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Late breaking abstracts

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Contents

Late breaking abstracts

LP-63

Towards Precision Toxicology - exploring toxicant-induced molecular perturbations in phylogenetically diverse model organisms via untargeted metabolomics.

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Chemical pollution is a multifaceted global challenge that poses significant risks to health, ecosystems, and economic prosperity. It is one of five 'planetary boundaries', out of a total of nine, that has now been breached, theoretically putting the world *en route* towards a future in which populations could struggle to survive. Solutions are urgently required to tackle the scourge of chemical pollution, with PrecisionTox – an EU Horizon 2020-funded research project – aiming to address this through development of New Approach Methodologies (NAMs) for chemical safety assessment. This will involve combining: 1) exposure of five phylogenetically disparate, 3Rs-compliant, non-sentient model organisms (*Daphnia magna*, *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Xenopus laevis*)and HepG2 cell line (collectively 'test systems') to up to 250 chemicals, with 2) phenotypic, transcriptomics & metabolomics analyses. Resulting data sets will facilitate exploration of the molecular basis of chemical-induced adverse health effects that may better guide regulator decision makers, while also paving the way towards (machine learning-guided) predictive toxicology, wherein toxicological outcomes might feasibly be predicted based on knowledge of a chemical's properties and an organism's genetic composition.

In this work, we present findings from untargeted metabolomics analyses performed as part of the PrecisionTox pilot study, in which PrecisionTox test systems were exposed over 48-hours to five distinct chemicals, at corresponding BMD_{10} and BMD_{25} levels. Samples collected throughout the exposure period, alongside blank controls, were extracted using a bespoke, semi-automated extraction workflow, established on a Beckman i7 workstation, that yields for each sample: 1) a fraction for RNAseq analysis, 2) a fraction for non-polar metabolomics (lipidomics) analysis, and 3) a fraction for polar metabolomics analysis. Untargeted metabolomics analyses of the latter fractions, derived from exposure control samples, followed by principal components analysis of resulting data sets, revealed clear metabolic differences throughout the exposure period for many PrecisionTox test systems, reflecting their underlying growth and development. Comparisons of this data against that for time-matched, chemically exposed samples revealed many instances of chemical-induced metabolic perturbations, though further work is now required to confirm the specific metabolites and/or pathways perturbed (if any) in each exposure scenario. This information must also be fused with insights from paired transcriptomics data sets, to build a more complete picture of the molecular key events underpinning chemically induced toxicological effects.

LP-64

California Proposition 65 Heavy Metal Exposure Assessment for Cultured Chicken (Gallus gallus) Products

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In the United States, human food produced from cultured animal cells (informally known as "cultured meat") must meet the same safety and facility registration requirements as conventional meat sources, including establishing the safety of the cultured food product, following Good Manufacturing Practices (GMPs), and registering the food production facility with the U.S. Food and Drug Administration (U.S. FDA) and the U.S. Department of Agriculture's Food Safety and Inspection Service (U.S. DA-FSIS). In addition to meeting U.S. federal regulatory requirements,

cultured meat products marketed in the State of California must also comply with California's Proposition 65. California Proposition 65 requires businesses to provide warnings to inform consumers about significant exposures to chemicals known by the State to be carcinogenic, reprotoxic, or developmentally toxic. This poster presents the results of a dietary exposure assessment for two cultured chicken products to estimate daily dietary exposure to three heavy metals that are listed on Proposition 65 (lead, cadmium, and arsenic) and assesses whether these cultured meat products have safe harbor from California Proposition 65 labeling.

As of 2023, the U.S. FDA has completed two pre-market consultations for "cultured chicken" products and the U.S. FDA had no questions about each firm's safety conclusions, despite the presence of detectable levels of lead, cadmium, and arsenic in both cultured chicken products. California law requires that a Proposition 65 warning be placed on products containing a Proposition 65 listed chemical unless the exposure to the average consumer is low enough to pose no significant risk of cancer or is below levels associated with reproductive or developmental toxicity. Thus, if an exposure subject to Proposition 65 can be shown to be less than a substance-specific acceptable exposure level, the responsible party has "safe harbor" from the Proposition 65 warning requirement.

Zheng et al. (2019) report weighted mean intakes of poultry among four different racial and ethnic groups (non-Hispanic white, non-Hispanic black, Hispanic, and other groups). Assuming that cultured chicken products are consumed at a similar rate and using published levels of heavy metals in each cultured chicken product submitted to the U.S. FDA, the resulting estimated daily lead exposure for one or more racial/ethnic groups exceeds lead's 0.5 µg/day Maximum Allowable Dose Level (MADL) specified by the State of California's Office of Environmental Health Hazard Assessment (OEHHA). In contrast, daily estimated exposure to cadmium and arsenic associated with the consumption of cultured chicken is below each chemical's respective safe harbor level of 4.1 and 10 µg/day, respectively.

This exposure assessment indicates that certain cultured meat products may result in lead exposures that require a Proposition 65 warning label in order to comply with California law.

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LP-65

Questioning Assumptions about the Safety and Abuse Potential of Medical Cannabis and Cannabinoids: A Critical Review and Rationale and Focus for Clinical Trials

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Background

While a rapidly growing literature focuses on efficacy in specific applications of medical cannabinoids, safety is not clear and much less well reported. Characteristic of the rapidly-increasing mass of data on cannabis and a limited number of cannabinoids are data of mixed quality, inconsistent results, and contradictory evidence for the questions being addressed. This situation is especially provocative because cannabinoids (CBs) have already been identified as putative adjuvant analgesics, anxiolytics, anti-neoplastic agents and there are scores of clinical trials currently under way. We review the nature of pain associated with a spectrum of diseases and suggest how the safety of principal classes of CBs might be more efficaciously addressed.

Objectives

We focus on the issue of abuse potential or liability by examining representative research data for validity and generalizability and conclude that there are a set of tacit assumptions that underlie much cannabis research and constitute threats to the validity of data collection and conclusions derived from that data, especially in the area of abuse liability. These admittedly broad assumptions include (a) a standard cannabis formulation, (b) standard routes of administration, standard potency dosing, (c) a standard pattern of use, (d) a standard user or patient, and (e) a standard vulnerability to misuse or dependence. Thus, our purpose was to identify gaps and densities in the evidence and opinions reported in an evolving literature in order to justify and design clinical trials in the area of abuse potential of medical cannabinoids.

Methods

We followed the scoping review methodological framework and guidelines proposed by Arksey and O'Malley (2005),and the Joanna Briggs Institute (Peters et al 2015) as well as suggestions offered by Tricco and colleagues (2016). Seven electronic databases [Google Scholar, Scopus, Web of Science, Embase, PubMed/MEDLINE, CINAHL, and the Cochrane Library] were searched to identify English-language peer-reviewed publications published generally within the last 20 years.

Results

Unpacking and questioning these assumptions leads to the conclusion that far more rigorous language and research design is needed to definitively address the question of cannabis abuse potential.

Conclusion

Based on the best available evidence, the general safety and abuse liability of medically supervised cannabis is comparable to any other class of pharmaceutical agents. However, the absence of scientific and regulatory standardization within the burgeoning cannabis landscape demands far more rigor and scrutiny than existing levels in industry and the academy.

LP-66

Effects of *Streptococcus Suis* Autogenous Vaccine application towards 20 antimicrobial residues in Swine Farm, Thailand

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Effects of *Streptococcus Suis* Autogenous Vaccine application towards 20 antimicrobial residues in Swine Farm, Thailand

Abstract

Antimicrobials residue in food constitute causes human health risk, particularly in the swine product in developing countries. Autogenous vaccine was applied against *Streptococcus suis* as program vaccine from piglets until finisher. The purpose of study was to determine the occurrence of antimicrobial drug residues after using autogenous vaccine against certain disease in the swine farm. Blood samples as well as drinking water, pig manure, wastewater at pre-biogas and post-biogas sludge on 20 antimicrobial drugs residue were evaluated. The samples were collected for 3 times on during December 2022 - March 2023 from a swine farm located in Nakhon Pathom province, Thailand. Twenty antibiotic drugs (Sulfadiazine, Lincomycin, Trimethoprim, Oxytetracycline, Amoxicillin, Norfloxacin, Ofloxacin, Levofloxacin, Ciprofloxacin, Enrofloxacin, Ampicillin, Chlortetracycline, Sulfamethoxazole, Doxycycline, Tetracycline, Erythromycin, Tylosin, Nalidixic acid, Tiamulin, Clarithromycin) were analyzed using LC-MS/MS, Thermo Scientific (TSQ Endura). The Mann-Whitney U test was performed for comparisons of the concentrations of antibiotic drug between non-autogenous vaccine and autogenous vaccine group. No significantly difference was detected in 20 antimicrobial drugs concentration between non-autogenous vaccine and autogenous vaccine group. However, amoxicillin in swine manure collected from non-autogenous vaccine group (5,233.06±7194.63 ng/mg) tended to be higher when compared with those of autogenous vaccine group (1,000.59±1326.51 ng/mg), whereas tiamulin still remains detected in manure and wastewater. These results demonstrate that the autogenous vaccine is a useful tool facilitating swine disease management and positively reduce the risk of antimicrobial drug residues in animals and environment. However, there was still a need to continue to collect more data on autogenous vaccine uses in the future for sustainable of livestock production and to safeguard human health.

