ICT2022

THE XVI[TH] INTERNATIONAL CONGRESS OF TOXICOLOGY

MAASTRICHT, THE NETHERLANDS

Late Breaking Abstracts

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LP-78

Genotoxicity Assessment in E-cigarette Consumers, Cigarette Smokers and Non-smokers- a pilot study

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The use of e-cigarettes is becoming increasingly common, especially among young adults and teenagers, partly because of the perception of their harmless effect compared to traditional cigarettes. Although several in vitro studies have evaluated the genotoxicity associated with e-cigarettes, human studies are still lacking. This pilot study aimed to assess the genotoxicity in e-cigarette users, cigarette smokers and non-smokers. A total of 84 healthy participants, including 20 e-cigarette users, 31 cigarette smokers and 33 non-smokers, were enrolled. Genotoxicity was evaluated by measuring DNA damage in blood as tail moment (TM) using the comet assay and detecting micronuclei (MN) in exfoliated buccal cells using Micronucleus assay. To evaluate nicotine exposure, cotinine (COT) which is a major metabolite of nicotine was measured. Cotinine (COT) was determined in urine (UCOT) and saliva (SCOT). Bivariate analyses showed that the levels of TM, UCOT and SCOT in e-cigarette and cigarette smokers were not statistically different. In contrast, the levels in both groups were significantly higher than those in non-smokers ($p < 0.001$). All parameters were significantly higher in e-cigarette users than non-smokers ($p < 0.02$). After adjusting the model for covariates, the results remained significant for TM, UCOT and SCOT ($p<0.001$). The prevalence of MN in e-cigarette users (40%) was higher than in cigarette smokers (27.5%). Overall, we conclude that the use of e-cigarettes increased DNA breakage in blood and induced micronucleus in the buccal cells in the same magnitude as in cigarette smokers. Similarly, e-cigarette users and cigarette smokers had elevated levels of urinary and salivary cotinine. It is possible to suggest that nicotine and other chemicals in e-cigarette induced genotoxicity, as reported in several in vitro studies. Our results highlight that using e-cigarettes can induce a genotoxic effect, suggesting that it is not as safe as it appears to the public.

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LP-79

A tiered approach for the testing of pharmaceutical intermediates for worker safety purposes; moving away from *in vivo* testing

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As a drug moves through the pipeline, the quantities of pharmaceutical intermediates (IMs) handled greatly increases. It is therefore imperative to understand the intrinsic hazards of these IMs to protect employees from adverse health effects. Traditional IM testing strategies were heavily reliant on *in vivo* studies. However, with *in vitro* and *in silico* techniques rapidly improving, testing strategies are changing. *In silico and in vitro* methods offer several benefits; lower costs; faster hazard evaluation; and a reduction in animal use, in alignment with the 3Rs principles.

Four critical endpoints are commonly assessed for worker safety purposes; mutagenicity; irritation; sensitisation; and acute oral toxicity (AOT). Assessment of such endpoints allows assignment of an in-house occupational hazard category (OHC) and appropriate control measures to minimise chemical exposure. GSK have adopted a tiered testing strategy for these, comprising read-across, *in silico, in vitro, in vivo* and physio-chemical data. *In silico* evaluations are performed for all IMs as a first-line approach. This facilitates the selection of the most suitable *in vitro* studies (in combination with physio-chemical data) and the waiving of *in vivo* studies where appropriate. For systemic effects, *in vivo* studies are still employed where further evidence is required to determine a chemical's true hazard. An oral toxicity, fixed dose procedure and a dual endpoint micronucleus-comet assay are conducted for AOT and mutagenicity purposes respectively. For local effects, the testing strategy is based solely upon *in silico*, *in vitro* and physio-chemical data. A bottom-up approach is primarily taken to assess irritation with the majority of IMs predicted to be non-irritants.

Typically following assessment of these endpoints, IMs are assigned to OHC 3 (>10 - ≤100 mcg/m^3); little is known about chronic health effects to warrant a less stringent OHC. NOAEL/NOELs (no-observed (adverse) effect levels) from historical repeat-dose studies, from our internal database, can however be used to move IMs to OHC 2 (>100 - ≤1000 mcg/m^3) by means of read-across. 97% of IMs, with 28-day rodent oral toxicity data, had a NOAEL/NOEL ≥5 mg/kg/day, resulting in an occupational exposure level (OEL) of ≥150 mcg/m^3. 69% had an OEL ≥1000 mcg/m^3. Case studies of two IMs – GSK01 and GSK02 - are presented to further explain the testing strategy as a whole.

Whilst this testing strategy has many benefits, it is not without its challenges. Results can be inconclusive due to out-of-domain IMs, low confidence intervals or lack of data. These challenges are being overcome in various ways including the use of multiple *in silico* models to assess each endpoint and the use of new *in vivo* data to improve the applicability domain of models.

