1], Kaitlyn

Coen[1], Timothy Landry[1], Jan Markus[2], Silvia Letasiova[2], Emily Reis[2], Alex Armento[1]
[1]*MatTek Corporation, Ashland, MA, USA*
[2]*MatTek Europe, Bratislava, Slovakia*

In vitro models of the respiratory tract have been available since 2000 and are widely used for toxicological, respiratory infection, tobacco safety, and inhaled drug delivery studies. They have been instrumental in understanding SARS-CoV-2 infection mechanisms and screening antiviral compounds. Despite these applications, there are large databases of animal toxicity data that are not directly translatable to data obtained from the human in vitro airway tissue models due to species differences. To close this gap, cells harvested from both rat and non-human primate (rhesus monkey) tissues were utilized to develop models similar to EpiAirway, the tracheobronchial tissue model offered by MatTek that is cultured with human cells. The tissues were characterized for structure, epithelial cell markers, barrier integrity, and functionality. Animal cell-derived 3D tissues exhibited similar characteristics to human tissues, including well-polarized epithelia with physiological transepithelial electrical resistance (TEER) values (>300 Ω*cm2), cilia formation, and mucin production. Acute exposure to four chemical toxicants showed species-specific changes in tissue viability and membrane integrity as measured by MTT and TEER assays, respectively. The tissue viability by 50% (ED-50) for vinyl acetate (VA) and chloroacetaldehyde (CA) were <2mg/tissue, and for propylene glycol (PG), it was >20 mg/tissue for all species. However, the ED-50 values for toluene (T) differed between species: human >20 mg, primate 16.2±1.7 mg, and rat 13.8±0.1mg. Based on MTT viability and TEER values, the chemicals were rank-ordered by toxicity: CA > VA > T > PG and controls (water and corn oil). Quality control data from tissue lots produced in the US and Europe showed no statistical difference in TEER values. Although more chemicals need to be tested, multispecies 3D airway tissue models are essential translational tools, predicting airway toxicity, bridging in vitro - in vivo gaps to reliably predict human responses, and offering a global alternative to animal testing.

KNOWLEDGE SHARING AND EDUCATION

P67
A new animal product free defined universal cell culture medium: Easy to use, do-it-yourself and beneficial for 2D and 3D culturing of normal and cancer cells
ABSTRACT #40

Stina Oredsson[1], Atena Malakpour-Permlid[2], Tilo Weber[3], Jeffrey Bajramovic[4]
[1]*Department of Biology, Lund University, Lund, Sweden*
[2]*Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Health Technology, Technical University of Denmark, Lyngby, Denmark*
[3]*Animal Welfare Academy of the German Animal Welfare Federation, Neubiberg, Germany*
[4]*3Rs Centre Utrecht, Utrecht University, Utrecht, The Netherlands*

Cell culturing methods are increasingly used to reduce and replace the use of live animals in biomedical research and chemical toxicity testing. Although live animals are avoided when using cell culturing methods, cell culture media often contain animal-derived components of which one of the most commonly used is fetal bovine serum (FBS). FBS is added to cell culture media among other supplements to support cell attachment/spreading and cell proliferation. The safety, batch-to-batch variation and ethical problems with FBS are acknowledged and therefore worldwide efforts are ongoing to produce FBS free media. Here, we present the composition of a new defined medium with only human proteins either recombinant or derived from human tissues. This medium supports long-term culturing/routine culturing of normal and cancer cells, plus it can be used for freezing and thawing of cells, i.e. for cell banking. It has been successfully tested with human normal and cancer-associated fibroblasts, human keratinocytes, several human breast and pancreatic cancer cell lines, human colon cancer CaCo-2 cells, L920 mouse fibroblasts as well as several other normal and cancer cells. We show growth curves and dose

response curves of cells grown in two and three dimensions as well as applications such as cell migration. Cell morphology was studied in real time by phase contrast and phase holographic microscopy time-lapse imaging. In conclusion, we present the composition of a defined medium without animal-derived components which can be used for routine culturing and in experimental settings for normal and cancer cells. This medium provides a leap towards a universal, animal component-free, cell culture medium.

P68
Fun with NAMs
ABSTRACT #122

François Busquet[1]
[1]*Altertox academy*

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, a new concept and format is expected to complement the current "arsenal" of tools available. TATAbox, the first serious 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. (1) https://joint-research-centre.ec.europa.eu/jrc-news/education-and-training-3rs-2018-02-27_en (2) Introducing the Three Rs into secondary schools, universities https://publications.jrc.ec.europa.eu › JRC123343

P69
Pioneering a Human-Centric Biology Research Database: The Age of Human Biology-Based Research.
ABSTRACT #164

Fabrizio Rossi[1], Wynand Alkema[2], François Busquet[3], Tomas Novotny[4], Nils Hijlkema[2], Giuseppe Castro[5], Pierre Deceuninck[6], Marco Straccia[1]
[1]*FRESCI by SCIENCE&STRATEGY SL*
[2]*Tenwise BV*
[3]*AlterTox srl*
[4]*EcoMole sro*
[5]*Ready2Use srl*
[6]*European Commission Joint Research Centre*

The biomedical research field is undergoing rapid transformation, with an emphasis on precision, accuracy, and human-centric approaches. The Joint Research Centre is spearheading this transition through its project that emphasizes human biology-based models as the cornerstone of health research advancements. The human body's intricacies cannot be fully replicated by other species, underlining the importance of direct human biology research. And human-based models offer insights into more relevant pathophysiology, genetics, and molecular pathways, ensuring more reliable predictions regarding new medical interventions, resulting in improved clinical trial outcomes. Recognizing the risk of potential overlaps or misinterpretations when comparing animal and human data, the significance of a dedicated human-centric database becomes evident. Our consortium is set to establish the EU's inaugural public database exclusively focusing on human biology-based models. Utilizing AI, this database will consolidate and offer a comprehensive view on human biology, ranging from in vitro methods using human cells to in silico models. This will be not just a data repository but an intuitive, AI-enhanced platform facilitating access to human biology-

specific literature, with a user-friendly interface where natural language requests are possible. While aligning with Directive 2010/63/EU's principles regarding animal testing, our primary dedication is human health research. The database is envisioned to stand alongside renowned platforms like PubMed and Clinicaltrials.gov, highlighting its potential impact on human-centric research. In summary, this project seeks to usher in an era where human health research is precise, pertinent, and ethical, truly heralding the dawn of human biology-based research.

P70
DEVELOPING AND EMBEDDING EDUCATION PROGRAMMES IN CO-CREATION WITH RELEVANT STAKEHOLDERS: THE TRANSITION TO ANIMAL-FREE INNOVATION UTRECHT EXPERIENCE (TPI UTRECHT)
ABSTRACT #337

Daniela Salvatori[12], Cyrille Krul[3], Anne Kienhuis[4], Merel Ritskes-Hoitinga[1], Paul, L. Carmichael[5], Janneke Hogervorst[6]
[1]*Veterinary Faculty, Utrecht University. The Netherlands*
[2]*on behalf of Transition to Animal-free Innovations Utrecht*
[3]*University of Applied Sciences, Utrecht. The Netherlands*
[4]*RIVM (Dutch National Institute for Public Health and Environment)*
[5]*Safety and Environmental Assurance Centre, Unilever, United Kingdom*
[6]*PETA UK, United Kingdom*

The Dutch government has established the Transition Programme for Innovation without the use of animals (TPI). Utrecht University, the University Medical Centre Utrecht, the University of Applied Sciences Utrecht and the National Institute for Public Health and Environment (RIVM) have joined forces in TPI Utrecht to effectively support and boost the transition. One of the core elements of our strategy is to create educational programmes for students, early career scientists and professionals in co-creation with the relevant stakeholders. Recently, we published the ambition statement striving towards innovation in higher education using fewer laboratory animals and focusing on the transformative value of education for acceptance of non-animal New Approach Methodologies (NAMs). Students and early career scientists are key stakeholders and can develop as effective change agents, but their perspective remains underrepresented. Our studies have explored students' viewpoints focusing on their values and their motivation to join TPI-inspired courses. We show that students share the ethical and scientific values that inspire the transition, and that their reflections on the socio-political landscape provide valuable insights on current and future challenges. The University of Applied Sciences Utrecht and TPI Utrecht are developing a practically-oriented master's degree in NAMs. The target group of the interdisciplinary master is the professionals in the field: they will obtain their degree through innovative education methods such as the challenge-based learning approach that relies on realistic and authentic assignments from the field. Systematic Review courses are offered to students and professionals together with specific coaching of researchers in their own working place. In co-creation with industry and government, TPI Utrecht is collecting needs for lifelong learning programmes. We have established a community working on the international Education HUB founded on the principles of sharing, co-creating, connecting and inspiring people, organisations, companies to accelerate the transition to animal-free research.

P215
Promoting ethical science through education: Bridging the gap with the 3Rs
ABSTRACT #376

Axelle Cooreman[1], Tamara Vanhaecke[1], Vera Rogiers[1]
[1]*Laboratory of In Vitro Toxicology and Dermato-cosmetology, Department of Pharmaceutical and Pharmacological Sciences, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel*

This project highlights the ethical considerations in science and emphasizes the goal of connecting different societal groups

through education on the 3Rs principles. In our current society, alongside concerns about the environment and sustainability, many questions arise regarding the use of laboratory animals in scientific research and its relevance to humans. Conversely, public trust in alternative methods remains low due to unfamiliarity. By implementing education on the 3Rs (Replacement, Reduction, Refinement), we aim to bridge the gap between society and scientists, ensuring that individuals from all societal levels understand the 3Rs and gain more confidence in alternative methods. Given the key role of synergy between science and education in significantly impacting society, we will introduce educational material for secondary school students to the principles of the 3Rs. We will visit various schools across Flanders and Brussels to disseminate this material and guide teachers through the process. Education on the 3Rs fits both basic science classes like biology and philosophy of life classes, as it addresses both the moral considerations of animal experimentation and the scientific principles behind alternative methods. This dual approach ensures that students understand the ethical and practical aspects of animal research and alternative methods. This initiative will encourage dialogue between scientists and citizens, engaging society in the 3Rs journey and collectively striving towards the common goal of advancing the 3Rs and achieving the best science.

LOCAL TOXICITY TESTING (SAFETY AND EFFICACY)

P71
NEW APPROACH METHODOLOGY TO SKIN EVALUATION FOR PESTICIDES: ADVANTAGES AND LIMITATIONS ABSTRACT #7

Julio Cesar Cianci[1], Thatiane Nunes Santana[1], Leandro Fernando Felix[2], Letícia Braggion Bacchin Lara[3], Carla Carolina Munari[1], Bruna Assunção Bechtold[4], Nádia Aline Corroqué[5], Carlos Alberto Goia[4], Daniela Viana[1], Luis Paulo Fava[6], Marcelo Toledo[7], Juliana Falcato Vecina[5]

[1]*Laboratory Analyst - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[2]*Laboratory Assistant - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[3]*Trainee - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[4]*Specialist - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[5]*Researcher - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[6]*Laboratory Coordinator - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[7]*Test Facility Manager - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*

Background and Objectives: Pesticides are widely used and indispensable in agriculture production. However, the human exposure to them may cause adverse health outcomes. Therefore, it is extremely important to evaluate the skin risks of these substances. Most pesticides are agrochemical formulations composed of a complex mixture of substances, usually the Contract Research Organization (CRO) knows only the active(s) ingredient(s), which shows us the need to investigate the entire compound. Our aim was to conduct a retrospective analysis of in vitro assays compared to in vivo GHS classification, and finally to conclude the advantages of strategies based on IATA and its limitations to agrochemical formulations. Material and Methods: 210 studies conducted over the period of 2 years (July 2020 to July 2022) were verified, repetitions were excluded, resulting in 187. The bottom-up strategy of IATA was chosen according to active(s) ingredient(s) characteristics. Results: Most pesticide (67%) were non-irritating. Only 33% were irritating, and after performing OECD 431, only 3% were Corrosive (1B/1C). According to results, 152 studies were classified, but less than 55% corroborate with the literature. Discussion and Conclusion: We found some discrepant results, which suggest the components influence or different test system used. Therefore, our results showed the importance to know all the formulation and how it is extremely essential to perform all the strategy to classify according to UN GHS. Another important detail to remember is that the previous classifications were performed in another test system, so divergences in the results may occur due to

histological differences between human and animal tissues. Consequently, it is essential to keep in mind that every new approach methodology has its own limitations, and it is fundamental to understand the conclusion of each experimental assay. Besides, the new classification and labelling will be necessary based on alternative methods in the near future.

P72
EYE DAMAGE, WHAT IS THE BEST STRATEGY TO REGULATE MY PESTICIDE?
ABSTRACT #11

Thatiane Nunes Santana[1], Julio Cesar Cianci[1], Letícia Braggion Bacchin Lara[2], Leandro Fernando Felix[3], Carla Carolina Munari[1], Bruna Assunção Bechtold[4], Nádia Aline Corroqué[5], Daniela Viana[1], Luis Paulo Fava[6], Marcelo Toledo[7], Juliana Falcato Vecina[5]
[1]*Laboratory Analyst - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[2]*Trainee - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[3]*Laboratory Assistent - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[4]*Specialist - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[5]*Researcher - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[6]*Coordinator - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*
[7]*Test Facility Manager - Mérieux NutriScience / Bioagri Laboratórios Ltda, Piracicaba-SP, Brazil.*

Significant progress has been made in replacement of the regulatory in vivo Draize rabbit eye test. A several alternative methods are recognized by the OECD to classify chemicals according to the UN GHS, but any one was not able stand-alone to predict eye hazard potential until 2022. The OECD 492B guideline describes the Time-to-Toxicity test using a commercially available reconstructed Human Cornea-like Epithelium (HCE), which mimics the histological, morphological, biochemical and physiological properties of the in vivo human corneal epithelium and is capable to classify the three main categories of UN GHS. Our aim was to conduct a retrospective analysis of in vitro assays based on OECD 491 (STE) and 437 (BCOP) and finally conclude the low prediction of these guidelines and how OECD 492B could be effective to classify agrochemical formulations. Thereby, 197 studies conducted between 2021 to 2023 have been verified: 153 performed STE, most were Category 1, otherwise 44 performed BCOP, most were No Category. Therefore, only 40% of results were conclusive results, which suggest the components influence or difference test system used or limitations of method. Mostly pesticides are agrochemical formulations composed of a complex mixture of substances, which shows us the need to have an efficient alternative method as OECD 492B. An important detail is to remember that the classifications were been performed in different test system, so divergences in the results may occur due to different endpoints of each method. We have performed 41 studies using HCE since our OECD 492B implementation, although few because the implementation is recently, it was able to classify every pesticide, independently if it is liquid, viscous or solid. Therefore, OECD 492B has capability to discriminate the three categories of UN GHS and could be consider a total replacement for classify pesticides in the next future.

P73
EPIGENETICS BIOMARKERS TO EVALUATE EARLY TOXICITY EFFECTS: A DERMAL TOXICITY CASE STUDY OF ALUMINUM DIETHYLPHOSPHINATE
ABSTRACT #31

Enzo Zini Moreira Silva[1], Irisdoris Rodrigues de Souza[1], Daniela Fiori Gradia[1], Daniel Pacheco Bruschi[1], Danielle Palma de Oliveira[23], Cynthia Bomfim Pestana[1], Daniela Morais Leme[13]
[1]*Department of Genetics, Federal University of Parana (UFPR), Curitiba-PR/Brazil*
[2]*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP/Brazil*
[3]*National Institute for Alternative Technologies of Detection, Toxicological Evaluation and Removal of Micropollutants and Radioactives*

(INCT-DATREM), Institute of Chemistry, Araraquara-SP/Brazil

Chemicals can cause toxicity at different levels of biological organization (i.e., cells, organs, organisms). At a cellular level, recent studies have explored the relationship between chemical exposure and epigenetic effects. In the skin, changes in normal epigenetic patterns (i.e., DNA methylation pattern, histone modifications, and expression of non-coding RNAs) have been related to premature skin aging and several skin diseases (e.g., psoriasis, skin cancer) (Orioli et al., 2018; Möbus et al., 2020). New Approach Methodologies (NAMs) have been developed to provide information on chemical hazards using mechanism-based data, avoiding or significantly reducing animal testing in chemical safety assessment. Thus, this study explores possible epigenetic effects caused by the phosphorus flame retardant Aluminum Diethylphosphinate (Alpi, 0.03, 0.06, and 0.12 mg/ml – non-cytotoxic concentrations)) in human keratinocytes (HaCaT cell line). Target genes related to DNA methylation (TET and DNMT family) and histone modifications (SWI/SNF complex) machinery were investigated by RT-qPCR. NEAT1 and MALAT1, two long non-coding RNAs (lncRNAs) involved with inflammatory skin responses, were also evaluated by RT-qPCR (Botchkarev et al., 2012). Global DNA methylation pattern on HaCaT cells exposed to Alpi was determined by quantifying 5-methylcytosine (5mC) and 5-hydroxymethyl cytosine (5hmC) by flow cytometry. Preliminary RT-qPCR data did not indicate significant changes in the expression of the target genes on HaCaT cells treated with Alpi. Significant alterations in the levels of 5mC and 5hmC on Alpi-treated HaCaT cells were also not verified in the G1 or G2 cell cycle phases. In summary, Alpi seems not to cause epigenetic effects on human keratinocytes, which can provide the first line of information for possible adverse effects on the skin.

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SKIN HYDRATION MEASUREMENT: AN IN VITRO APPROACH WITH TEER MEASUREMENT OF HUMAN EPIDERMIS RECONSTRUCTED COMPARED WITH IN VIVO METHODS ABSTRACT #59

Guillaume LACOSTE[1], Alexandra DIVET[2], Justine PENNEL[2], Charles REINIER[2], Sophie CATOIRE[1]
[1]*CELL ALTERNATIV, 60350 Trosly Breuil, FRANCE*
[2]*COSMEPAR, 44000 Nantes, FRANCE*

Moisturizing the skin is essential to its health and beauty. Indeed, repeated exposure to external aggressions disrupts the skin's natural hydration mechanisms and can deplete its water reserves. To remedy this, many moisturizing products on the market are designed to improve or maintain the skin's water balance. To assess the moisturizing potential of cosmetic products, two panel methods are commonly used: corneometry and tewametry (TEWL). We were interested in developing an in vitro method that would enable us to reduce the number of studies on volunteers, or to carry out an initial screening during the R&D phases, for a better selection of moisturizing ingredients or formulations. In this study, 8 finished cosmetic products with different galenic formulations (cream, serum, mask, etc.) were tested using in vivo and in vitro methods to obtain comparative data. The AlternaSkin in vitro model is a human reconstructed epidermis (RhE) in 3D in laboratory. The protocol consists of deliberately dehydrating the epidermis with clay powder and studying the impact of product application on rehydration by quantifying the evolution of trans-epithelial electrical resistance (TEER). TEER (in ohms) reflects the integrity and permeability of the cutaneous barrier. Like corneometry, this method analyzes hydration using the relationship between the tissue's electrical properties and its water content. Our preliminary results show that voluntary dehydration lowers TEER by 50-70%. We have also noticed that some products induce an increase in TEER as early as 30 minutes, which can reach 150%. Others require a longer contact time, up to 2 hours, to show an effect. Disparities can be observed between results obtained on volunteers versus in vitro, nevertheless increasing the number of products tested and refining a study protocol should smooth out these differences.

P75
DEVELOPMENT OF A METHOD FOR ASSESSING THE IRRITANT AND SOOTHING EFFECT OF GINGIVAL PRODUCTS USING RECONSTRUCTED HUMAN GINGIVAL EPITHELIUM
ABSTRACT #60

Laëtitia BEAUDEQUIN[1], Adeline JOSSEAUME[1], Fabien GUILLON[2], Sophie CATOIRE[1]
[1]*CELL ALTERNATIV, 60350 Trosly Breuil, FRANCE*
[2]*STERLAB, 06224 Sophia-Antipolis, France*

Present only in the mouth, the gingiva is a mucous membrane covering the base of the teeth, exposed to numerous daily aggressions such as toothpaste, mouthwash and various oral hygiene products, but also prone to infections directly linked to the individual's oral health. Good dental hygiene is therefore essential for healthy gingiva. Irritation testing techniques for oral hygiene products on monolayer cell cultures, however, do not provide for representative testing of their use. Sterlab®'s 3D biological model of in vitro reconstructed human gingival epithelium is chosen for this study. Its structure and characteristics make it possible to mimic in vitro, a human gingiva for the evaluation of oral products, both from a toxicity (irritation) and efficacy (soothing effect) point of view. The study of the irritant potential of oral products is based on the determination of ET50 (effective time to 50% cell death). The results obtained are compared with those of benchmarks and products already on the market. The efficacy study consists of testing soothing formulations to assess their potential, after determining the maximum time of use in the ET50 test, using an MTT cytotoxicity test and the IL-1alpha inflammatory interleukin assay. For this purpose, an inflammatory reaction of gingival epithelia is generated with 0.5% Sodium Lauryl Sulfate (SLS). The increase in IL-1alpha interleukins have to be more than twice that of the untreated control, enabling us to study the products' ability to reduce this inflammation after application. Sterlab®'s 3D biological model of human gingival epithelium, reconstructed in vitro, appears to be a model that can be used to test oral application products as closely as possible to the way they are actually used, in order to assess their toxicity and/or efficacy.

P76
RELEVANT END-POINTS TO DEFINE AN IN VITRO STUDY STRATEGY FOR NASAL APPLICATION PRODUCTS USING HUMAN NASAL EPITHELIUM RECONSTRUCTED
ABSTRACT #61

Adeline JOSSEAUME[1], Laëtitia BEAUDEQUIN[1], Johan GARDERES[2], Sophie CATOIRE[1]
[1]*CELL ALTERNATIV, 60350 Trosly Breuil, FRANCE*
[2]*LABORATOIRES GILBERT, 14200 Herouville Saint clair, France*

The nose is the gateway to everything we breathe, and is exposed to all kinds of environmental pollution: dust, pathogens, allergens... It is the respiratory system's first line of defense. As a result, the nasal hygiene marketing sector is expanding its range and diversifying its applications. Nasal cleansing moistens mucus and helps loosen secretions stuck to the mucous membrane. The aim of the project presented here is to determine the performance of different nasal solution in preventing damage to the nasal epithelium, and also to claim a moisturizing effect for the products. The choice of biological model and end points was crucial to this work. The model chosen was a three-dimensional model of human nasal epithelium reconstructed in vitro by Epithelix (MucilAirTM). Three lines of study were chosen to address the study of nasal solutions in their entirety: - Study of nasal epithelial integrity (Trans epithelial electrical resistance (TEER) measurement, Lactate deshydrogenase LDH dosage and Interleukine IL-8 assay), - Study of nasal mucociliary clearance (mucin quantification, ciliary beating frequency (CBF) and rheology), - Study of the hydrating effect (TEER and tissue morphology after hypertonic stress). Based on benchmark products with a well-known effect on epithelial integrity (Triton), mucociliary clearance (isoproterenol) or moisturizing effect (ectoine), it was possible to study cellular responses after treatment with nasal hygiene products, and to make comparisons between products and with benchmak products for each axis of study. This

enabled us to select the most relevant end-points in order to define a study strategy for nasal application products and confirm the relevance of the MucilAirTM model for this application.

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VALORIZING MARINE BYPRODUCTS FOR NUTRACEUTICAL APPLICATION
ABSTRACT #74

Giorgia Allaria[1], Lorenzo Dondero[1], Francesca Tardanico[1], Francesca Rispo[1], Giulia De Negri Atanasio[1], Federica Robino[2], Elisabetta Perata[2], Raffaella Boggia[3], Federica Turrini[3], Rebeca Vázquez Sobrado[4], María Mercedes Alonso Martínez[4], Celina Costas Iglesias[4], Matteo Zanotti Russo[2], Elena Grasselli[1]
[1]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa, 16132 Genoa, Italy*
[2]*Angel Consulting, 20122 Milano, Italy*
[3]*Department of Pharmacy, University of Genoa, 16148 Genoa, Italy*
[4]*ANFACO-CECOPESCA, Colexio Universitario, 36310 Vigo, Spain*

Background and Objectives In recent years, the production of fish-processing side streams by the fish supply chain has increased, inducing a significant impact on the environment and economy. This study belongs to the H2020 EcoeFISHent project (10103642) aimed to identify a pool of active ingredients from food fish-processing side streams with activity for human health. In particular, it is reported the PUFAs cell viability, and its antioxidant activity to investigate a possible nutraceutical application. Material and methods PUFAs were extracted from fish-processing products using green technology, a benchmark PUFA was used as standard compound. The sample was solubilized in absolute EtOH, related to the standard DHA content, and then was diluted in a solution of BSA (2mg/mL). Various concentrations were tested with this last solution using cell medium. HECV(human endothelial cells), HepG2 (human hepatocyte cells), and HaCat (human keratinocyte cells) were used as cellular models. Cell viability was investigated on HECV and HaCat for 24-48-72h using crystal violet assay testing different concentrations of PUFAs; moreover, the PUFAs antioxidant activity was evaluated on HepG2 and HaCat, by treating cells with H2O2, a known prooxidant agent. DPPH test was also carried out to evaluate PUFA's antioxidant effect. Results The obtained data reveal that the PUFAs, at the concentration tested, did not decrease the cells' viability at all the time points taken into account. The test of the antioxidant activity on HepG2 demonstrated that at the lower dilution, the PUFAs, present a protective action. The antioxidant power of the compounds was confirmed by the DPPH test. Discussion and Conclusion More data will be collected in the future to further confirm the safety of fish oils as bioactive ingredients from animal processing side stream, based on a circular economy idea. The best-performing extract will then undergo validation using 3D reconstructed skin and intestinal models.

P78
UPCOMING ADOPTION OF A DEFINED APPROACH FOR EYE HAZARD IDENTIFICATION OF NEAT SOLIDS UNDER OECD TG 467?
ABSTRACT #79

NATHALIE ALEPEE[1], ELS ADRIAENS[2]
[1]*L'Oreal R&I*
[2]*Adriaens Consulting bv*

The regulatory acceptance of Defined Approaches (DA) for assessing the hazard identification potential of chemicals remains an active topic for regulatory toxicology. Currently, two DAs for non-surfactant liquids have been adopted by the Organisation for Economic Cooperation and Development (OECD TG 467) to discriminate between three United Nations Globally Harmonized System of Classification (UN GHS) categories i.e., Category 1 (Cat. 1), Category 2 (Cat. 2) and No Category (No Cat.). The purpose of the current project is the inclusion of a a new DA for solid chemicals into OECD TG 467. The DA for neat solids (DAS) is based on a combination of the SkinEthic™ Human Corneal Epithelium (HCE) Eye Irritation Test (EIT) (OECD TG 492), and the Bovine Corneal Opacity and Permeability (BCOP) test method using the laser light-based opacitometer (LLBO) (OECD TG 437). The SkinEthic™ HCE EIT method is used to distinguish No Cat. from classified substances.

For classified substances the BCOP LLBO method is used to identify Cat. 1, the remaining solids are then predicted Cat. 2. The DAS is developed based on in-depth statistical analysis of a database on 109 solids for which in vitro and historically curated in vivo Draize eye test data exist. In summary, 77.4% Cat. 1 (N=31), 52.3% Cat. 2 (N=18) and 70.0% of No Cat. (N=60) solids were correctly identified, giving a balanced accuracy of 66.7% meeting the proposed minimum OECD performance of 75% (Cat. 1), 50% (Cat. 2) and 70% (No Cat). In addition, the uncertainty associated with the performance of the DAS based on 100,000 Bootstrap replicates, shows a 93.5% reproducibility. The DAS has shown to be successful for eye hazard identification of solids according to the three UN GHS categories. The DA for solids is currently under consideration by the OECD as Part III in TG 467.

P79
In vitro Hepatotoxicity Test for the Multiple Exposure to Functional Ingredients using AML-12 cells
ABSTRACT #119

Sohee LIM[1], Minho SHIN[1], Hoonjeong KWON[12]
[1]*Department of Food and Nutrition, Seoul National University, Seoul, Republic of Korea*
[2]*Research Institute of Human Ecology, Seoul National University, Seoul, Republic of Korea*

Recently, dietary supplements and functional foods have become familiar to consumers. Some products contain multiple ingredients, and each person has a chance to take various functional foods together, resulting in multiple exposure situations. However, in general, safety tests have been conducted using single ingredients, and safety studies about multiple exposure of functional ingredients are rare. Green Tea Extract(GTE), Ginkgo Biloba Extract(GBE), and Banaba Leaf Extract(BLE) are known to cause same side effect, hepatotoxicity. GTE, Garcinia Cambogia Extract(GCE), and Gynostemma Pentaphyllum Extract(GPE) are often used together. This study was designed to test hepatotoxicity of multiple intake of functional ingredients. Cell toxicity was measured using WST-1 method with mouse hepatocyte, AML 12. mRNA transcripts were measured to detect early signs of toxicity at low concentrations. Functional ingredient pairs with the same side effect(GTE, GBE, BLE) showed significant decrease in cell viability and concentration-dependent tendency. However, the dose used in the study was too high to apply the results to real intake conditions. Other functional ingredient pairs used in same product(GTE, GCE, GPE) didn't exhibit cytotoxicity. In the lower dose condition, mRNAs involved in protein synthesis, such as Etv5 and Ak4, as well as mRNAs in the apoptosis pathway, such as Megf10, Bcl2l14, and Clca3a2 increased in GTE-GCE pair compared to single treatment GTE. However, mRNAs involved in cell survival were also shown to increase. In conclusion, it is difficult to determine the effect of multiple exposures on cytotoxicity. Still, it was apparent that stress signals and repair systems were up-regulated in multiple exposure situation. Even though the in vitro cellular system can be easily employed to test multiple exposure scenarios, toxicity can be influenced by various ADME factors, so it can't be solely proven through cellular toxicity tests. Additional tests such as organoid or human-on-a-chip system would be desirable.

P80
Unraveling the Interplay of chemicals and UVA Light: Inflammatory Cytokines as biomarkers of photosafety assessment
ABSTRACT #152

Adriana S Maddaleno[1], Elisabet Teixidó[2], M Pilar Vinardell[1], Montserrat Mitjans[1]
[1]*Fisiologia, Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Spain*
[2]*Toxicologia, Departament de Farmacologia, Toxicologia i Química Terapèutica, Universitat de Barcelona, Spain*

Chemicals included in different products such as pharmaceuticals, cosmetic formulations, fragrances, and household items, can cause adverse reactions, including contact photosensitization, which encompasses both photoirritation and photoallergenicity. Photoirritation occurs when the chemical exposition to UV radiation leads to an acute inflammatory response resembling sunburn. In another way, photoallergenicity triggers an

immune response in the skin upon exposure to a chemical in the presence of UV radiation. Consequently, it is essential to conduct photosafety assessments of chemicals with potential for photosensitizing effects. In this sense, the development of new alternative tests to animal experimentation to predict these adverse reactions is of especial interest for cosmetics due to the European ban on animal use as well as for other product categories where there is a need to phase out animal testing. In this work, inflammatory cytokines were explored as biomarkers of photosensitization using different compounds categorised as photoirritants and/or photoallergens as Chlorpromazine, Benzophenone and 8-methoxypsoralen, as well as irritants and allergens (sodium lauryl sulphate, p-phenylenediamine). Thus, the commercial line of human keratinocytes HaCaT was exposed to different concentrations of compounds in the absence and presence of UVA light at 4J/cm2, and cell viability was determined by the NRU and MTT assays (1). Supernatants collected 24 hours after irradiation were analyzed using a multiplex array with the aim of semi-quantitatively measuring and identifying the levels of inflammatory cytokines and studying their potential use to discriminate between photoirritants and photoallergens. The results revealed changes in the levels of certain cytokines, such as MCP-1 and IL-6, depending on the type of product used. However, further quantitative studies are needed to confirm the utility of these cytokines as biomarkers for photosafety assessment.

P81
In vitro tolerance evaluation of cosmetic products: an overview of cutaneous and ocular irritation potential among different product categories
ABSTRACT #153

Charlène LEJAMTEL[1], Nathan STOCKMAN[1], Coralie PLACENTI[1], Eva-Anne SUBILEAU[2], Christophe CHESNE[12]
[1]*Eurosafe, Parc d'Affaires de la Bretèche, 35760 Saint-Grégoire, France*
[2]*Biopredic International, Parc d'Affaires de la Bretèche, 35760 Saint-Grégoire, France*

Local tolerance is a major concern in the cosmetic industry in relation to the potential adverse effects of products on human skin and eyes. Since the ban of animal testing, alternative in vitro methods have been developed [1]. They are now routinely used to investigate the irritation potential and are included in the regulatory safety assessment of cosmetic products [2]. Although the aim of the safety assessment is to minimize adverse effects, some products on the market are likely to cause irritation and this capacity may vary between product categories [3]. Using in vitro tests, we assessed skin and eyes irritation over 9773 products divided in 13 categories, representing the products found on the European market. In vitro models used range from reconstructed epidermis, mucosa and corneal epithelium to skin disc and hen's egg chorioallantoic membrane. Our aim in this study is to show the difference in irritation potential among different categories of cosmetic products. We found that 19% of the products appear to be potentially irritating for the skin and 35% for the eyes. The highest potential for skin or mucous membrane irritation is deodorants and intimate hygiene products with 34% and 43% of products respectively classified as potential irritants. With 97% and 72% of products likely to be eye irritants, soaps and perfumes appear to be the categories of greatest concern. Within the categories, 26% of products intended for babies present a risk of eye irritation, while 13% have a risk of skin irritation. All these data represent a global picture of the potential irritation risks associated with a wide range of cosmetic products. This comprehensive analysis serves as a valuable resource for both the cosmetic industry and regulatory authorities, enabling the development of safer cosmetic formulations.