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LP-67

Characterization of pollutant particle enhanced dust mite inflammatory effects in human primary CD34+ stem cell derived myeloid cells.

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Air pollutant material including diesel exhaust particles (DEP) have been suggested to have adjuvant behaviour towards inhaled aeroallergens such as house dust mite (HDM) resulting in enhanced inflammatory effects for allergic airway disease. The involvement of myeloid cells as a mechanistic contributor to this effect has not been fully investigated, particularly with regards to how myeloid cell responses to HDM are influenced by DEP. In this study, we used human CD34+ haematopoietic stem cell derived populations of macrophage and dendritic cells to test HDM responses. Initial characterisation was carried out using single cell RNA sequencing (ScSeq). This identified multiple sub-populations of immune cells within the broader classification of macrophage and dendritic cell differentiation protocols. ScSeq was also used to identify which sub-populations of macrophages responded to HDM exposure. Global responses were confirmed using Bulk-RNA-seq analysis. 4 of 6 sub-populations of macrophages demonstrated differential gene expression (DGE) with CXCL (CXCL5 and CXCL8) and CCL (CCL5 and CCL15) chemokine expression displaying for the most part exclusive population specific expression. Pearson corelation of DGE with baseline HDM receptor expression indicates that population specific induction may be attributable to the presence of TLR2, CD14, CD163, CLEC7A and TLR4. Exposure of macrophages to DEP resulted in a modest increase in toxicity assessed using LDH release. This was paralleled by an increase in CYP1B1 expression indicative of aryl hydrocarbon receptor activation and the presence of combustion chemicals such as PAHs. NQO1 and SLC7A11 were also induced indicating oxidative stress induction. DEP co-exposure resulted in enhanced HDM induced CXCL expression while having no effect on CCL expression. Oxidative stress gene expression was not influenced by HDM but was further increased above DEP levels upon co-exposure. These results point towards an enhanced effect of DEP on HDM induced CXCL chemokine expression through a mechanism that may involve increased oxidative or electrophilic stress. Finally, we examined the potential for DEP and HDM to modify antigen presentation machinery on macrophage cells by examining protein expression of CD40 and HLA-DRA using high content imaging analysis. No change in expression of HLA-DRA was observed. A modest increase in expression in

a small sub-population of cells was observed for CD40 in response to DEP. The impact of this modest change is unknown. In summary, macrophage sub-types exposed to DEP may enhance CXCL chemokine acute responses to HDM as a mechanism of enhanced allergic airway disease.

LP-68

An ex vivo human model for safety assessment of immunotoxicity of engineered nanomaterials

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The unique physicochemical properties of nanomaterials (NM) and engineered nanomaterials (ENM) have pushed their use in many applications ranging from medicine to the food industry, textiles, and many more fields. Thus, human exposure to NM and ENM is growing by the day. However, the current toxicity tests do not reflect the special characteristics of ENM and are not developed for ENM risk assessment. Here we propose a high-throughput cell-based assay using human peripheral blood mononuclear cells (PBMCs) that can monitor the effects of NM and ENM on cytotoxicity and innate immunity. The proposed assay is fully automated and miniaturized, with excellent assay performance parameters (Z-prime-score >0.5), amenable for large screening campaigns in an industrial setting. Immunotoxicity data for ENM safety assessment are collected in dose-response format. At different states, multiparametric readouts for cytotoxicity, and innate immunity are conducted in a combinatorial method, avoiding ENM-induced bias by endotoxin contamination. Integrating this high-dimensional data allows (i) holistic safety assessment of immunotoxicity effects caused by ENM, classifying safe and toxic ENM phenotypes, and (ii) deconvolving mode of action of the ENM effect on the PBMCs. As added value the data obtained can be used to troubleshoot ENM or for a safe-by-design approach in product development.

LP-70

Combining organ-on-a-chip and modeling to predict liver toxicity

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Despite the important contribution of animal experiments to the evaluation of drug safety in preclinical studies, ethical concerns and limited predictive power for clinical outcome drives the quest for alternative human-relevant models. Recent developments in organ-on-a-chip (OoC) microfluidic devices with interconnected tissue-engineered cultures have shown great promise to mimic human biology better than conventional systems.
Here, we present our exploratory study on the opportunities of combining OoC technologies with toxicokinetic and toxicodynamic (TK/TD) modeling to facilitate the prediction of the exposure-effect relationship and enhance safety assessment in preclinical studies for the endpoint of liver toxicity.
In a first step, we experimentally evaluated the OoC system using a human cell line-based gut co-culture model and qualified donors for the primary human liver model. We employed quantitative TK modeling to design experiments of well-known tool compounds on a gut-liver and liver-only OoC system with physiologically relevant exposure

conditions. Additionally, we mathematically assessed the clinical translatability of the data obtained from the OoC systems.
The intrinsic pharmacokinetic parameters of all studied tool compounds were determined by performing a model-based analysis of the obtained data. The mathematical modeling also addressed the common challenges associated with OoC systems, including media evaporation and compound loss due to non-specific binding to the chip material. To explore the potential of OoC systems in safety assessment, a TD model was added to the TK model to account for adverse effects occurring in drug induced liver injury (DILI). We showed that TK/TD modeling of the processes taking place on a gut-liver OoC system can better predict the relationship between drug concentration and its hepatotoxic effect.Our results show that combining OoC experiments with modeling & simulation can increase the translatability of this type of innovative preclinical methodology and thereby improve the safety assessments in preclinical development.

LP-71

Establishment of an in vitro method of rabbit embryo toxicity with toxicokinetics study

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This report introduces a novel method, rabbit whole embryo culture (WEC) combined with toxicokinetics (TK), for toxicity testing. Rodent WEC has been extensively used for in vitro screening of developmental toxicity. To improve the reliability of in vitro data, it is important to consider TK and species specificity. To test the utility and effectiveness of this method, we investigated the toxic effect of thalidomide on rab-bit embryos and its behavior in test systems both in vitro and in vivo under the same experimental condition. The data showed that thalidomide induced embryo malformations such as embryonic brain hypoplasia, short limb buds, and declined embryonic growth both in vitro and in vivo. The toxic effect increased with the increasing exposure of the embryo to thalidomide. In addition, we observed similar toxic effects and exposure–effect relationships in vivo and in vitro. Therefore, we preliminarily conclude that this new method can effectively predict and explain thalidomide toxicity. Furthermore, we investigated the behavior of test compounds in the WEC system for the first time, and this method is expected to be an important technique for in vitro toxicity study after extensive verification.

LP-72

Qilin Pill Exerts Therapeutic Effect on Resection-Induced Premature Ovarian Insufficiency Rats by Inhibiting the MAPK and PI3K-AKT Signaling Pathways

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Background: The Qilin pill (QLP) is a traditional Chinese compound prescription comprising 15 herbs that has demonstrated significant therapeutic effects on premature ovarian insufficiency (POI) in recent years. However, a pharmacological evaluation of QLP on ovarian function remains to be conducted, and the key mechanism of QLP treatment on POI is unclear.

Methods: Premature ovarian insufficiency rats were established by bilateral partial ovariectomy. The model rats were administrated with low (QLP-L), medium (QLP-M) and high (QLP-H) doses of QLP for 4 weeks to evaluate the ovarian function in terms of estrous cycle, hormone level, and follicle count. The mechanism of QLP in the treatment of POI was systematically explored by network pharmacology, and expression levels of the MAPK and PI3K-AKT signaling pathways were verified by Western blotting and molecular docking.

Results: The rat model of resection-induced POI was successfully established, and QLP could significantly recover the estrous cycle, decrease serum FSH levels, and decelerate follicle depletion after 4 weeks of administration. The optimal dose of QLP in the experiment was preliminarily determined to be 0.9 g/kg. Based on the network pharmacology methods, we constructed the compound-target network and protein protein interaction (PPI) network of QLP for the treatment of POI. The experimental verification of the enrichment analysis showed that QLP inhibited the MAPK and PI3K-AKT signaling pathways, and the key compounds and key targets involved were verified by molecular docking.

Conclusion: QLP exerted significant therapeutic effects on resection-induced POI rats, and this was achieved by the inhibition of the MAPK and PI3K-AKT signaling pathways. This study is the first to systematically investigate the effects and mechanism of QLP on POI rats, which will provide valuable guidance in clinic.