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care Welfare and Treatment of Animals.

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LP-80

The Effects of Food Matrix and Gastrointestinal Digestion on the Food Additive E171 (titanium dioxide): A study on particle characteristics and oxidative stress

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E171, consisting of TiO_2 micro- and nanoparticles is a widely used white food colorant, opacifying agent and can also be found in many pharmaceuticals. In recent years' various concerns about the safety of E171 were raised, including its potential to induce genotoxicity and possible contribution to the development of colorectal cancer. Many uncertainties around the potential toxicity of E171 emerged due to contradicting studies and poorly understood Mode of Actions. These discrepancies could be attributed to neglecting the influence of food matrices and the absence of information on the effects evoked on E171 during gastrointestinal digestion.

Our study aims to examine the impact of particle characteristics of E171 and the presence of yogurt, as an example for a relevant food matrix, on the potential induction of oxidative damage. Yoghurt was chosen as matrix as we are currently also executing a human dietary intervention study in which E171 is provided in yoghurt. Therefore, we investigated pristine E171 and E171 containing yogurt in their original state and after the application of an artificial upper gastrointestinal digestion model (TIM-1). Additionally, we examine the reactivity of E171 via the ability to produce Reactive Oxygen Species (ROS) in these different matrices and at various time points during the in-vitro digestion.

We utilized single particles Inductively Coupled Plasma Mass Spectrometry (sp-ICPMS) and Transmission Electron Microscopy (TEM) to characterize the particles. The production of ROS was detected via Electron paramagnetic resonance spectroscopy (ESR/EPR).

Our results demonstrated an increase of the median diameter of E171 in yogurt, as compared to the pristine materials, indicating increased aggregation and agglomeration, as well as the potential formation of a protein corona. Furthermore, the percentage of E171 particles < 100 nm decreased, when added to yogurt. Pristine E171 was capable of producing ROS, while the same material dispersed in yogurt, did no longer result in an increase in ROS compared to the control. After artificial digestion in the TIM model-1, E171 as pristine material and in yogurt showed an altered ROS production.

Our finding indicates the importance of a food matrix in the examination of adverse effects of E171 for the gastrointestinal tract. It furthermore highlights potential alterations of particle characteristics during the digestion upon arrival to the colon.

LP-81

Facilitating The Screening Of Large Data Sets Against Networks Of Adverse Outcome Pathways Through Digitalisation Of Knowledge

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Adverse outcome pathways (AOPs) represent a valuable construct for knowledge capture and evidence organisation in safety assessment. Data from in vivo, in vitro and in silico sources can be associated with events on AOPs, contextualizing the evidence and highlighting associations. This knowledge can then be used with a reasoning model to derive an overall call for each AOP, provide insight into the mode of action by which a compound may cause toxicity and screen for different toxic liabilities. However, until now, the approach has generally been low throughput, assessing evidence on a compound-by-compound basis and capturing the results in multiple different formats. This limits both the application and validation of AOPs. As a consequence, a method of digitizing this approach, allowing for a higher throughput, would prove extremely useful.

Firstly, being able to batch process large volumes of compounds with known adverse outcome (AO) liabilities against evidence contextualized on an AOP would allow us to probe the coverage of the network and data using a simple reasoning model. The output of the classifier could be compared to the AO classifications to look for gaps in any data or AOP knowledge implemented. Secondly, in certain instances, such as the early stages of drug discovery, it is common to have potentially thousands of compounds requiring assessment. These need to be prioritized and shortlisted and using evidence associated with AOPs could help make better decisions.

With this in mind, we took the digitalized AOP knowledge framework previously described, associated evidence, in the form of assay data and predictive models, and developed a batch processing screening tool to allow large volumes of compounds to be processed against an AOP network. The tool was then used to process a large data set of compounds with known carcinogenic potential against an AOP network relating to cancer. Analysis of the results was undertaken, looking at the coverage of this network and identifying gaps both in the available data and the knowledge of the modes of action captured within the AOPs. Improvements to the approach were identified and potential additions to the AOP network based on the findings suggested.

The work highlights that capturing AOPs and associated knowledge in a digital format allows for a higher throughput of query substances and association with the knowledge. We have shown that implementing batch processing can help with identifying knowledge and data gaps, so that they can be filled. In the future, it is hoped that this ability to batch process will also help facilitate the use of AOPs in high throughput screening applications.