P82
IN VITRO TEAR FILM INSTABILITY: A NEW PHYSICO-CHEMICAL APPROACH TO EVALUATE EYE DISCOMFORT
ABSTRACT #168

Laurent Guillet-Revol[1], Swati Kaushik[1], Thanh Pham[1], Koji Endo[2], Masanori Orita[2], Ingrid Gamba[1], Romain Cornet[1], Yoshimune

Nonomura[3], Caroline Rouas[1], Frédéric Amaral[1], Noémie de Crozé[1], Nathalie Alépée[4], Fleur Tourneix[5]
[1]*L'Oréal Research & Innovation, France*
[2]*L'Oréal Research & Innovation, Japan*
[3]*Yamagata University Faculty of Engineering, Japan*
[4]*L'Oreal R&I*
[5]*L'Oréal Research & Innovation*

The assessment of eye irritation potential is one of the most common safety issues in cosmetic industries. Despite the availability of well-established in vitro safety evaluation tests to predict eye irritation, the cosmetic industry lacks techniques to assess eye discomfort. Discomfort can be defined as a feeling of dryness, tingling or blurred vision, and is a subjective sensation. The surface of eye is protected by a natural barrier known as tear film. This film is composed of a lipid phase and an aqueous phase. It protects the eye from drying out as well as external aggressions such as pollution. Any change in the integrity of the tear film causes disappearance of this protective cushion which can often lead to discomfort. In this work, we used a model of tear film representative of the natural tear film. We have developed an in vitro method to measure the impact of a cosmetic formula on the stability/integrity of the model tear film. We studied the appearance and evolution of tear film instabilities by focusing on the spreading of the lipid phase in contact with the formula over time. Here, we evaluated 18 shampoos for discomfort using this new physico-chemical approach and compared its performance with the clinical study results. Our in vitro prediction seems to be consistent with the clinical results for eye discomfort. This results indicate that our innovative physico-chemical approach can be a potential in vitro method to help predict the discomfort potential of shampoo and can be further used to explore the discomfort potential of other cosmetic formulae and better understand ingredients contribution to discomfort. Key words: In vitro evaluation, Lachrymal film integrity, Eye discomfort, Physical chemistry

P83
3D toxicology models in animal-free nanofibrillar cellulose hydrogels. ABSTRACT #172

Johanna Lampe[1], Darren Heywood[2], Piia Mikkonen[1]
[1]*UPM Biomedicals, Finland*
[2]*Promega, UK*

Well defined, reproducible, biologically relevant, and cost-effective in vitro cell models that retain liver-specific cell functionality are needed for toxicology testing. These models should have the potential to provide clinically relevant toxicological profiles of drug compounds, and at the same time they should avoid the use of animals in research. Three-dimensional (3D) cell culture with hydrogels has become a powerful tool for the development of these models. GrowDex® hydrogels are plant-derived nanofibrillar cellulose hydrogels, which have been shown to provide an effective and experiment-reproducible 3D matrix for various healthy and cancerous cell types, including liver cells. The hydrogels physically resemble the extracellular matrix, support cell, spheroid, and organoid growth or formation, whilst allowing the cells to produce and remodel the matrix to best suit their needs. The hydrogels are shear thinning, temperature stable, without lot-to-lot variability, ideal to scale up automated 3D cell-based assays for e.g., drug discovery and development and toxicology testing. It is known that GrowDex hydrogels optimally support the growth and spheroid formation of liver cells like PHH and HepaRG (1), and that the cells retain their organ-specific functionality (2). In addition, GrowDex hydrogels support the 3D coculture of hepatocytes with Kupffer cells (3), allowing even more physiologically relevant models. We have developed a straightforward multiplexing protocol to quantify viability, cell death, CYP activity and albumin secretion all from the same sample, saving time and material. We are currently developing this protocol further to take it to automation and high throughput. This will allow animal-free toxicology testing and could also be used in the future as a control when qualifying primary samples.

P84
Skin sensitization assessment of pesticide formulations and their active components using the Integrated Testing Strategy
ABSTRACT #242

Mariela Lenze[12], Martina Daniela Benedetti[12], Juan Ignacio Pina[3], Julieta Roco[12], María Laura Gutiérrez[12]
[1]*National Research and Technological Council of Argentina (CONICET)*
[2]*Institute of Pharmacology, Buenos Aires University Medical School*
[3]*Atanor S.C.A*

Skin sensitization testing is mandated by regulatory authorities in various countries as part of safety assessments for pesticides. Internationally standardized testing protocols encompass in chemico and in vitro techniques, based on The Skin Sensitization Adverse Outcome Pathway (AOP), which minimize animal testing. While alternative methods are valuable, there is no single assay endorsed as a complete substitute for animal-based methods. To address this, Defined Approaches (DAs) have gained acceptance which implies the integration of multiple non-animal methods applied to a defined combination of test results with no expert judgment to interpret the outcome. However, the methods recommended by the OECD Guideline 497 on DAs for skin sensitization have been primarily evaluated using individual chemical components rather than mixtures or formulations. Since pesticides consist of complex mixtures of one or more active ingredients, which determine their biological activity, and adjuvants that facilitate their application, rigorous testing to characterize their skin sensitizing potential by DAs is necessary. Our goal is to compare the predictive capability of the Integrated Testing Strategy (ITS) DA on the skin sensitization assessment of 10 pesticides formulations vs. their active ingredients. Both final formulations and pure active components were provided by ATANOR S.C.A, along with their in vivo data. The ITS combines test methods that address the following key events (KEs) in the AOP: Protein binding (KE1) which is evaluated by the Direct Peptide Reactivity Assay (OECD TG 442C) and Dendritic cell activation (KE3) which can be assessed with the U-Sens method (OECD TG 442E); and it includes an in silico prediction which can be performed with the OECD QSAR ToolBox. So far, our preliminary results show that the U-SENS assay seems to have a good performance to predict in vivo non-sensitizers for both formulations and active components, but no definitive conclusion is possible using a "stand-alone" approach.

P85
Harnessing Fish Side-Streams for Sustainable Cosmetics: The EcoeFISHent Project
ABSTRACT #260

Elena Grasselli[1]
[1]*University of Genoa*

Background and Objectives: The escalation of fish-processing byproducts in the fish supply chain poses a significant environmental impact and economic challenge. This study aims to identify beneficial components from side streams generated during the processing of food fish, offering potential advantages for human skin. Notably, the H2020 EcoeFISHent project (101036428) focuses on six circular value chains, with one specifically dedicated to the extraction of collagen and fish oils from marine sources for potential application in human cosmetics. Materials and Methods: Within the EcoeFISHent project, active ingredients selected for testing include hydrolyzed collagen and fish oils. The initial step involved developing a tiered strategy for the extraction process setup and subsequent scaling. Results: Conducted tests identified specific screenings for evaluating the most effective extraction processes. Additionally, experiments with commercially available active principles refined and established our experimental procedures. Discussion and Conclusion: The five-year EcoeFISHent project culminates in the formulation of active principles, integrated into a cosmetic product ready for market placement. This comprehensive approach ensures not only effective extraction processes but also sets the foundation for the successful introduction of these active principles into the cosmetic market, contributing to sustainable practices in

the cosmetics industry.

P86
Unveiling Lung Cell Responses to Nanolignin Particles Contributing to New Insights
ABSTRACT #286

Dalia de la Fuente-Vivas[1], Emanuela Bellinetto[2], Eleonora Ruffini[2], Sofia P. Makri[3], Sara Collado[1], Tanja Meyer[4], Gianmarco E. Griffini[2], Laura Gómez-Cuadrado[1], Sonia Martel[1], Rocío Barros[1], Carlos Rumbo[1]
[1]*University of Burgos-ICCRAM*
[2]*Politecnico di Milano*
[3]*Creative Nano PC*
[4]*Bio Base Europe Pilot Plant*

Lignin, a biopolymer abundant in lignocellulosic material, holds promise for new materials like nanolignin, with diverse applications such as food packaging due to its unique properties. However, it's unclear if airborne lignin nanoparticles pose health risks. These particles are small enough to remain suspended in the air and penetrate biological barriers, may interact with cells, potentially causing health issues. This study aims to understand lung cell responses to nanolignin particles obtained through various biomass pretreatment methods, specifically organosolv-steam explosion and hydrothermal pretreatment of biomass, followed by an ultrasound-assisted process for organosolv and alkaline lignin nanoparticle production. The study investigates the physicochemical properties of Organosolv and Alkaline lignin nanoparticles, as well as lignin nanoparticles obtained from two commercially available lignins, Kraft and Soda for comparative analysis. Morphology, size, distribution, and porosity were characterized using scanning electron microscopy (SEM), dynamic light scattering (DLS), and Brunauer-Emmett-Teller techniques (BET). Additionally, cellular responses, including viability (Neutral Red-uptake) and oxidative stress (DCFH-DA fold quantification) effects on A549 human lung cells, were assessed at nanolignin concentrations ranging from 1 to 160 mg/L after 24 hours and 30 minutes of exposure, respectively. Although no dose-dependent cytotoxicity of nanoparticles was observed (1-32 mg/L), decreased cell viability was noted at 160 mg/L, particularly with Alkaline and Kraft-lignin nanoparticles (>50%). Moreover, oxidative stress was induced by all tested nanolignin types, with higher levels observed in Alkaline-lignin and Kraft-lignin nanoparticles. Overall, findings suggest that concentration and physicochemical characteristics, such as surface area, play a crucial role in potential toxicity. Further research is needed to fully understand intracellular responses, contributing to enhanced safety knowledge of these nanoparticles. This research was funded by the European Community's Horizon 2020 Framework Programme (grant number 952941 Project: BIOMAC, European Sustainable BIObased nanoMAterials Community).

P87
Inter-species different effect of anticoagulant rodenticides. An in vitro approach.
ABSTRACT #297

David Hernández-Moreno[1], Ana Valdehita[1], Azucena Bermejo-Nogales[1]
[1]*Department of Environment and Agronomy, National Institute for Agriculture and Food Research and Technology (INIA, CSIC)*

Anticoagulant rodenticides (ARs) are widely used to control rodent populations. However, their potential arriving to water sources leads to a concern about their effect on non-target organisms. Our aim was to evaluate the inter-species differences regarding the effect of ARs, considering cell lines from a target (murine hepatoma–Hepa1-6) and a non-target species (rainbow trout; hepatoma–RTH149– and gonads–RTG2). The assays were performed using 1st (coumatetralyl, warfarin and chlorophacinone) and 2nd generation ARs (brodifacoum, bromadiolone and difenacoum). Because of their known late effect, cells were exposed to a range of concentrations (0.78-100 mg/L) for 24, 48 and 72 h. Cytotoxicity was evaluated checking for mithocondrial, plasma membrane or lysosomal activity effects. Detoxification process was tested through the cytochrome P450-1a activity (EROD) evaluation in Hepa1-6 and RTG2 cells, using non-cytotoxic concentrations. Hepatic cell lines exhibited similar sensitivities. A time-dependant cytotoxicity was observed for brodifacoum, bromadiolone, chlorophacinone and

difenacoum (IC50 72h<24h). Coumatetralyl only showed effect after 72 h for Hepa1-6, while warfarin was not toxic for both cell lines even after 72h. A cytotoxicity ranking (lowest to highest IC50) was maintained over time: chlorophacinone< brodifacoum< difenacoum< bromadiolone. The EROD induction observed in Hepa1-6 after 24h was directly associated to the cytotoxicities observed over time, serving as an early warning about the effect of ARs. In general, lower effect was observed in RTG2 after 24h, and non-clear associations between EROD and IC50s were stablished for the rainbow trout cells. Results suggested a similar toxic effect on target and non-target organisms, showing the need to look for alternatives to traditional ARs, effective against rodents but less hazardous to wildlife. More experiments are ongoing to confirm the utility of the EROD activity as early warning biomarker in species with different sensitivities. Funding: Project TED2021-131186B-I00 supported by MCIN/AEI/10.13039/501100011033 and the EU "NextGenerationEU"/PRTR, and project RAR funded by PIE-CSIC.

P88
Technical applicability of polymers to in vitro test methods – the skin irritation test (SIT)
ABSTRACT #301

Ayse Aktalay Hippchen[1], Susanne Kolle[1], Dorothee Funk-Weyer[1], Robert Landsiedel[12]
[1]*BASF SE, Experimental Toxicology and Ecology, Ludwigshafen am Rhein, Germany*
[2]*Free University of Berlin, Pharmacy, Pharmacology and Toxicology, Berlin, Germany*

Polymers serve as functional materials in everyday life, with their applications ranging from hygiene articles to car parts. Polymers possess physicochemical properties differing from their monomeric precursors. Unlike their monomers and additives, a systematic evaluation of polymers is not yet required for REACh but is expected to be added to the regulation. To gain experience on the technical applicability of existing test methods for polymers, we tested polymers of different physicochemical properties with standardized, validated, and well-established SIT. 17 Polymers were applied directly on the reconstructed tissue as solids or liquids as given by their state under ambient conditions and tested according to the SIT (OECD Test Guideline No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Methods; oecd-ilibrary.org). 12 polymers yielded negative results and 5 yielded positive results (viability <50%). However, 5 of the 17 polymers were not applicable using the standard operating procedures and modifications of the standard protocols were needed. E.g.: A bead-formed waxy solid was pressed to form discs that cover the tissue surface. A foamy solid was punched to create a cylindric piece fitting on the tissue, but the required application mass was not reached, due to extremely low density. A gel-like solid was applied with a pin on top to assure contact with the tissue surface. Two liquid polymers showed adhesive properties and could not be removed fully from the tissue. While the application of a polymer appears rather simple in theory, the actual feasibility is not straight-forward, thus adaptation of existing procedures and development and standardization of additional steps are necessary to apply polymers to the SIT. Moreover, the modified methods will need a validation including assessment of their reproducibility and the relevance of the results obtained in vitro to the actual human exposure shall be assessed.

P89
EpiOcular time-to-toxicity – a test method for subcategorization of eye irritants
ABSTRACT #309

Silvia Letasiova[1], Lenka Hudecova[1], Jan Markus[1], Yulia Kaluzhny[2], Els Adriaens[3], Mitch Klausner[2]
[1]*MatTek In Vitro Life Science Laboratories, Mlynske Nivy 73, 82105 Bratislava, Slovakia*
[2]*MatTek Corporation, 200 Homer Ave, Ashland, MA, USA*
[3]*Adriaens Consulting BVBA, Bellemdorpweg 95, 9981 Aalter, Belgium*

In 2015 an OECD TG 492 "Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or

serious eye damage" was accepted and validated for the use of in vitro ocular tissue models. Initially,this TG only allowed for distinguishing between substances and mixtures not requiring classification and those that must be labeled for eye irritation or serious eye damage.Further specification of eye irritation severity was not included in the TG. More recently an OECD TG 492B was accepted,which allows for distinguishing between chemicals that:a)do not require labeling for serious eye damage or eye irritancy (No Cat),b)cause serious eye damage (Cat 1),and c)are eye irritants (Cat 2)according to the UN GHS ocular hazard categories.A new testing strategy was developed based on the results from 2 studies,CON4EI and ALT4EI projects.A robust final set of 144 reference chemicals–78 liquids and 66 solids,was obtained and the results provided confirmation of the new testing strategy.The performance criteria,established by the OECD expert group overseeing OECD TG 492B,were met for all 144 chemicals mentioned above.Using this data set we developed the EpiOcular™ time-to-toxicity test method for eye hazard identification of liquid and solid chemicals according to UN GHS.The new testing strategy for liquids correctly predicted 78.7% of Cat 1 (N=27),63.5% of Cat 2(N=26)and 82.0% of No Cat(N=25).The testing strategy for solids correctly predicted 75.0% of Cat 1(N=28),59.4% of Cat 2(N=16) and 80.3% of No Cat(N=22)materials.Overall,the new test method correctly predicted 76.8% of Cat 1(N=55),61.9% of Cat 2(N=42),and 81.2% of No Cat(N=47) test articles.The EpiOcular™ time-to-toxicity test method is a novel approach for subcategorizing of both liquid and solid compounds.The prediction models that were developed for liquids and solids are capable of distinguishing substances and mixtures into the 3 UN GHS ocular hazard categories:No Cat, Cat 2,and Cat 1.

P90
BETULINIC ACID GAMMA-CYCLODEXTRIN SUPRAMOLECULAR SYSTEM – AN IN VITRO ASSESSMENT ON 2D LUNG CELLS AND 3D RESPIRATORY TISSUE MODEL
ABSTRACT #318

Iasmina Marcovici[12], Iulia Pinzaru[12], Cristina Dehelean[12]
[1]*Toxicology, Drug Industry, Management and legislation University Clinic, "Victor Babes" University of Medicine and Pharmacy from Timisoara, 2 Sq. E. Murgu, 300041, RO*
[2]*Research Center for Pharmaco-toxicological Evaluations, "Victor Babes" University of Medicine and Pharmacy from Timisoara, 2 Sq. E. Murgu, 300041, RO*

Lung cancer (LC) stands out as one of the most urgent health issues worldwide, significantly contributing to the overall tumor-related increases in mortality rates. The available treatment regimens for LC lead to poor and unsatisfactory results, demanding the discovery of novel therapeutic strategies [1]. Betulinic acid (BA) constitutes one of the most researched pentacyclic triterpenes owing to its multispectral biological activities, including anti-tumor properties. However, the therapeutic applications of BA are compromised by its low solubility in water and scarce bioavailability [2]. To overcome the innate limitations of BA and concomitantly propose a potential novel treatment for LC, the present study aimed to explore in vitro the safety and pharmacological profiles of BA complexed with gamma-cyclodextrin (BA-GCD) – the most suitable cyclodextrin carrier for BA's bulky structure [2]. The respiratory toxicity of BA and BA-GCDs was assessed using the 3D EpiAirway™ reconstructed human tissues, while their anti-cancer effect was evaluated in NCI-H23 human lung adenocarcinoma cells. Both BA and BA-GCD showed an increased bioavailability in the EpiAirway™ 3D model, and a high cytotoxicity in NCI-H23 lung adenocarcinoma cells, effects that were indicated by the following results obtained at BA concentrations up to 50 µM, after 24 h of treatment: (i) a stimulatory effect on the viability of EpiAirway™ inserts; (ii) a significant and concentration-dependent reduction in NCI-H23 cells' viability to values under 70%; (iii) inhibition of NCI-H23 cells' proliferation; (iii) massive NCI-H23 cell shrinkage, rounding, detachment, and confluence loss; (iv) condensation of NCI-H23 cells' nuclei and F-actin filaments; and (v) upregulation of the mRNA expression of pro-apoptotic markers in NCI-H23 cells. The data obtained highlight BA and BA-GCD as promising structures for future in depth investigation due to their proper safety profile at

the respiratory level and selective cytotoxicity on LC cells.

P91
Aerosol Chemistry and in vitro evaluation of a Novel Herbal Heated Product Relative to Cigarette Smoke
ABSTRACT #321

Ian Crooks[1], Elinor Marwick[1], Amy Heath[1], Andrew Baxter[1], Maria Pinto[1], Kelly Pereira[1], Simone Hadley[1], Damien Breheny[1], Carolina Garcia-Canton[1]
[1]*B.A.T (Investments) Ltd.*

New products are emerging that support Tobacco Harm Reduction (THR), such as products that heat but do not burn tobacco. These have been shown to produce reduced toxicant emissions and reduced in vitro toxicity as compared to conventional cigarettes. Here, we evaluate a novel herbal heated product (HHP) by assessing its chemical emissions and in vitro activity compared to a reference cigarette, 1R6F. The tobacco-free HHP ingredients include non-tobacco substrate, nicotine and flavours. The HHP and 1R6F were puffed under the HCI regime. The ventilation holes on the HHP were unblocked. The composition of emissions from the HHP were analysed using targeted GC-MS-SIM or HPLC-UV. Compounds identified in the HHP emissions were compared to those from 1R6F. All targeted analyte emissions measured in the HHP were significantly reduced compared to 1R6F. A battery of in vitro assays assessed the mutagenic, genotoxic and cytotoxic potential of the HHP: Ames test (OECD 471), in vitro micronucleus (IVMN) assay (OECD 487) and Neutral Red Uptake (NRU) assay (BALB/c 3T3 cells, 24h -S9). 1R6F induced dose-dependent increases in revertants in strains TA98, TA100 and TA1537 +S9, genotoxic in the IVMN and cytotoxic in the NRU (IC50 of 82.92 mg/mL). The HHP did not induce increases in revertants in the Ames test. Although the HHP induced responses in the IVMN, the responses were significantly less than 1R6F, despite testing at significantly higher concentrations. In the NRU, the HHP showed an indication of cytotoxicity, but no IC50 value could be calculated. These results show that the emissions from the HHP are less mutagenic, genotoxic, and cytotoxic than cigarette smoke by the studied assays. The results of these studies suggest that the HHP has a potential role in tobacco harm reduction, with additional studies needed to determine its full harm reduction potential.

P92
c-SRC phosphorylation predicts skin irritation potential of chemicals using a tissue engineered skin model
ABSTRACT #335

Md Zobaer Hasan[1], Amy Leigh Harding[2], Hirofumi Nakanishi[1], Kiichiro Sohara[1], Tetsuo Furuno[1], Craig Murdoch[2], Helen Colley[2]
[1]*Rohto Pharmaceutical CO., Ltd*
[2]*University of Sheffield*

Background & Objectives: Chemical-induced skin irritancy or sensitivity to topically applied dermatological agents, cosmetics, skin-care products is a common problem. Several studies have used human skin equivalent (HSE) to identify increased expression of several genes in response to many chemicals and known irritants. However, these gene signature sets are often large to analyze for high-throughput screening. Analyzing the immediate up-stream signaling events upon treatment with chemical irritants may identify activation of specific kinases and could be tested for chemical irritancy in more rapid and high throughput manner. Materials and Methods: HSE was constructed in the laboratory (1).Fifty µL of chemicals were applied topically to HSE and incubated for 15 minutes at 37°C, after that protein lysate was prepared using RIPA buffer for phosphokinase array analysis. Results: The analysis of HSE protein extracts using a phosphokinase array revealed a significant increase in the abundance of phospho-c-SrcY419 when the skin irritant lactic acid (LA) was applied topically to the HSE for 15 minutes. This increase was not observed when non-irritants such as methylparaben (MP) and cocamide diethanolamine (Co-DEA) were applied, or when water was used as a carrier control. Previously, a panel of seven genes was identified to distinguish between chemical irritants and non-irritants (1). Among these genes, four were found to be regulated by the activation of transcription factors AP-1 (c-Fos/c-

Jun) and p65/NFκB. In line with this finding, increased phosphorylation of c-JunS63 and p65S536 was observed in response to irritants but not non-irritants. However, most kinases showed no difference in their phosphorylation status between irritant and non-irritant treatments. Conclusion: Our data shows specific activation of c-Src at Y419 position when exposed to chemical irritants. This can be used as a marker for predicting chemical irritation potential of chemicals used in cosmetic industry in a more robust, rapid and high throughput manner.

P207
In vitro Phototoxicity: Reconstructed Human Epidermis Phototoxicity test method – OECD 498 – Blinded Test
ABSTRACT #367

Christina Spohr[1], Markus Schulz[1]
[1]*ICCR-Roßdorf GmbH*

According to OECD TG 498, phototoxicity (photoirritation) is defined as “an acute toxic reaction induced by topically or systemically administered photoreactive chemicals following exposure of the body to environmental light”. The phototoxic potential of a test item can be determined using a three-dimensional model of the human epidermis (EpiDermTM). As this test allows the application of a test item to the air-exposed surface (stratum corneum), it mimics the in vivo situation and thus allows the prediction of the phototoxic potential of test substances applied in used concentrations. Prior to routine use of the test method, laboratories should demonstrate their technical competence by correctly classifying the six test items listed in OECD TG 498. The aim of this study was to establish and validate the OECD TG 498 phototoxicity test as a routine service in the field of contract research for regulatory purposes. The particular focus was on the suitability of the listed substances, the proposed solvents and the maximum concentrations specified for this purpose. In order to perform blinded testing, the top doses given in the OECD TG 498 and dilution factors suggested by the test kit supplier needed to be adjusted. The six phototoxic and non-phototoxic test items were determined correctly. Therefore, the selected substances are suitable for distinguishing phototoxic substances from non-phototoxic substances.

P213
EVALUATION OF SKIN CORROSIVITY PREDICTION USING THE 3D RECONSTRUCTED HUMAN EPIDERMIS MODEL, KeraSkin™
ABSTRACT #374

Jung-Eun Park[1], Jiyoung Lee[2], Sanghee Lee[2], Su-Hyon Lee[1], Lim Kyung-Min[3]
[1]*R&D Institute, Biosolution Co., Ltd., Republic of Korea*
[2]*Environmental Health Research Department, NIER, Republic of Korea*
[3]*College of Pharmacy, Ewha Womans University, Republic of Korea*

Growing support for alternatives like cell lines or 3D human epidermis models aims to reduce animal use in toxicity testing and research, addressing concerns about animal suffering and excessive sacrifice. The study assesses Skin Corrosion Test (SCT) predictive ability with RhE model KeraSkin™, derived from human skin cells. OECD TG 431 validates methods using five RhE models: EpiSkin™, EpiDerm™, SkinEthic™ RHE, epiCS®, and LabCyte EPI-MODEL24. National Institute of Environmental Research (NIER)'s Validation Management Team (VMT) guided the catch-up validation for KeraSkin™. The validation study followed OECD guidelines, including GD34 (2005), TG 431 (2015), and GD219 (2015), for me-too testing under TG 431. The test method categorizes substances based on cell viability treated for 3 and 60 minutes using the MTT assay, determining 1A, 1B/1C, or Non-corrosive status. To meet OECD GD219 criteria, we optimized the KeraSkin™ SCT SOP for a me-too test. The KeraSkin™ SCT SOP (version 1.0) underwent three revisions, including changes to test substance weighing, treatment volume, washing, and cut-off, resulting in version 1.3 update in December 2023. Out of 30 performance standards (PS) reference substances, 90% of 1A, 70% of 1B/1C, and 70% of non-corrosives substances were correctly classified, meeting the criteria with 77% accuracy. These results will lead to a validation study involving four laboratories to assess reproducibility and predictive capacity.

Key words: Skin corrosion test, RhE model, KeraSkin™, OECD TG431 Funding Source: This work was supported by a grant from the NIER, funded by the Ministry of Environment of the Republic of Korea

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MODELS, BIOMARKERS AND ASSAYS FOR DEVELOPMENTAL TOXICITY

P101
Epidemio-Toxicological profile of Colima. RESAS National Strategies in Government of Mexico.
ABSTRACT #12

Oliver Mendoza-Cano[12], Ana Luz Quintanilla-Montoya[12], Irma E. González-Curiel[3], Mónica Ríos-Silva[1], Octavio Gaspar-Ramirez[4], Andrea Camacho-de la Cruz[5], Maria Fernanda Romo-García[6]
[1]*Universidad de Colima*
[2]*CONAHCYT CEIBAAS Colima*
[3]*Universidad Autónoma de Zacatecas*
[4]*CIATEJ*
[5]*acamacho.tox@gmail.com*
[6]*Affmna@gmail.com*

Background and Objectives Environmental Emergency Regions (RESAS) in Mexico result from severe industrial-driven environmental degradation, not paradises but nightmares, linked to 78% of pandemic deaths. Activism spanning over a decade by environmental groups addresses issues like gas extraction, mining, GMOs, biopiracy, oil drilling, monocultures, and plant patents. A case in point is Colima, home to the vital Manzanillo port. Despite economic sectors like commerce and real estate, Colima faces the second-highest chronic kidney disease rates nationally and elevated cancer rates, notably prostate, breast, lung, ovary, and melanoma cases, exceeding national averages. The goal is to create an epidemiotoxicological profile, highlighting Colima's socio-environmental and health emergency. Material and Methods We define as action scenarios those highly contaminated strategic sites, which, due to their geography, convergence with other regions, and the uniqueness of economic agents (sugar cane, cement factory, Sea Port, Electrical and gas power generation, industrial activity, etc.) generate impacts that will lead to specific political instruments to be defined. Via ICP-MS and GC-MS, we conducted environmental analysis in well water, surface soil, sediments, and organisms in Colima. We conducted effect and exposure biomarkers n=250 children in the five action scenarios. Results We have results about TH1/TH2/TH17 inflammatory profile (Human IL-2, IL-10,TNF, IL-4, IFN-γ and IL-17A/flow cytometry). KIM (Kidney Injury/Enzyme Immunoassay). TSLP (Lung damage/enzyme immunoassay). Micronuclei (genetic damage/microscopy). Also: Age, Size, Weight, BMI, Circumference, Waist, CHF (Cardiovascular R.) 8. Nutritional status 9. Oxygen saturation. Hematological *(Complete biometry, with slide) Glucose Renal Profile Metals in urine and blood by ICP-MS Exposure to Benzene (Muconic Ac.) Urine Glyphosate. Discussion and Conclusion These crises have caused environmental devastation for more than three decades due to the activity of state, national, and international companies. The contaminated areas reach or exceed the levels of the worst pollution sites in China, Indonesia, the United States, and India.

P102
EVALUATION of EMBRYOTOXICITY of THIRAM with ALTERNATIVE METHODOLOGIES
ABSTRACT #32

Yu-Chi Ho[1], Chun-Wei Tung[2], Ming-Hsien Tsai[1], Chen-Yi Weng[1], Ju-Hsin Hsu[1], Hsien-Jen Cheng[1], B Linju Yen[3], Pinpin Lin[1]
[1]*National Institute of Environmental Health Sciences, National Health Research Institutes, Zhunan, Taiwan, R.O.C.*
[2]*Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan, Taiwan, R.O.C.*
[3]*Institute of Cellular and System Medicine, National Health Research Institutes, Zhunan, Taiwan, R.O.C*

Long term exposure to low dose food contact materials (FCMs) has been the concern of developmental toxicity. In objective of our present study is to identify potential embryotoxic PCM with alternative methods, inlcuding in silico tools, mouse embryonic stem cells (mESCs), human pluripotent embryonal carcinoma NT2 cells, and zebrafish embryos.

we prioritized FCMs of developmental toxicity concern with a weight-of-evidence computational model, and 127 chemicals were predicted to be of high concern. We selected 7 chemicals of high or low concern and evaluated their potential embryotoxicity in mouse embryonic stem cells test (mEST). Among selected chemicals, thiram was the strongest one for inhibiting cardiac differentiation in mEST. We further examined the effects of thiram on morphology and expression of differentiation-related genes in mESCs. Thiram delayed or repressed expression of three germ layer-related markers during cardiomyocyte differentiation, but promoted neuroectoderm differentiation in mESCs. Thiram also induced notochord malformation and developmental retardation in zebrafish embryos. In human pluripotent embryonal carcinoma NT2 cells, thiram suppressed the expression of the pluripotency ESC marker Nanog and increased expression of lineage-specific neuroectodermal markers, including Homeobox a1 and Nestin. Finally, utilizing transcriptome analysis, we found that thiram modulated the neural crest differentiation pathway in NT2 cells. In conclusion, we used some alternative approaches to identify a potential embryotoxic chemical, thiram. Furthermore, these findings provide information regarding the potential embryotoxic mechanisms of thiram.

P103
APPLICATION OF QUANTITATIVE IN VITRO-TO-IN VIVO EXTRAPOLATION TO REPROTRACKER DATA PROVIDES CONSERVATIVE ESTIMATES OF IN VIVO DEVELOPMENTAL TOXICITY
ABSTRACT #41

Amer Jamalpoor[1], Sabine Hartvelt[1], Marjory Moreau[2], Jeffrey Fisher[2], Andy Nong[2], Giel Hendriks[1]
[1]*Toxys B.V.*
[2]*ScitoVation*

The rise of animal alternatives for assessing compound-induced developmental toxicity has become evident over the past years. ReproTracker is a unique in vitro human stem cell biomarker-based assay that accurately predicts the teratogenicity hazards of pharmaceuticals and chemicals. However, the assay does not consider the exposure of chemicals or drugs to mother and fetus, hampering adequate extrapolation of in vitro findings to relevant clinical dosing scenarios. To obtain relevant human dose context from in vitro assays, in vitro-to-in vivo extrapolation (IVIVE) models are required to determine which dose would elicit a concentration in the body demonstrated to be developmental toxicants using in vitro assays. The goal of this pilot study was therefore to evaluate whether the ReproTracker assay can predict the in vivo developmental toxicity exposure levels of two known human teratogens, thalidomide, and carbamazepine. Here, we utilized Physiologically Based Pharmacokinetic (PBPK) modeling to describe the kinetic behavior of these drugs and applied quantitative IVIVE to derive human equivalent effect doses (HEDs) associated with adverse outcomes in the ReproTracker assay. HEDs derived from the ReproTracker readouts – lowest observed adverse effect level (LOAEL) values – were then compared with the reported teratogenic human clinical doses and the HED derived from animal studies (rat or rabbit). For both thalidomide and carbamazepine, quantitative IVIVE modeling of the ReproTracker data provided conservative estimates of in vivo developmental toxicity and revealed to be sensitive and protective of humans. Overall, this pilot study demonstrated the importance of application of IVIVE models in assessing developmental toxicity from in vitro data and showed that the combination of these tools could be used to facilitate animal-free safety assessment of novel chemicals and drugs.