LP-74

Use of alternative to animal testing for developmental and reproductive toxicity endpoints under REACH (EC 1907/2006): A need for guidance and validated integrated approaches

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Directive 2010/63/EU on the use of animals for scientific use and REACH (EC 1272/2008) regulation set animal experimentation for registration purpose as only bearable in last resort, for environment or health protection purpose. Yet, the number of animals used in the context of compliance to REACH regulation is still significant, mainly due to animal studies in standard data requirements. For instance, no less than 2500 animals are required in the three standard reproductive and developmental toxicity studies (i.e. OECD TG 443 and OECD TG 414 in two species) constituting a minimal data requirement for the highest tonnage band (substances registered above 1000 tonnes per

annum (tpa). Based on an analysis of the testing proposals submitted to ECHA, the current use of laboratory animals for investigating reproductive and developmental toxicity in the context of > 1000 tpa REACH registrations is estimated to be approximately 100 000 / year. This figure is not expected to decrease in the upcoming years due to a combination of different factors: the high number of substances registered above 1 000 tpa for which these endpoints are not clearly compliant (according to Cefic 2023 REACH Action Plan report), the lack of alternative methods to animal testing suitable for fulfilling the high tier data requirements, and difficulties encountered by registrants in applying other REACH dispositions for adapting the data requirement.
Weight-of-evidence analysis and read-across from analogue substances are two major adaptations of the data requirement under REACH. The existing dataset may need in some cases to be completed by newly generated *in vitro* data for which no standard integrated approach is available. Interestingly, an in vitro assays battery is close to validation for the evaluation of Developmental NeuroToxicity and another one is under development for Developmental ImmunoToxicity, two aspects optionally investigated in the OECD TG443 study. It opens the way to the routine use of new approach methodologies (NAMs) for regulation purposes.
However, the regulatory compliance for using these approaches is not as straightforward as by generating data in animal experiments. However, the acceptability of approaches that are different from standard data requirement is not warranted, especially when no guidance is available. In particular, guidance is needed to better characterize acceptable Weight-of-Evidence analysis implying *in vitro* data generation.

In this context, there is a clear **need for the development of *in vitro* batteries accepted in REACH context** for reproductive and developmental toxicology, and for further **guidance to improve the use of existing data**.

LP-75

3D *in vitro*-based Alternative Approaches for Ecotoxical Assessment

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In the context of bioethics and the principle of the 3Rs (replacement, reduction, and refinement), there is a growing need for reliable methods to evaluate the impact of endocrine-disrupting chemicals (EDCs) on the ecosystem, as alternatives to animal testing. However, conventional fish cell cultures have various limitations compared to primary cells. This study aims to explore the feasibility of using a zebrafish liver (ZFL) cell line in three-dimensional (3D) cell culture techniques, along with *in silico* systems, to develop innovative methods for assessing reproductive toxicity. The 3D structure of the zebrafish estrogen receptor was generated to explore modes of action upon estrogenic chemical exposure. The integration of the structure model and *in vitro* platforms proved to be valuable tools for screening estrogenic EDCs. Moreover, the 3D ZFL spheroids exhibited enhanced gene expressions related to liver functions and higher vitellogenin levels compared to monolayer cells. The 3D spheroid incorporated with nanofibers presented the regulation of physiological function and intercellular organization, improving the shortcomings of the spheroid culture. The findings demonstrate that engineering novel 3D platforms hold great promise as an alternative approach for eco-environmental assessments. As a result, these studies have successfully introduced robust alternative platforms to animal and primary cells for identifying potential EDCs, aligning with the 3Rs principle and contributing to the reduction of animal experimentation.

LP-76

Dioxin up-regulated the expression of neurofilaments in neuronal cells via AhR and MAPK pathways

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Dioxin and dioxin-like compounds form a group of persistent organic pollutants that can accumulate in humans and animals and persist for an extended period. Dioxin exposure is reported to affect nervous system development and increase the risk of neurodegenerative diseases. Generally, dioxin exerts its neurotoxicity via aryl hydrocarbon receptor (AhR). Neurofilament (NF) light (NFL) protein is a biomarker for both neuronal differentiation and neurodegeneration and its expression is controlled by the mitogen-activated protein kinase (MAPK) pathway. However, the effects of dioxin on NFL expression and involved mechanisms are incompletely understood. We aimed to investigate the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on NFL expression and elucidate the underlining signaling pathways and their potential crosstalk, specifically between MAPK and AhR pathway. We employed primary cultured rat cortical neurons to evaluate the effect of TCDD exposure on NFL expression. We also used nerve growth factor (NGF)-treated PC12 cells with specific inhibitors to investigate the involvement of and potential crosstalk between the MAPK pathway and the AhR pathway in mediating the effects of TCDD on NFL expression. After TCDD exposure, NFL mRNA and protein levels were upregulated in cultured neurons. NFL protein was preferentially found in the cell body compared with neurites of the cultured neurons. In PC12 cells, TCDD enhanced both NGF-induced NFL expression and phosphorylation of ERK1/2 and p38. The addition of MAPK-pathway inhibitors (PD98059 and SB230580) partially blocked the TCDD-induced NFL upregulation. CH223191, an AhR antagonist, reversed the upregulation of NFL and phosphorylation of ERK1/2 and p38 induced by TCDD. This study demonstrated TCDD-induced upregulation of NFL in cultured neurons, with protein retained in the cell body. TCDD action was dependent on activation of AhR and MAPK, while crosstalk was found between these two signaling pathways.

LP-77

Highly scalable and automation-compatible organ-on-chip platform

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Objectives

Recent developments in organ-on-a-chip (OoC) models have provided unprecedented tools as alternative to animal experimentation. However, their implementation has been hampered by low throughput and the absence of instrumentation-free systems.

We present a commercially available, standardized and scalable OoC platform termed the AKITA Plate. The microfluidic platform allows a relevant modeling of immune components of vascularized organs and biological barriers (blood-brain, epithelial-endothelial) and organoid/spheroid culturing. Compatible with standard multiwell plate formats, the AKITA Plate is an easy to use system.

Methods

The AKITA Plate layout is a standard 96 well (32 tests) or 384 well (128 tests) plate format which enables automated handling and confocal imaging. With an integrated microporous membrane this platform allows establishment of a co-culture with static conditions in one compartment and dynamic conditions in the other. The AKITA plate allows the

test of immune cell migration and the monitoring of biological barrier tightness upon inflammation either with fluorescent probes, confocal imaging or by measuring transepithelial electrical resistance with AKITA Lid device.

Results

The AKITA Plate has the ability to improve the structural and functional complexity of currently existing human organ models, such as biological barrier, air-liquid interfaces, and vascularized organoid models. With AKITA Plate, we have managed to monitor the migration of immune cells in vascularized in vitro tissues. Immune cell migration is mediated by the bilateral flow which also improves the tightness and maturation of the organ models.

Conclusions

In conclusion, we established a commercially available low to high throughput microfluidic platform for monitoring the inflammation and immune cell migration in multiple organ models. With our standardized and translational platform, we provide a better alternative to animal testing, and most importantly, accelerate therapeutic studies as well as drug discovery processes.

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In Silico Site-directed Mutagenesis of TAS2R Enzymes Integrated with Molecular Modelling Techniques to Predict Chemical Susceptibility to Toxic Compounds

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Bitter molecules can be perceived through a class of taste receptors belonging to the G-protein Coupled Receptor (GPCR) Taste Type 2 Receptor (TAS2R) family collecting about 6000 protein sequences. These receptors are expressed in various tissues, not only in the lingual epithelium one, and have recently been found to play roles beyond their primary function in bitter perception [1]. Indeed, the functional relationship between TAS2R and toxic molecules is most likely beyond sensing potentially noxious compounds as bitter and reasonably takes a part in their underpinning mechanism of action. Due to this fact, they attracted significant interest as potential targets for pharmacological treatment of various disorders. Focusing on humans, there are 25 expressed TAS2Rs and 2 putative TAS2Rs genes as per UniProt (https://www.uniprot.org/, last database access 20th of July 2023). Of note, the genes coding for TAS2Rs in humans are likely to be generated after recent duplication events and characterized by a high rate of amino acid substitution [3].

This work provides an *in silico* framework to study the impact of single point mutations on the human TAS2R46-ligand complex architecture of binding and stability taking strychnine, a well-known toxic alkaloid able to bind TAS2R46, as reference molecule. The 247 generated mutant structures were processed via molecular modelling and docking studies to verify the architecture of binding of strychnine upon comparison with the native strychnine-TAS2R46 crystal structure (PDB ID 7XP6). This revealed a series of TAS2R46 mutants theoretically ameliorating the binding of this alkaloid, alongside another set of mutants causing reduced binding capabilities. A bioinformatic approach was used to weight the mutants' likeliness to occur. In addition, molecular modelling studies were extended to a series of possible toxic TAS2R46 ligands retrieved from the T3DB database (http://www.t3db.ca/), for which this receptor may have a role regarding their toxic action.

This work presented an *in silico* new approach methodology to deepen our understanding on the capability of single point mutations occurring in a bitter receptor binding site of either enhancing or diminishing its capability of binding toxicant compounds. Moreover, it allowed us to investigate the possible role of bitter receptors in some mechanisms of toxicity highlighting how some mutants might affect the toxicity of certain toxicants.

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LP-79

A Bioinformatic Approach to Explore Zearalenone Estrogenicity: How a Single Nucleotide Variant in the Aromatase Enzyme Could Affect Its Inhibitory Activity

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Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin produced by *Fusarium* fungi. Its worldwide occurrence in cereal crops and in grain-based products represents a concern to human and animal health due to its endocrine disrupting effects such as precocious puberty, premature thelarche and carcinogenic actions [1]. In view of that, The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain established a tolerable daily intake for ZEN of 0.25 µg/kg body weight [2].
The chemical structure of ZEN resembles 17β-estradiol and it exerts its estrogenic activity competing with estradiol for activating estrogen receptors (ERs) and inhibiting aromatase (CYP19A1). ERs and aromatase are known to show single nucleotide variants (SNVs) among human population. These variations likely affect the capability of ZEN to interact with both of them, eventually resulting in a different activity compared to the wild type enzymes. Furthermore, some of these mutations have been previously reported as relevant from a clinical perspective being found in different kinds of cancer.
This work focused on human aromatase with the aim of investigating via 3D molecular modelling whether mutations occurring among individuals may affect the inhibitory potential of ZEN. Specifically, 434 SNVs were retrieved from the UniProt database (https://www.ncbi.nlm.nih.gov/snp; last database access 20th of July 2023). Then, molecular docking and dynamics simulations were performed in order to study the punctual effects of a selection of mutations on the inhibitory activity of ZEN.
The study highlighted the existence of different SNVs responsible for a different aromatase susceptibility to ZEN. As an example, depending on the amino acids substitution, mutations of T310 and D309 may have opposite effects either ameliorating the ZEN-aromatase interaction (likely resulting in an enhanced inhibitory activity) or in a loss of interaction.
In conclusion, this work showed the existance of aromatase variants with enhanced or reduced susceptibility of being inhibited by ZEN. Moreover, the clinical relevance of some of these variants pointed out the possibility that ZEN may have a huge impact on certain subjects. These findings could represent a sound starting point for further dedicated investigations.