LP-82

Role of the different domains of snake venom metalloproteinases in basement membrane pathology

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Snakebite envenoming provoked by species of the Viperidae family are characterized by local effects such as edema, pain, blistering, myonecrosis and hemorrhage and systemic pathological alterations such as hemorrhage and coagulopathies (1). The main toxins responsible for local and systemic hemorrhage are zinc-dependent proteinases named Snake Venom Metalloproteinases (SVMPs). These toxins also provoke other pathological effects such as inflammation, coagulopathies and tissue necrosis (2–5). The action mechanism of hemorrhage has not yet been fully elucidated; although, it has been proposed that BM protein degradation is one of the key steps in the pathology cause by SVMPs, since hydrolysis of this proteins results in a significant weakening of the mechanical stability of the capillary wall (3,6–8). However, it has not yet been possible to identify the BM proteins on which they interact, and the participation of the domains present in the different types of SVMPs (PI, which only have the metaloproteinase domain, and PIII, which have in addition the disintegrin-like and cysteine-rich domains - DC domain). Therefore, in this study, the interactions of hemorrhagic PI SVMPs BaP1, PIII SVMPs CsH1, and the DC domain of CsH1 with laminin and type IV collagen were investigated *in vitro* by Western Blot (WB), ELISA, and affinity chromatography. Also, the role of the catalytic domain in the binding were studied in the different assays by the used of batimastat, a metalloproteinase inhibitor. In the WB assay, only the CsH1 showed capacity for binding to type IV collagen and laminin. In agreement with this result, the CsH1 exerted the strongest binding to type IV collagen and to laminin in the ELISA assays, whereas BaP1 the lowest binding. In addition, it was observed that CsH1 inhibit by batimastat maintains the binding capacity to BM proteins, indicating that the binding is independent of the catalytic activity. Interestingly, the DC domain did not evidence binding to type IV collagen or laminin in the WB assay; consequently, in the ELISA assay the binding of the DC to this substrate was significantly lower than the CsH1, suggesting an important role of the metalloproteinase domain in the binding to BM proteins. The interaction of type IV collagen and the SVMPs was also studied by competition experiments between laminin and FITC- conjugated type IV collagen; in these experiments the preincubation of the CsH1 with laminin, significantly decrease its proteolytic activity on type IV collagen, suggesting the presence of exosites near the catalytic site. These results reveal the importance of regions within the metalloproteinase domain for the interaction of PIII SVMPs with BM proteins.

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LP-83

Mating of Göttingen Minipigs at Scantox for use in embryofoetal development studies

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The small size, microbiologically defined health status, and the many similarities to human make the Göttingen Minipig an ideal animal model in many types of biomedical studies. Early onset of sexual maturity compared to other large animal species, duration of gestation, and litter size, as well as the susceptibility of the Minipig to known human teratogens make the Minipig the logical scientific and economical alternative to other species for developmental and reproductive toxicology studies, especially if there is a need for a third species or using rats or rabbits is not applicable [1]. Knowledge of estrous synchronisation and mating procedures are essential for assuring the most optimal timelines for embryofoetal development studies.
At Scantox, mating is performed onsite on all days of the week after synchronisation, allowing for a study setup that facilitates the procedures to be performed all the way through the embryofoetal study from arrival, via synchronisation, mating, dosing, termination and on to assessment of test item related effects on fetuses. Data have been reviewed for control groups of embryofoetal development studies performed at Scantox within the last 6 years. The data show a high pregnancy rate of above 95%, with a mean litter size between 5 and 6 fetuses per litter and a low percentage of dead fetuses together with low rates of resorptions.
The data show that mating procedures at Scantox results in a high pregnancy rate allowing for fewer animals to be included to fulfil the guideline requirements [2]. Furthermore, the optimised procedures including synchronisation allows for optimised timelines ensuring a fast delivery of results.

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LP-84

13-week prenatal study on glyphosate and two glyphosate-based herbicides: first toxicological evaluations

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purpose
Glyphosate-based herbicides (GBH) are the world's leading products for weed management, but intense scientific, legal and public debate has been generated over their safety (1). There are several versions of GBHs, with different effects depending mainly on the co-formulants which can be far more toxic than the active ingredient itself. Notably one commonly known adjuvant is the toxic surfactant polyoxyethylene amine (POEA) a surfactant added to help glyphosate penetrate weeds, that is banned in Europe. The lack of data available in literature reinforce the need to perform toxicological studies that contemplate the presence of co-formulants in GBHs sold on a global scale (2). To remove any doubt on the safety of GBHs, the Ramazzini Institute, has planned an integrated project designed to test

not only the pure Glyphosate but also two commercial formulation, Roundup Bioflow (without POEA), and RangerPro (with POEA), at different doses referring to man-equivalent environmental exposures. Different endpoints are studied on the most relevant parameters for sub-chronic toxicity, carcinogenicity, genotoxicity, toxicity for development and reproduction etc.