P104
NEW APPROACH METHODOLOGIES FOR THE TERATOGENIC POTENTIAL: WHAT IF THE SOLUTION CAME FROM MECHANISTIC NETWORKS?
ABSTRACT #52

Matthew Burbank[1], Florian Gautier[1], Nicky Hewitt[2], Audrey Noel-Voisin[1], Nazanin Golbamaki[1], Sébastien Grégoire[1], Anne Riu[1], Ann Detroyer[1], Typhaine Bringel[1], Laurent Guillet-Revol[1], Léopold Carron[1], Emilie Le Mevel[1], Noémie De Croze[1], Gladys Ouédraogo[1]
[1]*L'Oréal Research & Innovation, Aulnay-sous-*

Bois, France
[2]*Cosmetics Europe, Brussels, Belgium*

New approach methodologies (NAMs) in the field of DART have been developed because of regulatory demands, starting with the cosmetic animal testing ban in the Europe in 2013 aiming to use NAMs to retain a high level of knowledge and safety, without the loss of promising new ingredients. Research into reproductive toxicity and teratogenicity is a high priority, with the overall objective of developing and evaluating NAMs in a decision tree approach for raw materials evaluation. There are several levels of complexity in pregnancy and embryo development with different windows of susceptibility and biological variability that can contribute to an incomplete understanding of teratogenic mechanisms. By analyzing the current knowledge (based on Key Characteristics, Adverse Outcome Pathways) and scientific gaps in literature and comparing them with existing NAMs, we believe that a more precise and specific define approach can be developed for this specific endpoint. To that end, we present here an overview of different AOPs on teratogenicity that could be combined to build an AOP network, therefore, representing a better depiction of biological processes and that could have mechanistic or predictive applications in risk assessment. From this mechanistic network, extraction of relevant biomarkers in humans was undertaken, enabling several mechanisms of action to be grouped together to assess this endpoint. We then proceeded to a global review of NAMs from both environmental and human health that could fill gaps regarding the analysis and the evaluation of teratogenicity. The objective was to have a more robust toolbox addressing endpoints in cellular processes and signaling pathways underlying its key stages with the objective to enrich evaluation strategies to predict teratogenicity.

P105
A GLOBAL APPROACH TO EVALUATE TERATOGENICITY, THYROID DISRUPTION, AND DEVELOPMENTAL NEUROTOXICITY PRODUCED BY ORGANIC CHEMICALS IN ZEBRAFISH EMBRYOS
ABSTRACT #92

Andrea Weiner[1], Ander Arbelaiz[1], Christoph Rummel[1], Arantza Muriana[1], Celia Rodriguez
[1]*BBD BioPhenix S.L.U (Biobide)*

The plethora of chemicals poses an increasing risk to human and environmental health. Special causes for concern are teratogenicity, neurotoxicity, and Endocrine-Disrupting Chemicals (EDCs). Using the zebrafish embryo as a New Alternative Method (NAM), we present a high content approach to identify the embryotoxic potential of reference substances as well as their thyroid disruptor potential and developmental neurotoxicity. Embryos of the transgenic fish line Tg(tg:mCherry) were exposed to a set of chemicals (Potassium perchlorate (PP), Benzophenone-2 (BP2), Propylthiouracil (PU), Acetaminophen (APA)) to assess the fluorescence of the reporter in the thyroid gland. By fluorescence measurements of the reporter for the thyroglobulin (tg), the compounds PP, BP2, and PU were correctly classified having a goitrogenic effect for concentrations < EC10 of the systemic toxicity. To identify potential downstream effects of the impaired endocrine system, T4 and T3 levels can be quantified by a newly developed LCMS method for whole-embryo homogenates, which enabled the detection of a T4 reduction for PP, BP2, and PU-treated embryos from ca. 0.5 µgT4/Lextract to around 0.1 µgT4/Lextract. Additionally, gene expression analyses of thyroid (tshβ, tpo, and/or tg) genes were assessed using RT-qPCR. A dose-dependent induction of tg mRNA for PP, BP2, and PU-treated zebrafish embryos was observed. This induction, however, could not rescue the low T4 level-phenotype as indicated by the LCMS analyses. This could potentially be attributed to one of the chemicals' common modes of action to inhibit the thyroid peroxidase which might have prevented the iodide oxidation required for functional T4. The results were complemented with common developmental toxicity (malformations; mortality) and DNT (Light/Dark transition Assay) in zebrafish embryos to highlight the controversial problem definition of EDCs. KW: Endocrine disruptors, thyroid hormones, teratogenicity, LCMS, photo locomotor transition test Presentation: Poster/Oral presentation

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P106
Chemical Mixture Calculator 2.0: A Web-Based Tool Combining Predicted Human Exposures with High-Throughput In Vitro Toxicity Data to Support Mixture Risk Assessment
ABSTRACT #135

Ehab Mustafa[1], Veronica von Saint Paul[2], Hilmar Ilgenfritz[2], Martin Scholze[3], Anne Marie Vinggaard[1]
[1]*National Food Institute, Technical University of Denmark, Denmark*
[2]*Labvantage – Biomax GmbH, Germany*
[3]*Centre for Pollution Research and Policy, Environmental Sciences Division, Brunel University London, United Kingdom*

Humans are simultaneously exposed to numerous chemicals from the environment, food, and daily life products. These complex chemical mixtures might pose a threat to human health, and one of the main challenges for conducting a mixture risk assessment (MRA) is the lack of exposure and hazard data for many of these chemicals (Mustafa et al. 2023). New Approach Methodologies (NAMs) provide an opportunity to overcome some of these obstacles with well-established high throughput in silico methods for population-based exposure and toxicokinetic predictions, as well as high-throughput screening (HTS) for biological activity. Here, we develop the Chemical Mixture Calculator 2.0 (CMC 2.0), a web-based tool with a user-friendly interface that allows the calculation of a potential mixture hazard (Hazard index, HI) for combinations of chemicals defined by the user. High-throughput dietary human exposure predictions are obtained from outputs of EPA's Systematic Empirical Evaluation of Models (SEEM) as part of the Exposure Forecasting (ExpoCast) platform and converted into human plasma concentrations by generic physiologically-based ("PBTK") models provided by the HTTK platform, resulting into internal steady-state plasma predictions for more than 7000 chemicals, and provided for the total population and various demographic-specific subgroups. Curated hazard data from HTS in vitro assays are collected from the Integrated Chemical Environment (ICE, US NTP), with the lowest in vitro activity concentration (benchmark concentration) chosen for the mixture hazard calculation. Pre-defined grouping criteria (chemical groups, AOPs) allow the focus on specific mixture assessment groups (CAGs), and exposure and hazard estimates of the databases can be replaced individually by the user. CMC 2.0 will be optimized for reproductive toxicity and (developmental) neurotoxicity, and typical applications are the identification of chemicals (or molecular initiating events, MIEs) expected to contribute most to a potential mixture risk ("mixture drivers") in support of a NAM-based chemical risk prioritization.

P107
High throughput platform for the validation and application of zebrafish embryo for ICH S5 (R3)
ABSTRACT #173

Claudia Miguel-Sanz[1], Irina Suárez[1], Sofia Barreiro[1], Valentina Schiavone[1], Vincenzo Di Donato[1], Anna Navarro[1]
[1]*ZeClinics, SL*

The use of zebrafish embryos in toxicology represents an alternative strategy in accordance with the 3Rs principles, seeking to reduce the impact of experimental procedures on animal welfare. Furthermore, given the substantial costs and prolonged assessments associated with developmental toxicology, zebrafish has emerged as a preferred model organism for conducting high-throughput developmental toxicity studies. Although zebrafish has been increasingly studied and used for developmental toxicity screening, an harmonization of protocols is needed. In this study we used a high throughput platform to validate and optimize the zebrafish development toxicity assay using a selection of cytostatic drugs from the reference compounds list of the ICH S5 (R3) guideline as starting point for its qualification as alternative assay. To assess the teratogenic effect, an AI integrated imaging analysis platform was used to evaluate the occurrence of malformations and mortalities. Some substances tested, such as 5-fluorouracil and cyclophosphamide were also tested for other endpoints as developmental neurotoxicity. Through the use of our deep learning algorithm, we achieved a precise detection and characterization of phenotypes. We prove that integrating the experimental

strengths of the zebrafish embryo model with AI enables high throughput fully automated detection of multi-organ compounds toxicity. Moreover, our results support the use of zebrafish embryos as an alternative to the animal models for the screening and assessing toxicity of candidate compounds for regulatory acceptance. It also lays the foundations for a faster and reliable environmental and human risk assessment based on New approach methodologies (NAMs).

P108
Bisphenol and its analogues induce neuroinflammation in BV2 microglial cells through the ROS-NF-κB signaling pathway
ABSTRACT #280

Jeong Doo Heo[1]
[1]*Korea Institute of Toxicology*

BPA has been used to coat plastic containers and beverage cans. Over the past 15 years, there has been ongoing controversy over its harmful effects, including hormone disruption, cancer, and asthma. Manufacturers have released "BPA-free" products as alternatives to BPA. Although the safety of these ingredients has not yet been verified, they are widely consumed. In particular, the effects of exposure to bisphenol A on cytokine production in the central nervous system or immune response are not well understood. In this study, the aim was to explore potential variances in toxicity and underlying mechanisms of BPA and its substitutes in microglial cells to fill these knowledge gaps. According to research findings, it has been confirmed that exposure to BPA and its substitutes (BPS, BPF, TMBPF) increases the expression of proteins such as NO, prostaglandin E2 (PGE2), and their upstream factors iNOS, COX-2 in BV2 cells. It has also been confirmed that this increases the production of inflammatory cytokines (IL-6, IL-1β, TNF-α) in a concentration-dependent manner and enhances the expression of inflammation-related genes. We discovered that phosphorylation of nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) is significantly induced in a concentration-dependent manner by bisphenol. Additionally, reactive oxygen species (ROS) were significantly increased by bisphenol compared to the control group. The results demonstrate that bisphenol is a chemical compound that increases inflammation in BV2 cells and enhances inflammation through the activation of the MAPK/NF-κB pathway.

P109
Developmental neurotoxic effects induced by PHMB exposure via oxidative stress mechanism: Integrated approaches with neuronal cells and zebrafish embryo models
ABSTRACT #292

Ha-Na Oh[1], Donggon Yoo[1], Seungmin Park[1], Sangwoo Lee[1], Woo-Keun Kim[1]
[1]*Korea Institute of Toxicology*

Poly(hexamethylenebisianoguanide-hexamethylene diamine) hydrochloride (PHMB) is a biocide with a wide range of antimicrobial activities. As a disinfectant and preservative in consumer products, PHMB can reach humans. Although toxicity studies for PHMB have mainly focused on systemic toxicity or skin irritation, its effects on developmental neurotoxicity and underlying mechanisms are not well-elucidated. In the present study, we evaluated the developmental neurotoxic effects of PHMB using IMR-32 and SH-SY5Y cell lines and zebrafish embryo model. In both cell lines, PHMB concentration ≥ 10 μM reduced neurite outgrowth, and cytotoxicity was observed at concentrations up to 40 μM. PHMB regulated the expression of neurodevelopmental genes and induced reactive oxygen species (ROS) production and also mitochondrial dysfunction. Treatment with N-acetylcysteine reversed the developmental neurotoxic effects of PHMB. Toxicity tests on zebrafish embryos showed that PHMB reduced survival and heart rate and caused irregular hatching. PHMB concentrations of 1-4 μM reduced brain and spinal cord width in transgenic zebrafish line and attenuated the myelination process. In addition, PHMB regulated the expression of neurodevelopmental genes in zebrafish embryos and induced ROS accumulation. Our results suggest that PHMB exerts developmental neurotoxic effects both in vitro and in vivo models via ROS-dependent mechanisms. Acknowledgement: This work

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P110
Cytotoxic and Neurotoxic Effects of Copper Pyrithione and Zinc Pyrithione on Neuronal/Astrocytic Co-Cultured Cells Through Oxidative Stress
ABSTRACT #293

Ha-Na Oh[1], Woo-Keun Kim[1]
[1]*Korea Institute of Toxicology*

In this study, we aimed to shed light on the neurotoxic effects of copper pyrithione (CPT) and zinc pyrithione (ZPT), commonly used as antifouling agents, particularly focusing on human exposure risks where understanding remains limited. We evaluated the cytotoxicity and neurotoxicity of CPT and ZPT on human SH-SY5Y/astrocytic co-cultured cells. Our findings revealed that both CPT and ZPT induced dose-dependent cytotoxicity in the co-culture model, with noticeable effects observed at concentrations around 400 nM. Additionally, these compounds suppressed neurite outgrowth parameters, indicating neurotoxic effects even at concentrations with minimal cytotoxicity (~200 nM). Furthermore, exposure to CPT and ZPT downregulated genes associated with neurodevelopment and maturation while upregulating astrocyte markers. Mitochondrial dysfunction and increased reactive oxygen species generation were also observed. Notably, treatment with N-acetylcysteine exhibited neuroprotective effects against CPT and ZPT toxicity, suggesting oxidative stress as a major mechanism underlying their toxicity in co-cultured cells. Acknowledgement: This work was supported by the Korea Environmental Industry & Technology Institute (KEITI) through the Core Technology Development Project for Environmental Diseases Prevention and Management [grant number 2021003310003]; and the Korea Ministry of Environment (MOE) and the Korea Institute of Toxicology [grant number 1711159817].

P111
Validation of a human 3D high-throughput vasculogenesis model for developmental toxicity screening
ABSTRACT #305

Ashwin Shah[1], Nicholas Dennison[12], Maximilian Fusenig[1], Solveig Klier[1], Tina Marie Hauser[1], Linda Sturm[1], Maria Alejandra Ramirez Martinez[1], Jannik Irmai[3], Uwe Freudenberg[1], Carsten Werner[13]
[1]*Leibniz Institute of Polymer Research Dresden, 01069 Dresden, Germany*
[2]*NeuronD, 01307 Dresden, Germany*
[3]*Technische Unversitaet Dresden*

Despite years of investigation, there is currently no officially approved in vitro method to replace animal testing for developmental toxicity. However, advanced in vitro models using human cells offer a valuable alternative for enhancing predictability and reducing the need for animal testing. Vasculogenesis and angiogenesis play crucial roles in both early and late embryo-fetal development, impacting organ and limb development, as well as extraembryonic tissues such as the placenta. Consequently, our objective was to create an in vitro vasculogenesis model using human umbilical vein endothelial cells (HUVECs) and mesenchymal stromal cells (MSCs) to detect embryotoxic and teratogenic substances. Accordingly, HUVECs and MSCs were cultured in a fully automated HTS-format using a Tecan-Fluent pipetting robot, utilizing a modular hydrogel matrix made up of 4-arm poly(ethylene glycol) (PEG)-peptide-conjugates and heparin maleimide building blocks. As readouts of the high-throughput culture, the morphological changes in capillary formation were examined using the high-content screening confocal laser raster microscope Opera Phenix. This analysis focused on evaluating filament length and branching degree of the capillaries. Furthermore, the assessment included characterization of cell viability and ROS accumulation within the cultures. To validate the assay a previously published compound library was used[1]. The vasculogenesis model successfully identified 80% of the toxic

compounds, with 87.5% falling within a log10 range of the in vivo peak plasma reference values. Additionally, a strong correlation coefficient of R^2=0.94 was found between the in vitro and in vivo data. In a sub-study investigating latent effects, short term exposure of Axitinib was leading to a prolonged morphological degeneration, senescence and delayed inflammatory response. In summary, the assay has demonstrated high predictivity and, when combined with additional assays, it has the potential to replace or reduce animal testing. This is significant for the field of drug discovery and chemical safety assessment.

P112
New insight into long-term effects of phthalates microplastics in developing zebrafish: Evidence from genomic alteration and organ development
ABSTRACT #313

Abdulkhalik Mansuri[1], Krupa Kansara[2], Dixit Raiyani[1], Dhairya Mazmudar[1]
[1]*Biological and Life Sciences, School of Arts & Sciences, Ahmedabad University, Central Campus, Navrangpura, Ahmedabad 380009, Gujarat, India*
[2]*Biological and Engineering Discipline, Indian Institute of Technology – Gandhinagar (IITGN), Palaj 382355, Gujarat, India*

The plasticizer leaches from the microplastics are one of the significant concerns related to plastic pollution. These plasticizers are known to be endocrine disrupters; however, little is known about their long-term effect on the development of aquatic vertebrates. Hence, the present study has been conducted to provide a holistic understanding of the effect of the three most common plasticizers, dibutyl phthalate (DBP), diethyl phthalate (DEP), and di-ethylhexyl phthalate (DEHP) leaching out from the microplastics in zebrafish development. Zebrafish larvae were exposed to different phthalates at different concentrations. The phthalates have shown significantly higher mortality and morphological changes in the larva upon exposure compared to the control. A significant change in the genes related to cardiovascular development (krit1, fbn2b), dorsoventral axis development (chrd, smad5), tail formation (pkd2, wnt3a, wnt8a), and floorplate development (foxa2) were also observed under the effects of the phthalates in comparison to control.

P211
THE METABOLOME OF CELL CULTURE SUPERNATANT AS A PROMISING COMPREHENSIVE BIOMARKER SOURCE FOR IN VITRO TOXICOLOGY
ABSTRACT #371

Christoph Magnes[1], Elmar Zügner[1], Fernanda Monedeiro[1], Heinz Hammerlindl[2], Helmut Schaider[2]
[1]*JOANNEUM RESEARCH, Institute for Biomedical Research and Technologies, Graz Austria*
[2]*Frazer Institute, The University of Queensland, Brisbane, QLD, Australia*

Background & Objectives Precise biomarkers for monitoring intracellular activation of biological pathways indicative of toxicity are essential to successfully establish New Approach Methodologies (NAMs) in toxicology. Our studies investigate the development of drug-induced resistance in cancer cells in an in vitro model of acquired cancer drug resistance characterized by four transition states (parent (drug sensitive), early persister, late persister, resistant state). The aim of the present study was to assess intracellular metabolic changes during the development of drug resistance and to identify associated changes in the metabolome of the culture supernatant that can be used as biomarkers. Materials & Methods A commercially available melanoma cell line (WM164) was used for cell culture experiments. Cells were treated with the BRAF inhibitor dabrafenib. Intracellular and supernatant metabolomes were analyzed by hydrophilic interaction chromatography coupled to high-resolution mass spectrometry (HILIC-HRMS). Metabolites were extracted by applying the boiling ethanol method. Results 138 intracellular and 111 supernatant metabolites were detected in the WM164 melanoma cell culture. Each of the four states showed specific intracellular metabolome profiles with separate clusters in the principle component analysis (PCA). Similar profiles were detected in the supernatant. PCAs of intracellular and

supernatant metabolome showed that parent and resistant states are more similar to each other than to the other states. Discussion and Conclusion We successfully detected specific metabolic changes in melanoma cells induced by a xenobiotic cancer drug treatment. Metabolic changes corresponded well with cell states during drug resistance development. We also detected corresponding metabolic profiles in the cell culture supernatant. The assessment of biomarker patterns in the supernatant is of great advantage enabling non-destructive, time-resolved analysis of intracellular processes. Thus, the metabolome of in-vitro assay cell culture media is a promising new source for biomarkers to assess drug candidate toxicity.

P212
Screening of Developmental Toxicity Using IPSCs for Agrochemicals
ABSTRACT #372

Kathryn Rudd[1], Yeyejide Adeleye[1], Richard Currie[1], Theresa Placke[2]
[1]*Syngenta, Jealott's Hill International Research Centre, Bracknell, UK*
[2]*Syngenta Crop Protection AG, Basel, Switzerland*

There is an increasing demand from farmers for effective, safe and sustainable products to manage disease, weed or pest species that are resistant to commercially available agrochemical products. The invention and registration of new crop protection active ingredients requires suitable safety testing to identify uses that are safe to humans and the environment. Included in this safety testing are tests in rats and rabbits that investigates the potential for developmental toxicity. Current in vivo developmental toxicity tests are resource intensive, involving a large number of animals, are low throughput and expensive, highlighting the requirement for rapid screening methods to assess and prioritize chemicals for further investigation. Syngenta used the devTOX quickPredict assay from Stemina (Stemina Biomarker Discovery Inc., Madison, WI, United States) to investigate developmental toxicity for a subset of compounds. The devTOX quickPredict assay successfully predicted developmental effects in a concentration-dependent manner consistent with the in vivo responses.

MODELS, BIOMARKERS AND ASSAYS FOR ENDOCRINE DISRUPTION

P113
TIME FRAME COMPARISON IN STEROIDOGENESIS ASSAY
ABSTRACT #53

Andrea RIVERO ARZE[1], Elise GRIGNARD[1], Pauline LELANDAIS[1], Philippe HUBERT[1]
[1]*PEPPER - Paris (FR)*

Pepper is carrying the validation of a method aimed at enhancing the OECD Test Guideline (TG) 456 for the H295R Steroidogenesis Assay. The objective was to reinforce the regulatory utility of TG 456 by obtaining more comprehensive information concerning the disruption of steroidogenesis. The method is based on the publication by Ahmed et al. (2018) which utilized LC-MS/MS to measure 19 distinct steroids. In November 2021 a Standard Project Submission Form (SPSF) was submitted to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) of the OECD for inclusion in their work plan. Based on the comments received during the submission, it was agreed that an extra step to the validation would be included : "comparison of 2 timepoints (48h and 72h) for the secretion of the 19 hormones, for Forskolin and Prochloraz (full-dose responses) in one laboratory". The validation group went beyond the requirements, with two laboratories running this comparison. The cells were cultured as described in TG456. The only modification of the protocol was the duration of the exposure. Some plates were exposed during 72h while others were stopped after 48h. The results showed that during the MTT assay, cells were more prone to detach after 72h, resulting in higher variance of the MTT based values of the quality control plates. Furthermore, the analytical methods proved to be more accurate when analysing the 48h samples. One of the problems with the existing TG is that the concentrations of the oestrogens in the vehicle group are often very low after 48h, leading to fail the acceptability criteria. But the longer incubation time did not change this and even led to a further decrease in estriol and

estrone concentrations. In conclusion there are no significative benefits extending the incubation time from 48h to 72h.

P114
EVALUATION OF CYTOTOXICITY AND METABOLISM OF T-2 TOXIN IN HEPATIC SPHEROIDS CULTURED IN MICROFLUIDIC DEVICES
ABSTRACT #58

Mercedes Taroncher[1], Jose M. de Hoyos-Vega[2], Alan M. Gonzalez-Suarez[2], Kihak Gwon[2], Yelko Rodríguez-Carrasco[1], Maria Jose Ruiz[1], Alexander Revzin[2]
[1]*Research Group in Alternative Methods for Determining Toxics Effects and Risk Assessment of Contaminants and Mixtures (RiskTox). Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100, Valencia, Spain*
[2]*Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA*

The T-2 toxin (T-2) is a secondary metabolite produce by Fusarium fungi. It belongs to the A trichothecene group and is the most toxic of the group. The T-2 infects cereals and cereal-based commodities. Therefore, there is a strong need to develop models of T-2 effects on human organs. Given the central role played by the liver in metabolism of toxicants or xenobiotics, we wanted to establish an in vitro liver model of T-2 toxicity. Our team has previously shown that hepatic cells placed into microfluidic devices exhibited better phenotype and function compared to standard larger volume cultures. In this work, we used microfluidic devices to create three-dimensional (3D) cultures of hepatic cells. The objectives of this work were: (i) to ascertain benefits of microfluidic spheroid cultures compared to standard (large volume) 3D cultures (Aggrewell plates), (ii) to compare cytotoxicity of T-2 in microfluidic monocultures of hepatic spheroids versus spheroids containing hepatic cells and stellate cells, and (iii) to characterize induction of metabolizing enzymes and other markers of hepatic phenotype upon exposure to T-2. The results demonstrated that spheroids formed faster and released more albumin in microfluidic spheroid cultures compared to standard large volume 3D cultures. The cytotoxicity increased with increasing T-2 concentrations (15, 30 and 60 nM) in both monoculture and co-culture, being more pronounced in monoculture and reaching toxicity up to 4-fold greater than the control. The RT-PCR analysis of CYP1A1, CYP1A2, CYP2E1, CYP3A4, UGT1A1, MDR1 and MRP2 showed that exposure to T-2 induced higher expression for all genes with the exception of CYP3A4 and UGT1A1. In conclusion, we demonstrated that hepatic spheroids formed faster and were more functional in the microfluidic device compared to the standard 3D culture plate and that microfluidic cultures metabolized T-2. Acknowledgements: PID2020-115871RB-100, grant PRE2021-096941 and CIAICO/2022/199 Conselleria innovació (GV).

P115
BUILDING CONFIDENCE IN IN VITRO BATTERIES FOR ENDOCRINE DISRUPTION TESTING
ABSTRACT #80

Vera Engelbrecht[1]
[1]*PETA Science Consortium International e.V.*

Transitioning to non-animal methods for endocrine disruption (ED) testing is a significant shift away from traditional approaches that use animals. Non-animal methods offer the potential to overcome the limitations of animal tests by providing faster, more reproducible, and relevant data for assessing endocrine-disrupting chemicals.1 However, this transition comes with complex social and regulatory challenges. The current in vitro Organisation for Economic Co-operation and Development (OECD) ED test guidelines primarily focus on molecular initiating and key events and don't cover all the physiological processes that occur in intact organisms. One way to overcome these challenges is to use in vitro test batteries addressing several important questions with independent measurements. However, developing and validating these methods currently limits the regulatory use of these combinations of tests. This work provides an overview of the current status of available non-animal approaches for ED testing and delves

into why their acceptance for regulatory purposes may face resistance due to concerns surrounding technological advancements. These doubts may be partly rooted in the fact that these methods are not a one-to-one replacement for animal tests but rely on mechanistic information from several assays rather than one apical outcome. This work also addresses several limitations of current animal test methods used in an attempt to assess ED substances,2 including transient fluctuations, high variability, interspecies differences, and other confounding factors. This calls into question the continued reliance on current animal methods as the unequivocal gold standard in the development and confidence-building process for modern in vitro test batteries designed to assess ED substances.

P116
ASSESSING THE IMPACT OF PHYTOESTROGENS ON SKIN VIA NEXT-GENERATION RISK ASSESSMENT
ABSTRACT #107

Francesca Rispo[1], Lorenzo Dondero[1], Giulia De Negri Atanasio[1], Giorgia Allaria[1], Francesca Tardanico[1], Erica Lertora[1], Ilaria Demori[2], Giosuè Costa[3], Emanuela Marchese[3], Simón Perera-del-Rosario[45], Eva Serrano-Candelas[4], Martina Palomino-Schätzlein[4], Elisabetta Perata[6], Federica Robino[6], Pier Francesco Ferrari[7], Sara Ferrando[1], Matteo Zanotti-Russo[6], Jan Markus[8], Silvia Letasiova[8], Elena Grasselli[910]
[1]*Department of Earth, Environment and Life Science, University of Genoa, Genova, Italy*
[2]*Department of Pharmacy, University of Genoa, Genova, Italy*
[3]*Department of Health Sciences, University "Magna Graecia", 88100 Catanzaro, Italy*
[4]*ProtoQSAR SL, Centro Europeo de Empresas Innovadoras (CEEI), Parque Tecnológico de Valencia, 46980 Valencia, Spain*
[5]*Departament de Medicina i Ciències de la Vida, Institut de Biologia Evolutiva (CSIC-UPF), Universitat Pompeu Fabra, Barcelona, Spain*
[6]*Angel Consulting S.a.s. 20122 Milano Italy*
[7]*Department of Civil, Chemical and Environmental Engineering, University of Genoa, via Opera Pia, 15, 16145 Genoa, Italy*
[8]*MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia*
[9]*Department of Earth, Environment and Life Science, University of Genoa, Genova, Italy*
[10]*Interuniversity Center for the Promotion of 3R Principles in Teaching and Research (Centro 3R), Italy*

Background and objectives According to the European Commission, an endocrine disruptor (ED) is an "exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations". Scientific concern exists about the nature and the safety of the ingredients used by the cosmetics industry regarding their endocrine-disrupting effects. Phenolic compounds present in soy (genistein and daidzein) are considered the most powerful phytoestrogens due to their ability to interact with estrogen receptors; this ability is associated with the structural similarity to 17β-estradiol, a steroid hormone. These two are the only natural compounds included in the call for data published by EU regarding the concern of the presence of ED in cosmetic formulations (https://ec.europa.eu/newsroom/growth/items/651201/en). We designed an innovative procedure based on Next Generation Risk Assessment, a hypothesis-based that integrates in silico and in vitro approaches. Materials and Methods In the simulated exposure scenario, the phytoestrogens were applied onto the 3D reconstructed skin models (EpiDerm) followed by evaluation of viability using MTT assay at different concentrations (from 10-3M to 10-9M) for 24h and 48h. Quantitative RT-PCR was performed to analyze the effects of genistein and daidzein on the mRNA expression of estrogen receptors (ERs). Molecular docking calculations were performed to elucidate the binding modes of the two natural compounds with ER alpha and beta. Results The two polyphenolic compounds showed no significant harmful effects in tissue viability. Gene expression of ER alpha and ER beta were differently affected by estradiol, genistein and daidzein at different concentrations and time points. Discussion and conclusion We have designed a tiered approach to evaluate effects of potential ED that involves exposure of a target organ (skin)

and evaluation of multiple parameters including viability, regeneration and gene expression.

P117
sodium iodide symporter as target for endocrine disruptors - novel in vitro bioassays with optional biotransformation step
ABSTRACT #139

Puja Kumari[1], Sebastian Lungu Mitea[234], Jiří Novák[1], Klára Hilscherová[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[3]*Department of Pharmaceutical Biosciences, Drug Safety and Toxicology, Uppsala University (UU), SE-75124 Uppsala, Sweden*
[4]*Department of Biology and Environmental Science, Linnaeus University (LNU), SE-39182 Kalmar, Sweden*

Thyroid hormones (THs) play a pivotal role in maintaining physiological equilibrium across vertebrates, influencing critical processes from metabolism (1) to neural development and cardiovascular health. Dysregulation of the hormonal system by endocrine-disrupting compounds (EDCs) can lead to adverse health effects (2). One of the priority targets of EDCs is the synthesis of THs and the sodium-iodide symporter (NIS) activity. It is responsible for the uptake of iodide to thyrocytes, and without its activity, synthesis of THs is not possible (3). A bioassay was designed to assess the impact of chemicals on iodide uptake by NIS-expressing cells using a colorimetric Sandell-Kolthoff reaction. Two stably transfected human cell lines, overexpressing human NIS, were juxtaposed with a rat thyroid cell model. The NIS inhibition was screened for a set of model chemicals, and several NIS inhibitors have been identified. Yet, for some chemicals, the effect potential could be masked by their cytotoxicity. To enhance the physiological relevance, we integrated an external biotransformation system (BTS) into our assay, addressing the limitation of in vitro models. This BTS was based on rat S9 and optimized for compatibility with live cells. The results show that BTS capacity has an impact on the toxic potential of identified NIS inhibitors. To determine the conservation of NIS protein across vertebrate species and assess the applicability of our findings beyond mammals, we utilized the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool in conjunction with different cell models. The SeqAPASS analysis suggests that NIS is relatively well conserved across vertebrates, and thus also, the results from the developed bioassays have good cross-species relevance. The obtained results show that the bioassay is suitable for screening purposes. The project received funding from the EU H2020 research and innovation program under the ERGO project (grant agreement No. 825753).

P118
IDENTIFICATION OF MIXTURE RISK DRIVERS OF ANTIANDROGENICITY IN EUROPEAN BLOOD SAMPLES USING HIGH-THROUGHPUT EFFECT-DIRECTED ANALYSIS
ABSTRACT #142

Maria João Valente[1], Maria Margalef[2], Christina Rørbye[3], Marja Lamoree[2], Anne Marie Vinggaard[1]
[1]*National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark*
[2]*Amsterdam Institute for Life and Environment (A-LIFE), Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*
[3]*Hvidovre Hospital, Hvidovre, Denmark*

Humans are continuously exposed to complex mixtures of chemicals arising from the environment, food, and consumer products. Many of these chemicals display endocrine disruption potential. As they travel through the environment-food-human continuum, and from the mother to the child, they may lead to adverse reproductive health outcomes in children [1]. To define limits of human exposure to hazardous chemicals, it is important to determine their bioactivity as components of complex mixtures and identify the drivers of mixture effects. This work aimed at showing the applicability of a high-throughput effect-directed analysis approach [2], combined with an in vitro reporter gene assay with suspect and non-target screening, for the identification of chemical drivers of antiandrogenic activity of extracts of pooled European human blood

samples. Enriched extracts of pooled samples representing the adult European and Danish population, as well as cord blood of Danish origin, were tested for antiandrogenic activity in the AR EcoScreen™ assay [3]. Active pooled samples underwent fractionation and non-targeted chemical profiling through high performance liquid chromatography coupled to a fraction collector (FractioMate™) and a qTOF mass spectrometer. The bioactivity of fractions was screened using the same in vitro assay. Whole extracts from adult and cord blood showed specific antiandrogenic potential. Although there was no overlap of active fractions between all three pooled samples, common active fractions were detected between adult European/Danish blood and Danish adult/cord blood, indicating that these populations may share common chemical drivers of mixture effects. Chemical profiling of active fractions highlights the distinct classes of potential contaminants responsible for the observed bioactivity. This study had financial support from the European H2020 Green Deal project PANORAMIX (Grant Agreement No. 101036631).

P119
CLIMBAZOLE AND BISPHENOL A MIXTURE EFFECT ON STEROIDOGENESIS IN MAMMALIAN CELLS
ABSTRACT #177

Dieynaba NDIAYE[1], Mylène LORCIN[1], Marie PERCEAU[1], Flavien DENIS[1], Laurent GATE[1]
[1]*French Research and Safety Institute for the Prevention of Occupational Accidents and Diseases (INRS), Toxicology and Biomonitoring Division, 1 rue Morvan, F-54519 Vandoeuvre les Nancy, France*

Bisphenol A (BPA) is a well-known plasticizer ubiquitously present in the environment of the general population and in many occupational settings. Indeed, BPA has been widely used in Europe, alt-hough its use is more restricted. It's established that BPA exposure decrease testosterone levels in human causing infertility, and impairing male tract development. Azoles are a class of antifungal used worldwide in agriculture and personal care products. Human exposure occurs through consumption of drinking water but also through application of personal care products where azoles are used as preservatives. Among the azole family, Climbazole (CBZ) is a synthetic chemical which exposure alters reproduction and steroidogenesis in fish models. Using OECD test guideline 456 we showed in a previous study that CBZ treatment reduces testosterone levels in H295R cells. Here we decided to test the effects a theoretical mixture of BPA and CBZ on H295R cells in order to characterize the interaction of these two chemicals which share a similar mode of action (testos-terone decrease). Cells were treated according to the following experimental combinations: - Condition 1: fixed concentration of BPA (IC30) and increasing concentrations of CBZ - Condition 2: fixed concentration and CBZ (IC30) and increasing concentrations of BPA. We then measured 10 hormones in the steroidogenesis pathway but also testosterone and estradiol, in order to study the toxicity of the mixture. Our results showed for the first time in H295R cells, that even if the effects of the two mixtures is greater than the effect of each chemical alone, BPA and CBZ interaction does not conform with the model of additivity. We are here in presence of an antagonism. These results give additional insights regarding the toxicological mechanism of action of mixtures of two chemicals sharing similar modes of action in H295R cells.