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LP-80

Feeding rate as a phenotypic endpoint for pollution assessment

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The toxicological hazard and safety assessment of chemical substances relies on the outcome of animal testing. A combination of mortality, phenotypic and molecular endpoints are employed to assess this, however, animal welfare considerations, societal concerns, and regulatory action highlight the need for novel approaches methodologies in risk assessment. In this context, non-invasive tests and model species not categorized as "animals" can be used to reduce the use of higher animals according to the 3Rs principle. Such tests can provide comparative conclusions with faster and more economical approaches. Focusing on the freshwater ecosystem, daphnids have been extensively used for toxicological studies, and their feeding rate following exposure to pollutants is a common phenotypic endpoint in ecotoxicology assessment. Feeding impairment indicates early alterations in animal physiology, thus providing insight for further investigation. The feeding rate is usually assessed with extended incubation periods and large volumes of media, resulting in increased waste generation and use of animals, which highlights the need for improved standardized methods. In this study, we developed a robust approach based on tracking the ingestion of fluorescent microparticles. Parameters such as the total volume, the concentration of particles, and the number of daphnids were optimized to study the impact of a selection of metals and pharmaceuticals on feeding. There was a concentration-dependent decrease in feeding rates for most of the pollutants used, indicating their strong effect on the physiology of the animal. This method demonstrates an efficient means of assessing toxicology to guide future studies in working concentrations of chemicals and the assessment of water quality.

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Comparative of *Actinobacillus pleuropneumonia* Autogenous and Commercial Vaccine Application Towards Antimicrobial Residues in Pig Farm, Thailand

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The antimicrobial resistance and antimicrobials residue in livestock, particularly in the swine products, are major concern in public health and causes human health risk. Autogenous vaccination during nursery piglets until finisher as routinely vaccine program enable solution towards reduction of antimicrobial resistance and antimicrobial residue. This study aimed to explore the difference of antibiotic residues between a commercial and autogenous *Actinobacillus pleuropneumonia* (APP) vaccine application in a pig farm in Thailand. Pig blood samples as well as drinking water, pig manure, wastewater at pre-biogas and post-biogas sludge were collected for 4 times from January to June, 2023. The 20 antimicrobial drugs including Sulfadiazine, Lincomycin, Trimethoprim, Oxytetracycline, Amoxicillin, Norfloxacin, Ofloxacin, Levofloxacin, Ciprofloxacin, Enrofloxacin, Ampicillin, Chlortetracycline,

Sulfamethoxazole, Doxycycline, Tetracycline, Erythromycin, Tylosin, Nalidixic acid, Tiamulin, Clarithromycin were then analyzed using LC-MS/MS, Thermo Scientific (TSQ Endura). Doxycycline and Tetracycline found in the blood of autogenous group of which tended to be lower when compared with a commercial one. Only amoxicillin, doxycycline and tetracycline were found in manure. Although there is no significant different of certain antibiotic found in blood between autogenous and commercial APP vaccine used in pig farm. However, the further study on impact of autogenous vaccine uses should be done in a greater number of farms.

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LP-82

Chronic exposure of healthy participants to semi-volatile organic compounds model using an optimized aerosol delivery system

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Semi-volatile organic compounds (SVOCs) are present in a plethora of professional and household products (i.e., pesticides, cosmetics, plasticizers, floor coverings and furnishings)[1]. Several SVOCs have shown endocrine disrupting effects and are labeled as chemicals of concern[2,3]. They are often present in the indoor environment where adults spend an estimated 19 to 21 hours a day[4]. SVOCs are mainly found in the air as aerosols and settled on dust particles[5]. Although inhalation is an important route of exposure, there is a lack of human studies to understand SVOCs toxicokinetics. The reason is the absence of reliable techniques for measuring the inhaled dose and practical device for use with human participants. Our pilot study is the first step to fill this gap. We optimized an aerosol delivery system (ADS) for SVOCs to generate known inhalation exposures. Our first study objective was to assess the feasibility of recruiting participants for a repeated inhalation SVOC exposure over a period of one week with the ADS. The second objective was to improve an experimental design for future comprehensive toxicokinetic study with participants. Our chosen model SVOC was di(2-ethylhexyl) phthalate (DEHP). Ring-deuterated DEHP (DEHP-d4) was used to differentiate the experimental exposures to DEHP from environmental contaminations. Occupational exposure limits (OELs) are time-weighted average air concentrations of hazardous substances for which workers can be repeatedly exposed (8-hour workday and 40-hour workweeks) for a working lifetime without adverse effects[6]. The exposure dose in our study was calculated (0.45 mg/week) after quantification of DEHP-d4 in the generated aerosol and set below the Swiss OEL for DEHP. Participants (N=4) used the ADS with DEHP-d4 at home twice daily (morning and evening; 40 puffs in 10 minutes) for four days. Urine samples were collected before exposure (day 1), and then four times per day (upon waking, at noon, late afternoon and at bedtime) the rest of the study week. Four urinary DEHP-d4 metabolites were quantified: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP) and mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP).The participants reported that the optimized ADS was easy-to-use and that the study protocol was understandable. Labelled metabolites were successfully found in the urine samples following the DEHP-d4 exposure concentration. The metabolite concentrations increased over the exposure week indicative of a possible accumulation. This pilot study shows that future toxicokinetic studies with healthy participants can be conducted with controlled exposures to SVOCs using this optimized aerosol delivery system.

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A Novel Microwell Platform for Organoid Culture Standardization: Intestinal Toxicity Assessment via High-Content Imaging and Phenotypic Analysis

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In recent years, organoids have emerged as a game-changer for disease modeling and drug screening. These organoids are three-dimensional, miniaturized, and simplified versions of an organ that mimic some of the key features of the native tissue *in vitro*[1]. Traditional organoid culture methods embed these structures in solidified extracellular matrix (ECM) thus introducing an intrinsic lack of reproducibility and creating highly heterogeneous organoid populations. Moreover, organoids are randomly distributed within the ECM, complicating subsequent readouts and image analyses[2].

To overcome these challenges, we used Gri3D®, a ready-to-use platform for high-throughput and reproducible organoid culture[3]. Based on a standard 96-microtiter plate, each well contains a dense microwell array patterned in a cell-repellent hydrogel. The platform enables homogenous cell seeding, efficient cell aggregation, and subsequent formation of a single organoid per microwell in suspension-like conditions. A uniquely designed pipetting port, adjacent to each well, allows safe media exchange for long-term cultures. The resulting organoids are positioned in predefined locations in the same focal plane, allowing simultaneous tracking at high resolution.

Combined with the ImageXpress® Micro Confocal system, we follow the development and self-organization of healthy human rectal organoids over time with brightfield imaging. Using an AI-based approach, we efficiently detect every single organoid and characterize its size, diameter, as well as complex morphological features such as the lumen. Finally, we investigate the concentration-dependent toxicity of a small panel of compounds on human rectal organoids using AI-based brightfield image analyses and fluorescence-based readouts.

The combination of a high-density microcavity array culture approach and a high-content imaging system together with machine-learning algorithms allows the assessment of phenotypic features at a single-organoid level in an automatable high-throughput fashion. The presented approach has high potential in solving key challenges related to disease modeling and compound assessment at large scale using organoids.

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LP-84

Liver toxicity of therapeutic antisense oligonucleotides in 3D primary liver cell spheroids– a promising novel *in vitro* test for hazard identification

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Antisense oligonucleotides (ASOs) are a promising therapeutic modality to modulate the expression of disease-causing RNA molecules, but their clinical development is hampered by hepatotoxicity. In preclinical and clinical safety evaluation, ASOs concentrate in the liver and can trigger Liver enzyme leakage. Investigative toxicology studies ASOs can induce a range of deleterious effects such as the induction of apoptosis, off-target effects, and the saturation of RNA processing machinery. 3D primary liver cell spheroids are regularly used as in vitro tools to study the hepatotoxic potential of small molecule drugs, however their relevance to study the hepatotoxic potential of ASOs has yet to be established. This work evaluates the relevance of the 3D primary liver cell spheroids to study the hepatotoxic potential of ASOs using a set of preclinical and clinical ASOs. In the first part of this work, the uptake of ASO by the model and the resulting downregulation of the RNA target was assessed. Histochemistry results show that ASOs are readily and uniformly taken up by the model, reaching the inner core of the cell spheroid. Also, quantitative PCR analyses show that treatment with ASO leads to a strong downregulation of the target RNA molecule lasting up to 14 days. In the second part of this work, the in vitro cytotoxicity of the ASOs was assessed by monitoring viability of the model (Cellular ATP content), cytosolic leakage (LDH leakage) and the induction of apoptosis (cellular Caspase 3/7 activity). Interestingly, the in vitro cytotoxicity of the ASOs correlates with their hepatotoxicity reported previously, with the viability measurement being the most sensitive biomarker. Finally, several ASOs led to an induction of apoptosis in the model, albeit at very different magnitudes, suggesting that this is a common hazard for ASOs. Altogether, these results suggest that 3D primary liver cell spheroids are a promising in vitro tool to assess the hepatotoxic potential of ASOs. In future investigations, more clinical ASOs will be assayed using the same approach.