methods

Sprague-Dawley rats were exposed to pure glyphosate or GBHs and in this preliminary work we evaluate 4 groups (the 3 high doses + control) over a total of 10. The doses of glyphosate selected ranged from the EU acceptable daily intake to the EU no observed adverse effect level. The high doses presented here are 50 mg/Kg bw/day of glyphosate or glyphosate equivalent for the 2 GBHs tested. The treatment started from prenatal life, goes through lactation and then 13 weeks after weaning. Animals were sacrificed and a complete necropsy was performed. At necropsy, blood was collected for hematological and biochemical analyses. The organs and tissues collected during necropsy were fixed, paraffin embedded, sectioned to 4 µm, and processed in alcohol-xylene series to be stained with hematoxylin and eosin for microscopic evaluation. All slides were evaluated by a junior pathologist and a senior pathologist. Fisher exact test and Dunnett's' test were mainly used for the statistical analysis of data.

results

Here we present the first preliminary data on sub-chronic toxicity to high treatment doses. During the experiment we did not observe any difference in survival between groups at the end of the 13 weeks of post weaning treatment. Gestation and lactation were not affected by the treatment. At the end of the in vivo study differences in body weight, food and water consumption in the treated animals were within the normal range of 10% compared to unexposed control animals. Organ weights at the end of the treatment were also not significantly different between the treated and control groups. Non-neoplastic lesions of varying degrees were observed in all the 3 high dose groups, in males or females, with particular attention to the liver and kidneys.

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LP-85

Statins decrease cardiomyocyte viability and mitochondrial function via intracellular acidification

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Introduction

Cholesterol-lowering statins have proven to significantly reduce major cardiovascular events and are used by 180 million patients worldwide. Musculoskeletal complaints are experienced by 7–29% of all users and associated with decreased mitochondrial function. Cardiomyopathy has not been described for statins, probably due to a much higher mitochondrial content compared to skeletal muscle. However, statins are usually taken lifelong and as mitochondrial function reduces with age, cardiac tissue may also become susceptible to side effects in elderly.

Objectives

We aimed to investigate the metabolic effects of statins on induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs).

Methods

CM viability, intracellular pH, mitochondrial morphology and membrane potential were determined using high-throughput microscopy after 48h exposure to 0.3-100 µM of the acid and lactone form of various commonly used statins. Next, oxygen consumption (OCR) and extracellular acidification rates (ECAR) were determined using the Seahorse XF-96 Flux Analyzer.

Results

All statins decreased cell viability dose-dependently, most prominently by simvastatin lactone (99.9±0.5%, mean±SEM, p<0.0001 at 100 µM) and most potently by cerivastatin acid (IC_{50}-value of 3.6 µM (95%-CI 0.1 - 206.0)). All statins decreased both ECAR and OCR, again most strongly by simvastatin lactone, which declined basal and maximal respiration by 53±11% (p<0.05) and 80±8% (p<0.01), respectively. Statins increased the intracellular acidification up to 52±9% (p<0.05). Finally, statins reduced mitochondrial membrane potential by 22±14% (p=0.28).

Conclusion

Statins decrease CM viability at higher concentrations and probably decrease mitochondrial respiratory capacity, extracellular acidification and membrane potential via an increased intracellular acidification. These results may have clinical implications for patients with a decreased mitochondrial capacity, where low statin concentrations in the therapeutic range could be harmful. Our results shed new light on possible effects of statins on cardiac tissue. Future studies should investigate whether this could aid a more personalized statin treatment.

LP-86

The role of ADP/ATP carrier activity in drug-induced mitochondrial dysfunction in renal proximal tubular cells

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Introduction

Mitochondrial dysfunction is an underestimated cause of drug-induced kidney injury. Transport proteins in the mitochondrial inner membrane form a significant class of potential drug off-targets, as these account for approximately five percent of the mitochondrial proteome. So far, most transporter-drug interactions have been reported for the mitochondrial ADP/ATP carrier (AAC), which mediates the exchange of cytosolic ADP for mitochondrial ATP.

Objectives

As it remains unknown to what extent mitochondrial carriers, and AAC in particular, contribute to adverse drug effects, we aimed to investigate its role in drug-induced mitochondrial toxicity *in vitro* in human proximal tubular cells.

Methods

CRISPR/Cas9 was applied to generate *AAC3*$^{-/-}$human conditionally immortalized renal proximal tubule epithelial cells. Mitochondria were isolated to study functional AAC-mediated ATP and ADP transport. Cells were exposed to the AAC inhibitors bongkrekic acid, carboxyatractyloside, suramin and CD437 (0.1-100 µM), after which effects on respiratory capacity was assessed using various substrate conditions. Additionally, mitochondrial morphology and function were studied by fluorescence microscopy (TMRM) and citrate synthase (CS) activity.

Results

Two *AAC3*$^{-/-}$ clones were successfully generated and showed residual ADP import rate of 55±7% and 33±8% (mean±SEM, p<0.05) and ATP export rate of 71±10% (p=0.062) and 54±11% (p<0.05). Compared to WT, *AAC3*$^{-/-}$ clones showed a reduced ATP production rate and oxygen consumption, particularly metabolic spare capacity was

affected. All AAC inhibitors, except for suramin, reduced basal and maximal respiration, compared to vehicle. Additionally, CS activity was reduced in *AAC3*$^{-/-}$, without influencing overall mitochondrial morphology.