P120
Pancreatic beta cell signaling and function disruption by metabolic disrupting chemicals.
ABSTRACT #179

Katreece Feiertag[1], Philip Marx-Stölting[1], Tewes Tralau[1]
[1]*The German Federal Institute for Risk Assessment (BfR)*

Background: The pancreas is a crucial organ in the maintenance of metabolic homeostasis. It contains endocrine beta cells that produces stores and secretes insulin, making it one of the key regulators in glucose homeostasis. Beta cell dysfunction is linked to both diabetes mellitus type 1 and type 2; hence, several studies have investigated the circumstances leading to this outcome. Although diabetes

mellitus type 2, obesity and the metabolic syndrome are considered lifestyle diseases, mounting evidence suggests chemicals, termed metabolic disrupting chemicals can contribute to the progression of these diseases. A human-derived beta cell capable of secreting insulin in response to glucose, endoc- βH1 could give insight into the possible mechanism in metabolic disruption in human beta cells. Aim: To determine the effects of metabolic disrupting chemicals on beta cell signaling using a human derived beta cell model, endoc-βH1. Methods: Neutral Red uptake assays and WST-1 assays were used to determine the cytoxicity of bispehenol A (BPA), its alternatives, pesticides and their mixtures to endoc--βH1. Dose response curves were subsequently generated. Glucose-stimulated insulin secretion assays were performed to assess the effects of the aforementioned chemicals on the insulin capability of the cells. Furthermore, RNA sequencing was done as well as peptide analyses using a human metabolic hormone panel. Results: Bispehenol A (BPA), its alternatives, pesticides and their mixtures decreased the viability of the cells and affected insulin secretion. Conclusion: Endoc-βH1 is a valid model for the investigation of metabolic disrupting chemicals on the function and signaling of the human beta cells.

P121
Battery of in vitro testing of deiodinases inhibition by thyroid hormone disrupting chemicals and environmental pollutant mixtures
ABSTRACT #183

Veronika Janečková[1], Tereza Nešporová[1], Ondřej Brózman[1], Sabina Hřibová[1], Jiří Novák[1], Klára Hilscherová[1]
[1]*Masaryk University, Faculty of Science, RECETOX*

Thyroid hormone (TH) regulation is a vital and complex process for proper organism functioning that involves different organs in many species. Disruption of the thyroid hormone system (THS) can result in a variety of adverse effects, especially during development. Iodothyronine deiodinases (DIOs) represent key TH metabolism enzymes that are responsible for the conversion between active and inactive forms of TH. The disbalance of DIOs function has been described as an important molecular initiating event within the adverse outcome pathway network for thyroid hormone disruption leading to adverse effects in various vertebrates. DIOs occur in three isoforms - DIO1, DIO2, and DIO3 - each prevalent in different tissues and with diverse affinities to various substrates. Despite the importance of THS balance, there are missing in vitro high throughput tests, respecting 3R principles, for the assessment of TH regulation disruption, namely focused on the inhibition of different DIO isoforms. Our study aims to develop a set of in vitro human cell lines with lentivirally-transfected overexpression of DIO2 and DIO3. We adopted and modified a previously published method including a combination of substrates and inhibitors for screening across multiple chemical agents included in the H2020 ERGO project. Additionally, the developed assay battery is optimized towards testing environmental samples. The method quantifies iodine release from the deiodination process by the Sandell-Kolthoff reaction in microtiter plate format, serving as an index of DIOs activity. We are optimizing the models and methodology for specificity enhancement towards the assessment of the effect of test compounds on the selected DIO isoforms, which enables us to determine the influence of endocrine disruptors in a DIO-specific manner. This research has received funding from the EU H2020 research and innovation programme under grant agreement No. 825753 and Czech Science Foundation (GACR) under grant agreement GX20-04676X.

P122
Refinement of a classical in vitro endocrine activity assay by integrating skin barrier function and skin metabolism to mimic topical application
ABSTRACT #190

Emery Sylvie[1], Sayah Ines[2], Lereaux Guillaume[2], Viallard Chloé[2], Gregoire Sebastien[2], Burbank Matthew[2], Bury Dagmar[3], Rigaudeau Anne-Sophie[4], Rouas Caroline[3], Grall Romain[1], Riu Anne[1]
[1]*L'Oréal Research and Innovation, Aulnay-*

sous-Bois, France
[2]*L'Oréal Research and Innovation, Aulnay-sous-bois, France*
[3]*L'Oréal Research and Innovation, Clichy, France*
[4]*EPISKIN, Lyon, France*

Next generation risk assessment evaluating the systemic toxicity of cosmetic ingredients needs to develop robust new approach methodologies, which also cover potential endocrine activity with physiological relevance. To this end, we supplemented our endocrine activity testing toolbox with a co-culture 3D-Transactivation Assay (TA), combining a reconstructed human epidermis (RHE) with TA cell lines, thereby modeling consumer-relevant topical application. Few years ago, we developed the 3D-TA model using the 1cm² RHE EpiSkinTM as skin barrier model after a comparison to other available reconstructed skin models such as full thickness skin model and the 1cm² or 0.11cm² SkinEthic RHE. The Full thickness model showed fibroblasts' migration into cells used in the estrogenic or androgenic activation TA, and the insert format of the 1cm² SkinEthic RHE was uncompatible with cells seeded underneath the skin model. Finally, the results using the high throughput solution with the 0.11cm² SkinEthic RHE showed high variability. Within the Green Deal context, the refinement of 3D-TA model using the 1cm² EpiSkinTM is needed allowing high throughput testing, using the 0.5cm² SkinEthic RHE for miniaturization. First, the 3D-TA with 1cm² SkinEthic RHE model was used for validation with reference compounds, compared to results obtained using the 3D-TA 1cm² EpiSkinTM in which endocrine activities were significantly decreased due to the skin barrier model and metabolism from EpiSkinTM. The quantitative absorption of parent compound and metabolites formation was monitored by LC-MS using 1cm² SkinEthic RHE and compared to the results obtained from current 3D-TA for endocrine activities testing. Then the transposition to a semi high throughput system using the 0,5 cm² SkinEthic RHE will be made. Overall, the 3D-TA method incorporating topical application into classical in vitro assays may lead to a considerable refinement of the results which better resemble real-life consumer conditions.

P123
Different metabolizing systems improved the informative value of in vitro endocrine disruption assays: a comparative study
ABSTRACT #191

Emery Sylvie[1], Budin Clemence[2], de Boer Tjalf[2], Brouwer Abraham[2], Lereaux Guillaume[3], Burbank Matthew[3], Gregoire Sebastien[3], Bury Dagmar[4], Grall Romain[1], Riu Anne[1]
[1]*L'Oréal Research and Innovation, Aulnay-sous-Bois, France*
[2]*BioDetection Systems, Amsterdam, The Netherlands*
[3]*L'Oréal Research and Innovation, Aulnay-sous-bois, France*
[4]*L'Oréal Research and Innovation, Clichy, France*

Metabolically competent in vitro cell-based assays allow us to assess either potentially toxic metabolites, or potential detoxification of the parent compound. Regulatory bodies emphasized the need for developing appropriate metabolically competent in vitro assays also for the assessment of potential endocrine active substances, even more within the European Green deal context for classification of ingredients and mixtures. Thus, we updated our toolbox with metabolically competent transactivation assays incorporating S9 fractions. In contrast to the commonly used rat liver S9, human liver S9 is rarely used for endocrine activity assays due to possible strong interference with estrogen receptor transactivation readouts because of the intrinsic presence of hormones. To investigate this further, we compared the performance of S9 fractions from rat liver, human liver and human skin incorporated into the classical gene reporter transactivation assays (TA) for estrogenic and androgenic activities assessment. For this purpose, we used well-known estrogenic and androgenic active compounds, such as Methoxychlor, Bisphenol A (BPA), Flutamide and Vinclozoline, using phase I or phase I and II co-factors in order to capture both potential toxic metabolites formed and potential detoxification of the parent compound. The qualitative formation of metabolites with rat liver S9 was confirmed by LC-MS or GC-MS. In fact, we observed

differences in metabolic capabilities of the investigated S9 fractions, with rat liver S9 showing stronger metabolic activity compared to human skin S9, triggering different outcomes in the estrogenic and androgenic transactivation assay. Work using human liver S9 is still on going. This study demonstrated the feasibility of incorporating human liver S9 into classical transactivation assays for endocrine activity testing and the relevance of the incorporation of metabolic systems in in vitro assays, to optimize the characterization of a potential endocrine activity of chemicals.

P124
Poly-fluoroalkyl substances (PFASs) detectable in waste of electrical and electronic equipment (WEEE) plants – preliminary metabolomics and toxicological data from the VAISAL project.
ABSTRACT #205

Stefano Lorenzetti[1], Milena Mikhail[1], Laura Di Benedetto[1], Anton Vremere[1], Stefano Di Bona[2], Alessandra Di Veroli[2], Emanuele Artino[2], Carolina Barola[3], Francesca Buiarelli[4], Patrizia Di Filippo[5], Roberta Galarini[3], Franco Lucarelli[6], Simone Moretti[3], Giulia Pazzi[6], Donatella Pomata[5], Carmela Riccardi[5], Gabriele Cruciani[2], Laura Goracci[2]
[1]*ISS - Istituto Superiore di Sanità*
[2]*University of Perugia*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche*
[4]*University Sapienza of Rome*
[5]*DIT-INAIL*
[6]*University of Florence*

The VAISAL project (INAIL BRiC2019 - ID13 grant) aimed to identify chemicals of emerging concerns in three Italian plants recycling waste of electrical and electronic equipment (WEEE) and to perform a cell-based toxicological investigation in two human-derived cell lines, A549 (epithelial bronchial cells) and HepG2 (hepatocytes). Using a 14-stage cascade impactors to collect particle matters (PMs) samples, inorganic and organic compounds (23 and 149 analytes, respectively) were characterized. Furthermore, using liquid-chromatography coupled to high-resolution mass spectrometry on the same collected samples, non-targeted analysis methods detected further compounds. Some targeted and non-targeted organic compounds have been subjected to cell-based bioassays in A549 and HepG2, in order to perform in a dose-dependent manner (1pM-100microM range): i) cytotoxicity by MTS assay, ii) preliminary untargeted lipidomics analysis. In addition, metabolic degradation by human liver microsomes, human skin microsomes and human liver hepatocytes were also performed. Besides others, targeted and untargeted approaches assessed the presence of 8 (eight) different Poly-FluoroAlkyl Substances (PFASs). Hence, data on their role in the two in vitro models (A549 and HepG2), representatives of two PM routes of exposure, will be presented concerning their metabolomics and toxicological role, including effects on cell viability and hormone-dependent biomarkers.

P125
Increases in disinfection byproduct generation potentiate to trigger anti-estrogenic endocrine disruption in zebrafish estrogen receptor alpha
ABSTRACT #208

Sang-Ah Lee[1], Maranda Esterhuizen[2], Chang Seon Ryu[3], Hyunki Cho[3], Indong Jun[3]
[1]*Jeju National University*
[2]*University of Helsinki*
[3]*KIST Europe*

The escalating use of disinfectants in wastewater treatment plants, prompted by the detection of residual viruses in sewage, has led to an increased generation of disinfection byproducts (DBPs). The release of treated effluent into aquatic environments introduces concerns regarding the potential adverse effects of DBPs, particularly on zebrafish estrogen receptor α (zERα), a key player susceptible to DBP exposure. This case study investigates the endocrine-disrupting properties of four DBPs—chloroiodomethane (CIM), dibromochloromethane (DBCM), bromodichloromethane (BDCM), and trichloroacetic acid (TCA)—focusing on their impact on zERα. DBP-induced estrogenic and anti-estrogenic activities were assessed using the Luciferase Reporter Assay System in HEK293-ERE-zEsr1 cells. The estrogenic

activities of all DBPs were lower than 20% in zERα. The findings reveal a notable anti-estrogenic activity of all DBPs against zERα, with CIM, BDCM, DBCM, and TCA exhibiting anti-estrogenic effects of 80.8%, 78.4%, 49.0%, and 64.1%, respectively. Additionally, the DBPs displayed negligible estrogenic effects on zERα. This study sheds light on the less-explored realm of DBP-induced endocrine disruption, particularly within the dihalomethanes group, emphasizing the environmental concerns associated with DBPs as potential endocrine disruptors in aquatic ecosystems.

P126
Adipocytes 3D scaffold free microtissues for toxicology and preclinical applications
ABSTRACT #213

Francesca Rescigno[1], Francesco Carriero[1], Christian Pellevoisin[2], Marisa Meloni[1]
[1]*VitroScreen, Milan, Italy*
[2]*Urbilateria, Tours, France*

White adipose tissue (WAT) is a complex organ composed mainly of differentiated adipocytes responsible for the body energy homeostasis, the metabolism of sex steroids and glucocorticoids, and the modulation of important biological processes through adipokines acting at both local and systemic level. Many evidences have supported the toxicological function of the adipose tissue, in particular with respect to the hydrophobic endocrine disruptors(1) and persistent organic pollutants(2), which tend to distribute into lipophilic compartments of humans, animals and fishes. Moreover, metabolic syndrome, obesity and hormonal disfunctions have WAT as primary biological target and the brite adipocytes discovered in WAT as potential target for therapeutic treatments. VitroScreen has developped human 3D scaffold free adipocytes microtissues by using the hanging drop technology starting from suspension of human primary preadipocites of single/pool donors with different BMI. A multiple endpoint approach has been adopted to characterize the established “microadipe model” at biochemical, morphological, gene and protein expression levels compared to a 2D monolayer: the 2 models were compared in term of intracellular lipid droplets (adipogenesis), amount of glicerol release in medium (lipolysis) and for the transcriptional activity of specific markers of mature adipocytes (e.g. PPARy2, FABP4, ADIPOQ, LEPTIN) by qRT-PCR. Several experimental protocols were thus defined to model different preadipocyte differentiation stages (e.g. differentiation towards the brite adipose tissue called “browning of WAT”) up to 14-20 days after seeding. The modulatory effects derived from treatment with reference molecules (e.g. forskolin, caffein) were investigated. Given that WAT inflammation as a critical step in the pathogenesis of obesity and metabolic syndrome(3), an inflammatory status has been induced on the “microadipe model” dosing in the culture medium pro-inflammatory cytokines. The use of established “microadipe model” represents a promising, relevant and predictive biological tool for preclinical and toxicological assessment of active ingredients, including food supplements, toxicants and hormone modulators.

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REPURPOSING TRANSGENDER TESTICULAR TISSUE: ANDROGENIC TESTICULAR ORGANOIDS FOR HIGH-THROUGHPUT APPLICATIONS
ABSTRACT #274

Samuel Madureira Silva[1], Guillaume Richer[1], Andrea Errico[2], Katerina Papageorgiou[13], Tamara Vanhaecke[3], Ellen Goossens[1], Yoni Baert[13]
[1]*Biology of the Testis laboratory - Genetics, Reproduction and Development, Vrije Universiteit Brussel, Belgium*
[2]*Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy*
[3]*In Vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Belgium*

New approach methodologies should include human-derived models for a safer and faster replacement of animals. In reprotoxicity and endocrine disruption, human testicular organoids can be an important tool. In testicular organoid formation, prepubertal tissue is the most efficient. However, human immature

testicular tissue is scarce, making it irrelevant for high-throughput applications. Conversely, transgender tissue is abundantly available and it has been shown to exhibit similar characteristics to immature tissue. To investigate whether transgender tissue allows organoid formation, we derived organoids from transgender (n=5), prepubertal (n=3), pubertal (n=3) and adult cisgender (n=3) testicular tissue and evaluated architecture and testosterone production. To enlighten the testosterone profile in transgender organoids, we stimulated the cultures with 1, 50 and 100 IU/L of human chorionic gonadotropin (hCG) and analysed testosterone concentration over 63 days. Protein markers confirmed the presence of the main testicular cells in transgender organoids: spermatogonia, Sertoli, Leydig, and peritubular myoid cells. Although transgender organoids did not show a seminiferous tubule-like architecture, compartmentalisation was present: core composed of peritubular myoid cells and extracellular matrix proteins, and lining periphery of Sertoli, germ and Leydig cells. This architecture was comparable to pubertal. Prepubertal organoids presented a closer architecture to native tissue. Contrarily, adult cisgender organoids showed no organisation and limited cell assembly. Moreover, transgender cultures showed the highest levels of testosterone at day fourteen (715.14±667.51 µg/L) and stabilised after day 35 at ≈73 µg/L. The cultures did not respond to hCG stimulation. Transgender testicular tissue can form compartmentalised organoids able to produce testosterone and, thus, hold potential for endocrine disruption testing. Although transgender testicular organoids did not show ideal organisation, these are still a valuable alternative to organoids derived from immature tissue in high-throughput applications. Future research should investigate how to correct the organoid's architecture, promote germ cell differentiation, and validate endocrine disruption testing.

P128
Toxicity of microplastics and additives in a complex cellular liver model.
ABSTRACT #278

Lucas Gaillard[12], Léo Sportes-Milot[12], Béatrice Legrand[12], Kévin Bernal[12], Jules Imler[12], Etienne Blanc[12], Antonietta Messina[3], Jean-Charles Duclos-Vallée[3], Xavier Coumoul[12], Karine Andréau[12]
[1]*INSERM UMR-S 1124 - T3S, environmental Toxicity, Therapeutic Targets, cellular Signaling and Biomarkers, 45 rue des Saints-Pères, 75006 Paris, France*
[2]*Université Paris Cité, UFR des Sciences Fondamentales et Biomédicales, 45 rue des Saints-Pères, 75006 Paris, France*
[3]*INSERM UMR-S 1193, Physiopathogénèse et Traitement des Maladies du Foie, Centre Hépatobiliaire, Hôpital Paul Brousse, 94800 Villejuif, France*

Defined as particles smaller than 5mm, microplastics (MPs) are currently a source of intense concern because of their presence in everyday products. Humans are exposed to MPs, as well as to their additives bisphenols (BPs) and perfluoroalkyl substances (PFAS), mainly by ingestion of contaminated food and water. Once ingested, these pollutants reach distant organs such as the liver and may cause damage related to metabolic dysfunction-associated steatotic liver disease (MASLD)1. This clinicopathological syndrome involves well-known steps from steatosis to the metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and finally cirrhosis and hepatocellular carcinoma (HCC). The aim of our study is to evaluate the toxicological impacts of mixtures of MPs and their additives in order to decipher involved mechanisms in a complex 3D liver cellular model including the three cell types involved in MASLD steps: hepatocytes, stellate cells and Kupffer cells modeled respectively by HepaRG, LX-2 and THP-1 cell lines. Our initial cytotoxicity results from the 3 cell types, independently grown in 2D, revealed differential susceptibility towards MPs (5 - 150 µg/mL) and additives BPA, BPS, PFOS, PFOA (100 pM - 100 µM). Lipid droplet quantification assay by microscopy performed on HepaRG cells also showed a steatosis induction only after 5 days exposure to 10 µM BPA or PFOS. Our spheroids generated by mixing HepaRG, LX-2 and THP-1 cells in agarose micro-molds is being developed and is currently being validated in terms of viability, hepatic marker gene expression assessed by RT-qPCR, and spatial distribution of the different cell types

visualized by confocal microscopy analyses. The main prospect of these results is the use of our complex 3D liver model to investigate the adverse impacts of MPs and their additives, alone or in combination on several readouts such as induction of steatosis, inflammation and fibrosis.

P129
Assessment of endocrine disruptor impacts on carbohydrate metabolism in the HepaRG human hepatic cell line
ABSTRACT #288

Kévin Bernal[12], Béatrice Le-Grand[21], Arnaud Tête[21], Min Ji Kim[31], Xavier Coumoul[21], Etienne Blanc[21]
[1]*INSERM UMRS 1124, Team Metatox, Paris, France*
[2]*Université Paris Cité, Paris, France*
[3]*Université Sorbonne Paris Nord, Paris, France*

Metabolic disorders are among the main adverse health outcomes that have been associated with chemical exposure. To fill the gaps to assess such disrupting properties, it is crucial to develop new approach methodologies (NAMs) for regulatory testing and still necessary to understand their underlying mechanisms of action. In the frame of metabolic dysfunction-associated steatotic liver disease (MASLD), using the HepaRG differentiated human hepatic cell model, we assessed the impact on the carbohydrate metabolism of 10 selected endocrine disruptors, from various chemical families, suspected of having diabetogenic properties : bisphenols (BPA, BPF, BPS), phthalates (DBP, DEHP), butylparaben (BP), perfluoroalkylated substances (PFOA, PFOS), organochlorides (p,p'-DDE, a metabolite of the pesticide DDT) and one metal (CdCl2) (1). Using non-cytotoxic concentrations, we assessed the level of expression of genes belonging to carbohydrate metabolism (glycolysis, gluconeogenesis, glycogenesis and glycogenolysis) after acute exposure for 5 days. The effects of the chemicals were analyzed also for several endpoints: uptake and release of glucose, production of lactate, glycogen content, mitochondrial respiration. Among the 10 selected compounds, p,p'-DDE, was the most effective. SLC2A2, G6PC and GYS2 gene expressions and glycogen content were altered. Therefore, using siRNA, we further assessed the role of PXR and CAR in the adverse effects induced by this molecule. The underlying mechanisms are still under investigation. The results of the experiments conducted with the p,p'-DDE suggest that gluconeogenic-related parameters and glycogen content could be considered as endpoints for NAMs despite the need to test other reference compounds.

P130
Impact of endocrine disruptors on prostate organoid structure and integrity
ABSTRACT #300

Jules Imler[1], Amel Ait Mouffok[2], Sylvie Bortoli[1], Stephan Clavel[2], Xavier Coumoul[1], Arnaud Tête[1]
[1]*Inserm UMR S-1124, Université Paris Cité, 75006 Paris, France*
[2]*CNRS UMR 7275, Institut de Pharmacologie Cellulaire et Moléculaire, 06560 Valbonne, France*

The prostate, an accessory gland in the male reproductive system, is prone to a number of common diseases, including hormone-dependent cancers. Prostate is notably sensitive to the action of androgens and oestrogens, two steroid hormones acting through the binding to the androgen receptor (AR) and the oestrogen receptors (ERα and ERβ) respectively. Aberrant signalling of these nuclear receptors has been linked to the development of prostate cancer. Exposure to endocrine-disrupting chemicals (EDCs) may interfere with the proliferation and differentiation of prostate cells through various mechanisms, including illegitimate binding to AR or ERs, thus mimicking or antagonizing the function of natural hormones. Among EDCs, organochlorinated compounds such as chlordecone and Di(2-ethylhexyl) phthalate (DEHP) have been reported to interact with AR and ER signalling. Chlordecone, an insecticide extensively used in French West Indies, has been classified as possibly carcinogenic (Group 2B) by the International Agency for Research on Cancer (IARC). Epidemiological

studies have associated chlordecone exposure with an increased risk of developing prostate cancer [1]. Additionally, recent studies have suggested a potential link between exposure to DEHP, a commonly used plasticizer ubiquitous in the environment, and the occurrence of prostate cancer [2]. Mouse prostate organoids, 3D cell assemblies originating from a stem cell progenitor through self-organization, mimic prostate architecture and function. The formation of prostate organoids occurs through several specific stages, from proliferation to functionalization, requiring AR signalling pathway. In this study, we investigated the impact of a 5-day exposure to chlordecone and DEHP on the formation of mouse prostate organoids by evaluating morphological changes, cell death induction, and the expression of AR and ERs target genes. Prostate organoids emerge as a relevant model to characterize the molecular and cellular events triggered by EDCs on the prostate, and as a powerful tool to assess endocrine disruption potential of new chemicals.

P131
THYROID HORMONE DISRUPTING POTENTIAL OF ENVIRONMENTAL POLLUTANTS AND SAMPLES ASSESSED BY MOLECULAR-INITIATING EVENTS-BASED IN VITRO SCREENING BATTERY
ABSTRACT #339

Jiri Novak[1], Marie Smutna[1], Zuzana Tousova[1], Puja Kumari[1], Saskia Finckh[23], Werner Brack[23], Klara Hilscherova[1]
[1]*RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Department of Effect-Directed Analysis, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany*
[3]*Department of Evolutionary Ecology and Environmental Toxicology, Faculty of Biological Sciences, Goethe University Frankfurt, Max-Von-Laue-Strasse 13, Frankfurt am Main, 60438, Germany*

The thyroid hormone (TH) system has been recognized as a significant target for endocrine disruptive compounds (EDCs). However, the study of these modes of action is negatively affected by the lack of suitable specific high-throughput assays. Thus, a battery of in vitro bioassays addressing TH system-relevant molecular initiating events (MIEs) has been developed within the H2020 ERGO project [1]. The MIEs have been prioritized based on the adverse outcome pathway (AOP)-driven strategy, and they cover endpoints in TH synthesis (NaI symporter inhibition, NIS; thyroperoxidase inhibition, TPO), TH transport (T4-transthyretin displacement, TTR), TH metabolization (aryl hydrocarbon receptor-related metabolism, AhR), and TH receptor interactions (ThR). The bioassay battery has been applied to assess THD potential of selected human exposure-relevant chemicals representing different chemical and use categories, including industrial chemicals, pharmaceuticals, personal care products or pesticides. The results demonstrate that some widespread pollutants can interfere with TH system via multiple modes of action. While there is information regarding THD effects of some compounds, there is limited knowledge on the effects of environmental exposure mixtures. We investigated THD potential of mixtures in treated wastewaters from 15 European countries. Effluent samples from 20 wastewater treatment plants with different capacities and treatment technologies were obtained using on-site large-volume solid phase extraction and assessed using the bioassay battery. The most frequently detected effects were AhR activation, TTR-binding inhibition, and TPO inhibition. ThR-mediated agonism or antagonism were detected at a few sites, while NIS inhibition was shown only exceptionally. Nevertheless, these effects could be masked by the cytotoxicity of the complex samples. The bioassay battery was shown to be applicable not only for screening potential TH disruptors but also for complex environmental samples. The research received funding from the EU H2020 program (ERGO project; No. 825753) and from the Czech Science Foundation (project GX20- 04676X).

P132
DEVELOPMENT OF A HUMAN STEM CELL-BASED IN VITRO MODEL TO ASSESS DEVELOPMENTAL LIVER TOXICITY INDUCED BY THYROID HORMONE DISRUPTORS

ABSTRACT #352

Lorna Marchandise[1], Robim Marcelino Rodrigues[1], Yoni Baert[1], Tamara Vanhaecke[1]
[1]*In Vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Brussels, Belgium*

Thyroid hormone system disruptors (THSDs) pose a significant threat by disturbing the intricate processes of thyroid hormone (TH) production, distribution, metabolization, and secretion, crucial for fetal development. Their interference extends to the liver, a key player in TH transport and metabolism. Despite some elucidated mechanisms of THSD action, many remain poorly understood, compounded by the reliance on rodent models, which do not fully mirror human physiology. To address this gap, a novel in vitro toxicity model leveraging human stem cells is created, focusing on alterations caused by THSDs during the early phases of liver development. As such, multipotent human skin derived precursor cells (hSKPs) were differentiated into “hepatic progenitor cells” (hSKP-HPCs) to mimic in vivo liver development in vitro and enable studying ‘human foetal’ hepatic TH signalling. Triiodthyronin and thyroxin - essential THs to regulate the basal metabolic rate of all cells including hepatocytes - were incorporated into the culture throughout differentiation at levels comparable to systemic in vivo concentrations. Current culture methodology involves 2D monolayer formation, which is known to fall short in capturing the full complexity of the in vivo microenvironment and induce structural and functional alterations to the cells. Hence, a transition to a 3D setup was additionally studied to better mimic natural conditions and functional alterations. Characterization efforts are ongoing and encompass gene expression and protein profiling to elucidate hepatic aspects and TH signalling pathways in the presence or absence of THs and in relation to the cells’ spatial configuration. Additionally, metabolic function evaluation involves quantifying TH metabolite concentrations in the supernatant via LC/MS/MS analysis. Once the optimization and characterization of the hSKP-HPC cell system is completed, it will be evaluated for its potency to confirm known and identify new potential THSDs. This in vitro system aims to support next generation animal-free identification of THSDs.

MODELS, BIOMARKERS AND ASSAYS FOR SYSTEMIC AND IMMUNE TOXICITY

P133
Human Cells as New Approach Methodologies for Immunotoxicity Testing
ABSTRACT #252

Victor Johnson[1], Emanuela Corsini[2], Fenna Sillé[3], Norbert Kaminski[4]
[1]*Burleson Research Technologies, Inc.*
[2]*Università degli Studi di Milano*
[3]*Johns Hopkins University, Bloomberg School of Public Health & Center for Alternatives to Animal Testing*
[4]*Michigan State University*

Toxicology, including the discipline of Immunotoxicology, is a field of science that is at a crossroads between decisions to adopt in vitro and alternative methods versus the need for in vivo models. Approval of the ban for toxicity testing of cosmetic constituents using in vivo models by the EU in March 2013 marked a turning point for the necessity to consider and develop alternative methods, coined New Approach Methodologies (NAM). This initiative led to the development and approval of the first in silico and in vitro alternatives to animal testing for immunotoxicity testing that are now accepted for regulatory purposes in many countries worldwide. The pressure to reduce animal use in toxicity testing is not limited to cosmetics and enormous scientific efforts have been expended to develop non-animal alternative approaches. The immune system by design is a highly complex and integrated system that presents challenges for the development of in vitro approaches. Challenge accepted – the field of immunotoxicology has worked arduously in pursuit of appropriate and informative in vitro models. Utilization of human cells for development of NAMs provides direct translatability to human health and risk assessment. The focus of this symposium is to highlight some of the innovations that are leading to breakthroughs in the incorporation of in vitro alternatives for hazard identification and risk assessment. The overarching goal of this symposium is to provide attendees with a sense

of confidence that the field of immunotoxicology is successfully developing NAMs address safety concerns for xenobiotics and pharmaceuticals with the potential to harm the immune system.

P134
IN VITRO ASSESSMENT OF mRNA-DRUGS OFF-TARGET EFFECTS: FOCUS ON OXIDATIVE STRESS, ENDOPLASMIC RETICULUM STRESS, MITOCHONDRIAL STRESS AND AUTOPHAGY
ABSTRACT #258

Gloria Melzi[1], Valeria Bettinsoli[12], Valentina Galbiati[1], Marina Marinovich[1], Emanuela Corsini[1]
[1]*Department of Pharmacological and Biomolecular Science (DiSFeB) "Rodolfo Paoletti", Università degli Studi di Milano, Via Balzaretti 9, 20133, Milan, Italy.*
[2]*Department of Pharmacy, Università degli Studi di Napoli Federico II, Naples, Italy.*

Among new generation drugs, Nucleic Acid-based therapeutics and vaccines are emerging. Together with efficacy, their potential adverse effects on organ toxicity and immunotoxicity must be evaluated. This research aimed to provide an in vitro toxicological approach to evaluate possible off -target adverse effects of new RNA-drugs, developed within the Centro Nazionale di Ricerca "Sviluppo di terapia genica e farmaci con tecnologia a RNA". The proposed approach is based on the evaluation of the ability of selected mRNA-drugs to trigger toxicity pathways. These pathways provide a mechanistic understanding of how exposure to a particular substance or stressor can result in toxicity. Toxicity pathways are crucial in toxicological studies and risk assessments, helping scientists and regulators identify key events and biomarkers associated with adverse effects. The toxicity pathways investigated included oxidative stress, endoplasmic reticulum stress, mitochondrial stress, and autophagy. For each, it was selected a compound that will act as positive control for all the analysis: tert-Butyl Hydroperoxide (tBHP) for oxidative stress, Thapsigargin (TG) for endoplasmic reticulum stress, Rotenone (Rot) for mitochondrial stress, Rapamycin (Rapa) for autophagy. Toxicological evaluation was performed on peripheral blood mononuclear cells (PBMC) obtained from anonymous buffy coats of 5 male and female healthy donors, purchased from the Niguarda Hospital (Milan, Italy). PBMC were treated for 24 hours with the RNA-drugs and the four positive controls. Lactate dehydrogenase release and apoptosis analysis were performed to determine possible cytotoxicity. RNA-sequencing analysis was conducted using the positive controls, and genes involved in the main toxicity pathways selected and analyzed through Real-Time PCR following treatment of PBMC with the selected RNA-drugs. The results obtained on the analysis of these toxicity pathways will be presented. Our approach offers the opportunity to screen new RNA-drugs for potential toxicity, possible gender effects, variability in the response and markers identification possibly translatable to clinics.