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Primary human liver spheroids are scalable, industrial grade micro physiological systems (MPS) for liver safety assessment of small molecule drugs

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Background: Hepatotoxicity is a severe safety concern that can cause the discontinuation of the development of drug candidates. MPS raised high expectations that their use in industrial practice would improve hepatoxicity assessment. However, only a few MPS effectively made it beyond proof-of-concept into the regular industrial workflow. Among the available 3D MPS, primary human hepatic spheroids accurately model the essential features of the native liver and their scalability, ease-of-use, reproducibility, and miniaturization make them compatible with standardized industrial high-throughput applications. Moreover, primary human hepatic spheroids predict hepatotoxicity more accurately than 2D primary human hepatocyte cultures.

Objective: It was the objective to further evaluate the relevance of the 3D liver microtissues by testing drugs from FDA DILIrank database after normalization by the corresponding plasma exposure, in a 7 day cellular ATP based assay.

Methods: Drugs from the FDA DILIrank dataset were selected for cytotoxicity testing in primary human hepatic spheroids from InSphero AG Switzerland. The 7-day cellular ATP IC50 values of 82 drugs were determined and normalized by their corresponding human exposure data (total plasma Cmax). The ATP IC50 to total plasma Cmax ratios (Ra/c) were compared to the FDA classifications of “Most- and No-DILI-Concern” as well as to the available “Severity Scores from 1-8” and “Drug Label Information”.
Results: The Ra/c of the 82 drugs correlated well to the FDA reported *in vivo* hepatotoxicity. Ra/c scores below 90 flag 80.6 % of the “Most-DILI-Concern” drugs, whereas 84.2% of “No-DILI-Concern” drugs are correctly predicted as safe. Beyond the clear separation of “no-DILI” and “Most-DILI-concern” drugs, the “DILI Severity Scores from 0 to 8” and “Drug-label Information” provided additional specific information which further increased Sensitivity up to 92 %.
Conclusions: The unbiased selection of 82 DILI validation drugs from the FDA DILIrank database and the normalization of the obtained ATP IC50 values by the corresponding human plasma concentration were considered as major advance of this study showing the high predictive value and relevance of the model. Beyond Hazard identification in the Drug Discovery Phase, Toxicologists see high value of the regular application of the InSphero DILI test for supporting their Medicinal Chemists from all Therapeutic Areas. The standardisation and high data quality of the 384 well plate experiments allow to investigate large volumes of compounds in an appropriate turnaround time. This matches the key industrial requirements as needed for QSAR studies in the lead optimization phase.

LP-86

Possible teratogenic effects mediated by seminal plasma exposed to thalidomide in rabbits

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In this study, the risk of teratogenesis in female rabbits via male rabbit semen was evaluated using thalidomide as a representative teratogen. Liquid chromatography-mass spectrometry was performed to detect thalidomide and its 5-hydroxylated (major metabolite in humans), and 5’-hydroxylated (major metabolite in rodents) metabolites in seminal and blood plasma to analyze the seminal transition after oral thalidomide treatment at 250 mg/kg in male rabbits. Thalidomide concentrations and the two metabolites in seminal plasma were equivalent to those in plasma. Based on this toxicokinetic information, we administered 0.4 mg/kg of thalidomide, which was 100 times the maximum seminal plasma transfer concentration, intravaginally to female rabbits on gestational days 1 to 13. These findings demonstrate that intravaginal thalidomide administration did not induce any teratogenic effects in rabbit fetuses. Additionally, thalidomide concentrations and two metabolites in the placenta, yolk sac, and fetus were not influenced by implantation position in the uterus. The Physiologically based pharmacokinetic model developed based on a single oral study revealed that the measured and estimated thalidomide concentrations and its metabolites were in close agreement shortly after administration. These results revealed that after vaginal administration, thalidomide could reach the uterus via the systemic circulation and not via direct exposure through the external cervix. The comparison of the pharmacokinetics of intravaginal and oral administration showed significant differences in the maximum concentration and the area under the curve up to the last quantifiable time-point, that is, differences of 2,500 and 5,000 times, respectively. Thus, the exposure concentration to living organisms by vaginal administration is considered extremely low compared with oral administration. Therefore, this study concluded that semen-mediated thalidomide teratogenic effects are absent in rabbits.

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Fumonisin B1 inhibits p53 dependent apoptosis via HOXA11-AS/miR-124/DNMT axis in human liver cells

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FB_1 is a hazardous mycotoxin that induces toxic and carcinogenic effects in humans and animals. FB_1induces changes to the epigenome which provide insight into its toxic and carcinogenic nature. The lncRNA HOXA11-AS influences the epigenome by modulating DNA methylation functioning as a competing endogenous RNA (ceRNA) or molecular scaffold. However, the role of HOXA11-AS in FB_{-1}-toxicity is unknown. Therefore, we investigated the effect of FB_{-1} on p53-dependant apoptosis via the HOXA11-AS/miR-124/DNMT axis. HepG2 liver cells were treated with various concentrations of FB_1 (0, 5, 50, 100 and 200 µM; 24 h). qPCR and/or western blotting was used to determine expression of HOXA11-AS, miR-124, SP1, , DNMT3B and p53. p53 promoter methylation was assessed, whilst luminometry was used to measure caspase activity. FB_1 upregulated HOXA11-AS ($p \leq 0.05$) leading to the subsequent decrease in miR-124 ($p \leq 0.01$) and increase in SP1 ($p \leq 0.001$), and DNMT3B ($p \leq 0.001$). This promoted the hypermethylation of p53 promoters ($p \leq 0.001$)thereby reducing p53 expression ($p \leq 0.001$) and caspase activity ($p \leq 0.001$). Taken together the data suggests that FB_1 inhibits p53-dependent apoptosis via HOXA11-AS/miR-124/DNMT axis in HepG2 cells.

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LP-89

Etanol neurotoxicity in SH-SY5Y cells is prevented by harmine but notdimethyltryptamine

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Introduction: Abusive ethanol consumption may lead to alcohol use disorder (AUD). Its neurotoxic effects are still not clear, although oxidative stress is involved in neuronal apoptosis induced by ethanol, its neurotoxic effects remain unclear. Ayahuasca is a traditional tea with psychoactive effects used in spiritual rituals. Some studies have demonstrated that tea extracts block the expression of ethanol sensitization, showing its possible neuroprotective effect against ethanol, and may be considered as a possible therapeutic intervention in the treatment of AUD. However, *in vitro* studies regarding the neuroprotective effect of the main compounds of ayahuasca tea are still scarce.

Objective: This study aims to evaluate the neurotoxicity and neuroprotective potential of dimethyltryptamine (DMT) and harmine (HRM) against ethanol-induced neurotoxicity in SH-SY5Y neuroblastoma cells.

Methodology: A concentration-response curve (CRC) was obtained for both DMT and HRM (0,1, 1, 10, 100 and 1000 µM of each), and also for their combination (1:1, 1:2, 1:5 and 1:10 – DMT: HRM). The highest concentration without

neurotoxic effect (NOAEL) was determined for both drugs. Incubations were performed for 48h in SH-SY5Y human neuroblastoma cells. Subsequently, the NOAEL of DMT and HRM were incubated for 48 hours in the presence of the lethal concentration 50 of ethanol (250 mM). Cell viability tests were performed using MTT assay, with absorbance reading at 595 nm.
Results: The NOAEL for DMT and HRM were 100 µM and 10 µM, respectively. Thus, the concentration chosen for both substances to continue the experiment was 10 µM. The NOAEL for the substances fraction was 1:2. DMT–ethanol association did not show statistical significance compared to ethanol alone. However, HRM–ethanol association and DMT/HRM fraction-ethanol association were able to prevent ethanol neurotoxicity.
Conclusion: These data suggest that HRM and DMT/HRM fraction, but not DMT, have demonstrated neuroprotective potential, and further studies are needed to understand these interactions.

LP-90

AIRATox: An AutoML platform for *in vivo* toxicity prediction

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Objective

Compound toxicity and adverse effects resulting from chemical exposure are essential regulatory factors for the pharmaceutical industry. There is an increasing demand for computational models to replace or augment traditional in vivo toxicity tests conducted on laboratory animals to reduce animal sacrifice (for example, European Union REACH/3R principles, Tox21 and ToxCast by the U.S. government). This study provides a multi-modality, multi-endpoint AutoML platform for predicting in vivo toxicity.