Conclusion

In conclusion, (genetic) mitochondrial AAC inhibition mainly impacts renal cell metabolic spare capacity, which may especially impair renal function under energy-demanding conditions. Our generated model is a unique and valuable tool to investigate the role of AAC in adverse drug effects. Our findings highlight mitochondrial transport proteins as a yet largely unexplored class of potential drug off-targets. However, further research should elucidate exact mechanisms of AAC-mediated toxicity.

LP-87

Extended silver nanoparticle-induced pulmonary inflammation in a metabolic syndrome mouse model and resolvin D1 treatment

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Metabolic syndrome (MetS) exacerbates susceptibility to inhalation exposures such as particulate air pollution, however, the mechanisms responsible remain unelucidated. Previously, we determined a MetS mouse model exhibited exacerbated pulmonary inflammation 24 h following AgNP exposure compared to a healthy mouse model. This enhanced response corresponded with reduction of distinct resolution mediators. We hypothesized silver nanoparticle (AgNP) exposure in MetS results in sustained pulmonary inflammation. Further, we hypothesized treatment with resolvin D1 (RvD1) will reduce exacerbations in AgNP-induced inflammation due to MetS. To evaluate these hypotheses, healthy and MetS mouse models were exposed to vehicle (control) or AgNPs and a day later, treated with resolvin D1 (RvD1) or vehicle (control) via oropharyngeal aspiration. Pulmonary lung toxicity was evaluated at 3-, 7-, 14-, and 21-days following AgNP exposure. MetS mice exposed to AgNPs and receiving vehicle treatment, demonstrated exacerbated pulmonary inflammatory responses compared to healthy mice. In the AgNP exposed mice receiving RvD1, pulmonary inflammatory response in MetS was reduced to levels comparable to healthy mice exposed to AgNPs. This included decreases in neutrophil influx and inflammatory cytokines, as well as elevated anti-inflammatory cytokines. Inefficient resolution may contribute to enhancements in MetS susceptibility to AgNP exposure causing an increased pulmonary inflammatory response. Treatments utilizing specific resolution mediators may be beneficial to individuals suffering MetS following inhalation exposures.

LP-88

Complex-Mixture Uptake and Integrated Organismal Effects in Fish Exposed to a PFAS-Impacted Hydrological Gradient

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We deployed mobile fish-exposure laboratories to a legacy fire-training area (FTA) in 2018, 2019, and 2021 using fathead minnows to evaluate environmental PFAS mixture uptake in the background of complex contaminant chemistry. Our experiments provide evidence of site-specific effects on PFAS mixture uptake and biomarker responses. For those PFAS detected at both the REF and FTA sites, with few exceptions BCFs were generally consistent between sites. Previous laboratory studies of PFAS uptake investigated single compounds or a simple mixture and may not accurately reflect the effects of complex environmental mixtures on uptake dynamics. This project yields high quality, kinetic-based BCF values for a variety of PFAS with a common model aquatic vertebrate across under conditions representing site-specific environmental complexity. During our investigation, we identified a suite of organism-level effects at the FTA site consistent with PFAS exposure, including the disruption of hepatic lipid profiles, testis cell proliferation and apoptosis, and sperm performance, as well as inter-individual differences in PFAS mixture uptake. Future studies proposed here will build on previous work by conducting dose-responsive integrated effects assessments to establish linkages between aqueous PFAS concentrations, PFAS body burden, cellular and molecular biomarkers, and adverse organismal impacts.

LP-89

A new *in silico* NAM to discriminate classified/not-classified chemical substances for acute oral toxicity

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In a regulatory context, acute oral toxicity (AOT) studies are required for classification according to the CLP/GHS criteria. These studies were originally designed to determine the median lethal dose (LD_{50}). Based on 3R principles, OECD guidelines were updated in order to refine the protocols and decrease the animal suffering or reduce the number of animals used. Although ethically defensible, these refinements have compromised the scientific integrity of LD50 estimates becoming more conservative. Recent OECD guideline updates prevent accurate determination of LD_{50} and may lead to classify using Acute Toxicity Estimates (ATE) at <50% mortality. This is exacerbated by premature sacrifice at the first signs of serious suffering, which also artificially inflates the death count as animal recovery was often observed in older studies. Furthermore, the precise cause of animal death was often unclear in past studies as acute adverse effects can arise from either local or systemic toxicity and results were not always well described in reports. Inversely, *in silico* methods, are ethical, more rapid and of lower cost compared to *in vivo* testing, and provide a scientifically based alternative to the implied AOT. During this work, we developed a new QSAR for AOT classification for rat LD_{50} using chemical family and water solubility as descriptors.
At this stage the NAM comprises a pilot study where the training set was built with LD_{50} values aggregated from REACH substance dossiers. The dataset contains 56 alcohols, 23 aldehydes and 19 carboxylic esters. For each study, we gathered the information relative to the test protocol (*e.g.* guideline, species). Data were thoroughly curated