P135
Micro-/nano-plastics activate the NF-κB pathway in an in vitro model of inflammatory bowel disease.
ABSTRACT #295

Alice De Groote[12], Julie Laloy[2], Vincent Maloteau[2], Jonathan Decarpentrie[12], Jean-Michel Dogné[12]
[1]*Clinical Pharmacology and Toxicology Research Unit (URPC), University of Namur, 5000 Namur, Belgium*
[2]*Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium*

Background and Objectives: The risk linked to the ingestion of micro-/nano-plastics has raised concern among authorities. Studies on in vitro mammalian cells are rising and indicate microplastic toxicity including inflammation, oxidative stress, or metabolic changes. However, most of them are focusing on a single type of plastic. Few of them demonstrated a link with intestinal inflammation, this suggest that a preliminary inflammatory intestinal state could promote toxicological effects of some micro and nano-plastics. We tried to find out if micro- and nano-plastics could have a greater toxicological impact in people with intestinal inflammation. To do this, we first developed an in vitro model

of inflamed intestine. Material and Methods: We generate a pro-inflammatory cytokine cocktail by stimulating differentiating and LPS stimulating THP-1 monocyte-like cells to create a repeatable and close-to-physiopathology inflamed gut model. To evaluate the impact of different micro-/nano-plastics on Inflammatory bowel disease ant their capacity to exacerbate the inflammation, we use the NF-κB reporter (Luc)-HCT-116 cell line exposed or not to our pro-inflammatory cocktail. Then, we exposed our intestinal cell model to polystyrene beads (100 & 500 nm) and PVC microparticles at concentrations range from 10 to 500µg/ml. We tested plastics particles alone or in mixture. Results: Our results showed a production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-8 and IL-6) by THP-1 in their supernatant. This cocktail induces the activation of NF-κB on HCT-116 cells. PS and PVC increased the inflammation on the inflamed intestinal model but not in a healthy model from 100 or 500 µg/ml. Plastics mixtures show a significant increase in inflammation at lower doses than those taken alone. Discussion and Conclusion: Taken together, our results clearly highlighted the exacerbation of inflammation by micro-/nano-plastics through the NF-κB pathway under the conditions of our innovative in vitro model of IBD.

P136
Evaluation of the impact of microplastics on neutrophil activation.
ABSTRACT #303

Alice De Groote[12], Jean-Michel Dogné[12], Jonathan Douxfils[123], Constant Gillot[12]
[1]*Clinical Pharmacology and Toxicology Research Unit (URPC), University of Namur, 5000 Namur, Belgium*
[2]*Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium*
[3]*Qualiblood s.a., QUALIresearch, 5000 Namur, Belgium*

Background and Objectives Previous research has demonstrated the role of microplastics in the inflammation process. NETosis, a form of neutrophil death involved in the pro-inflammatory response to different kinds of stimulus such as infections or reaction to ~~foreign matter. This study aims to assess the~~ effect of three types of plastics particles on NETosis under healthy and inflammatory conditions. Material and Methods: Neutrophil activation by polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) at 50µg/ml was investigated. Inflammatory conditions were induced using a cocktail of pro-inflammatory cytokines (TNF-α, IL-1β, IL-8 and IL-6) derived from differentiated and LPS activated THP-1 cells. Human neutrophils were isolated from whole blood obtained from a healthy donor. Neutrophil activation was monitored over an 8-hour period by measuring the release of DNA and neutrophil elastase (NE). Time to reach 50% release (T50) and the area under the curve (AUC) were calculated. Results: Significant differences were observed between controls, both positive and negative, for PET under inflammatory conditions for both biomarkers analyzed. The AUC of DNA release for PET in the inflammatory condition was 33.6% (95%CI: 31.7–35.6%), compared to 8.4% (95%CI: 7.8–9.1%) for the negative control. Similarly, for the AUC of NE release, significant differences were observed in the inflammatory condition, with 42.8% (95%CI: 40.1–45.6%) for PET compared to 2.1% (95%CI: 1.6–2.6%) for the negative control. No significant differences were observed for PET under healthy conditions. Discussion and Conclusion: This study successfully evaluated the impact of plastics under inflammatory conditions, demonstrating a significant effect of PET on neutrophil activation for both biomarkers analyzed. However, PS and PVC did not exhibit a significant impact on neutrophils at the concentrations tested.

P137
Assessment of the respiratory sensitizing potential of substances on in vitro macrophage model
ABSTRACT #319

Rhamiya Mahendran[1], Louis Scott[1], Ewelina Hoffman[1], Victoria Hutter[12]
[1]*ImmuONE Limited, Sycamore House, Stevenage, Herts, UK, SG1 2BP*
[2]*Centre for Topical Drug Delivery and Toxicology, University of Hertfordshire,*

Hatfield, Herts, UK, AL10 9AB

Chemical respiratory allergy poses significant health risks, with sensitization of the respiratory tract being the initial step leading to immune-mediated hypersensitivity reactions. Despite its regulatory, industrial, and social implications, the detection and discrimination of respiratory sensitizers remain challenging,1,2. Current methods focus on skin sensitization, lacking standardized approaches for respiratory allergens. Alveolar macrophages (AM) play a pivotal role in recognizing sensitizing agents, triggering stress responses and cellular signals, culminating in dendritic cell activation and migration. This study employs both high content image analysis (HCIA) combining AM morphology and viability endpoints, and microarray proteomics screens as in-vitro tools with the potential to mechanistically characterise individual cells and discriminate chemical respiratory sensitizers from irritants. Immortalized human macrophages were exposed to a panel of sensitisers and non-sensitisers. HCIA utilized fluorescent dyes to assess cellular morphology and viability. Inflammatory markers were evaluated via cytokine/chemokine expression assays, including semiquantitative membrane arrays and dual-colour antibody microarrays. From cytokine arrays alone, looking at 36 targets, it was difficult to identify a single cytokine that would be able to be used to discriminate between sensitizers and non-sensitizers3. To this end, a broader proteomic approach was taken to identify if expanding the protein fingerprint profile would enable discrimination between sensitizers and non-sensitizers. Proteomic analysis, supplemented by protein-protein interaction studies, identified distinct alterations between sensitizers and non-sensitizers. While individual cytokines lacked discriminatory power, a panel of 30 proteins, subjected to k-means clustering, accurately differentiated between the two groups. Our findings highlight the necessity of employing a panel of factors rather than relying on a single cytokine for sensitization discrimination. The exposure to respiratory sensitizers induced AM activation and a specific cytokine release pattern, while exposure to irritants did not. With this in-vitro model, we can postulate a set of promising markers that allow the discrimination of chemical respiratory sensitizers from irritants.

MODELS, BIOMARKERS AND ASSAYS FOR SYSTEMIC TOXICITY

P138
EVALUATION OF SENESCENCE GLYCOBIOMARKERS IN TWO DIFFERENT IN VITRO AGING MODELS ABSTRACT #50

Lucia Rackova[12*], Rebeka Kodrikova[2], Maria Sediva[2], Marek Nemcovic[2], Zuzana Brnoliakova[1], Marek Lepáček[1], Zuzana Pakanova[2]

[1]*Centre of Experimental Medicine, Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic*
**Corresponding author e-mail: rackova.luci@gmail.com*
[2]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovak Republic*

Background. Augmented β-galactosidase activity is commonly used as a biomarker for senescent cells. This enzyme is located in lysosomes and converts β-galactosides into monosaccharides under acidic pH. The activity is optimal under lysosomal pH 4, but conventional assays measure this activity at pH 6. Coincidentally, the alterations in N-glycosylation of glycoproteins have emerged as promising biomarkers of aging, with the most evident age-related increase in the agalactosyl N-glycans in human blood proteins [1].
Methods. Our study employed positive-ion MALDI-TOF mass spectrometry to compare the N-glycome changes in human dermal fibroblasts developing replicative senescence (RS) and premature senescence implemented by non-lethal exposure to H2O2 (SIPS). Senescence-associated beta-galactosidase (SAbgal)-positive staining was used as the standard senescence marker. Data were processed with FlexAnalysis, glycan structures were assigned using GlycoWorkbench, and relative intensities were analyzed with matchMass software.
Results and Discussion. A decrease in the relative intensities of bi-galactosylated core-fucosylated biantennary N-glycans (NA2F) was

observed in both RS and SIPS cells. Consistently, we confirmed an increase in the SAbgal activity marker in both in vitro aging models. Additionally, an increase in oligomannose-type N-glycans (M3-M8), both with the onset of RS and SIPS, was found. Still, less profound increases were determined for SIPS cells with a mild particular influence of high-mannose-type N-glycans (up to 20% vs. non-senescent control) compared to one- to two-fold higher increases measured for M6 and M5 in RS cells. Unlike SIPS cells, RS cells showed a reduced portion of ERAD-linked free oligosaccharides GN1 compared to low-passage cells. This may reflect decreased quality control accompanying cellular aging.
Conclusion. These results indicate the suitability of cell-based glycoprofile markers for studying aging processes in vitro. Still, factors such as time following gerontogen exposure or its specific mechanism can shape this glycobiomarker, thus creating its added value over the standard ones.

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[1] Rackova L, Mach M, Brnoliakova Z. An update in toxicology of ageing. Environ Toxicol Pharmacol. 2021;84:103611.

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BIOACTIVE COMPOUNDS EXTRACTED FROM TUNA BY-PRODUCTS
ABSTRACT #95

Mª Mercedes Alonso Martínez[1], Rebeca Vázquez Sobrado[1], Diego Méndez Paz[1], Giorgia Allaria[2], Elena Grasselli[2], Celina Costas Iglesias[1]
[1]*ANFACO-CECOPESCA, Ctra Colexio Universitario 16, 36310 Vigo, Spain*
[2]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa, 16132 Genoa, Italy*

The enhancement of actions inside the productive chain for optimization of energy, time, resources, reducing waste production and increasing yields is a powerful premise of the circular economy in the food supply chain. An example of such action is the study developed within the H2020 EcoeFISHent project (10103642), aimed to identify a pool of active ingredients from food fish-processing side streams with healthy bioactivity. Protein hydrolysates and gelatin, obtained from tuna industry by-products, were applied to several assays to determine its in vitro bioactivity. Diverse concentrations were tested to assess cell viability and cytotoxic effect in cellular cultures, using MTT assay. Functional activities were determined in vitro, in cellular assays. The cellular models used were RAW 264,7 (murine macrophage), MC-3T3 (murine osteoblast), and HepG2 (human hepatocyte) for testing antiinflamatory1, antiosteoporotic2 and antioxidant capacity3 respectively. The tests reflect different cytotoxic effects of samples for each cell line. For cellular assays, the highest concentration that did not produce a significant reduction in viability was used. The results obtained from the functional assays confirm the potential of protein hydrolysates as bioactive ingredients from tuna by-products allowing the consolidation of the circular economy process.

P140
Human Cell Line Activation Test (h-CLAT) with alternative experimental design and fluorophores
ABSTRACT #123

Eliska Pacalova[1], Lada Svobodova[1], Marketa Dvorakova[1], Kristina Kejlova[1], Alena Vlkova[1], Marian Rucki[1], Hana Bendova[1], Dagmar Jirova[1], Gabriela Goffova[1]
[1]*Centre of Toxicology and Health Safety, National Institute of Public Health, Prague, Czech Republic*

The human Cell Line Activation Test (h-CLAT, OECD 442E) represents one of the new approach methodologies (NAMs) leading to human-relevant non-animal testing of skin sensitization potential. The method is based on overexpression of cell surface markers associated with the activation of monocytes and dendritic cells (CD86 and CD54 membrane proteins in THP-1 human monocytic leukemia cell line). The expression levels of CD86 and CD54 are quantified following 24h exposure to serial dilutions of chemicals selected on the basis of the CV75 (i.e. the concentration that allows 75% of cell viability). Chemicals are classified as sensitizers if the relative fluorescence intensity (RFI) of either CD86

and/or CD54 exceeds a defined threshold (i.e. RFI CD86≥150 and RFI CD54≥200) compared to the vehicle control. RFI values are considered for the prediction if the cell viability is above 50%. The method can be used in combination with previously implemented methods (i.e., Direct Peptide Reactivity Assay – DPRA, LuSens assay). Proficiency chemicals listed in TG442E were tested in three independent runs. CD86 (B7-2)-APC-Alexa Fluor 750 and CD54-PE labeled antibodies were used as alternative fluorophores to FITC-labelled antibodies (described in the standard protocol), in order to prevent the autofluorescence background interferences, the fluorescence spill-over, and the spectral overlapping at the FITC-specific wavelengths. DAPI was used as an alternative for the determination of viability instead of Propidium Iodide staining. Troubleshooting options have been suggested to prevent inconclusive or false negative predictions, including the description of representative cell morphology, dot-plot patterns, recommended gating strategy, 96-wells plate design, etc. Due to the 96-well plate design high-throughput testing may be implemented in the laboratory. The research was supported by the Ministry of Health, Czech Republic - conceptual development of research organisation ("National Institute of Public Health - NIPH, IN: 75010330").

P141
The BMDC model, a performant cell-based test to assess the sensitizing potential and potency of chemicals
ABSTRACT #181

Fabrice Battais[1], Sandrine JACQUENET[1], Mélanie Mourot-Bousquenaud[1]
[1]*French Research and Safety Institute for the Prevention of Occupational Accidents and Diseases (INRS)*

Background Chemical-induced allergies are major occupational diseases with significant impact on workers. Every year, new chemicals appear in the industrial field. Therefore, in order to improve health prevention in the workplace, a better understanding of the allergic risk associated with the chemical sensitizers placed on the market is needed. The European Chemicals Agency (ECHA) addressed this concern through various regulations. Traditionally, chemical sensitization has been assessed using laboratory animals. More recently, in vitro models have been developed and validated for the evaluation of the hazard of sensitizers. Among them, changes induced by chemical sensitizers on dendritic cells (DC) have been extensively studied (1). Objectives INRS has developed an original model based on the use of DC derived from mouse bone marrow (BMDC) (2). These cells have the advantage of being easy to obtain with homogeneous phenotype and leading to reproducible results. Methods The readout from the BMDC model is based on expression levels of six phenotypic markers measured by flow cytometry. Results In a study including over 120 industrial substances, the BMDC model has demonstrated a strong sensitivity, a specificity, and an accuracy to differentiate between sensitizers and non-sensitizers, and above all to classify them according to their sensitizing potency (3). Conclusion Since then, the BMDC model has been used to assess the sensitizing potential of bisphenol A (BPA) and its potential substitutes (4). The sensitizing potential of a large number of biocidal substances was also tested with the BMDC model. Thanks to its very good performances, this model could complete the battery of existing regulatory tests for assessing chemical substances.

MODELS AND METHODS

P93
Beyond the Ink: Understanding Cellular and Molecular Effects of Iron-Based Tattoo Pigments on Macrophages
ABSTRACT #24

Marianne Vitipon[1], Véronique Collin-Faure[1], Bastien Dalzon[1], Thierry Rabilloud[1]
[1]*Chemistry and biology of metals, UMR 5249 CNRS/CEA/UGA*

Tattoos are becoming increasingly popular across the generations, with 10-20% of the population in Europe and the United States bearing at least one. Tattoos represent an intradermal injection of pigments (i.e. coloured insoluble micro- and nano-particles), which remain in place throughout life. Although

tattoos have been around for centuries, the question of their health impact has really arisen rather lately. The implementation of REACH legislation has prompted manufacturers to scrutinize the safety of tattoo pigments and inks. However, there remains a significant gap in our understanding of the cellular and molecular long-term effects of these pigments on immune skin cells. In the TATTOOINK Project, we investigate a wide range of tattoo pigments on macrophages. Macrophages, pivotal in pigment persistence through phagocytic processes and capture-release-recapture cycles, are also key players in the inflammatory response and tissue homeostasis. Dysregulation in macrophage functionalities can potentially lead to substantial health consequences such as sarcoidosis. To assess the long-term effects of tattoos, we are testing iron-based pigments using two exposure schemes: an acute exposure for 24 hours and a 7-days post exposure recovery period. Iron-containing pigments are still used in tattooing, particularly in the medical field for dermopigmentation of nipple-areola complex after mastectomy. Proteomic analyses are conducted at both exposure times, 24 hours and a 7-days post exposure recovery period, to understand the underlying cellular mechanisms. Prior functional research has demonstrated that high but nontoxic concentrations of iron-based particles can significantly alter macrophage functions. These impacts include a decrease of the phagocytic capacity and the ability to respond to bacterial stimuli by secreting nitric oxide and pro-inflammatory cytokines. Another study revealed that the effects induced on macrophages are pigment-dependent (e.g. compound, size, etc). Our study brings valuable insights into the intricate relationship between tattoo pigments, macrophages, and potential long-term health effects.

P94
TOXICOLOGICAL IMPACT OF DEGRADED ORGANIC TATTOO PIGMENTS ON HUMAN CULTURED KERATINOCYTES
ABSTRACT #38

Lise Aubry[1], Lucie Breuillé[1], Thierry Douki[1]
[1]*Univ. Grenoble Alpes, CEA, CNRS, IRIG, SyMMES, F-38000 Grenoble*

Tattooing is increasingly popular but the safety of tattoo remains to be fully evaluated. Tattoos involve insoluble pigments that persist for years in the dermis. The colored pigments are currently mostly organic compounds, which may be altered by sun exposure or dermic macrophage activity and release soluble molecules able to diffuse into the epidermis and affect keratinocytes. Interestingly, a link is suspected between tattoos and cutaneous diseases like basal-cell carcinoma. Our work is thus focused on the toxicity of degraded pigments in keratinocytes, a topic on which only limited information is available. High-performance liquid chromatography coupled to mass spectrometry was used to investigate the degradation of pigments and dynamic light scattering to measure the mean hydrodynamic diameter of pigment particles. In vitro studies using the HaCaT keratinocytic cell line allowed us to unravel the effects of degradation on the toxicological profile of pigments. Three pigments were unambiguously cytotoxic (Po13, Pr122 and Pr254) while other were much less (Py74, Pg7, Pv23, Pb15:3). Emphasis was then placed on an azo pigment, Pigment orange 13 (Po13). Simulated sunlight exposure of Po13 can generate photoproducts and induce a decrease in the size of Po13 particles. Sunlight aged-Po13 induced a decrease in cell viability of HaCaT cells compared to control, but did not affect the release of reactive oxygen species. The major photoproduct of Po13 was isolated and was shown to be cytotoxic in the micromolar range. Similar studies were performed with Pigment red 254 (Pr254). Thus, photoaging seemed to modify Po13 in terms of physico-chemical properties which could lead to the modification of its toxicological properties in HaCaT cells whereas Pr254 was found to be much less sensitive to photoaging. Ongoing studies involve the possible induction of an inflammatory response by photodegraded pigments, and the characterization of the effects of macrophage activity on pigments.

P95
Is Schild method and the Gaddum equation still valid for receptor antagonist research?

ABSTRACT #93

Martin Ezechiáš[1]
[1]*Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, Czech Republic*

Background and Objectives: The Schild methodology, based on the Gaddum equation, has traditionally been a common tool for estimating equilibrium dissociation constants of competitive receptor antagonists. While widely used and generally considered correct, the Gaddum equation is derived from the law of mass action and thus may not account for dose-response curves with slopes differing from one. In this research, we investigate a novel extended form of the Gaddum equation, incorporating a slope parameter for the curve, and evaluate it using various modifications of Schild analysis. Materials and Methods: To assess the method's efficacy, we utilized mixtures of estrogen receptor competitive agonists and antagonists measured through the standardized recombinant yeast assay BMAEREluc/Erα (Leskinen et al., 2005). Employing the Schild methodology, we calculated the equilibrium dissociation constants of competitive antagonists using both the standard Gaddum equation and the novel extended form. The derivation of this novel Gaddum equation has been previously published (Ezechiáš and Cajthaml, 2019). Results: In cases where the Gaddum equation falls short and fails to produce accurate values, our extended equation consistently provides correct estimates of antagonist affinity. Discussion and Conclusion: The results of our study suggest a need to reconsider our current paradigm and mathematical formulation for competitive receptor antagonism, such as the Gaddum equation. Our mathematical model has proven to be a valuable tool for obtaining precise measurements of antagonist affinity. This discovery holds significant implications not only for the field of toxicology but also for pharmacology, as the affinity of receptor-ligand interactions represents a fundamental characteristic for many therapeutic drugs and toxic compounds. This research can pave the way for more accurate and insightful assessments of competitive receptor antagonists, contributing to advancements in drug development and safety evaluation.

P96
IMAGE-BASED PHENOTYPIC PROFILING OF FISH GILL CELLS UNDERGOING CHEMICAL EXPOSURE
ABSTRACT #159

Barbara Jozef[1], Sven Mosimann[12], Miha Tome[3], Anze Zupanic[3], Kristin Schirmer[124]
[1]*Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland*
[2]*ETH Zurich: Swiss Federal Institute of Technology, Zürich, Switzerland*
[3]*NIB: National Institute of Biology, Ljubljana, Slovenia*
[4]*EPFL: Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland*

Fish are pivotal in evaluating the environmental impact of chemicals, where the effects on survival and growth are commonly assessed in laboratory experiments as indicators of fish population health. Applying fish cell-based models using permanent fish cell lines offers a promising avenue to significantly reduce, and possibly eliminate, the need for animal testing (1). Despite this premise, the ecotoxicology community has yet to provide a comprehensive, mechanistic explanation for how chemicals with distinct structures and presumed different modes of action can yield a shared outcome: reduced cell proliferation and, consequently, diminished fish growth (2). One emerging assay to shed light on such mechanisms is the high-content multiplexed image-based phenotypic cell profiling (3). The according assay uses up to five fluorescent stains to mark major components of the RTgill-W1 cells, namely the nucleus, mitochondria, lysosomes, cytoskeleton and endoplasmic reticulum. Briefly, 24h after the cell seeding in a 96-well plate, cells are subjected to chemicals in a minimum of five concentrations in a time-resolved manner. The impact of chemicals is assessed during the lag phase, exponential phase, and stationary phase of cell population growth. The images are acquired using Bio Tek Cytation 5 using 20x high-contrast objectives. Nine fields of view are monitored per well to capture a sufficient amount of cells per chemical insult. Images are processed by segmenting cell organelles and various cell compartments (membrane, cytoplasm, and perinuclear space), followed by quantifying

intensities, texture, shape, and morphology using Cell Profiler. The approach shown here has the potential to serve as a robust source of data for further investigating chemical-induced alterations in fish cells. The phenotype of a targeted cell proves sensitive, with a subset of phenotypic features deviating from those of healthy cells, serving as a distinctive fingerprint to characterise the early biochemical profile of cells undergoing chemical toxicity.

P97
Impact of Ellagic Acid and Pomegranate Extract on Oxidative Stress in In Vitro and In Vivo Models
ABSTRACT #170

Francesca Tardanico[1], Giulia De Negri Atanasio[1], Lorenzo Dondero[1], Francesca Rispo[1], Giorgia Allaria[1], Guendalina Olivero[2], Annamaria Pittaluga[2], Federica Turrini[2], Raffaella Boggia[2], Erica Lertora[1], Sara Ferrando[1], Gianluca Damonte[3], Annalisa Salis[3], Katia Cortese[3], Elena Grasselli[14]
[1]*Department of Earth, Environment and Life Science, University of Genoa, Italy*
[2]*Department of Pharmacy, University of Genoa, Genoa, Italy*
[3]*Department of Experimental Medicine, University of Genoa, Italy*
[4]*Interuniversity Center for the Promotion of 3R Principles in Teaching and Research (Centro 3R), Italy*

Background and Objectives Oxidative stress represents an imbalance between antioxidant and oxidant species, with a tendency towards the latter. Such an increase can lead to various alterations, including inflammatory disorders, such as the animal model of multiple sclerosis known as experimental autoimmune encephalomyelitis (EAE). EAE is an autoimmune-mediated degenerative disease of the central nervous system characterized by an induced response to inflammatory and oxidative stress. Ellagic acid (EA), a widely recognized polyphenol with antioxidant properties. In this study, ellagic acid and pomegranate juice were evaluated both in vivo, using mice with EAE, and in vitro, using HECV cell line and 3D reconstructed intestinal models to monitor the oxidation process. Material and methods The by-products of lipid peroxidation were assessed in cerebral cortex and intestinal tissues from both wildtype and EAE mice treated with both ellagic acid and pomegranate juice. Furthermore, the antioxidant effects of EA on endothelial cells were evaluated in vitro by inducing oxidative stress with H2O2. Additionally, the beneficial effects of EA's secondary metabolites, known as urolithins, were also examined in the EpiIntestinal model. Results Both ellagic acid and pomegranate juice lead to a marked decrease in oxidative stress in cerebral cortex and intestinal tissues of EAE mice when compared to controls. The lipid peroxidation values of treated mice are like those of wild mice. Moreover, EA showed protection from oxidative damage in vitro at a concentration of 3 µM. Discussion and conclusion The results, as expected, suggest an antioxidant effect that in HECV. Regarding nervous and intestinal tissues, the decrease of oxidative stress is associated with treatment with EA and pomegranate juice, inserted into the water drunk by mice for a less invasive treatment; moreover, the number of animals involved has been limited as much as possible, to follow the 3R principle.

P98
Daphnia magna model in the toxicity assessment of gliotoxin, ochratoxin A and its combination
ABSTRACT #200

Raquel Penalva-Olcina[12], Cristina Juan[1], Monica Fernández-Franzón[1], Ana Juan-García[1], Eeva-Riikka Vehniäinen[2]
[1]*Laboratory of food Chemistry and Toxicology, University of Valencia*
[2]*Department of Biological and Environmental Science, University of Jyväskylä*

Mycotoxins are low molecular weight compounds present in food and feed and although their effects on human health have been widely described their mechanisms of action are still undefined. Gliotoxin (GTX) and Ochratoxin A (OTA) are some of the most dangerous Aspergillus spp produced mycotoxins. Daphnia magna has a huge capacity to assess ecotoxicity being comparable to the mammalian spp model (Guillhermino et al., 2019). Here the subsequent tests were performed on D. magna

exposed to GTX, OTA, and its combination following the standardized OECD guidelines 202 and 211 (OECD 2008): a) acute toxicity tests by treating D. magna at serial dilution concentration during 96h; b) heart rate test by counting the number of heartbeats recorded from D.magna treated at the highest concentrations; c) immobility assessment, reproduction and growth rate of D. magna after 21 days of exposure; and d) transcript levels of genes involved in xenobiotic metabolism (mox, gst, abcb1, and abcc5), and oxidative stress (vtg-SOD) analyzed by qPCR. GTX showed an acute toxicity decrease in heart rate when compared to OTA which manifested a delayed effect shown at the immobility test. Both GTX and OTA showed to increase in genes involved in xenobiotic metabolism whereas only the mycotoxin mixture showed to increase in oxidative stress genes. From these results, we can say that these mycotoxins at the concentrations assayed do have an effect over D. magna being able to cause environmental or human damage. This work has been supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-100, FORTHEM Alliance FIT-FORTHEM project MyInBioTox and Conselleria d'Educació, Universitats I Ocupació from Generalitat Valenciana project CIAICO2022/199. RPO would like to thank the University of Valencia for being awarded by the Ph.D. grant "Atracció de Talent" and FORTHEM Alliance for the possibility of her research stay in JYU.

P99
Application of miniaturized Ames assays to assess mutagenicity of nitrosamine impurities
ABSTRACT #201

Csaba Boglàri[1], Cécile Koelbert[1]
[1]*Xenometrix AG*

Background and Objectives. The presence of genotoxic impurities in pharmaceuticals and in drug or food packaging material is a significant health concern, therefore, efficient and reliable test methods are required for the batch monitoring. Detailed regulatory guidance (ICH M7) for genotoxic impurities includes a recommendation for the application of miniaturized Ames test systems, which can be used for the mutagenicity assessment of nitrosamines. Miniaturized versions of the traditional Ames test have the advantage that a lower quantity of chemical or impurity, and liver microsomal S9 fraction is required, providing a resource-friendly alternative to the Petri dish-based Agar Plate test. Existing experimental data showed that there is high concordance between the results gained with miniaturized Ames tests and the traditional Petri dish-based method, however, further testing is required to investigate the performance of miniaturized Ames assays in the context of testing nitrosamines. Methods. We investigate the mutagenicity of selected nitrosamines using two miniaturized Ames assay versions, an agar-based 6-well plate test, the MicroAmes6 Kit, and a liquid microplate fluctuation format assay, the Ames MPF. The results acquired with the miniaturized assays are compared to the data generated with the traditional Agar Plate test. Results and Discussion. Our data suggests that the miniaturized Ames assays are applicable to reliably assess the mutagenicity of nitrosamines. The results obtained with the miniaturized Ames assays and the Petri dish-based Ames data are in good concordance for the tested nitrosamines. The miniaturized Ames tests can also be considered as resource-efficient assays that can be readily applied for the mutagenicity assessment of nitrosamines. Furthermore, the high-throughput testing provided by the miniaturized Ames assays can facilitate the generation of robust mutagenicity data on nitrosamine impurities, thus a better understanding of the concerns associated with it, and ultimately, the establishment of straightforward risk assessment strategies.

P100
TOXICITY ASSESSMENT OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS ISOMERS: A STUDY FROM CYTOTOXICITY TO OXIDATIVE STRESS WITH DNA DAMAGE
ABSTRACT #225

Lara Ferreira Azevedo[1], Cecilia Cristina de Souza Rocha[1], Marília Cristina Oliveira Souza[1], Ana Rita Thomazela Machado[1], Paula Pícoli Devóz[1], Bruno Alves Rocha[1], Lusania

Maria Greggi Antunes[1], Andres Campiglia[2], Fernando Barbosa Júnior[1]
[1]*University of Sao Paulo, School of Pharmaceutical Sciences of Ribeirao Preto, Department of Clinical Analyses, Toxicology, and Food Sciences. Av. do Café s/nº, 14040-903, Ribeirao Preto, Sao Paulo, Brazil*
[2]*Department of Chemistry, University of Central Florida, Orlando, FL 32816, USA*

Polycyclic aromatic hydrocarbons (PAHs) represent one of the most extensive classes of known carcinogenic and genotoxic compounds widely distributed across the globe. As PAH molecules increase in size and angularity, there is also an increase in their hydrophobicity and electrochemical stability. Besides, evidence suggests PAHs genotoxicity also increases with size, up to at least four or five fused benzene rings1. Therefore, understanding the relative toxicity of different high molecular weight (HMW)-PAHs isomers is essential for assessing their potential risks to human health and the environment at concentrations found in environmental samples. It is noteworthy that most of the HMW-PAHs are still not investigated regarding their toxicity potential, despite the great number of compounds ubiquitously in the environment. In this context, this study aimed at investigating and comparing the potential toxic effects of increasing concentrations - 2.5; 5.0; 10.0; 20.0 and 40 µM - of four HMW-PAHs isomers (MW=302 g/mol), namely dibenzo(b,l)fluoranthene (DB(b,l)F), dibenzo(a,j)fluoranthene (DB(a,j)F), dibenzo(a,l)fluoranthene (DB(a,l)F) and naphtho(1-2j)fluoranthene (N(1-2j)F) following exposure and metabolic activation in HepG2 cells. Based on PAHs compounds' carcinogenic and mutagenic potential, some assays were selected to investigate the potential to induce cytotoxicity, apoptosis/necrosis, genotoxicity, and oxidative stress with DNA damage. Following 48h exposure to the different HMW-PAHs isomers, DB(a,l)F was the only isomer which induced concentration-dependent cytotoxic effects. Apoptosis was the main mechanism of HepG2 cell death following exposure to HMW-PAHs isomers, which could be induced by the significant increase in DNA damage and in 8-hydroxy-2'-deoxyguanosine (8-OHdG) adduct level formation following exposure to all HMW-PAHs. The highest concentration of DB(a,l)F exhibited the greatest potential to induce DNA damage and 8-OHdG formation compared to all other HMW-PAH isomers. This study evidence that the distinct arrangements of atoms in HMW-PAHs isomers can impact in their toxic potential and that DB(a,l)F was the most toxic HMW-PAHs isomer investigated.

NON-ANIMAL METHODS FOR SAFETY TESTING OF BIOPHARMACEUTICALS/BIOTHERAPIES/VACCINES

P142
Implementation of primary culture systems to investigate substance related toxicity in steatotic hepatocytes ABSTRACT #99

Tanja Krimmling[1], Anett Ullrich[1], Tim Brecklinghaus[2], Jan G. Hengstler[2], Dieter Runge[1]
[1]*PRIMACYT Cell Culture Technology GmbH, Schwerin, Germany*
[2]*Leibniz Research Centre for Working Environment and Human Factors at the Technical University of Dortmund (IfADo), Dortmund, Germany*

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 pre-validation 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, cholesterol). 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 standardized 2D culture, experiments on fatty degeneration were also performed in 3D spheroid culture to investigate differences in susceptibility between 2D and 3D cultures. 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. Bodipy fluorescence dye was used to quantify and visualize lipid droplets in hepatocytes in 2D and 3D cultures. Studies in rat hepatocytes indicated the visualization of steatosis in paraformaldehyde fixed cells by free fatty acids. In addition, Bodipy was used in 2D rat hepatocyte cultures to determine the steatotic effect of tetracycline and dexamethasone in a dose dependent manner. In summary, the results of both 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.

P143
CELL PAINTING ASSAY AS HAZARD ASSESSMENT TOOL IN THE EARLY DRUG DISCOVERY PIPELINE ABSTRACT #161

Jeroen Esselink[1], Gurvan Mahe[1], Andre van Marle[1], Ludovico Buti[1], Peter Imre Racz[1]
[1]*Charles River Laboratories*

The high attrition rate in the drug discovery pipeline underscores the urgent need to improve early models that can accurately predict adverse effects in humans. Cell Painting assay, a multiplexed, high-content image-based profiling assay, holds great potential in this regard by offering an unbiased approach to detect subtle changes in cellular homeostasis. In this study we investigated the correlation between the concentration and exposure time of test compounds and their predicted mode of action in Cell Painting assay, using fifteen reference compounds. We exposed U2OS cells, in eight (8) concentrations of the reference compound set at 24 and 48 hours. Cell Painting images were analyzed by automatic segmentation and battery of quantification strategies developed by the JUMP Cell Painting consortium. This resulted more than 3000 phenotypic measures at the single cell level. Data was pre-processed and analyzed in KNIME v.4.1.3 analytics platform by reconfiguring selected readouts into three dimensions Principal Component Analyses (PCA) plot to reveal dose-dependent morphological effects of the toxicity triggers. The compound Berberine for example at low concentration exhibited effects similar to the solvent controls but high concentration exposure (100µM) resulted in non-specific cytotoxic response. Berberine exposure at 10µM induced mitochondria redistribution specific effect, in alignment with the literature data. The established quality control criteria such as the use of nuclei count to assess potential plate effect; statistical analyses of uniformity plates; visualization of the expected effect of control compounds in t-SNE plots and validation of the XGBoost trees model enable the successful implementation of the assay as a screening tool in drug discovery. Importantly, Cell Painting is adaptable for various cell lines, compatible with high-throughput read-out. Adaptation Cell Painting early in the compound development program could identify hazard risk, reduce the attrition rate and provide better candidate selection during drug discovery process.