Materials and Methods

We developed a desktop platform in order to facilitate the training, validation, and testing of several different toxicity models. The platform employs an innovative feature extraction technique by bringing together user-defined and non-user-defined characteristics. A self-supervising Graph Neural Network [1] model is trained using a considerable quantity of the SMILES database that is available in the public domain so that a compound feature library may be trained. The system contains baseline models that were created from data that is available in the public domain for the purpose of predicting liver and kidney in vivo toxicity, drug-induced liver injury, liver carcinogenicity, skin sensitization, and toxicogenomic for four different endpoints. Do-it-yourself (DIY) features are included in the system, allowing users to retrain baseline models or construct new models without having prior knowledge of programming or machine learning. The system is able to take information from a wide variety of sources, such as SMILES, biochemistry, haematology, and genomics databases.

Results

The AUC ranged from 74% to 86% for the various baseline models generated by the system. We predict toxicity outcomes for individual rats solely based on their genomic data, achieving AUCs of over 79% for Necrosis, 86% for Hypertrophy, 83% for Microgranuloma, and 83% for Cellular Infiltration using data from rodents.

Conclusions

We offer a platform that allows users to train, validate, and evaluate machine learning models using SMILES data on their own using a do-it-yourself approach. The technology is meant to ensure optimal data security because there is no information exchange regarding molecules outside of the pharmaceutical company. It is feasible that in the future, the platform will be expanded to include a federated learning framework in order to achieve scale efficiencies.

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LP-91

Unveiling Drug Responses in Liver Spheroids: Multiplexing 3D Cell-Based Assays and Imaging in a Microwell Platform

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The critical need to minimize animal testing and reduce drug attrition rates has resulted in the development of more complex *in vitro* models. Notably, **3D cell cultures** have emerged as a significant alternative due to their ability to mimic the complex *in vivo* microenvironment, providing more physiologically relevant results compared to traditional 2D cell cultures. Liver spheroids were among the initial applications of 3D models for toxicity assessment, significantly enhancing the safety and efficiency of potential drug candidates' evaluation[1].

However, the widespread adoption of 3D cell cultures faces challenges related to **reproducibility**, **automation**, and **tissue loss** using conventional technologies. To address them, we present an innovative solution - **Gri3D®: a novel hydrogel microwell 96 wellplate**, where each well features a microwell array in a cell-repellent hydrogel[2]. This design promotes **uniformcell seeding, efficient aggregation,** and the formation of a single microtissue per microwell under **suspension-like conditions**. The resulting microtissues are positioned within the same focal plane, enabling simultaneous high-resolution tracking. The platform also incorporates a unique pipetting port for safe media exchange and allows to perform **multiple on-plate assays**.

In the study, we grew Upcyte® hepatocyte spheroids[3] simultaneously in Gri3D® and U-bottom 96 well plates to compare ease of use, performance in terms of volume, required steps and time, sensitivity, detection window, and scalability for multi-assays. We then exposed the microtissues to inducers and inhibitors of liver enzyme activity and assessed CYP (cytochrome P450) activity and microtissue viability. The plates were evaluated on a Tecan Spark® Cyto, with the readouts encompassing fluorescence and luminescence intensity measurements, and brightfield and fluorescence imaging in each well.

Our innovative **3D multiplexing approach** allowed us to gain valuable insights into the impact of different drugs on CYP activity and microtissue viability, all within a single experiment. By adopting the Gri3D® platform and a compatible multimode plate reader, along with multiplexed cell-based assays, allowed for efficient simultaneous analysis of multiple factors, advancing our understanding of liver spheroid's response to drug exposure. This approach shows immense potential in addressing critical challenges related to **compound assessment and drug screening on a larger scale** using 3D cell cultures.

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LP-94

Pharmacokinetics of polystyrene nanoparticles in the chicken embryo

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Nanomaterials have a particle size in the nano range (≤ 1 µm). They have been widely used in different fields such as electronics and medicine. One type of nanomaterials are nanoplastics, and they are being actively considered as candidates for drug delivery vehicles in nanomedicine. The vascular injection (intravenously) of nanomedicine is one of the routes of administration being considered. It will therefore be important to explore the distribution and excretion (pharmacokinetics) of nanoplastics in the body before they can be approved as therapeutic treatments. Very little is known about the pharmacokinetics of plastic nanoparticles. Here, we describe the fate of (i) 150 nm europium-tagged polystyrene nanoparticles and (ii) 1 µm polystyrene nanoparticles with a green fluorescent tag (Ex/Em = 460/500 nm) injected into the vitelline vein of stage 17–25 chick embryos *in ovo*. The embryos were analyzed, at stage 25–35 consistently. Using quantitative image analysis we find that the nanoplastics are mostly concentrated in the heart, liver and kidneys with much less being found in eyes, brain, intestine and liver. We also find that nanoplastics adhere to migratory cardiac neural crest cells, which later contribute to the ventricular septum of heart. Finally, we found no evidence that chick embryos eliminate the plastic nanoparticles by secreting them into the allantoic fluid. These data suggest that the chicken embryo is a useful model for studying the pharmacokinetics of plastic nanoparticles and could help in the pre-clinical phase of nanomedicine testing. Moreover, performing pharmacokinetic experiments provide us great opportunity to do quantitative assessments on biodistribution of plastics nanoparticles.

LP-95

SaferSkin Case Studies: Comparison of Skin Sensitization Risk Assessment Results based on different Integrated Approaches to Testing and Assessment

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Purpose

Characterising known and new chemical compounds for skin sensitisation provides a basis for the development of safer products where ingredients are exposed to skin. By including new approaches such as tiered testing strategies and integrated data analysis it is possible to develop next generation products adhering to emerging regulations, scientific evidence and animal welfare principles. To ensure data integrity during such assessment the OECD provides characterisation guidelines and Defined Approaches (DA) to uniform workflows. In this study we developed and applied an integrated characterisation tool called «SaferSkin» to compare the results of different DA for eight compounds and included results obtained from current OECD guidance and emerging methods. We tested two compounds with unclear or indeterminate results with the SENS-IS assay to explore the value of the experiment in strengthening the weight of evidence and arriving at a clearer conclusion.

Methods

To evaluate compounds as possible skin sensitisers, we used integrated data analysis with different approaches applied to eight compounds: farnesal, safranal, 2-butoxyethyl acetate, 3-(diethylamino) propylamine, furil, benzyl alcohol, squaric acid and ethyl (2E, 4Z)-deca-2,4-dienoate. We applied Bayesian network, multiple regression, a

TIMES SS (GHS) computation, the SkinDoctor application and the MultiCASE CASE Ultra Toolbox and evaluated the data against existing human patch assay and Local Lymph Node Assay (LLNA) data. We included data from DPRA, KeratinoSens*TM,* h-CLATassays and the ITSv2 workflow as well as GARD assays in our *in silico* predictions adhering to the OECD Guideline 497 and related defined approaches. For contradicting results, we used experimental SENS-IS assays as an additional tool to predict sensitisation.

Results

With our integrated approach we obtained clear results for farnesal and safranal with slight predictive variations and contradicting results for the six compounds 2-butoxyethyl acetate, 3-(diethylamino) propylamine, furil, benzyl alcohol, squaric acid and ethyl (2E, 4Z)-deca-2,4-dienoate. Squaric acid and ethyl (2E, 4Z)-deca-2,4-dienoate were additionally studied with SENS-IS. With this work we draw attention to the importance of integrated approaches during risk assessment of chemicals and materials by defined approaches and the careful analysis and comparison of results from different methods as provided by the SaferSkin application. We show that some methods might lead to unclear results and highlight that in such cases it is essential to extend obtained results by further techniques such as the SENS-IS assay to clarify prediction outcomes.

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LP-96

Integrating Safety and Sustainability by Design into the Lifecycle of Pharmaceutical Products

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Sustainability by Design (SbD) is a program that aims to improve the environmental performance of pharmaceutical products throughout their life cycle. The program assesses the product sustainability metrics including the use and safety impacts of substances of concern, efficiency and consumption of energy, water, and chemicals, and waste generation. The program is based largely in accordance with the European initiative Safe and Sustainable by Design (SSbD), which promotes innovation to replace hazardous substances in products and processes with safer alternatives, as well as to apply green chemistry principles.

In this poster, we look at two different pharmaceutical modalities (biologic and small molecule) and show how both approaches can improve the safety and sustainability of pharmaceuticals. We compare our SbD program with the EU's SSbD and showcase similarities and differences. We also highlight the scientific and technical challenges and limitations of both analyses. Those include workload of the assessment reports, lack of cross-industry standardization and comparison, and the heavy reliance on hazard classification without considering risk assessment and weight-of evidence concepts in toxicology. We outline some recommendations to address these issues to enable the continuous improvement of the safety and sustainability of pharmaceutical products.

LP-97

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

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Being the first line of defense of the organism against airborne pathogens the airway respiratory epithelium is also a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections.

Here we established a new co-culture model using well characterized, standardized human airway epithelium such as MucilAirTM, SmallAirTM and human lung primary macrophages (CD45+,HLA-DR+, CD206+, CD11b+and CD14-) for studying bacterial and viral infections. The alveolar macrophages were not only able to adhere to the epithelial cells, but also functional: The macrophages were capable of phagocytosis, evaluated using pHrodo™ Red (S cerevisiae Bio-particles Conjugate). Moreover, the co-culture models respond to pro-inflammatory stimuli such as LPS, TNF-a and Poly(I:C) with an increased IL-8 secretion.