and relevant studies on mono-constituent substances were selected based on guideline (OECD 401, 420, 423 or equivalent). A geometric mean LD_{50} was calculated for substances with more than one validated LD_{50} value. Water solubility was predicted using iSafeRat® software. A preliminary model was developed by plotting water solubility against LD_{50} for three chemical families as a proof of concept. For carboxylic esters and aldehydes, we determined the worst-case LD_{50} delimiting AOT categories for each chemical family. At this stage the model provides CLP classification categories only [1], although for alcohols, a linear regression was determined and is used to predict specific LD_{50} values.

For the validation set, a group of 18 substances (8 alcohols, 5 carboxylic esters and 5 aldehydes) with validated AOT studies was selected. One alcohol was out of the applicability domain. The overall accuracy of our pilot model was 94.1% and the specificity was 93.8%. The results from the pilot model are encouraging and further work will rapidly extend the applicability domain of our model and include a supplementary descriptor which takes into account local effects based on mechanisms of action. In addition, the model will be 'tuned' to predict either LD_{50} or more conservative ATE values.

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LP-90

Organotypic 3D primary human nasal tissue model for toxicological studies

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The nasal mucosa serves as the first line of defense against inhaled chemicals, drugs, and respiratory infections. Here we developed a novel in vitro primary human cell-based 3D human nasal epithelial tissue (NET) cultured on a cell culture insert with a porous (0.4 µm) membrane bottom at an air-liquid interface (ALI). The NET model was characterized by histology, barrier function (TEER), viability (MTT), toxicity, and infection with viruses.

Histological and immunohistochemical evaluation of the in vitro NET showed a polarized, multilayered tissue that expresses markers of epithelial cells (CK19), tight junctions (ZO-1 and Claudin-1), mucin (MUC5B and MUC5AC), SARS-CoV-2 entry-related /proteins/genes (ACE-2 and TMPRSS2), and cilia (alpha-tubulin). Single cell sequencing of the differentiated NET also confirmed the presence of differentiated cell types (goblet, club cells, basal, multiciliated cells, and not previously identified cells (NPIC). The NPIC were enriched in response to allergic rhinitis/respiratory syncytial virus infection (increase in IL1RL1, MMP9, MMP10) and some laminins and integrins. Infection of the NET with viruses also showed high expression of IDO1, which is associated with innate antiviral immune functions. To monitor reproducibility, the MTT and TEER results from N=9 lots were analyzed: MTT OD values were >1.0 (mean OD =1.7±0.2) and TEER values were 300.9±42.4 Ω*cm2 (n=9 lots). To evaluate the utility of the nasal tissue model for toxicological studies, we tested the effect of a known mucous membrane irritant, butylamine, following 4 hr topical exposure to 0.5 mg/mL and 2 mg/mL of the test article. The irritant reduced the barrier integrity to 33.2% ± 0.0 and 12.3% ± 5.8, respectively, compared to the vehicle control (corn oil), which is indicative of toxicity of the test article. In short, this novel NET model can be added to the toxicologist's toolbox of 3D respiratory tissue models to predict safety of chemical inhalants, therapeutic candidates, viral and bacterial infections at the inhalation entry site.

LP-91

Investigating the impact of gap scheduling on the toxicity of PARP1-selective AZD5305 combined with carboplatin using bone marrow MPS and QST modelling

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Oncology drug combinations as personalized cancer treatments have been a popular strategy to target tumour heterogeneity. However, finding tolerable and efficacious schedules can be challenging, particularly due to overlapping bone marrow (BM) toxicity. In this study, we aimed to predict the tolerability of clinical schedules and support clinical trial design by integrating bone marrow microphysiological system (BM MPS) data with our quantitative systems toxicology (QST) modelling framework. Our results suggested that the sequential administration of AZD5305 after carboplatin with gaps of 24 or 48 hours would not mitigate the toxicity of concurrent schedules and informed decision-making to open two parallel cohorts with PARP1-selective AZD5305 in combination with carboplatin.