P144
When high-resolution chemical imaging bridges toxicology testing: the example of PFOA ABSTRACT #219

Charlotte B. A. Stoffels[12], Sébastien Cambier[2], Tom Wirtz[1], Muriel Mercier-Bonin[3], Arno C. Gutleb[2], Jean-Nicolas Audinot[1]
[1]*Advanced Instrumentation for Nano-Analytics, Materials Research and Technology (MRT) department, Luxembourg Institute of Science and Technology (LIST), Belvaux, Luxembourg*
[2]*Environmental Health, Environmental Research and Innovation (ERIN) department, Luxembourg Institute of Science and Technology (LIST), Belvaux, Luxembourg*
[3]*Toxalim, Université de Toulouse, INRAE, INP-ENVT, INP-EI-Purpan, Université de Toulouse 3 Paul Sabatier, Toulouse, France*

Perfluoroalkylated substances (PFAS), such as perfluorooctanoic acid (PFOA), have been extensively used for many industrial purposes and are therefore omnipresent in our environment. However, these chemicals are

persistent and accumulate in living organisms, leading to major health issues. Upon oral exposure, the gastrointestinal tract is the first physical barrier against these toxicants. Unexpectedly, their impacts on the intestinal wall are largely unknown. This study aims to discover the uptake, intracellular fate and toxicity of PFOA using state-of-the-art in vitro assays combined with new imaging methodology. Notably, we correlate electron microscopy (EM), which reveals the inner morphology of the cells, with mass spectrometry (MS), which provides analytical information. In this last respect, we use secondary ion mass spectrometry (SIMS) that has both an excellent spatial resolution and an excellent detection limit, allowing sub-cellular localization at low concentrations. An intestinal epithelial cell line (Caco-2) was grown on inserts and acutely (24h) exposed to PFOA (0-100 µM). Various toxicity assays investigating cell redox metabolism, apoptosis, oxidative stress or respirometry were performed. Moreover, cells were chemically fixed for EM and SIMS imaging. The samples were analyzed using a focus ion beam scanning electron microscope (FIB-SEM) combined with a SIMS (FIB-SEM-SIMS) for in situ correlative microscopy at sub-20 nm lateral resolution. We successfully localized and quantified PFOA inside intestinal cells. PFOA is only localized in the cell cytosol, but not in cytosolic lipid droplets. The cytosolic concentration of PFOA increases until reaching a plateau above 1 µM. Interestingly, cell redox metabolism increases at low concentrations (max. at 5 µM) while cell apoptosis decreases, characterizing the hormesis. No impact was observed on oxidative stress nor respiration. The combination of powerful imaging techniques with usual toxicology assays provides insightful information about the uptake, fate and toxicity of PFOA on intestinal cells and opens new opportunities in toxicology.

P145
Size-dependent pulmonary mucus penetration and cellular uptake of liposomes: insights for application in pulmonary drug delivery
ABSTRACT #221

Juliana Carrillo Romero[12], Laura Fernández Méndez[234], Alberto Katsumiti[1], Jesús Ruiz-Cabello[235], Susana Carregal Romero[235], Felipe Goñi-de-Cerio[1]
[1]*GAIKER Technology Centre, Basque Research and Technology Alliance (BRTA), Zamudio, 48170 Spain*
[2]*Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), San Sebastián, 20014 Spain*
[3]*CIBER de Enfermedades Respiratorias (CIBERES), Madrid, 28029 Spain*
[4]*Euskal Herriko Unibertsitatea (UPV/EHU), Donostia, 20018 Spain*
[5]*Ikerbasque, Basque Foundation for Science, Bilbao, 48013 Spain*

Liposomes are extensively investigated as potential drug delivery systems in the lungs, owing to their therapeutic effectiveness, biocompatibility, and safety. However, limited information exists regarding their interaction with pulmonary mucus barrier and cellular uptake. Therefore, gaining a deeper knowledge on how their physicochemical properties such as size may influence pulmonary mucus penetration and cellular uptake of liposomes could significantly improve their application in drug delivery. The aim of this study was to evaluate the pulmonary mucus penetration and cellular uptake of liposomes of different sizes (100 nm, 300 nm, and 800 nm). For it, artificial pulmonary mucus (APM) was synthesized and examined for its rheological properties when exposed to the different liposomes. Subsequently, the penetration of these liposomes into APM was investigated. Cellular uptake of liposomes was assessed in two lung models: A-549 and Calu-3. After confirming that APM exhibited no significant rheological changes, the assays demonstrated that both 100 nm and 300 nm liposomes exhibited higher mucus penetration compared to 800 nm liposomes, which were predominantly retained in the mucus layer. Similarly, cellular internalization of 100 nm and 300 nm liposomes was higher than 800 nm liposomes. Comparing the two cell lines, cellular uptake was higher in A549 than in the mucus-secreting Calu-3 cells. Results indicate that liposomes with sizes ranging from 100 nm to 300 nm appear to be promising candidates for use as cellular drug delivery systems, while 800 nm liposomes may find their application in the mucus layer, where

they are primarily retained. Overall, the size of liposomes significantly influences their ability to cross lung barriers, and it must be a fundamental factor for drug delivery.

P146
DEVELOPMENT OF RECONSTRUCTED INTESTINAL MICRONUCLEI CYTOME (RICYT) ASSAY IN 3D HUMAN GUT MODEL FOR GENOTOXICITY ASSESSMENT OF ORALLY INGESTED SUBSTANCES
ABSTRACT #249

Hui Kheng LIM[12], Christopher Owen HUGHES[12], Choon Wee Joseph TAN[1], Michelle Jing Sin LIM[2], Jia'En Jasmine LI[3], Moumita RAKSHIT[4], Calvin YEO[3], Kern Rei CHNG[3], Angela LI[3], Joanne Sheot Harn CHAN[3], Kee Woei NG[456], David Ian LEAVESLEY[27], Benjamin Paul Chapman SMITH[12]

[1]*Future Ready Food Safety Hub (a joint initiative of A*STAR, SFA & NTU), Nanyang Technological University, Singapore*
[2]*Innovations in Food and Chemical Safety (IFCS) Programme, Agency for Science, Technology and Research, Singapore*
[3]*National Centre for Food Science, Singapore Food Agency, Singapore*
[4]*School of Materials Science & Engineering, Nanyang Technological University, Singapore*
[5]*Environmental Chemistry & Materials Centre, Nanyang Environment and Water Research Institute, Singapore*
[6]*Harvard T. H. Chan School of Public Health, Harvard University, USA*
[7]*Skin Research Institute of Singapore (SRIS), Agency for Science, Technology and Research, Singapore*

The micronucleus (MN) assay is widely used as part of a battery of tests applied to evaluate the genotoxic potential of chemicals, including new food additives and novel food ingredients. Micronucleus assays typically utilise homogenous in vitro cell lines which poorly recapitulate the physiology, biochemistry and genomic events in the gut, the site of first contact for ingested materials. Here we have adapted and validated the MN endpoint assay protocol for use with complex 3D reconstructed intestinal microtissues; we have named this new protocol the reconstructed intestine micronucleus cytome (RICyt) assay. Our data suggest the commercial 3D microtissues replicate the physiological, biochemical and genomic responses of native human small intestine to exogenous compounds. Tissues were shown to maintain log-phase proliferation throughout the period of exposure and expressed low background MN. Analysis using the RICyt assay protocol revealed the presence of diverse cell types and nuclear anomalies (cytome) in addition to MN, indicating evidence for comprehensive DNA damage and mode(s) of cell death reported by the assay. The assay correctly identified and discriminated direct-acting clastogens, aneugens and clastogens requiring exogenous metabolic activation, and non-genotoxic chemicals. We are confident that the genotoxic response in the 3D microtissues more closely resembles the native tissues due to the inherent tissue architecture, surface area, barrier effects and tissue matrix interactions. This proof-of-concept study highlights the RICyt MN cytome assay in 3D reconstructed intestinal microtissues is a promising tool for applications in predictive toxicology.

P147
Assessing Immune-Related Toxicity Using a 3D In Vitro Hepatotoxic Model
ABSTRACT #269

Mi-lang Kyun[1], Hyewon Jung[12], Inhye Kim[13], Ji-in Kwon[13], Jeong Ha Kim[1], Ju-Kang Kim[1], Myeongjin Choi[1], Jung Yun Lee[1], Minseong Jo[14], Seungyeon Lim[1], Dong Ho Woo[1], Chang Hoon Choi[1], Seo Yule Jeong[1], Hyun-A Oh[1], Ye-Ji Kim[12], Yu Yeong Jeong[15], Hye-Won Lim[1], Daeui Park[6], Yu Bin Lee[1], Kyoung-Sik Moon[1]

[1]*Department of Advanced Toxicology Research, Korea Institute of Toxicology, Daejeon 34114, Republic of Korea*
[2]*Human and Environmental Toxicology, University of Science and Technology, Daejeon 34113, Republic of Korea*
[3]*Department of Food and Nutrition, Hannam University*
[4]*Department of Microbiology and Molecular Biology, Chungnam National University*
[5]*Department of Biological Science, Chungnam National University*

[6]*Department of Predictive Toxicology Research, Korea Institute of Toxicology, Daejeon 34114, Republic of Korea*

Current drug researches on evaluating liver toxicity often employ two-dimensional cell cultures, but pose challenges in elucidating intricate immune mechanisms. Herein, our study seeks to address these limitations by mimicking in vivo liver tissue conditions through 1) simulate human liver environment via 3D co-culture of parenchymal and non-parenchymal cells, 2) analyze responses upon induced hyperinflammation resembling cytokine release syndrome (CRS), and 3) overcome constraints of in vitro immunotoxicity evaluation. We co-cultured parenchymal (human hepatocyte cell line, HepaRG) and non-parenchymal vascular cells (human umbilical vein endothelial cells, HUVEC) using hydrogel encapsulation techniques and employed peripheral blood monocytes (PBMC) to simulate human immune responses. An immune hyperactivity was generated by treating with PolyIC (Polyinisinic-polycytidilic acid), a synthetic analogue of dsRNA (double stranded RNA) to simulate viral infections and trigger immune responses by TLR3 activation. The immune hyperactivity triggered by PolyIC resulted in elevated expression of CRS-related genes in HepaRG, along with an increase in the expression of hepatotoxic genes like CRP and C3, and influenced the expression of CYP and drug transporter genes. In addition, gene expression of CRS indicators and permeability increased in HUVEC, indicating immune hyperactivity due to PolyIC treatment affects vascular permeability. In supernatant from the co-culture model, CRS indicating cytokines (IL-6, IL-1b, TNF-a) increased notably. Theses alterations associated with the induced hyperinflammation were mitigated by treating with corticosteroid. Collectively, we observed immune-mediated toxicity occurring in both parenchymal and non-parenchymal cells of 3D in vitro liver model accompanied by an immune environment. Therefore, it is suggested that our 3D in vitro liver model inducing immune hyperactivity will be employed valuably in the development of future biotherapeutics. Keywords: 3D, Hydrogel, immune hyperactivity, Cytokine release syndrome, hepatotoxicity

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P148
EVALUATION OF CYTOTOXICITY AND REGENERATION CAPACITY OF ARTSKIN™, A NOVEL HUMAN DERMAL SKIN SUBSTITUTE ABSTRACT #272

Manuella Godoi[1], Caroline Peruzzi[2], Guilherme Colla[2], João Hoepers[1], Janice Koepp[2], Juliano Ferreira[1]
[1]*UFSC*
[2]*Biocelltis*

Autologous skin and tissue-engineered skin grafts have become crucial in treating burns and wounds, serving as effective epidermal and dermal substitutes. These substitutes play a pivotal role in pharmacological and basic research, contributing to the hazard assessment of chemical compounds and advancing our understanding of fundamental processes related to skin regeneration. In response to evolving global regulations, the development of in vitro testing tools aligns with the 3Rs principle: "replacement, reduction, and refinement." This study focuses on developing a human dermal skin substitute, ARTSkin™, and applies in vitro pre-clinical tests in ensuring the safety assessment of Advanced Therapy Medicinal Products (ATMPs). This guarantees the reliability of results and has the potential to minimize or substitute the reliance on animal testing for safety assessments. To achieve this aim, ISO 10993-5 served as a guide for conducting the cytotoxicity test. Additionally, the regenerative capacity was assessed through scratch cell assays. Methods involved the cultivation of L929 mouse fibroblast cells in DMEM medium with specific supplements. Cytotoxicity was evaluated using the MTS assay, with positive and negative control tests for comparison. Absorbance measurements at 545 nm provided insights into cell viability. Results were categorized as toxic or non-toxic based on the percentage of controls, indicating viability levels. The findings revealed the absence of cytotoxicity in ARTSkin™ e ARTSkin™ SI ($p<0.05$) in comparison with positive control, demonstrating its safety for potential use as a skin substitute. Furthermore, ARTSkin™ and ARTSkin™ SI exhibited a commendable ability to regenerate after mechanical friction ($p<0.05$), reinforcing its potential as a promising option for wound

healing applications. Our findings indicate that ARTSkin does not exhibit a cytotoxicity profile. Nonetheless, to validate this result using a different mechanism of action, the Neutral Red Uptake (NRU) cytotoxicity test will be conducted.

P149
A primary human podocyte assay enables sensitive in vitro detection of dedifferentiation and effacement by high-content imaging
ABSTRACT #282

Nicholas Brittain[1], Ben Coker[1], Kirsten Ramsay[1], Kate Gilroy[1], Kathryn Garner[1]
[1]*Newcells Biotech Limited, The Biosphere, Draymans Way, Newcastle Helix, Newcastle upon Tyne, NE4 5BX, UK*

The glomerulus mediates the first stage of renal filtration, functioning to prevent leakage of serum proteins into the ultrafiltrate. Podocytes are specialised epithelial cells that form a key component of the glomerular filtration barrier (GFB) via the intercellular slit diaphragm (SD) and foot processes. Podocytes can respond to stress stimuli by undergoing dedifferentiation and foot process effacement (FPE), characterised by downregulation of SD proteins, disruption of SD integrity and a simplification of cellular architecture, impairing GFB function. Newcells Biotech have developed an in vitro model of primary human podocytes, which forms a size- and charge-selective barrier comparable to the in vivo GFB and expresses the full range of differentiated podocyte markers and SD components including CD2AP, podocin, and nephrin in over 95% of cells. By measurement of transepithelial electrical resistance (TEER) and 70 kDa FITC-Dextran permeability, assessment of podocyte monolayer function is enabled and has been validated across multiple podocyte toxins including adriamycin, puromycin and palmitate. Here, our primary model is leveraged in high-sensitivity detection of subtle alterations that impact podocyte function. Treatment of the model with tumour necrosis factor (TNF), an inflammatory cytokine known to be elevated in diabetic nephropathy, characterised by GFB impairment, results in marked podocyte monolayer disruption by TEER measurement ($p < 0.001$) without a significant loss of cell viability as detected by ATP content using the CellTiter-Glo® assay. Through SD protein immunofluorescence and use of high-content imaging and analysis software, including CellReporterXpress and CellProfiler, we have demonstrated that TNF exposure consistently results in downregulation of SD proteins or a more compact cell morphology across multiple independent human podocyte populations . Furthermore, phalloidin staining demonstrates remodelling of the actin cytoskeleton. Therefore, this primary cell imaging approach enables rapid, sensitive, in vitro assessment of SD perturbations and FPE that may occur independently of podocyte toxicity.

P151
Silkworms as a novel invertebrate model for toxicity testing: bridging the gap between traditional and alternative methods
ABSTRACT #333

Atsushi Miyashita[1], Masaki Ishii[2], Kazuhisa Sekimizu[1]
[1]*Teikyo University*
[2]*Musashino University*

Background and Objectives: In the quest to find ethical alternatives to mammalian animal testing, our research has been pioneering the use of silkworms (Bombyx mori) as a viable invertebrate model. This innovative approach aims not only to reduce reliance on traditional mammalian models but also to enhance the capabilities of existing in vitro and in silico methods. Material and Methods: A list of 59 standard cytotoxic compounds were orally administered to silkworm larvae, and LD50 value was determined for rach compond. Redults: Our findings reveal a promising correlation between the acute toxicological responses of silkworms and mammals following oral administration of chemical substances. Specifically, our study involving 59 OECD-listed standard cytotoxic compounds demonstrated a significant correlation (R-square value of 0.66) between the LD50 values obtained from silkworms and those from mammalian data. Furthermore, the reproducibility of these toxicity assessments across different laboratories underscores the

reliability and potential of silkworms as a standardized model for toxicological studies. Discussion and Conclusion: Building on this foundation, we have launched a new project focused on refining an oral acute toxicity testing protocol using silkworms. This protocol extends beyond LD50 estimations to include comprehensive evaluations of systemic health and detailed histopathological analyses of organ tissues, aiming to provide a more nuanced understanding of compound toxicity. This presentation will outline our groundbreaking work with silkworms, highlighting both the proven efficacy of this model in previous studies and the ambitious goals of our ongoing research initiative. Acknowledgment: This research was supported by grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (Research program on development of innovative technology).

ORGAN-ON-A-CHIP & MICROPHYSIOLOGICAL SYSTEMS

P152
A novel exposure device for airborne materials to investigate toxic potential and uptake after inhalation exposure using innovative organ-on-a-chip technology
ABSTRACT #64

Tanja Hansen[1], Detlef Ritter[1], Nico Sonnenschein[1], Carsten Brodbeck[2], Cristian Hoyer[34], Uwe Marx[4], Katharina Schimek[4]

[1]*Fraunhofer ITEM, Hannover, Germany*
[2]*Fraunhofer SCAI, Sankt Augustin, Germany*
[3]*TU Berlin, Berlin, Germany*
[4]*TissUse GmbH, Berlin, Germany*

An innovative device-based method has been developed to assess the toxic effects of airborne (nano)materials and chemicals on the lungs and secondary organs. For this purpose, an exposure unit was established to expose human alveolar lung models at the air-liquid interface. In parallel, an integrated microfluidic two-organ chip was developed combining the human alveolar lung model with a human liver model and ensures long-term co-culture by continuous medium perfusion of the organ models. We present the layout of the prototype and first results with positive and negative control chemicals. The physical principle of thermophoresis is employed to ensure relevant and reproducible deposition of aerosol particles to the cell surface. Preliminary deposition experiments revealed that deposition rates of 30% or higher are achieved with aerosols in the size range below 1000 nm. Lung cell models (hAELVi and A549) were shown to tolerate clean air exposure to flow rates up to 3 mL/min in a perfusion setup without impairment of viability, as judged by WST-assay. Dose-response relationships could be obtained for formaldehyde, yielding an EC50 dose of 2700 or 7500 ppm x ml for A549 cells, depending on perfusion or static conditions respectively, which is in line with historical data obtained with an established test setup. The first proof-of-concept that the system can be successfully used with hAELVi cells as alveolar model and HepaRG cells as liver equivalent was provided by exposure to formaldehyde, SDS and lactose. Based on selected readouts (e.g. Albumin, IL-8) it was proven that the liver equivalent is responsive to exposure via the lung barrier equivalent. In conclusion, this platform allows for repetitive exposure of lung cell models to (nano)particles, chemicals, and gases, enabling examination of their toxic effects on both the lungs and secondary organs. This project was funded by the German BMBF, NanoINHAL Grant No. 03XP0226.

P153
COMPARATIVE STUDY OF SPHEROIDS (3D) AND MONOLAYERS CULTURE (2D) FOR EVALUATING THE TOXICITY OF CYCLOPIAZONIC ACID IN in vitro USING HUMAN NEUROBLASTOMA SH-SY5Y CELLS
ABSTRACT #77

Carmen Martínez-Alonso[12], Yelko Rodríguez-Carrasco[12], Maria-Jose Ruiz[12]
[1]*Research Group in Alternative Methods for Determining Toxics Effects and Risk Assessment of Contaminants and Mixtures (RiskTox)*
[2]*Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of*

Valencia

Mycotoxins are a wide and heterogeneous group of secondary metabolites produced by filamentous fungi that can contaminate food and feed and threat to human and animal health. Cyclopiazonic acid (CPA) is a mycotoxin produced by several species of Penicillium and Aspergillus that are capable of growing in many food matrices. Given the growing importance of establishing better risk assessments for mycotoxins, new three-dimensional (3D) in vitro cell models, such as spheroids, are required because can better recapitulate the in vivo architecture of natural tissue and organs. The objective of this work was to compare the cytotoxicity (IC50) of CPA (500-1500 nM) in spheroids (3D) and in monolayers culture (2D) of human neuroblastoma (SH-SY5Y) cells at 24, 48 and 72 Hours of exposure by the MTT assay. The results showed that the IC50 of CPA in spheroids model were 1132.37 ± 46.33 nM, 1069.02 ± 278.76 nM, 567.22 ± 34.42 nM after 24, 48 and 72 h of exposure, respectively. Otherwise, the IC50 of CPA in monolayer culture were 864.01 ± 164.09 nM, 436.73 ± 22.12 nM, 392.33 ± 10.95 nM after 24, 48 and 72 h of exposure, respectively. Our results demonstrated what we expected: the evaluation of CPA cytotoxicity in spheroids, as opposed to monolayer cultures, is anticipated to provide a more realistic representation of cell behavior that mimic in vivo conditions. These findings could be justified due to the numerous intracellular junctions in the 3D models, imitating physiological barriers, as well as a dense extracellular matrix with small pores, which influence xenobiotic transport by decreasing its penetration. Therefore, 3D cell culture is a more realistic model than traditional cell culture but efforts to develop a more robust 3D cell culture models are needed. Acknowledgements: Spanish Ministry of Science and Innovation Project (PID2020-115871RB-I00). Ministry of Education FPU Grant (FPU21/04950).CIAICO/2022/199

P154
IN VITRO IMMUNE-COMPETENT, VASCULARIZED SKIN MODEL DEVELOPED IN PHYSIOXIA CONDITIONS USING A MICROPHYSIOLOGICAL SYSTEM FOR TESTING AND EVALUATION OF SKIN SENSITIVITY
ABSTRACT #162

Edith Filaire[1,2], Marina Simon[1], Pierre Gaudriault[3], Dario Fassini[3], Christian Poinsot[1]
[1]*ICARE Group*
[2]*UMR 1019 INRAE-University Clermont-Auvergne*
[3]*CherryBiotech*

Background and Ojectives: Prevalence of allergic contact dermatitis is increasing, justifying the need to assess skin sensitization potential for any chemical products [1]. Current assessments of potential sensitization events of new compounds are performed according to three guidelines and a battery of in vitro tests. None of them consider the interplay between the epidermis/dermis cells and the resident immune cells. There is an urgent need for a complete in vitro skin model containing the resident cell types to better understand the role of immune and endothelial cells in skin and to be able to use it for drug testing. Moreover, advances in 3D cell culture, microscale fluidic control, and cellular analysis have enabled the development of more physiologically relevant engineered models of human organs with precise control of the cellular microenvironment. The aim of our study was to develop a SME under physioxia conditions using a microphysiological system (MPS) that includes an endothelial barrier and some relevant resident immune cells involved in the skin sensitization cascade. Material and Methods: The model integrated NHEK, NHDF, HUVEC cells, THP-1, and THP-1 derived macrophages. Structure, cells and tissue-specific markers were revealed by immunocytochemistry techniques. Microenvironment were controlled by the MPS. Results: Our approach resulted in a multilayered, differentiated epidermis proliferating on top of the dermis as revealed by immunolabelling analyses. Presence of specific markers of human epidermis/dermis as well as presence/activation of immune resident cell phenotype was verified using immune labelling techniques. Conclusion: We developed an immunocompetent, differentiated, and vascularized full-skin in vitro model envisaging safety/sensitization assessments of cosmetic ingredients. The applications to the cosmetic

industry are significant and bring hope of optimized evaluation of cosmetic safety before their commercialization. This 3D skin model should allow in future to obtain more translatable indications regarding possible inflammatory events induced by drugs and medical devices.

P155
TRACK-ETCHED MEMBRANES FOR DRUG PHARMACEUTICAL RESEARCH ABSTRACT #246

Karina Cuanalo-Contreras[1], Dennis Benkmann[1]
[1]*SABEU GmbH & Co. KG*

Over the last decades, the incorporation of membranes into systems used for pharmaceutical research has grown significantly. One example is the use of microporous track-etched membranes in microfluidic systems, organs-on-a-chip and cell culture inserts for tissue engineering. Track-etched membranes have attracted attention due their good mechanical properties, parameter reproducibility, easy handling and commercial availability. They can be tailored into a wide range of requirements such as pore size, pore shape, pore angle, pore density, optical characteristics, membrane thickness, and surface modifications (1). Tissue-culture treated track-etched membranes serve as excellent support for cell growth and efficient nutrient supply, with the added benefit of precise control of pore parameters, a critical pre-requisite to achieve reproducible results. Track-etched membranes have been successfully integrated in multiple in vitro systems, playing roles that include not only the formation of physical interfaces, but allowing the growth of cells in in vivo-like microenvironments. A plethora of tissue engineering approaches require suitable scaffolds to support cell growth and mimic physiological compartments (2). Synthetic membranes are widely used to create various epithelial and endothelial tissue barriers in vitro. Porous polymeric scaffolds, such as track-etched membranes, provide biocompatibility, reproducibility, and mechanical stability to enable tissue culture. They also facilitate the diffusion of nutrients and substances into cells. Track-etched membranes create apical and basolateral compartments – unlike traditional 2D cell culture methods – while forming robust interfaces. These features enable cell polarization, communication, and tight junction formation. Overall, the transport and permeability properties of tissue barriers are mimicked. With the current explosion of human-relevant methods, one should keep in mind the commercial availability and standardization potential of the materials and devices used in those systems. Finally, the creation of in vitro methods of industrial interest, will allow the assessment of chemicals, drugs and cosmetics, and reduce the high drug attrition rate.

P156
COMPARATIVE STUDY OF THE IMPACT OF STATIC AND DYNAMIC CULTIVATION CONDITIONS ON THE CYTOTOXICITY INDUCED BY THE MYCOTOXIN PATULIN ON SH-SY5Y SPHEROIDS ABSTRACT #271

Veronica Zingales[123], Elisa Cimetta[23], María-José Ruiz[1]
[1]*Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100, Valencia, Spain.*
[2]*Department of Industrial Engineering (DII), University of Padua, Via Marzolo 9, 35131, Padova, Italy.*
[3]*Fondazione Istituto di Ricerca Pediatrica Cittá Della Speranza (IRP) – Lab BIAMET, Corso Stati Uniti 4, 35127, Padova, Italy*

Mycotoxins are natural toxic food contaminants with known adverse effects on human health. In recent years, great efforts have been underway to develop in vitro cell-based systems aimed at closely mimicking the physiological conditions of human cells in vivo. This has resulted in a shift from traditional monolayer cell culture to more complex three-dimensional (3D) cultures, with their integration into microfluidic devices representing a pivotal step towards creating more biomimetic in vivo-like models [1]. The focus of the present study was to evaluate the cytotoxic effects induced by the exposure to the mycotoxin patulin (PAT) on human neuroblastoma SH-SY5Y spheroids cultured

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under static and dynamic conditions. Spheroids were exposed to PAT at concentrations ranging from 3.12 to 12.5 µM for 24 h. Under static conditions, the effect of PAT exposure on spheroid viability was investigated by ATP assay; the results were then compared with those obtained in dynamic conditions. To evaluate the effects induced by continuous exposure, the spheroids were integrated into a chip composed of an inlet and outlet tank and 3 wells hosting the spheroids connected to each other by microchannels. Interestingly, a different sensitivity to PAT was detected between the two culture models. Microfluidic systems have emerged as a valuable tool for enhancing the physiological relevance of 3D cell culture, enabling the study of continuous exposures to toxicants under controlled, adjustable, and replicable conditions. The proposed use of spheroids integrated in a microfluidic device as a model for assessing continuous exposure to mycotoxins is fairly novel. This work is the first step towards a realistic risk assessment scenario for mycotoxins, which has a high societal importance as exposure to them through food intake is ubiquitous. Acknowledgments: Spanish Ministry of Science and Innovation grant (PID2020-11587RB-100 MCIN/AEI/10.13039/501100011033; Generalitat Valenciana post-doctoral grant (APOSTD/2022; Ref.CIAPOS/2021/228); ERC Starting Grant – MICRONEX UERI17_01.

OTHER NAMS RELEVANT TOPICS

P157
DYES UNDER THE REACH REGULATION: A STRATEGY FOR GENOTOXICITY ASSESSMENT USING NAMs
ABSTRACT #44

Elena Campagnoli[1], Monica Locatelli[1], Andrea Mauri[2]
[1]*Kahlberg Consulting srl*
[2]*Alvascience*

• Background and Objectives REACH Regulation 1907/2006 requires at Annex VII and VIII level, to produce in vivo test in the field of genotoxicity when at least one of the in vitro tests of the standard battery displays a positive result. Organic dyes are a special and vast class of chemicals very often showing positivity when tested in vitro for bacteria mutagenicity. Specifically, the positivity of the Bacteria Reverse Mutation Assay (OECD471) is not confirmed by further in vitro studies on mammalian cells and by preliminary in vivo results. It is therefore necessary to develop and use New Approach Methodologies (NAMs) that can be accepted at regulatory level providing reliable and relevant results. • Material and Methods In silico methodologies, such as Quantitative Structure-Activity Relationship (QSAR) modelling, clustering of chemicals and read across approach are considered reliable alternatives to reduce in vivo testing. The present work explores the development of an in silico system based on genotoxicity alerts identified among those available in the literature and on new findings, and evaluated on a massive proprietary database of in vitro and in vivo genotoxicity studies. • Results The aim of this in silico system is to group similar dye substances considering the selected genotoxicity alerts. The system can then be used to select one or more substances to be tested in vivo. The selected substances are chosen to be representative of each group of dyes. • Discussion and Conclusion The in vivo results can then be used to improve the system and gain insight on the relationship between specific structural features and the in vivo genotoxicity of dyes.

P158
A CRITICAL REVIEW ON THE UTILISATION AND CHARACTERISATION OF EXTERNAL BIOTRANSFORMATION SYSTEMS IN IN VITRO TOXICOLOGY
ABSTRACT #73

Sebastian Lungu-Mitea[123], Matilda Stein Åslund[4], Inska Reichstein[5], Felipe Vidal[3], Andreas Schiwy[56], Henner Hollert[56], Miriam Naomi Jacobs[7], Klára Hilscherová[3]
[1]*Linnaeus University, Department of Biology and Environmental Science, Kalmar, Sweden*
[2]*Uppsala University, Department of Pharmaceutical Biosciences, Toxicology and Drug Safety, Sweden*
[3]*Masaryk University, Faculty of Science,*

RECETOX, Brno, Czech Republic
[4]*Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden*
[5]*Goethe University Frankfurt, Department of Evolutionary Ecology and Environmental Toxicology, Germany*
[6]*Department of Environmental Media Related Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (FhG-IME), Schmallenberg, Germany*
[7]*UK Health Security Agency, Harwell Science and Innovation Campus, Chilton, OXON, UK*

In high-throughput in vitro toxicological assessments, the lack of intrinsic biotransformation capacities often necessitates incorporating external biotransformation systems (BTS), such as S9 fractions and microsomes [1]. However, the reliability and reproducibility of these systems remain contentious [2]. To elucidate these issues, we conducted a critical review in alignment with PRISMA and EFSA guidance [3]. The study focuses on the utilisation and characterisation of BTS alongside eukaryotic test systems, particularly in the context of endocrine disruption, mutagenicity, and genotoxicity. Employing previously determined eligibility criteria, Boolean search operators, and selection processes, we identified 229 publications from established literature databases. A coding book comprising 21 data item domains was utilised for systematic data extraction, emphasising BTS characterisation, reaction components, and experimental setup. We employed a grading scheme to assess the methodological rigour of BTS reporting within these studies. The scores and extracted data were subjected to multiple component analyses to identify patterns, followed by meta-analyses of quantitative data via regression analysis and frequentist statistical interference to consolidate the findings. Overall, our findings indicate a pervasive lack of methodological rigour in the investigated literature. Furthermore, no stringent concentration-response or spatiotemporal relationships involving BTS protein concentrations and cofactors were identified. However, we identified alternative application patterns, found in fish or in-house-derived systems, that hint towards potential solutions. We outline a regulatory framework for standardising BTS reporting, which could alleviate the current perplexity in BTS utilisation and secure quality standards for future applications. The project has received funding from the EU H2020 project ERGO (No.825753).

P159
Centro 3R: Advancing 3Rs Principles in Education, Research, and Beyond
ABSTRACT #82

Elena Grasselli[1], Arti Ahluwalia[1], Valeria Chiono[1], Paolo Fabene[1], Gianfranco Fiore[1], Monica Mattioli Belmonte[1], Gabriella Nicolini[1], Alberto Rainer[1], Sonia Scarfì[1], Livia Visai[1]
[1]*3R Centre, Inter-University Centre for the Promotion of the 3R Principles in Education and Research, University of Pisa, Pisa, Italy.*

The Italian Centro 3R, founded to promote the 3Rs in university teaching, has formed a collaborative network to share resources and teaching methods. An initial survey assessed researchers' and students' attitudes toward animal experiments and alternatives, revealing that while Italian researchers were aware of the 3Rs, they often viewed animal tests as indispensable and considered replacements as complementary models. Science students, on the other hand, had limited knowledge of animal protection legislation and the 3Rs concept. In response, the Centro 3R's university network introduced courses and credits emphasizing ethics, one-health, preclinical models, invertebrate models, organ-on-a-chip, and in silico models to mainstream replacement through comprehensive education. These courses, initially elective, have tripled in number since 2018 and are in high demand, indicating the success of the pervasive education strategy. Centro 3R, with its mission to promote the 3Rs and responsible research, is actively engaging the next generation of scientists through expanded course offerings and increased awareness. This complements its broader goal of advancing the "One Health" approach and influencing the well-being of people, animals, and the environment, both at the national and European levels. The Center is also striving for institutional recognition and support from the Italian Ministry of Health and the Ministry of University and Research, recognizing its pivotal role in shaping current

thinking on critical issues of planetary well-being.