Upon bacterial infection with methicillin-susceptible Staphylococcus aureus strain (MSSA), compared to MucilAir™ monocultures, MucilAir™-macrophages showed stronger immune responses: (i) a reduction of bacterial growth (up to 1.5Log10 CFU) and (ii) decreased upregulation of IL-8 and b-defensin-2 secretions. Interestingly, greater difference was observed for Streptococcus pneumonia (Sp19F): The presence of macrophages led to a decrease of 3.5Log10 CFU after 24 hours of culture versus MucilAir™ alone.

These novel in vitro models might find applications in understanding the role of immune-epithelial cell interactions in infection disease.

LP-98

"ProtoAquaTox and COMBASE: QSAR models for assessing acute and chronic aquatic toxicity"

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Industrial products, including cosmetics, pharmaceutical products and biocidal active substances, can end up easily in different environmental compartments during manufacturing, usage, and disposal. Some are adsorbed to the soils and get deposited, while most are water-soluble and have low volatility, and thus are transported to water compartments in the environment. Notably, pharmaceutical drugs and cosmetics are primarily intended to elicit specific desired effects in humans, but their release can lead to unforeseen adverse effects on non-target species in the environment.

The aim of the present work has been the development and implementation of computational tools and models to support the prediction of the ecotoxicological effects of industrial products, with special focus on aquatic toxicity. On one side, in the context of COMBASE project (https://webgate.ec.europa.eu/life/publicWebsite/project/details/4472) we have developed QSAR models to predict sludge and algae acute toxicity of biocides. These models were complemented and extended to other chemical types, including pharmaceutical drugs and cosmetic ingredients, under the context of the project EcoCosmePharm (https://cordis.europa.eu/project/id/845373). The extended models have been implemented in our software platform 'ProtoAquaTox' (https://sites.google.com/view/ecocosmepharm/software), which utilizes multi-tasking QSTR models to predict acute and chronic aquatic toxicity across various test species alongside the three trophic levels and chemical types including pharmaceutical drugs and cosmetic ingredients. These tools will support risk assessors and relevant stakeholders to quantitatively evaluate the risk and will help to reduce the need for animal testing by promoting the use of computational advanced tools.

LP-99

The role of autophagy in betaine-promoted hepatoprotection against non-alcoholic fatty liver disease induced by CDAHFD in mice

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Betaine, a compound found in foods such as sugar beet, spinach, and shrimp, has shown beneficial effects on non-alcoholic fatty liver disease (NAFLD). Several mechanisms have been proposed as the hepatoprotective and anti-steatogenic actions of betaine, but their associations have been not fully understood. In the present study, male ICR mice were fed with choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) with or without betaine (0.2-1% in drinking water) for 1 week. Betaine dose-dependently normalized CDAHFD-induced fatty liver via the restoration of *S*-amino acid (SAA) metabolism such as SAM/SAM ratio, and the phosphorylations of AMPK and ACC. CDAHFD-induced endoplasmic reticulum (ER) stress (BiP, ATF6, and CHOP) and apoptosis (Bax, cleaved caspase-3, and PARP) were inhibited, but down-regulated autophagy (LC3II/I and p62) was stimulated by betaine. To examine the role of betaine-induced autophagy in the NAFLD prevention, we injected chloroquine (CQ), an autophagy inhibitor, to CDAHFD- and betaine (0.5 % in drinking water)-supplemented mice. CQ treatment did not affect hepatic SAA metabolism, but suppressed the betaine-promoted liver protection and anti-steatogenic effect. The autophagy

inhibition by CQ increased ER stress and apoptosis which were prevented by betaine. Intriguingly, proteins involved in lipid metabolism including p-AMPK, p-ACC, PPARα, and ACS1 were also reversed by CQ. Thus, the results of present study suggest that the activation of autophagy can be an important upstream mechanism of the inhibition of steatosis, ER stress, and apoptosis by betaine in NAFLD. Grant No. NRF-2019R1I1A3A01058584

LP-100

Development of an Allergy Test for Tropomyosin in Novel Foods

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Introduction: A number of novel foods based on insects such as yellow mealworm, migratory locust or house cricket, have recently undergone authorisation within the EU. However, consumption of insect protein may cause allergic reactions in certain individuals. Data provided by Broekmanet al. (2016)[1] suggest a high (87 %) probability for an allergic history to shrimp to be associated with allergic cross-reactivity to protein from mealworm. The assessment of allergy-related risks plays a crucial role towards protecting consumers from allergic (anaphylactic) reactions; however, a predictive non-clinical assay for assessment of the allergic potency of insect-based foods has hitherto not been available.

Purpose: Thegoal was to develop a predictive assay for food allergy from foods based on complete or processed insects as ingredients. Tropomyosins from shrimp and house dust mite were tested as model allergens.

Methods: Peripheral blood mononuclear cells were isolated from blood of non-allergic donors and shrimp (Pen a1) allergics by density gradient centrifugation. Monocyte-derived dendritic cells (MoDCs) were generated by adherence (Gramlich et al., 2019)[2], followed by 5-day cell culture in RPMI 1640/10% FCS with GM-CSF (100 ng/mL) and IL-4 (10 ng/mL). 48 h after stimulation, costimulatory CD86, adhesion molecule CD54 and MHC-II molecule HLA-DR were stained using corresponding fluorochrome-coupled monoclonal antibodies and analysed by FACS. Release of cytokines was detected in culture supernatants by analyses using multiplexing technique (Luminex).

Results: Expression of CD86, CD54 and HLA-DR were dose-dependently increased on MoDCs by stimulation with 0.1, 1 and 10 µg/ml natural shrimp or house dust mite (Der p10) tropomyosin. Results were calculated as RFI (rel. fluorescence intensity) and fold change (FC) comparing to unstimulated cells. In healthy donors (n=6), CD86 was increased by a FC > 5 and a FC > 10 after stimulation by 10 µg/ml of natural shrimp or house dust mite tropomyosin, respectively. In MoDCs from patients with shrimp allergy (n=3), CD86 was increased by a FC > 20 by both forms of tropomyosin. For HLA-DR and CD54, increased expression comparing to control was observed, however in people with allergy and healthy donors at similar level. Considering that a RFI value ≥ 150 % for CD86 would be regarded as a positive result in the dermal sensitisation assay h-CLAT (OECD TG442E), the new assay proposed here would suggest tropomyosin to be a strong allergen. In addition, natural shrimp and dust mite tropomyosin induced high release of inflammatory cytokines IL-6, IL-12, MIP-1α, MIP-1β and TNF-α into the supernatants of MoDCs.

Conclusion: The present food allergy test dose-dependently detected tropomyosin from different arthropod species and may contribute to animal-free testing for specific key events within the AOP259 Food Allergy. (Internal funding by BfR-LMS-08-1322-803)

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LP-101

Pulmonary toxicity of iron oxide nanoparticles following intratracheal instillation in mice

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With the increased production and usage of superparamagnetic iron oxide nanoparticles (SPIONs) in both industrial and biomedical applications, there is a concern of the potential adverse health effects induced by these materials. Workers in production and research are potentially exposed to SPIONs through inhalation. Occupational exposure is of particular concern, as workers may be exposed for longer time periods and at higher exposure levels of iron oxide nanoparticles (IONPs) compared to end-consumers. The main aim of this study was therefore to examine the pulmonary toxicity of IONPs at occupationally relevant doses. In support of the application of new alternative methods (NAMs) in hazard assessment of nanomaterials, the IONPs were tested with both *in-vivo* and *in-vitro* 3D lung models. Briefly, 7 weeks old female wild type mice (C57BL/6J) were exposed to IONPs (100nm) through intratracheal instillation. 50 µL of 180 µg/mice, 60 µg/mice and 20 µg/mice IONPs or saline was instilled followed by 150 µL air. Following a post-exposure time of 1, 3 or 28 days, the mice were euthanized and the lungs were flushed twice with 0.8 mL saline to recover the bronchoalveolar lavage (BAL) fluid. The number of inflammatory cells, especially polymorphonuclear neutrophils (PMNs), was determined by microscopic evaluation (differential leukocyte count). The lungs were harvested for histopathological evaluation and gene expression analysis of inflammatory and oxidative stress markers. In addition, a 3D lung model of human epithelial cells (A549) and differentiated monocytes (dTHP-1) was exposed to 3.4 µg/cm2 or 20.5 µg/cm2 IONPs at the air-liquid interface (ALI) in a VITROCELL 6 cloud system. Cytotoxicity was measured using Alamar Blue and LDH assay after 24h or 72h exposure. A reduction in viability was seen after exposure to both doses of IONPs at ALI compared to the saline exposure control. Preliminary *in-vivo* data suggest no behavioral morbidity up to 28 days after exposure to the particles. Analysis of pulmonary inflammation (BAL differential leukocyte count and gene expression) is on-going, and the results are to be presented.

LP-102

Towards mimicking the human alveolus with co-cultures of epithelial types I and II and endothelial cells.