LP-92

Evaluation of *in silico* method for Ames mutagenicity of pesticides and their metabolites

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Introduction:
In silico methods, such as quantitative structure-activity relationship (QSAR) models, have been applied to the mutagenicity evaluation of pesticide metabolites at the recent pesticide evaluation in the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as well as the European Food Safety Authority (EFSA). Currently, we are investigating the applicability of *in silico* methods to the pesticides evaluation in Japan. In this study, as a case study, *in silico* methods for predicting Ames mutagenicity were applied for pesticides and their metabolites based on the evaluation reports from the Food Safety Commission of Japan (FSCJ). The reliability of QSAR prediction was evaluated.
Method:
The 15 pesticides, for which *in silico* methods were applied in the evaluation reports from JMPR or EFSA, were selected, i.e. ethiprole, etoxazole, quinclorac, chlorfenapyr, cyfluthrin, dimethenamide, spinosad, bifenazate, Pyriproxyfen, flupyradifron, flumioxazin, florpyrauxifenebenzyl, mandipropamide, mandestrobin, mepanipyrim. 2D chemical structures (SMILES) of their parent pesticides and metabolites were prepared for Ames QSAR analysis. *In silico* assessment of Ames mutagenicity was performed using complementary two different types of QSAR software

(statistical-base: CASE Ultra, rule-base: Derek Nexus) and reliability of prediction results was evaluated compared to the reported results of Ames test for the parent substance and some metabolites.
Results and Discussion:
Within the 15 pesticides, quinclorac and bifenazate had positive prediction results with one and two QSAR software, respectively. These two pesticides were predicted to be positive based on alert substructures, i.e. quinoline of quinclolac and hydrazine of bifenazate, but the Ames test results reported in FSCJ reports were negative. Consequently, QSAR results were judged false positives. In addition, the large number of metabolites of quinclolac and bifenazate were predicted as positive in contrast to that only a few metabolites were predicted as positive in remaining 13 pesticides. All of quinclolac and many of bifenazate metabolites have same structural alert with parent compounds. This may lead to false positive prediction. Positive prediction results for other metabolites based on another alert structures may indicate mutagenicity, whose positive may need to be confirmed by the Ames test.
Conclusion:
From the results of our evaluation, 1) *in silico* methods are applicable for evaluating Ames mutagenicity of pesticide metabolites and 2) the possibility of false positives can be rule out by comparison with the Ames test results obtained for the parent pesticide and some metabolites.

LP-93

Comparison of hepatic cellular models for toxicity testing: effect of central metabolism inhibitors on mitochondrial respiration

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The liver is a main metabolic hub in the body, being responsible for central metabolic pathways, as well as xenobiotic metabolism. Having cellular systems able to mimic human hepatic metabolism is a priority in studying bioavailability and potential toxicity of drugs. Despite the existence of several hepatic cell models, many lack essential metabolic activities, influencing toxicity outcomes in many case studies. This research aims to metabolically characterize different human liver cell models using metabolic and toxicity assays. Well-established cell systems as HepG2 and HepaRG were compared with the newly implemented human-induced pluripotent cells derived into hepatocytes (Hep-like cells).

To perform the metabolic characterization, model compounds targeting various metabolic pathways were used as metabolic challenges. These compounds were UK-5099 (mitochondrial pyruvate carrier blocker), etomoxir (inhibitor of the fatty acid oxidation), 2-deoxy-D-glucose (glucose analog inhibiting glycolysis), 3-nitropropionic acid (inhibitor of the succinate dehydrogenase in the TCA cycle), and rotenone (complex I inhibitor of the mitochondrial electron transport).

Cytotoxicity of each compound over 24-hour incubation was evaluated with the resazurin-based assay in each cellular model: HepG2, HepaRG, and Hep-like cells. In addition, a respiration (Mitostress) assay using the Seahorse analyzer was used to assess the effect of the selected compounds on mitochondrial respiration.

Upon compound exposure, a higher sensitivity was observed in Hep-like cells compared to the other two models, in line with previous findings [Boon et al]. From these preliminary findings, Hep-like cells show potential in recapitulating a metabolic behavior closer to human primary hepatocytes. Improving hepatic *in-vitro* models to assess drug toxicity is an essential milestone towards replacing animal models.

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LP-94

Production of secondary microplastics using a physical abrasion technique for toxicity studies

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Microplastics (MP) are found in the environment and humans are exposed to an estimated 86 to 168,000 particles per day orally. To facilitate study of MP effects on human health MP reference materials are required. For their production a protocol was developed that uses physical abrasion of polymers via by stainless-steel beads within an ultrasonic tissue homogenizer, with liquid nitrogen pre-treatment and dry ice exposure during production to reduce temperature and maintain brittleness. Polyethylene terephthalate (PET) “virgin” and 3 different branded water bottles were used for the production. Virgin PET MPs had an average median diameter of 44.01µm (+6.24). PET branded bottles produced a MP of smaller average median diameter; bottle A 30.82µm (+4.97), bottle B 28.57µm (+7.37) and bottle C 25.32µm (+9.70). Virgin PET MPs were more irregular compared to those from the branded bottles. Metal analysis using ICP-MS was conducted to assess potential contamination from the production method. Several metals were detected in the first batches of MPs in particular aluminum. It was hypothesized that the aluminum originated from aluminum foil trays that had been used to avoid plastic contamination during the freeze-drying process. Glass containers were implemented instead, and this change resulted in the aluminum content being almost totally eliminated. As aluminum binds to PET MPs this provides an opportunity to look at the effect on toxicity of aluminum bound to PET. The MPs are being used for toxicity studies focused on in vitro human gastrointestinal fluid and cell model exposures, cell uptake studies and to generate knowledge for risk assessments of the effect of environmental MPs on human health.