P160
TOWARDS THE APPLICATION OF NEW APPROACH METHODOLOGIES (NAMs) TO FOOD MIXTURES
ABSTRACT #34

Maricel Marin-Kuan[1], Breanne Holmes[1], Paul Rogeboz[1], Helia Latado[1], Anna Voiland[1], Claudine Cottet-Fontannaz[1], Bastien Gentili[1], Julie Mollergues[1], Virginie Bechmann[1], Danilo Basili[1], Amaury Patin[1]
[1]*Société des Produits Nestlé*

The development of new approach methodologies (NAMs) is a prominent and promising initiative to improve the understanding of the toxicological effect of chemicals in humans generating more human centric data while reducing animal testing according to the 3R principles. This initiative is consequently promoting a shift of the toxicological paradigm to ease prioritization relevant for the human situation. Despite the complexity, progress has been demonstrated to assess safety of chemicals of toxicological concern, however, the assessment of mixtures including environmental or food-related mixtures remains challenging. In alignment with the development of a roadmap for action on NAMs in Risk Assessment proposed by EFSA (1), a Proof of Concept (PoC) was set-up to evaluate the feasibility to apply a fit-for-purpose battery of in vitro, in chemico and omics assays to water, food, and feed samples. Benchmarking the readiness of selected models to generate sound toxicokinetics data for risk assessment will be evaluated. For that, a panel of in vitro assays covering cell stress, endocrine activity and genetic damage were applied to characterize a food-related mixture as case study. As quality control, the sample was spiked with selected substances of toxicological concern to confirm the validity of the approach. Conditions for sample preparation and for fractionation to facilitate the identification of the bioactive compounds were developed for the specific matrices. In vitro assays using 2D and 3D organoids were tested as well as bioassays coupled to High-Performance Thin Layer Chromatography as alternative targeted approach for the identification of substances of concern in mixtures (2,3). Preliminary definition of the end-to end process from Point-of-Departure (PoD) to biological profiling of the selected case study will contribute to elucidating the feasibility of the NAMs approach for food safety applications and the encountered limitations and underlying gaps to be solved.

P161
Reactivity of Acrylamides Causes Cytotoxicity and Activates Oxidative Stress Response
ABSTRACT #6

Julia Huchthausen[1], Beate I. Escher[12], Nico Grasse[3], Maria König[1], Stephan Beil[4], Luise Henneberger[1]
[1]*Helmholtz Centre for Environmental Research – UFZ, Department of Cell Toxicology, Permoserstr. 15, 04318 Leipzig, Germany*
[2]*Eberhard Karls University Tübingen, Environmental Toxicology, Center for Applied Geosciences, 72076 Tübingen, Germany*
[3]*Helmholtz Centre for Environmental Research – UFZ, Department of Analytical Chemistry, Permoserstr. 15, 04318 Leipzig, Germany*
[4]*Technische Universität Dresden, Institute of Water Chemistry, 01069 Dresden, Germany*

Acrylamides are widely used industrial chemicals that cause adverse effects in humans or animals, like carcinogenicity or neurotoxicity. The excess toxicity of these reactive electrophilic chemicals is especially interesting, as it is mostly triggered by covalent reactions with biological nucleophiles, such as DNA bases, proteins, or peptides. The cytotoxicity and activation of oxidative stress response of 10 (meth)acrylamides measured in three reporter gene cell lines occurred at similar concentrations. Most acrylamides exhibited high excess toxicity, while methacrylamides acted as baseline toxicants. The (meth)acrylamides showed no reactivity toward the hard biological nucleophile 2-deoxyguanosine (2DG) within 24 h, and only acrylamides reacted with the soft nucleophile glutathione (GSH). Second-order degradation rate constants (kGSH) were measured for all acrylamides with N,N′-methylenebis(acrylamide) (NMBA) showing the

highest kGSH (134.800 M−1 h−1) and N,N-diethylacrylamide (NDA) the lowest kGSH (2.574 M−1 h−1). Liquid chromatography coupled to high-resolution mass spectrometry was used to confirm the GSH conjugates of the acrylamides with a double conjugate formed for NMBA. The differences in reactivity between acrylamides and methacrylamides could be explained by the charge density of the carbon atoms because the electron-donating inductive effect of the methyl group of the methacrylamides lowered their electrophilicity and thus their reactivity. The differences in reactivity within the group of acrylamides could be explained by the energy of the lowest unoccupied molecular orbital and steric hindrance. Cytotoxicity and activation of oxidative stress response were linearly correlated with the second-order reaction rate constants of the acrylamides with GSH. The reaction of the acrylamides with GSH is hence not only a detoxification mechanism but also leads to disturbances of the redox balance, making the cells more vulnerable to reactive oxygen species. The reactivity of acrylamides explained the oxidative stress response and cytotoxicity in the cells, and the lack of reactivity of the methacrylamides led to baseline toxicity.

P162
DEVELOPMENT OF SKINPIX, A HUMAN SKIN PERMEABILITY DATASET ABSTRACT #19

Lisa Chedik[1], Shamkhal Baybekov[2], Frédéric Cosnier[1], Gilles Marcou[2], Alexandre Varnek[2], Catherine Champmartin[1]
[1]*Institut national de recherche et de sécurité pour la prévention des accidents du travail et des maladies professionnelles (INRS), Dept Toxicologie et Biométrologie, 1 rue du Morvan, 54519 Vandœuvre-lès-Nancy, France*
[2]*University of Strasbourg, Laboratory of Chemoinformatics UMR 7140 CNRS, 4, rue B. Pascal, Strasbourg 67081, France*

Accurate comprehension of cutaneous absorption parameters for xenobiotics plays a crucial role in drug and cosmetic development, as well as in the assessment of environmental and occupational chemical risks. In line with this objective, SkinPiX (Skin Permeation of identified Xenobiotics) is a new dataset that meticulously consolidates and enhances existing knowledge on skin permeability. By curating research published between 2012 and 2021, SkinPiX complements and expands upon existing databases in the field. Rigorous inclusion and exclusion criteria have been implemented to ensure the creation of a harmonized and reusable dataset. SkinPiX encompasses comprehensive skin permeability data for 110 compounds of diverse structures, including drugs, industrial toxics, flame retardants, and pesticides. Substance-specific parameters, such as logP and molecular weight, are reported alongside key skin permeability endpoints, including steady-state flux (Jss), maximum flux (Jmax), lag time (tlag), and skin permeability coefficient (Kp). The dataset offers convenient filtering options based on specific criteria, such as compound names and skin donor types, and is compatible with the previously established HuskinDB database(1). The resulting SkinPiX dataset is made openly accessible (https://doi.org/10.57745/7FHQOY), providing researchers with a valuable resource for data extraction, comparative analyses, and the construction of predictive models. Through detailed analysis of the dataset, common practices in skin permeability research are revealed, including preferred sources of skin, specific skin layers used, and formulations employed as acceptor and donor types. As a result, SkinPiX highlights the fact that experimental conditions can significantly influence the data, underscoring the need for standardized reporting and harmonized experimental conditions in this field. In addition, the Generative Topographic Mapping method is applied to SkinPiX and compared to HuskinDB to monitor the progress in skin permeability research and identify chemotypes of notable concern. Importantly, the analysis underscores the limited understanding of emerging chemicals, emphasizing the need for further investigation in this area.

P163
Impact of Tiger Nut products on the bioavailability of mycotoxins: An In Vitro Assessment Using Caco-2 Cells ABSTRACT #196

Paula Llorens[1], Paloma Arjona-Mudarra[2],

Raquel Penalva-Olcina[1], Olga Pardo[2], Ana Juan-García[1], Juan Carlos Moltó[1], Cristina Juan[1]
[1]*Laboratory of Food Chemistry and Toxicology, University of Valencia*
[2]*Department of Analytical Chemistry, University of Valencia*

Ensuring food safety, especially concerning the presence of mycotoxins, is crucial due to the associated health risks. This study primarily aims to investigate the effect of tiger nut polyphenols on mycotoxin bioavailability using the Caco-2 cell model, which simulates the human intestinal epithelium, thereby providing insights into the interaction between these compounds and their absorption in the human intestine. Tiger nut beverage (TNB) and the solid by-product (TNBP) were prepared in the laboratory. Both were subjected to in vitro digestion, following the Infogest protocol (Brodkorb et al., 2019). The bioavailability of mycotoxins, in the presence or absence of digested tiger nut products, was assessed using differentiated Caco-2 cells following the Juan-García et al. (2019) methodology. The results demonstrated variability in the bioavailability of individual mycotoxins in the solvent: AFB1 (23±2%), OTA (3±1%), and ZEA (78±3%). The presence of TNBP and TNB influenced the bioavailability of mycotoxins. For AFB1, bioavailability decreased over time when combined with the other mycotoxins and tiger nut products (from 29-32% to 10-12%). OTA absorption remained constant in the solvent (~2.5%), while it exhibited a decreasing trend in the double combinations (25-27% to 12-14%) except for OTA+AFB1 and triple combination with TNBP. ZEA exhibited consistent percentages (ranging from 8-15%) albeit lower in combinations compared to the solvent. These results suggest potential interactions that may influence intestinal absorption, hinting at the protective role of polyphenols in tiger nut against the bioavailability of certain mycotoxins, especially against ZEA, signifying a promising approach for improving food safety and reducing health risks associated with mycotoxin exposure. This work has been supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-100, Conselleria d'Educació, Universitats I Ocupació from Generalitat Valenciana project CIAICO2022/199. PL would like to thank the University of Valencia for being awarded by the Ph.D. grant "Atracció de Talent".

P164
Acceleration of scientific confidence-building in non-animal approaches in Germany
ABSTRACT #250

Tina Stibbe[1], Annette Bitsch[2], Simin Chelopaz Amini[3], Jan Hengstler[4], Robert Landsiedel[35], Marcel Leist[67], Andreas Schepky[8], Andreas Stucki[1]
[1]*PETA Science Consortium International e.V., Stuttgart, Germany*
[2]*Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany*
[3]*BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany*
[4]*Technical University Dortmund, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany*
[5]*German Toxicology Society (GT e.V.), Mainz, Germany*
[6]*University of Konstanz, CAAT-Europe, Konstanz, Germany*
[7]*University of Konstanz, Department of In Vitro Toxicology and Biomedicine, Konstanz, German*
[8]*Beiersdorf AG, Hamburg, Germany*

Background: The implementation of the EU's Chemicals Strategy for Sustainability and consequent updates to regulations will increase regulatory testing requirements for new and already-marketed chemicals. This will result in more requests for animal tests. Simultaneously, calls to demonstrate product safety without using animals are getting louder within the scientific and non-scientific communities. Thus, the European Commission recently committed to developing a roadmap for the transition to an animal-free regulatory system for chemicals. Methods: Representatives from industry, academia, and non-governmental organisations in Germany have formed an alliance to accelerate scientific confidence-building and regulatory application of non-animal methods for toxicity assessment of chemicals. In vitro and in silico methods offer novel solutions to protect human health and the environment. However, few of these

methodologies developed in research laboratories proceed to regulatory application. From this alliance's perspective, the main reasons for the inadequate uptake of new methods are lack of political prioritisation of the issue, lack of systematic coordination strategies to facilitate coherent research and development to enable later regulatory application, and insufficiently targeted funding for the development and validation of test methods to transfer from academia to industry and regulatory acceptance. Results: The alliance suggested to the German government steps for taking action towards achieving this goal by presenting a concept for a roadmap to transition to non-animal approaches. These included (i) formulating a national strategy to make animal use for regulatory testing redundant, (ii) establishing an independent body responsible for the transition of non-animal methods from development to application, and (iii) providing resources for activities targeted at building scientific confidence in these methods. Conclusion: Following these steps will be essential to transition to a safer, more sustainable future without the use of animals, which can in turn also support the activities of the European Commission and of other EU member states.

P165
SKIN IRRITATION AND GENOTOXICITY OF MICROFIBRILLATED CELLULOSE AND SILICA NANOPARTICLES
ABSTRACT #264

Juliana Varella Cruz[12], Viviana Costa Gagosian[1], Emanoela Lundgren Thá[1], Washington Luiz Esteves Magalhães[3], Pedro Henrique Gonzalez de Cademartori[4], Cynthia Bonfim Pestana[1], Danielle Palma de Oliveira[2], Daniela Morais Leme[1]

[1]*Department of Genetics, Federal University of Paraná, Curitiba, PR, Brazil*
[2]*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil*
[3]*Embrapa Florestas, Colombo, PR, Brazil*
[4]*Graduate Program in Engineering and Science of Materials – PIPE, Federal University of Paraná, Curitiba, PR, Brazil*

Background: The demand for greener and safer products has increased recently, especially for daily-use products, such as cosmetics and disinfectants. In this context, microfibrillated cellulose (MFC) and silica nanoparticles (SiO2NP) have been proposed to develop sustainable and renewable products. MFC presents a universal ligand property that allows interactions with polymers and other nanoparticles, and SiO2NP can be used as a chemical carrier. However, there is still a lack of knowledge on nanoparticle toxicity. Therefore, this study aims to evaluate the skin irritation potential of MFC and SiO2NP after acute and repeated exposure using a reconstructed human epidermis (RHE) and assess the genotoxicity of both nanoparticles. Material and Methods: The skin irritation test was performed using the SkinVitro-RHE, an RHE model developed by our group. Exposure of MFC and SiO2NP followed the SkinEthic protocol described in the OECD TG 439, with adaptations for repeated exposure. Nanoparticle permeability in the RHE model was evaluated through autofluorescence assay using confocal microscopy to support the interpretation of skin irritation data. For genotoxicity evaluation, the in vitro micronucleus (MN) assay was performed using V79-4 cells, according to the OECD TG 487. Also, the phospho-histone H2AX was quantified by flow cytometry assay as a biomarker of DNA damage (double-strand breaks). Results: Our data showed that neither nanoparticle is a skin irritant in acute and repeated exposure, and the threshold of cell viability remained above 50% compared to the negative control. Neither tested nanoparticles permeated the epidermis barrier, and no histological changes were observed. Regarding the genotoxicity, neither nanoparticle increased the MN formation or levels of γ-H2AX. Discussion and Conclusion: Therefore, this study showed that MFC and SiO2NP are not skin irritants or genotoxicants and may add value to the sustainable process of developing nanotechnology products.

P166
Chemical and biological assessment of a Heated Herbal Product reveals marked reductions in aerosol toxicants

and in vitro toxicity compared cigarette smoke
ABSTRACT #266

Robert-Daniel Vasile[1], Ourania Komini[1], Georgiana Cava[1], Fiona Chapman[1], Mike Brown[1], Roman Wieczorek[2], Edgar Trelles Sticken[2], Ole Dethloff[2], Liam Simms[1], Matthew Stevenson[1]
[1]*Imperial Brands PLC*
[2]*Reemtsma Cigarettenfabriken GmbH*

Heated Herbal Products (HHPs) are an emerging category of potentially reduced harm nicotine products for adult smokers who would otherwise continue smoking. The aim of the present study was to compare the chemical composition and in vitro toxicological activity between the aerosol of a novel HHP (generated using Pulze 2.0 and iSenzia sticks) and cigarette smoke (generated using a 1R6F reference cigarette). HHP aerosol and 1R6F cigarette smoke were generated using the ISO 20778 regime (55ml puff volume, 30 sec puff interval, 2 sec puff duration). No ventilation blocking was applied to the HHP product. For the assessment of the chemical composition of the HHP and the 1R6F reference cigarette, selected analytes, including WHO 9 priority toxicants, were measured. To assess the biological activity of the products, the HHP aerosol and 1R6F whole smoke were assessed using the CORESTA regulatory in vitro toxicology assays: neutral red uptake (BEAS-2B) for cytotoxicity, Ames (TA98, TA100) for mutagenicity and in vitro micronucleus assay (V79) for genotoxicity. Chemical analysis of the HHP aerosol revealed substantial reductions in aerosol constituents when compared to reference cigarette smoke. Specifically, levels of the WHO 9 priority toxicants were reduced by an average of 97% in the HHP aerosol when compared to reference cigarette smoke on a per puff basis. The HHP aerosol demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis (98%). The HHP aerosol also exhibited no mutagenicity and marked reduction in genotoxicity under the conditions of the tests, when compared to the response seen from the reference cigarette. These results indicate the potential for the tested HHP to offer substantially reduced exposure to toxicants, as well as a reduction in biological toxicity compared to reference cigarette.

P167
Policy for science in 3Rs: What was done? What is ahead of us? What changes can we expect?
ABSTRACT #267

Francois Busquet[12], Leonie K Mueller[3]
[1]*altertox*
[2]*caat-europe*
[3]*Altertox*

Over the last decades, progress towards a regulatory system based on alternatives to animal testing in the EU is walking hand in hand with policy making and research funding (public or private) to implement this goal into a reality. The last policy term (2019-2024) has seen unprecedented initiatives to modernize the regulatory landscape from the European institutions, (e.g. EU Green deal zero pollution non toxic environment, CLP revision, detergent, pharma package...) to the EU agencies (e.g. EFSA roadmap, EMA scoping paper on 3Rs, ECHA workshop on NAMs…) and at the European Parliament (Motion of resolution on plans and actions to accelerate the transition to innovation without the use of animals in research, regulatory testing and education). In addition, the private sector, the NGOs (European Citizen Initiative on cruelty free), and of course the scientific community at large thanks to EU projects and the break out of disruptive technologies such as AI and organoids/microfluidic systems drive change towards the regulatory uptake of alternatives to animal testing. This presentation aims to map some of the key initiatives at the interface of science and policy during the last policy term and reflect on successes and remaining gaps, to prepare for the chances that lay ahead. Particularly, it will elaborate on the upcoming challenges to pursue the policy making of key pieces of legislation (e.g. the delayed REACH revision and one substance one assessment) critical for regulatory implementation of alternatives to animal testing efforts that have been halted in the past months by Member States asking for a “regulatory pause” (1) on environmental laws. Lastly, this presentation will reflect on the upcoming European Parliament election that will define the work plan of the European Commission and thereby

future priorities that can be expected to affect our work as well.

P168
GENOMARK: a transcriptomic biomarker in human HepaRG cells to modernize genotoxicity assessment
ABSTRACT #270

Anouck Thienpont[1], Eunnara Cho[2], Andrew Williams[2], Matthew J. Meier[2], Carole L. Yauk[3], Vera Rogiers[1], Tamara Vanhaecke[1], Birgit Mertens[4]
[1]*Department of In Vitro Toxicology and Dermato-cosmetology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*
[2]*Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada*
[3]*Department of Biology, University of Ottawa, Ottawa, ON, Canada*
[4]*Department of Chemical and Physical Health Risks, Sciensano, Juliette Wytsmanstraat 14, 1050 Brussels, Belgium*

To modernize genotoxicity assessment and reduce reliance on experimental animals, high-throughput new approach methodologies (NAMs) in human-relevant test systems are needed. An interesting group of NAMs are transcriptomic biomarkers, consisting of subsets of genes that robustly and consistently respond to chemicals from specific mechanistic classes. Previously, we developed an 84-gene transcriptomic biomarker, GENOMARK, that identifies genotoxic substances in metabolically competent human HepaRG™ cells. GENOMARK shows a very high predictive performance to classify genotoxicants based on gene expression data collected with microarrays, RT-qPCR and high-throughput technologies such as RNA-Seq and TempO-Seq®. Here, we demonstrate how GENOMARK gene expression data can be used quantitatively, i.e. for potency ranking. We compared potency rankings of chemicals predicted as genotoxic using whole genome or targeted gene panel (i.e., +S1500 panel) transcriptomic analysis between larger gene sets and smaller subsets such as the GENOMARK genes and the 64 genes included in TGx-DDI, another transcriptomic biomarker for genotoxicity. HepaRG™ cells were exposed to 13 chemicals in an adequate concentration range and gene expression data were collected with TempO-Seq®. Hazard classifications for genotoxicity were conducted using the GENOMARK and TGx-DDI prediction models. Potency ranking of the chemicals was performed using benchmark concentration (BMC) modeling in BMDExpress with a benchmark response value of 1 standard deviation. Interestingly, regardless of the gene set used for the BMC analysis, similar potency rankings based on median gene BMCs were obtained. Overall, this work highlights the potential of transcriptomic biomarkers such as GENOMARK and TGx-DDI to facilitate a rapid and efficient human-relevant identification of genotoxicants while simultaneously providing information on potency. Currently, the use of gene expression data to derive point of departure values for genotoxicity assessment is also being explored.

P169
ALTERTOX MARKETPLACE: UNITING THE HEROES OF 3RS
ABSTRACT #275

francois busquet[1]
[1]*altertox*

Since more than ten years, Altertox academy (www.academy.altertox.be) is deeply involved in promoting the use of alternatives to animal testing; either by organizing trainings; via communication tools (podcasts, videos, webinars) or by making scientific voice heard through policy contact. After the creation of a serious game (the TATAbox), tool designed to open conversation about New Approach Methodologies (NAMs), Altertox is launching its own Marketplace gathering all partners involved in the 3Rs community. Altertox Marketplace is centralising all partners involved in 3Rs substituting animal experimentation, as well as services providers or animal-free consumables. It includes Contract Research Organisations (CROs), Lab equipmentequipements suppliers, consultants, consumables, REACH compliance and more. This new tool is user-friendly and is a free service! Furthermore, to increase the value of the database and be prepared for future regulatory demands, we have implemented a

possibility to search by AOPs (Adverse Outcome Pathway, which is a sequence of events from the molecular initiating events to the adverse outcomes in the whole organism). This search allows to identify partners offering test(s) targeting specific key event(s) in the biological chain of the AOP (full or key event), to support the use of NAMs in chemical safety assessment. AOPs list is coming from the AOP-Wiki (aopwiki.org), hosted by the Society for the Advancement of Adverse Outcome Pathways (SAAOP)*.It represents the central repository for all AOPs developed as part of the OECD AOP Development Programme, launched in 2012. It is connected to the Altertox marketplace thanks to an API (Application Programming Interface) to keep it up-to-date. We think this tool will be more than useful for EC to implement their roadmap on phasing out animal testing.

P170
The COST Action Networking Activity IMPROVE: 3Rs concepts to improve the quality of biomedical science
ABSTRACT #285

WINFRIED NEUHAUS[12]
[1]*AIT Austrian Institute of Technology GmbH, Competence Unit Molecular Diagnostics, 1210 Vienna, Austria*
[2]*Danube Private University (DPU), Department of Medicine, 3500 Krems, Austria*

COST Actions are European projects that promote the formation of networks. A range of resources are available for this purpose, such as the organisation of events, meetings, workshops and training schools, as well as the awarding of Virtual Mobility Grants, Dissemination Grants, ITC (inclusiveness target countries) grants and short-term scientific missions. The COST Action IMPROVE was developed from a bottom-up approach initiated by members of European 3Rs centres and its network platform EU3Rnet. This activity will be presented in detail with its possibilities, plans and instruments. The working groups focus on Quality and Translatability of Science, Implementation, Dissemination and Education, whereby ethics will be an integral part in the work of the single working groups. Currently, over 200 participants reflecting several stakeholder groups take part in the activities of this project. Cooperation and active involvement in the COST Action will be invited within the framework of this open bottom-up network approach. Further information can be found via following link: https://cost-improve.eu/

P171
In vitro based testing strategy for the identification of non-genotoxic carcinogens
ABSTRACT #289

Mariam SALEH[1], Ludovic LE HEGARAT[1], Kevin HOGEVEEN[1]
[1]*ANSES: French Agency for Food, Environmental and Occupational Health and Safety, Fougeres Laboratory, Toxicology of Contaminants Unit, Fougères, France*

In vitro based testing strategy for the identification of non-genotoxic carcinogens (NGTxC): SALEH Mariam1, HOGEVEEN Kevin1, LE HEGARAT Ludovic1 1ANSES: French Agency for Food, Environmental and Occupational Health and Safety, Fougeres Laboratory, Toxicology of Contaminants Unit, Fougères, France Carcinogenic compounds are generally categorized into two classes, genotoxic (GTxC) and non-genotoxic (NGTxC) carcinogens. GTxCs induce cancer via direct DNA effects, whereas NGTxCs promote carcinogenesis via a series of diverse Modes of Action (MOA). While GTxCs can be detected based on a battery of in vitro and in vivo genotoxicity assays, there is no in vitro-based testing strategy to identify NGTxC, Therefore, their identification requires 2-year in vivo rodent studies that are time consuming, expensive, pose ethical concerns, and may not be directly extrapolated to humans due to species-specific responses. Thus, the transition into in vitro New Approach Methodologies (NAMs) could be an alternative method for human-relevant chemical safety assessment. In this context, and as a part of the PARC project, we aim to develop an in vitro testing strategy using different models of the human hepatic HepaRG cell line in combination with Transcriptomics and High Content Analysis (HCA) to assess different NGTxC MOAs. So far, our results from 24h acute treatments with NGTxC compounds

in a 2D HepaRG model show a significant changes in different MOAs related to Histone H3 tri-methylation (m3H3K9), neutral lipid accumulation, and cytotoxicity detected by High content analysis. Changes in the pro-inflammatory response (IL-8) were also observed with some NGTxC compounds. Currently, we are assessing the NGTxC MOA using HepaRG 2D, 3D, and 3D co-culture models in 3 day chronic treatments, which could be more informative in these assessments and more similar to the in vivo situation.

P172
Toxicological assessment of porous silica nanoparticles: cytotoxicity, genotoxicity and immunogenicity
ABSTRACT #298

Trisha Patel[1], Zeeshan Ahmad[2], Umakhanth Venkatraman Girija[1], Tarsem Sahota[2], Neenu Singh[1]
[1]*De Montfort University, Leicester School of Allied Health Sciences, Leicester, UK*
[2]*De Montfort University, Leicester School of Pharmacy, Leicester, UK*

Porous silica nanoparticles (PSNs) hold immense promise as drug delivery carriers owing to their high surface area, accessible silanol groups, customisable pore and particle sizes, and facile surface modification capabilities. However, before their clinical translation, a thorough assessment of PSN toxicity is imperative to ensure their biocompatibility. This study undertook a comprehensive evaluation of various toxicological endpoints, encompassing cytotoxicity, genotoxicity, and immunogenicity, using three cell lines representing potential exposure routes of PSNs. Physicochemical characterisation of PSNs was conducted to identify the size, charge and surface functionalisation. Lymphoblastoid TK6, monocytic THP-1, and liver cancer HepG2 cells were exposed to five functionalised PSNs, polyethylenimine (PEI), amine, carboxyl, thiol or silanol at varying concentrations (0 to 200□g/ml) for 24, 48, or 72 hours. Cytotoxicity assessment via the MTT assay revealed varying toxicity among PSN types and cell lines. Notably, PSN-PEI showed significant toxicity towards THP-1 cells compared to TK6 cells. PSN-Carboxyl exhibited toxicity in HepG2 cells, whereas PSN-Thiol demonstrated minimal cytotoxicity across all cell types, suggesting differential cellular responses. Genotoxicity analysis using the cytokinesis block micronucleus assay identified an increased micronuclei frequency induced by PSN-PEI and PSN-Carboxyl in TK6 and THP-1 cells, underscoring the significance of DNA damage evaluation. This correlated with the cellular uptake observed in THP-1 cells. Immunogenicity studies using western blotting revealed interactions between PSNs and C3b protein. PSN-Silanol showed binding to multiple complement proteins C1q, C3 and MBL, thereby suggesting activation of all three complement pathways – classical, alternative and lectin, respectively. Complement activation could generate anaphylatoxins and PSN opsonisation, affecting the therapeutic effect of PSNs. This study provides crucial insights into PSN toxicity, ensuring the use of safe and effective nanocarriers for drug delivery. Future research will investigate intracellular signalling events and protein corona associated with toxicity to better understand the impact of PSNs, thus supporting safe nanocarriers.

P173
CHARACTERIZATION OF HUMAN ISOGENIC EPITHELIAL CELL LINES AS A RELEVANT TOOL TO STUDY COLON CARCINOGENESIS AND INTERACTION BETWEEN GENES AND ENVIRONMENT
ABSTRACT #307

Arnaud Tête[1], Arnaud Liana[2], Hélène Le Mentec[3], Nathalie Poupin[2], Isabelle Gallais[3], Noémie Tournade[3], Carolina Duarte-Hospital[1], Yannick Lippi[2], Fanny Mathevet[4], Gaelle Lefort[4], Catherine Lavau[3], Agnès Burel[5], Reggie Surya[6], Jerry W. Shay[7], Xavier Coumoul[1], Nathalie Vialaneix[4], Sylvie Bortoli[1], Dominique Lagadic-Gossmann[8], Laurence Huc[93]
[1]*INSERM UMRS1124 - Université Paris Cité*
[2]*INRAE UMR 1331 ToxAlim*
[3]*Université de Rennes - INSERM - EHESP - IRSET - UMRS 1085*
[4]*INRAE UR875 MIA-T*
[5]*Université de Rennes - Biosit - UMS 3480*

[6]*Department of Food Technology - Faculty of Engineering - Bina Nusantara University*
[7]*Department of Cell Biology - University of Texas Southwestern Medical Center*
[8]*Université de Rennes - INSERM - EHESP - IRSET - UMR_S 1085*
[9]*LISIS UMR CNRS INRAE - Université Eiffel UGE*

Colorectal cancer (CRC) is the fourth most common cause of death from cancer worldwide. CRC is a multistep and progressive disease where genetic factors are important in the initiation, the development and the progression of the disease. CRC can arise from sequential steps including the acquisition of mutations in the adenomatous polyposis coli (APC), followed by the mutational activation of oncogene KRAS and the inactivation of the tumor suppressor gene, TP53. The occurrence of CRC is largely influenced by the environment, including food contaminants, lifestyle and nutrition. However, the influence of mutations on the response to environmental pollutants is poorly evaluated. Environmental carcinogenesis lacks robust models to explore the interaction between genes and environment and to determine whether genetic mutations associated with colon carcinogenesis generate a particular susceptibility to the harmful effects of pollutants. Our aim was to characterize an innovative cell model consisting of 6 isogenic human colon epithelial cell lines carrying mutations in key driver genes involved in CRC progression and metastasis. Altogether, these cell lines recapitulate colon carcinogenesis from the healthy, preneoplastic, adenoma and carcinoma stages in a simplified way. We showed that all the cell lines express a battery of detoxification enzymes. They exhibit differences in cell and mitochondrial morphology, in proliferation and migration capacities, and in clonogenicity. They also display a flexible energy metabolism, and differences in sensitivity to genotoxic stress, to mitotoxic stress and to cell death stimuli. In conclusion, this in vitro model of colon carcinogenesis may be a powerful and relevant tool to study the effects of environmental pollutants on the colorectal carcinogenesis from the early to the late and metastatic stages, and to evaluate gene-environment interactions in food toxicology.

P174
Comparison of neurotoxic organophosphorous compounds through in vitro determination of the biochemical half maximal inhibitory concentration
ABSTRACT #322

Manon Verger[1], Elsa Cantabella[1], Jennifer Millerioux[1], Anne Bossée[2]
[1]*DGA CBRN Defence, Toxicology Department*
[2]*DGA CBRN Defence, Chemistry Division*

Schedule 1 of the Chemical Weapons Convention (CWC) amendment decided by the State Parties at the end of 2019 to include additional nerve agents (Novichoks and carbamates) that implies the need to characterize their toxicity. Our laboratory aims at developing an in vitro method that allows the comparison of new nerve agents' toxicity in few days with other already studied nerve agents. This method involves the calculation of inhibition rate constant (Ki) by identifying the biochemical half maximal inhibitory concentration (IC50) in the plasma of Wistar rat. Our method is resumed by three major steps. First, identification of optimal concentrations to inhibit cholinesterases by the toxic agent is conducted. Then, assays to adjust the incubation time needed to inhibit cholinesterases are performed. Finally, experiments undertaken in triplicates are required to accurately plot cholinesterases inhibition relative to toxic concentration. The IC50 can thus be determined and Ki calculated. Three Ki have been established. While results for VX and A-232 were similar (4.83 e-11 and 3.04e-11 M respectively), the calculated Ki from sarin (3.53e-09 M) was lower than those of VX and A-232. Consequently, VX and A-232 inhibit 50% of cholinesterases at lower concentrations than sarin and thus are considered as more toxic by this developed method, as already demonstrated by numerous in vivo studies on rats. In one week, our laboratory can efficiently conclude in neurotoxic organophosphorous compound toxicity of a new agent by comparing its IC50 or Ki with those already determined. The IC50 and Ki determination of other newly scheduled nerve agents is currently an ongoing process (families 1.A13, 1.A14… as appointed by CWC) which will further allow to rank the agents by their toxicity. Furthermore, exploring a possible correlation between in vitro

determination of IC50 and in vivo median lethal dose could reduce animal use in the future.