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The respiratory tract is continuously exposed to inhaled air and all its possible components. Exposure to particles deriving from human activities is, therefore, unavoidable. The size of those particles will dictate the parts of the lung that may be at risk. In the case of nanoparticles, they may overcome all the obstacles posed by the respiratory tract and reach the alveolus. Therefore, in the context of nanosafety assessments and considering the ethical constraints regarding the use of animals in science, it is crucial to have a human cells-based model, able to mimic the human

lung barrier, for nanotoxicological applications. In this work, we are setting up a model of the human lung barrier by co-culturing type I (hAELVi cells) and type II (A549) alveolar cells with endothelial (EA.hy926) cells.
Mono-, double- or triple- cultures of the different cell types were seeded on 0.4 mm pore inserts and characterized by measuring the transepithelial electrical resistance (TEER), permeability to Lucifer Yellow, and cellular metabolism, throughout time in culture.
The expression of relevant markers of barrier formation and cell type [e.g. tight junctions, surfactant production, and others] was studied by immunocytochemistry. Whenever pertinent, submerged conditions were compared with air-liquid interface (ALI) conditions.
As expected, monocultures of A549 cells displayed very low TEER values (<35 $\Omega.cm^2$) throughout the culturing period, but high metabolism and a punctuated pattern of ZO-1. On the other hand, hAELVI monocultures stably exhibited TEER values above 1000 $\Omega.cm^2$ after one week in culture, with low metabolism rates and widespread, continuous, expression of ZO-1. When double cultures were prepared, the TEER values were initially low, but increased after 10 days in ALI, reaching interesting resistance values (>300 $\Omega.cm^2$)towards the third week of culture.
The experiments on the triple cultures and the detailed immunocytochemical characterization of the different cultures are ongoing, but the results obtained so far look promising in terms of barrier formation. The following steps will be to grow the triple cultures in an organ-on-chip with breathing motion ability, to evaluate its impact on barrier formation, and start testing it for nanotoxicological applications.

LP-103

New non-invasive, label-free monitoring approach for 2D and 3D cell culture

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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.

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LP-104

Refined dermal absorption for the biocidal active substance Diamine using newly generated human and rat in vitro dermal absorption data

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The biocidal active substance N- (3-aminopropyl)-N-dodecylpropane-1,3-diamine (CAS 2372-82-9), also referred to as Diamine, is currently under regulatory review as part of the EU Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) review program. The substance is strongly basic and positively charged at physiologic pH. It is water soluble, has surfactant properties and is strongly adsorptive owing to its positive charge and C12 alkyl chain.

Historical human *in vitro* dermal absorption data for [^{14}C]-Diamine are difficult to interpret, in part because levels in receptor fluid following a 24h exposure to a 1% dilution were non-quantifiable (<0.01% of applied dose). Historical rat *in vivo* dermal absorption data on a 0.1% dilution of [^{14}C]-Diamine have been evaluated by EU regulatory authorities and, following the approach in the EFSA guidance on dermal absorption, considered to support a dermal absorption value of 2% for human risk assessment purposes.

Rat dermal absorption is generally considered to be greater than human dermal absorption although the degree of interspecies difference will in part depend on the route of absorption (e.g., intercellular, intracellular and follicular). To better understand rat and human differences in dermal absorption of Diamine, human and rat *in vitro* dermal absorption studies were conducted on a 0.1% dilution of [^{3}H]- Diamine. The use of a tritium label increased the specific activity of the substance by several orders of magnitude compared to a single [^{14}C] label, thereby lowering the limit of quantification. The human *in vitro* dermal absorption results for 0.1% [^{3}H]-Diamine showed that despite adsorption to the outermost layers of skin, human *in vitro* absorption to receptor fluid was very low and far below the existing risk assessment value of 2%.

Furthermore, the triple pack approach, which considers the rat *in vivo* as well as the paired human and rat *in vitro* studies, provides a similar dermal absorption estimate to that of the human *in vitro* dermal absorption study alone. These results confirm that this strongly adsorbing and positively charged substance has very low potential to penetrate human skin.

LP-105

Determination of the chemical composition in the combustion of biomass of plant origin

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In the last decade, the importance of the circular bioeconomy has increased. In 2021, Regulation (EU) 2021/695 defines areas for possible missions and areas for possible institutionalised European partnerships to be established under article 185 or 187 TFEU, one of which it is - Sustainable, inclusive and circular bio-based solutions. The main goals of are to: accelerate the innovation process, developing novel bio-based solutions; accelerate market deployment of existing mature and novel bio-based solutions and ensure a high level of environmental performance by bio-based industrial systems.

At the same time, however, the importance of safety and health risk assessment when using alternative biofuels should not be neglected. Since there are combustion processes, the generation of fine and ultrafine particles and the presence of polycyclic aromatic hydrocarbons, classified as carcinogens, are expected. That is why we set ourselves

the goal of present study – to assess the chemical composition of aerosols formed during the combustion in laboratory conditions of plant biomass, such as coffee, sunflower flakes, straw, alfalfa, cherry pone.
Samples were generated in a cascade combustion system on filters to sample 9 particle size categories. a GC method was used for the analysis of polycyclic aromatic hydrocarbons (PAHs) in samples of experimentally generated fine particles between 10 and 0.016 microns in size:
1. The efficiency of extraction of PAHs with different extraction techniques - Soxhlet and ultrasonic (UZ) extraction was investigated;
2. The extraction efficiency of PAHs with solvents of different polarity was investigated;16 priority PAHs have been identified. Observes the presence of at least 6 unidentified PAHs, which constitute about 23% of the total PAH mass with unclear toxic properties.
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LP-106

Rosmarinic acid supramolecular cyclodextrin complexes – an assessment of the cytotoxic effect on human breast adenocarcinoma cells

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Rosmarinic acid (RA) is a well-known plant metabolite, a polyphenol frequently found in medicinal and aromatic plants, traditionally used as food source. More recently, its pharmacological properties were thoroughly investigated by innovative *in vitro* techniques, indicating a phytochemical compound with significant antitumor potential, evidenced by multiple targeted mechanisms [1]. The limitation of the biological action is related to the low solubility and permeability in biological environments. A promising option to improve these limiting parameters is to obtain supramolecular systems. Cyclodextrins (CDs) are cyclic oligosaccharides, capable of forming host-guest complexes with hydrophobic molecules, resulting complexes with increased solubility, stability and permeability [2]. The main objectives of this study were to synthesize and characterize RA complexes with beta and gamma cyclodextrins, and to evaluate their cytotoxic potential on non-invasive (MCF-7) and invasive (MDA-MB-231) breast cancer cells.
RA-CD supramolecular systems were obtained by the crystallization method from ethanol-water solution. The FT-IR (Fourier transform infrared) analysis highlighted shifts or decreases in intensity of the bands characteristic of phenolic

carbonyl and hydroxyl groups and the presence of the characteristics bands of cyclodextrins. Scanning electron microscopy (SEM) provided useful information on the approximate sizes and crystal morphologies of the complexes. The Differential Scanning Calorimetry (DSC) analysis revealed the endothermic effect of the dissociation of smaller water molecules and the lower appearance temperature. The antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and rosmarinic acid and its supramolecular systems showed a significant antioxidant activity, over longer periods of time. The cytotoxic potential assessment on breast adenocarcinoma cells (MCF-7 and MDA-MB-231) indicated the importance of concentration,exposure time, and cell type on cell viability and morphology. The most significant effects were observed in the case of supramolecular systems with gamma cyclodextrin (at the highest concentration tested) on MCF-7 cells (36.57% viability) compared to MDA-MB-231 cells (59.11% viability). Morphological changes, cell migration and evaluation of apoptotic processes confirmed the data obtained for cell viability. These results are different as compared to those observed for the extract in previous research, that assessed the effects of *Thymus vulgaris* extract (rich in rosmarinic acid) encapsulated in cyclodextrins on human breast cancer cells [3].
The obtained supramolecular systems have the potential of controlled release, show antitumor effects on cells in monolayer and require further investigation of the underlying mechanisms using three-dimensional cells complemented by angiogenic effects *in ovo*.

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LP-107

MEHP exposure alters survival and growth of human ovarian follicles in vitro

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Phthalates are compounds found in everyday items like plastics and personal care products. Our constant exposure is suspected to adversely affect female fertility. However, human experimental proof is lacking. We studied the effects of the most common phthalate metabolite, mono-(2-ethylhexyl) phthalate (MEHP), on adult human ovaries using ovarian tissue culture and epidemiologically defined concentrations (2.051-20 510 nM).
Histomorphological and steroid production analyses were performed on human ovarian tissue exposed to MEHP for 7 days *in vitro*. In addition, impact of MEHP on cell viability and gene expression (bulk RNA sequencing) were investigated upon 7-day exposure using human granulosa cell lines (KGN, COV434), germline tumor cell line (PA-1), and human ovarian primary cells.
Ovarian tissue exposure to MEHP reduced follicular growth (20.51 nM) and increased follicular degeneration (20 510 nM). Analysis of transcriptomics data of MEHP-exposed ovarian cells revealed altered gene ontology (GO) terms including cytoskeleton, adherens junctions and Hippo pathway. Selected genes related to these GO terms were

validated using qPCR in cell culture and immunofluorescent staining of RNA and protein in exposed ovarian tissue, validating CSRP2, CTNNB1 and YWHAE as targets of MEHP.

Collectively, our findings indicate that exposure to phthalates can adversely affect female fertility by hindering the development and survival of human follicles likely through a mechanism involving cytoskeleton, adherens junctions and Hippo pathway. The next steps will involve deciphering the cell type specific effects of MEHP in ovaries by single-cell RNA sequencing.