P175
A toxic Western diet that causes liver damage can also lead to Alzheimer's disease.
ABSTRACT #328

Anna Mietelska-Porowska[1], Angelika Więckowska-Gacek[1], Justyna Domańska[1], Dominika Tkaczyk[1], Dominik Chutorański[1], Urszula Wojda[1]
[1]*Nencki Institue of Experimental Biology Polish Academy of Scences in Warsaw*

Nowadays, the Western dietary (WD) model, which originated in the USA but is rapidly spreading in developed and developing countries, both in Europe and around the world, is becoming more common. This change in diet is due to lifestyle changes associated with technological advances and a rapidly increasing pace of life. WD is based on ultra-processed ready-to-eat foods made from refined substances, rich in simple carbohydrates, salt, fat and cholesterol. This dietary pattern is also poor in cereals, fibre and mono- and polyunsaturated fatty acid content, including the anti-inflammatory omega-3, -6 and -9 fatty acids. We conducted experiments in which we fed WD wild-type C57BL/6 mice and a transgenic mouse line with a mutation in the gene encoding the amyloid precursor protein, APP, as a model for Alzheimer's disease. In our study, we showed that WD consumption in the short term leads to the development of NAFLD, followed by NASH. NAFLD is characterised by fat accumulation in the liver and infiltration of immune cells into the liver parenchyma, where they secrete pro-inflammatory cytokines causing hepatocyte damage and subsequent steatosis to steatohepatitis and progresses to various degrees of liver fibrosis, cirrhosis and hepatocellular carcinoma. Furthermore, we have shown that WD-induced liver damage can influence the acceleration or appearance of neuropathological changes in the brain, characteristic of AD. One of the main functions of the liver is detoxification, essential for the removal of circulating Aβ. Hepatocytes can directly remove circulating Aβ by uptake and degradation or by excretion in the bile. NAFLD as a consequence of impaired WD-induced lipid homeostasis is accompanied by low expression of hepatic proteins involved in the clearance of circulating Aβ. Impaired hepatic Aβ degradation leads to increased levels of circulating Aβ, which may contribute to increased Aβ accumulation in the brain and the development of AD.

P176
DRUG INDUCED FATTY LIVER DISEASE (DIFLD): A REVIEW OF THE MOST REPORTED CLINICAL MANIFESTATIONS AND MECHANISMS, AND THEIR CONSISTENCY WITH CURRENT ADVERSE OUTCOME PATHWAYS.
ABSTRACT #330

Ernesto Lopez[1], Anna Rapisarda[1], Ivan Rienda[2], Thomas Luechtefeld[3], José Vicente Castell[1], Ramiro Jover[1]
[1]*Joint Research Unit in Experimental Hepatology, University of Valencia, Health Research Institute Hospital La Fe & CIBEREHD, Spain.*
[2]*Pathological Anatomy Service, La Fe University and Polytechnic Hospital, Valencia, Spain.*
[3]*ToxTrack, Baltimore, MD, USA.*

Background and Objectives: Drug-induced fatty liver disease (DIFLD) is a hepatic condition characterized by the accumulation of intracellular lipids in hepatocytes, leading to micro- and macro-vesicular steatosis. Despite comprising over 20% of drug-induced liver injury (DILI) cases, due to the poor standardization of clinical reports and epidemiological studies, the exact incidence of DIFLD is unknown. The difficulty in diagnosis and the potential progression to steatohepatitis, liver failure, and death underscore the urgency to improve diagnostic and prognostic protocols. Material and Methods: In the present study DIFLD related articles were searched by keywords combined with boolean operators, across several databases. Sysrev, a web-based collaborative platform aided by machine learning models, was used for selection of relevant review articles. Finally, clinical and mechanistic data were extracted, and analyses

performed. Results: Data from review articles on DIFLD and associated bibliography allowed to build a database including steatogenic drugs, and their associated major pathological findings and mechanisms. The analysis allowed the identification of connections between clinical data/outcome and mechanisms in DIFLD, as well as the identification of different categories of steatogenic drugs, being one of them of high concern. Some DIFLD drugs exacerbate pre-existing metabolic steatosis. Others directly cause steatosis (macro or microsteatosis) or steatohepatitis. Nine mechanisms were identified in DIFLD development, acting at different levels and resulting in steatosis. Through our comprehensive review, we observed that the existing AOPs, which are mainly based on nuclear receptor MIEs, inadequately capture the biological intricacies underlying chemically induced liver steatosis. Discussion and Conclusion: Use of retrospective information extracted from bibliography enabled novel categorization of steatotic drugs based on their clinical outcome and mechanisms, opening avenues for identifying characteristic DIFLD subphenotypes and getting closer to a better diagnosis and outcome prediction. Moreover, there is an urgent need for incorporating novel steatosis MIEs into more comprehensive AOPs.

P177
Comparing the transcriptomes of Daphnia and zebrafish for the evolutionary conservation of a gene network for fatty liver disease
ABSTRACT #342

Shaleen Glasgow[1], Yavor Hadzhiev[1], Ferenc Mueller[1], John .K Colbourne[1]
[1]*University of Birmingham*

Genes that govern fundamental biological processes are deeply rooted in evolutionary history. Comparative evolutionary biology demonstrates that disease-associated genes are evolutionarily conserved across metazoans. The peroxisome proliferator-activated receptor α (PPARα) is a major transcription factor that regulates hepatic fatty acid and lipid metabolic processes in vertebrates. These metabolic processes can become perturbed by environmental chemicals, leading to fatty liver disease, fibrosis and hepatocellular carcinoma. Environmental pollution is a global health crisis, and rodents have been the primary models for human chemical safety testing due to our shared mammalian biology. However, the importance of mammalian systems is being challenged by ethical and scientific considerations. Comparative transcriptomics facilitates our understanding of evolutionarily shared responses to toxicants and broadens the utility of alternative model species in predicting adverse human and environmental health. Despite the absence of a liver in invertebrates, the molecular networks governing liver fatty acid metabolism may be evolutionarily conserved and possibly predictive of adverse health in humans. The micro-crustacean Daphnia possesses amino-acid sequence orthologs of human genes for 70% of the PPARα signalling pathway. Moreover, Daphnia signals human pathways regulated by PPARα when exposed to a PPARα agonist. Here, we use comparative single nuclei transcriptomics of Daphnia and Zebrafish embryos to investigate the tissue-specific response to a PPARα agonist and identify homologous liver-like tissues in Daphnia. This research shows promise for the reduction and replacement of mammals, and through conserved molecular biology, it proposes Daphnia as an alternative model for chemical safety testing of liver toxicity.

P178
Applicability of Standard in vitro Alternative Testing Method for Acute Fish Toxicity
ABSTRACT #348

Jing Yang[1], Xiaoqian Chen[1], Feng Tao[1]
[1]*Shanghai Academy of Public Measurement*

As a predicting method of acute fish toxicity (AFT) test or an alternative of AFT range-finding test, a standard in vitro testing method (OECD 249, 2021) by using gill epithelial cell lines of rainbow trout (RTgill-W1) has been published in 2021. Due to its small test system and short test period, this standard method showed good potential for application. However, it also has its limitations, e.g. may have predictive bias for neurotoxicants, has no

application for metal compounds. In addition, the correlation of cytotoxicity and acute fish toxicity, as well as its applicability in practical application is still unclear. Therefore, ten test chemicals including reference substance commonly used in aquatic toxicity test, metal compounds and neurotoxic substances have been used to evaluate the applicability of OECD 249. With the exception of carbendazim, which cause toxic effects in fish through the apoptotic pathway, there is a good linear relationship between the EC50 values of cytotoxicity and the LC50 values of acute toxicity by using standard fish species (i.e. test species recommended by OECD 203 and Chinese standard species Gobiocypris rarus) (with the correlation coefficient r2 of 0.7009 to 0.7975). Although deviations in toxicity classification of the same chemical in different test are inevitable, a good conversation relationship between cytotoxicity and in vivo acute toxicity by using standard fish species could still be established in the same laboratory, which means accurately prediction of fish maybe feasible in different laboratory based on its own in vitro-in vivo correlation of different chemicals. Furthermore, more sensitive or effective endpoints of OECD 249 can be developed to improve its toxicity prediction, significant toxic effects can be also used as the envidence of insoluble high molecular chemicals passing through biomembranes which requried for data exemption in chemical registration.

P179
Exploring the use of NAMs for the identification and characterisation of biomarkers of effect applied in risk assessment
ABSTRACT #350

Georgia Bompola[1], Sara Levorato[1], Lucian Farcal[1], Claudia Rocancio-Peña.[1]
[1]*European Food Safety Authority (EFSA)*

The European Food Safety Authority (EFSA) is implementing a project aiming to develop guidance on the use of biomarkers of effect in regulatory risk assessment of chemicals. In the first phase, the work focused on examining definitions and description of biomarkers of effect, several aspects related to the context of application, the scope of the future guidance and other scientific principles to be considered for its development. To support these activities, relevant initiatives/projects and knowledge in this area were identified, collected and analysed within a complementary mapping study. This included a) comprehensive searches and reviews (e.g. publications, projects, databases/tools) and b) a survey addressed to stakeholders. The survey aimed to collect general information around biomarkers of effect and specific input and views on different aspects related to their use, especially in regulatory contexts. One aspect explored in the study was the topic of "how new approach methodologies (NAMs), including in vitro and in silico methods can be used in the context of biomarkers of effect (e.g. for their optimisation, validation, measurements)". The outcome of the mapping study includes i) a set of representative examples of biomarkers of effect where in vitro studies were used for the identification, characterisation and selection of biomarkers, and ii) an inventory of resources including references to NAMs-related projects, publications or databases. The above outcomes should support further the prospects on exploiting NAMs in the qualitative and quantitative evaluation of the causal relationship (biologically plausible link) between the biomarkers and the adverse outcome/apical effect, especially by providing links to key events of the adverse outcome pathways (AOP).

P203
Unlocking the intestinal transport of low molecular mass AGEs: Insights from a new approach methodology
ABSTRACT #362

Xiyu Li[12], Sebastiaan Wesseling[1], Yaxin Sang[2], Ivonne Rietjens[1]
[1]*Wageningen University & Research*
[2]*Hebei Agricultural University*

The absorption of food-borne advanced glycation end-products (AGEs) may potentially add to the accumulation of AGEs in the body from endogenous sources, and the extent of this contribution to the overall AGE exposome may vary depending on the specific type of AGE. Thus, the aim of the present study was to elucidate the intestinal absorption, intracellular

accumulation and transport of a series of selected low molecular mass (LMM) AGEs using a new approach methodology (NAM) to obtain insight in potential differences in their bioavailability. The use of a Caco-2 transwell cell model can be considered as the gold standard for studying the intestinal transport in vitro, providing results that closely mimic those obtained under in vivo conditions. Furthermore, the method allows for application of different inhibitors and promoters of intestinal transport to explore the underlying mode of action. The results obtained reveal that among the 10 LMM AGEs tested, glycolic acid amide (GALA) is transported at the highest rate, whereas N-ε-(carboxymethyl)lysine (CML) demonstrates the highest accumulation inside the intestinal cells. In contrast, cross-linked AGEs showed minimal absorption and intracellular accumulation. Although all tested AGEs can cross the intestinal barrier through the paracellular route, their uptake is limited. GALA is able to utilize the PEPT-1 for transport, whereas CML and glyoxal-derived lysine dimer (GOLD) can employ alternative active transporter mechanisms. This study provides valuable insights into potential variations in bioavailability and intracellular accumulation of various LMM AGEs. It also demonstrates the potential for employing a NAM to fingerprint the intestinal transport of AGE mixtures.

P205
Investigating the impacts of hyperglycemia and gemcitabine co-exposure on mitochondrial genotype in human lymphoblastoid cell lines
ABSTRACT #364

Shaurya Mathur[1], Katherine Chapman[1]
[1]*In Vitro Toxicology Group, Swansea University, Swansea, UK*

Currently, little is known about how hyperglycaemia in combination with mutant p53 status and chemotherapeutic treatment impact drug resistance with respect to mitochondrial pathways. Mitochondrial DNA mutations may play a role in drug resistance during long-term chemotherapy [1]. Activation of cancer-associated pathways in p53-null cells may contribute to chemoresistance [2]. The impact of hyperglycaemic conditions and gemcitabine exposure on mitochondrial-associated gene expression was evaluated across a panel of 84 genes in TK6 (wild-type p53) and NH32 (p53-null) isogenic cell lines. Cell cultures were administered gemcitabine (between 0.25 nM and 10 nM) for 24h. Selected concentrations aligned with the 50% Relative Population Doubling limit. Cultures were exposed to 'normal' glucose (NG, 11 mM), high glucose (HG, 30 mM), NG and gemcitabine, and HG and gemcitabine prior to RNA extraction. Reverse transcription-quantitative polymerase chain reaction of TK6 RNA samples revealed downregulation of CPT1B (~0.53-fold control) and SLC25A16 (~0.62- fold control) in treatments with HG and gemcitabine, but not with NG and gemcitabine. Our preliminary investigations indicate hyperglycaemia and gemcitabine co-exposure may impair the expression of genes involved in mitochondrial functioning. Future research will focus on mitochondrial-associated gene expression analysis of NH32 cultures and consequently specific genes of interest by targeted next-generation sequencing.

P206
Influence of Static and Dynamic Conditions on the Morphology and Viability of Cell and Tissue Cultures
ABSTRACT #365

Júlia Kubalcova[12], Peter Pôbiš[1], Helena Kandarova[12]
[1]*Center of Experimental Medicine, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia*
[2]*Faculty of Chemical and Food Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovakia*

Complex in vitro models (CIVMs) have been defined as systems in a 3D multi-cellular environment within a biopolymer or tissue-derived matrix, which incorporates primary or stem cell-derived cells, immune system components, and mechanical factors, such as stretch or perfusion, or at least two of these elements. Among technologies categorised as CIVMs, we can find microfluidic technology, or Organ-on-Chip, that enables researchers to replicate blood circulation and better simulate drugs' ADME (Clapp et al., 2021; Langhans,

2018; Mastrangeli & van den Eijnden-van Raaij, 2021). Despite numerous projects targeting microfluidics and "Organ-on-Chip" technology, standardisation and validation of these systems remain a significant challenge. Our research aimed to evaluate the impact of static and dynamic conditions on the cultivation of selected 2D and 3D in vitro models used in preclinical research. This study verified the feasibility of using the microfluidic system MIVO (React4Life) for cultivating the VERO E6 cell line and the EpiIntestinal 3D tissue model. The liquid flow rate, together with cell seeding density, had a significant impact on the cells. The next step focused on the EpiIntestinal tissue model and changes in its barrier properties in both conditions. Subsequently, the 3D model was exposed to selected drugs, Cemtirestat and Ibuprofen. The results for Cemtirestat showed no impact on the viability or barrier properties of the 3D tissue models. At the same time, in the experiment with ibuprofen, we have seen different impacts on barrier properties in static and dynamic conditions and possible recovery from an initial barrier decrease. The results confirmed that correctly set dynamic conditions can contribute to a better understanding of interactions in biological systems, which is critical for the development of an accurate and relevant in vitro toxicological testing platform. This work was supported by the following grants: Vega 20/0153/20, DS-FR-19-0048, and APVV-19-0591

P208
PHYSIOLOGICALLY BASED KINETIC MODELING REVEALS AN INFLUENCE OF DOSE AND STRUCTURAL CHARACTERISTICS ON THE RELATIVE POTENCY (REP) VALUES FOR PA N-OXIDES.
ABSTRACT #368

Yasser Alhejji[12], Frances Widjaja[1], Sebastiaan Wesseling[1], Ivonne Rietjens[1]
[1]*Division of Toxicology, Wageningen University, PO Box 8000, Wageningen, 6700 EA, The Netherlands*
[2]*Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, 51452, Saudi Arabia*

Pyrrolizidine alkaloids (PAs), often occur in their N-oxide forms (PANOs), are natural toxins produced by plants against herbivores. PANOs are less toxic than their parent PAs. However, upon their reduction to the corresponding PAs they can induce hepatotoxicity. Establishing the relative potency (REP) values for PANOs relative to their parent PAs (REPPANO-to-PA) can aid the risk assessment of the combined exposure to these toxicants. Therefore, the aim of this study was to define the REPPANO-to-PA values for a series of PANOs using new approach methodologies (NAMs) including physiologically based kinetic (PBK) modeling and in vitro assays. The present study predicted REPPANO-to-PA values for open-chain diester and monoester PANOs, which were then compared to the REPPANO-to-PA values previously predicted for macrocyclic diester PANOs, using similar PBK modeling based approach. The simulations revealed that REPPANO-to-PA values are dose dependent being higher at low exposure scenarios that better reflect human dietary exposure than dose levels used in animal studies. The predicted REPPANO-to-PA values for open-chain diester and monoester PANOs ranged from 0.43-0.63 while for macrocyclic diester the values ranged from 0.68-0.92. The heliotridine-type PANOs, lasiocarpine N-oxide and heliotrine N-oxide, showed REPPANO-to-PA values of 0.43, notably lower than that derived for retronecine-type PANOs. These results indicate that not only the esterification type but also the stereochemistry configuration of the necine base could influence the REPPANO-to-PA values. To conclude, our PBK modelling highlights the role of dose and structural characteristics on the REPPANO-to-PA values of the various classes of PAs and provide insights in the underlying kinetics causing such differences. This approach is in-line with the 3Rs principle, and contributes to improving the current risk assessment of PAs and PANOs, enabling quantification of REPPANO-to-PA values for a large number of PANOs at dose levels relevant for human exposure scenarios without the need for animal data.

P216
NAMs for EFSA's Nano regulatory risk assessment
ABSTRACT #351

Adriana Scattareggia Marchese[1], Maria Chiara Astuto[1], Irene Cattaneo[1], Claudia Roncancio-Peña[1]
[1]*European Food Safety Authority (EFSA)*

In the EU context of regulated food and feed products, the European Food Safety Authority (EFSA) carries out scientific assessments to evaluate their safety to support risk managers in the decision-making. When a dossier submitted to EFSA concerns nanomaterials or materials containing small particles including nanoparticles requiring nano-specific assessment, the 2021 EFSA Scientific Committee Guidance on Risk Assessment of Nanomaterials must be followed. This Guidance provides indications on how to conduct assessment of nanomaterials in areas within EFSA's remit of activity in the context of human and animal health risk assessment. Within this framework, EFSA's Nano Guidances highlight the relevance in using New Approach Methodologies (NAMs) as part of Integrated Approaches to Testing and Assessment (IATA) for evaluating nanospecific toxicokinetic and toxicodynamic aspects. In particular, the development of IATA is proposed for addressing the identified gaps for nanospecific assessment and/or for integrating the assessment of the fraction of small particles in the conventional assessment. Several NAM-based options are proposed and, in general, the third parties are encouraged to address the gaps using alternative methods in light of 3Rs (Reduce, Refine, Replace animal testing). With the aim to develop further recommendations on the use of NAMs for nanoparticles/nanomaterials risk assessment, EFSA launched in 2021 a project on the use of NAMs for the hazard assessment of nanofibers (NANOCELLUP). Recently, EFSA launched the NAMS4NANO project, still ongoing, articulated on three main lots: one on the Review of NAM-based tools for nano-specific risk assessment and developing a 'Qualification System for NAMs', and the other two lots on 'Designing and conducting a set of risk assessment case studies in specific areas and for horizontal topics'. These projects pave the way for the inclusion of NAMs in the EFSA's risk assessment of nanomaterials, connecting research and regulatory risk assessment while staying abreast of innovation.

P217
HUMAN IN VITRO PERCUTANEOUS ABSORPTION OF BPA ANALOGUES: BPAF AND TGSA
ABSTRACT #15

Catherine Champmartin[1], Claire Seiwert[1], Matthieu Aubertin[1], Emmy Joubert[1], Frédéric Cosnier[1], Lisa Chedik[1]
[1]*Institut national de recherche et de sécurité pour la prévention des accidents du travail et des maladies professionnelles (INRS), FRANCE*

The decrease in bisphenol A (BPA) usage in several countries, driven by its recognized endocrine-disrupting effects, has given rise to numerous alternative chemicals such as bisphenol S (BPS), bisphenol AF (BPAF) and 2,2'-diallyl-4,4'-sulfonyldiphenol (TGSA), three BPA analogues. BPAF is use for polymer synthesis, while TGSA is used in technical papers. It has been reported that the common chemical structure of bisphenols gives them estrogenic properties that could prove to be harmful to health. BPAF is classified as toxic to reproduction 1B by the European Union and was found in river, soils and the urine of residents near a BPAF manufacturing plant. TGSA toxicity is poorly understood. However it was found in indoor dust and quantified in the urine of doctors, teachers and water plant staffs. Although skin is a significant occupational exposure route, there is no data for TGSA and BPAF skin permeation. Our aim was to generate percutaneous absorption data for these two substances following OECD guidelines. [14C]-BPAF or TGSA was dissolved in water then applied in vitro to fresh human skin using static Franz cells, for 20 hours minimum. Steady-state flux (Jss), lag time (tlag), and skin permeability coefficient (Kp) were determined. At the end of the experiment, the distribution of dose in the different compartments, particularly in skin, was assessed. In order to predict how much of the dose remaining in the skin could be further absorbed, strippings and an epidermis-dermis separation were also carried out on skin

samples, to obtain a concentration profile by skin layer. The permeability coefficients of BPAF and TGSA are around 1,000 times higher than the BPS one, making the skin an important potential exposure route. These entirely new percutaneous absorption data will definitively contribute to a better assessment of occupational risks related to these chemicals.

P218
Protective effects of H.triquetrifolium against oxidative stress and inflammation and assessment of anti-cancer potential
ABSTRACT #222

Ece Sabuncu[1], Dilara Güreşçi[1], Yağmur Özhan[1], Meriç Aras[1], Etil Güzelmeriç[2], Hande Sipahi[1]
[1]*Yeditepe University, Faculty of Pharmacy, Department of Toxicology*
[2]*Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy*

Hypericum triquetrifolium is endemic to the Mediterranean and Eastern Europe and grows mostly in the Aegean region of Turkey. It was used as an anti-inflammatory agent to act in reflux and burn treatment as well as against infection by local people (1). Anti-cancer activity has also been shown using various cell lines however the effects of the extract against pancreatic tumor lines are not well-known. Transformation from normal cells into tumor cells can be exacerbated by chronic inflammation through inflammatory pathways induced by oxidative stress (2). In the light of this information, in the present study we investigated, protective effect of H.triquetrifolium extract against oxidative stress and inflammation. The cytotoxicity of the extract doses until 1 mg/mL was studied by MTT assay on the RAW 264.7 murine macrophage cell line. The protective role of the extract against LPS-induced inflammatory biomarkers NO, PGE2 and IL-6 were determined with non-cytotoxic doses (3). The results revealed a promising antioxidant effect with using DPPH (205,23 ± 9,49 mg/g TE), CUPRAC (297,65±3,16 mg/g TE) and, FRAP (158,26±4,03 mg/g TE) methods. NO assay was performed on extract concentrations where cell viability was above 70%. 0.5 mg/mL showed 86% nitrite inhibition and, in both IL-6 and PGE2 assays the same potent dose showed a near complete inhibition of inflammation compared to control group. Potential anti-cancer activity was revealed through the selective cytotoxicity mechanism since the IC50 of healthy HDF cell line is 6-fold over compared to MIA PaCa-2 human pancreatic carcinoma cell line. Our ongoing study is the determination of oxidative stress biomarkers (SOD, CAT activities and GSH level) as well as lipid peroxidation resultant MDA level on LPS-induced cells. Selective anti-cancer activity of H.triquetrifolium will also be shown 3D spheroid formation assay in MIA PaCa-2 and HDF cell lines.

P219
SODIUM DODECYL SULFATE AEROSOL GENERATION AND CHARACTERISATION OF TOXICITY FOLLOWING IN VITRO AEROSOL APPLICATION TO MUCILAIR™
ABSTRACT #23

Jo Wallace[1], Kamil Czuchrowski[1], Taseef Khalid[1], Stephen Huish[1], Iain Crombie[1], Hazel Paulo[1], Blane Stobbs[1], James Baily[1], Mary McElroy[1]
[1]*Charles River*

In vitro organotypic air liquid interface (ALI) models of the lung including MucilAir™ (Epithelix) are used to inform inhalation risk assessment and provide data to support regulatory review. Sodium Dodecyl Sulfate (SDS) applied directly (liquid application) has historically been used as a toxicity positive control substance (Welch et al 2021). However, aerosol application methods better match the in vivo dose route and are more physiologically relevant. The objective was to optimise aerosol delivery of SDS and subsequently characterise its toxicity using MucilAir™ with an In-vitro Continuous flow Exposure System (ICES). During optimisation, efficiency, deposition rates, flow rate, humidity, and nebuliser choice were among the aspects explored. SDS aerosol at three dose levels was then applied to MucilAir™ using the ICES. The same masses of SDS were applied to MucilAir™ as liquid doses, for comparison. Aerosol SDS was quantified gravimetrically and using analytical methods. Following 24 h exposure to SDS, toxicity was assessed using

a panel of markers including TEER, cilia beat frequency, release of inflammatory markers and histopathology. Results for reference groups (including aerosol controls) were comparable to historical data indicating the ongoing good condition of the MucilAir™ tissues in the ICES. Aerosol application of SDS (low and high dose levels) demonstrates a comparable dose response to the toxicity from direct liquid applications of SDS as expected. The highest aerosol delivered dose of SDS resulted in marked toxicity by every measure: a reduction in TEER, IL-8 and ATP levels, increased LDH release and a considerable reduction in CBF. Histopathology review (cilia loss, epithelial thinning, apoptosis/necrosis) were consistent with quantitative assays of damage. Overall, aerosol application using ICES delivered reproducible and accurate levels of SDS to MucilAir™. Aerosol SDS toxicity corresponded to liquid application and indicates that the ICES is appropriate for delivery of aerosol doses to ALI lung models.

P220
INHALATION TOXICITY ASSESSMENT OF POWDER COSMETIC PRODUCTS WITH AN IN VITRO PULMONARY MODEL
ABSTRACT #16

Béatrice BUI[1], Marie-Pierre GOMEZ-BERRADA[1], Pierre-Jacques FERRET[1]
[1]*Safety Products Department, Pierre Fabre Dermo Cosmetique, Toulouse, FRANC*

Background and Objectives: Evaluation of toxicity of cosmetic products generating cloud of particles and its ingredients must consider the different exposure routes. Inhalation is a potential exposure route for this type of cosmetics. It was interesting to assess local toxicity in the upper respiratory tract to powder cosmetic products, using in vitro pulmonary model. The aim was to assess the repeated dose toxicity of two powder cosmetic products: face powder and dry shampoo, on a three-dimensional model of fully differentiated human upper airway epithelium: MucilAir™. Material and Methods: The toxic effects after 4 days of daily exposure were evaluated on epithelium reconstructed from nasal donors pool. The cells were exposed to 3 concentrations determined to reflect the consumer's level of inhalation exposure to the products. Several endpoints were analyzed: cytotoxicity, cilia beating frequency, mucociliary clearance, airway inflammation and histology. Results: Face powder: Exposures to 0.028, 0.28 and 2.8µg per tissue didn't affect TEER, LDH release, CBF or morphology. A minor increase (limited and reversible) of IL-8 at 2.8 µg was observed. At 0.028µg, an increased MCC mimics physiological response to xenobiotic. Dry shampoo: Exposures to 3.1 and 31µg per tissue didn't affect TEER, LDH release, CBF, MCC, IL-8 or morphology. At 310µg, a statistically but not biologically significant increase of IL-8 was observed. A dose-response trend is observable in several measurements performed (TEER, CBF, MCC and IL-8) suggesting that increasing the number of exposures or doses could affect the epithelium. Discussion and Conclusion: Overall, these results indicate no local toxicity from face powder up to 2.8 µg and from dry shampoo up to 310 µg, in the upper respiratory tract. These highest exposures remain conservative situations (respectively 100 and 10 times higher than level of potential inhalation exposure of the products).

ADDITIONAL ABSTRACTS

ADDITIONAL ABSTRACTS

Tuesday, 4 June 2024
13:00 - 14:00

Session LS1: Models, biomarkers and assays for systemic toxicity

Lunch session 1
Simulating kidney tubular crystallopathy in vitro
ABSTRACT #224

Devon Barnes[1], Mara Vonk[1], Chaja Klein[1], Manoe Janssen[1], Rosalinde Masereeuw[1]
[1]*Utrecht University*

Background and objectives Kidney crystallopathy is characterized by the presence of crystals within renal tubules with detrimental effects. The interplay of crystal-induced tubular obstruction, cytotoxic effects, and inflammatory responses is central to the pathogenesis of crystallopathy, with specific histological features and associated challenges in diagnosis and treatment1. The research objective was to investigate the mechanisms underlying crystal-induced renal inflammation and identify the process involved in inflammasome activation within proximal tubules. Our study focuses on simulating kidney crystallopathy and its impact on inflammasome components, caspase activity, cytokine production, and the NF-κβ pathway using in vitro models. Materials and Methods Conditionally immortalized proximal tubule epithelial cells (ciPTECs) were used to investigate the role of uric acid, a known crystal-forming metabolite, and the influence of acidification on crystal formation. A series of in vitro assays were explored to assess cytotoxicity, oxidative stress, mitochondrial function , inflammation as well as the expression of NLRP3 inflammasome related markers on the gene and protein level. Results Exposing ciPTECs to uric acid under reduced pH conditions reduced cell viability and induced oxidative stress and inflammation compared to a physiological pH of 7.4 and led to crystal formations at 800 µg /ml. Our findings also showed that uric acid induced the expression of inflammasome-related markers, including caspase-1, ASC and TNFα at the mRNA level, and IL-1β at the protein level. These effects were most pronounced with higher uric acid concentrations and lower pH levels. Discussions and Conclusions The results of the study will be utilized as a conceptual basis toward establishing and expanding upon a test battery of in vitro assays to further characterize crystal-forming and potentially nephrotoxic chemicals and measuring individual key events toward the generation and evaluation of adverse outcome pathways for crystallopathy-related kidney failure.

Lunch session 1
ASPIS Academy and the Early-Stage Researchers' transformative potential in toxicology
ABSTRACT #341

Eliska Kuchovska[1], Luiz Ladeira[2], Gaelle Hayot[3], Ruben Martinez[4], Barira Islam[5], Kirsten Veltman[6], Julia Dominika Zajac[7], Agata Ormanin-Lewandowska[8], Martijn J. Moné[9], Helena Kandarova[10], Silvia Tangianu[11], Giorgia Palloca[11], François Busquet[12]
[1]*IUF - Leibniz Research Institute for Environmental Medicine, Germany*
[2]*University of Liège, Belgium*
[3]*Karlsruhe Institute of Technology, Germany*
[4]*Leitat Technological Center, Spain*
[5]*Certara, UK*
[6]*RIVM, The Netherlands*
[7]*VUB, Brussels, Belgium*
[8]*University of Birmingham, UK*
[9]*Leiden University, The Netherlands*
[10]*Slovak Academy of Sciences, Slovakia*
[11]*University of Konstanz, Germany*
[12]*Altertox, Belgium*

Within the dynamic realm of chemical risk assessment, the implementation of New Approach Methodologies (NAMs) represents a revolutionary framework that substitutes conventional testing methods with innovative, effective, and ethically conscious alternatives. Early-Stage Researchers (ESRs) have a crucial role in influencing the future of NAMs for chemical next-generation risk assessment. Nurturing this developing generation is of utmost importance, as it provides the foundation for innovation and progress. The ASPIS Academy (AA) acknowledges the importance of ESRs and highlights their significance in the field of NAMs. The AA

functions as a well-organised networking platform, supporting more than 120 ESRs from the three H2020 European projects of the ASPIS cluster (ONTOX, RISK-HUNT3R, and PrecisionTox). The AA is dedicated to promoting ESR careers through specific programs that include training, mentorship, mirroring senior research groups, promoting laboratory exchanges, communication and dissemination, and sustainability. These activities are guided by ESR representatives and advised by senior researchers. The AA actively promotes inclusivity by extending a warm welcome to individuals from many backgrounds, beliefs, and identities, so creating an environment that facilitates equal opportunity for the ideas and aspirations of an emerging generation of scientists to thrive. The AA is a prime example of creating collaborative and supportive conditions for early-stage researchers as it faces challenges and envisions a future of dynamic growth. This work represents a step forward in the discussions between ASPIS ESRs and the toxicology community, highlighting the work done by ASPIS ESRs and showcasing the transformative potential of the youth.

Wednesday, 5 June 2024
13:00 - 14:00

Session LS2: Session organised by The Centre for Animal-Free [Proefdiervrij] Biomedical Translation: Co-creating a life long training program

Lunch session 2
An introduction to the CPBT (The Centre for Animal-Free [Proefdiervrij] Biomedical Translation) – workshop: co-creating a lifelong learning program.
ABSTRACT #361

Paul Carmichael[1], Daniela Salvatori[2], Merel Ritskes-Hoitinga[2], Cyrille Krul[2], Anne Kienhuis[3]
[1]*Unilever SEAC, UK*
[2]*Utrecht University*
[3]*RIVM, Netherlands*

The Dutch National Growth Fund is investing EUR 125 million in a new centre for animal-free biomedical testing. Known as the Centre for Animal-Free Biomedical Translation, its aim is to generate safer, more effective treatments, while eliminating animal suffering. The Centre will use this generous funding from the Netherlands National Growth Funds (NGF) to establish a focus for the accelerated development and dissemination of animal-free biomedical innovations and testing expertise. This will offer economic and social benefits with improved medicines, ultimately, without animal testing. Within the CPBT, new creative efforts will accelerate novel routes of validation and qualification of NAMs (new approach methodologies) including MPS (microphysiological systems) for chemical safety assessments and we will implement the developed methods, tools, and expertise together with partner researchers and companies. Standardization, validation, qualification, and implementation of animal-free methods will be integral elements in this process. The new centre will also offer education, training, advice, and support to enhance the acceptance and use of animal-free biomedical innovations. There are many players and stakeholders involved in the implementation of NAMs. We will aim to use the RIVM framework described in figure 1, to understand the specific needs of stakeholders and to identify which educational and training activities will have priority, to create viable networks able to facilitate development and implementation of NAMs. Our starting question will focus on specific stakeholder positions on the NAM implementation curve. We will work and reflect on concrete cases, with context of use, applying co-creation methods to design specific processes and needs. The ESTIV session will also aim to collect the suggested needs for future lifelong-learning programs applicable to specific networks.

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