LP-95

Assisting toxicological risk assessments of chemicals with VHP4Safety.

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The Virtual Human Platform for Safety Assessment (VHP4Safety) is a project from the Dutch national science agenda with the ultimate goal of providing solutions to perform precision safety assessments solely based on human data, without the use of laboratory animals. The aim is to improve the prediction of potential harmful effects of chemicals, including pharmaceuticals, based on the integration of data on human physiology, chemical characteristics and perturbations of biological processes. The project works on the development of a cloud-based infrastructure that integrates in silico models combined with databases and new approach methodologies for in vitro evaluations, to perform chemical risk assessments relevant to humans.

Within the VHP4Safety project, various in silico models are developed to predict the toxicokinetics and toxicodynamics of chemicals and pharmaceuticals and their effect on human physiology. For example, PBPK models and metabolism predictions are developed to provide insights into the toxicokinetics, linking external exposure and internal concentrations in specific organs. In the platform, the Adverse Outcome Pathway (AOP), a risk assessment framework that captures mechanistic information of toxicological processes, linked by measurable endpoints that progress toward an adverse outcome, is a key element. Furthermore, QSAR models, MIE predictions and quantitative AOPs provide insights into toxicodynamics.

A central idea of the VHP4Safety project is the integration of in silico models, in vitro data and biological knowledge, for providing safety estimates and a thorough understanding of potential risks. Another objective of the platform is to manage access to existing clinical and in vitro data from external resources, including patient, biomonitoring and epidemiological data, as well as experimental data generated in the project. To integrate the large variety of data and models in a virtual infrastructure, core data management concepts such as Data Management Plans and FAIR principles are applied where possible, providing all essential information on the data life cycle and metadata, linked to ontologies and persistent identifiers for optimal integration and use in computational workflows.

The aim of this poster is to share the progress within VHP4Safety with the Toxicology community and to have open discussions on the implemented approaches in modelling, experimental analysis, integration, and communication, which we will initiate by formulating questions for the audience. This way, we will be able to create a more inclusive community and optimise future developments within the project for application in risk assessments.

LP-96

Sub-acute Oral Toxicity Study of Nano-Bentonite, Co-solvent Bentonite, Micro-sized clay in ICR mouse

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This study is a safety test to develop as a candidate material for primary plastic alternative materials from Nano-Bentonite(NB), Co-solvent Bentonite(CB) and Micro-sized clay(MC) derived from mineral resources. We performed sub-acute oral administration (4 weeks repeated) in ICR mouse. The NB, CB and MC were administered to male and female ICR mice at oral doses of 0 and 1000 mg/kg (body weight). Experimental animals were monitored for the mortality, body weight changes, food intake, clinical signs gross findings during 4 weeks after dosing. It was also accompanied by hematological, biochemical analyses and histopathology. In the above studies, the no-observed adverse effects level (NOAEL) is considered to be 1000mg/kg/day for both sexes. Therefore, we concluded that NB, CB and MC have weak toxicity in ICR mice.

LP-97

Novel mechanistic assessment of hematotoxicity induced by CRBN-based PROTACs using CRISPR tools

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Immunomodulatory imide drugs (IMiDs) are successfully used in the clinic against multiple myeloma and act as molecular glues bringing together the E3 ligase Cereblon (CRBN) and various neo-substrates for targeted proteasomal degradation. The affinity of IMiDs for CRBN has been exploited to enable linking of novel proteolysis-targeting chimera (PROTAC) targets to this E3 ligase. However, degradation of the IMiD neo-substrate Ikaros, which has been linked to IMiD induced hematotoxicity, can still occur with PROTACs containing an IMiD-based warhead. Hence, we applied CRISPR knockout tools to investigate the role of Ikaros in human hematopoietic stem and progenitor cells (HSPCs) differentiation/proliferation assays.

Knocking out CRBN via CRISPR confirmed that the IMiDs lenalidomide and pomalidomide caused CRBN-mediated degradation of Ikaros in HSPCs. Furthermore, the knockout of Ikaros itself affected HSPC differentiation/proliferation into the erythroid or granulocyte/monocyte lineage whereas megakaryocyte markers were increased in accordance with the known role of Ikaros as an inhibitor of megakaryopoiesis. Similar effects were observed after treatment with the IMiDs pomalidomide or iberdomide providing confidence that Ikaros degradation is a key molecular event driving hematotoxicity. Finally, the availability of CRBN KO HSPCs for our *in vitro* hematotoxicity assay has provided the capability to discriminate CRBN-mediated events vs off-target hematotoxicity of IMiD-based PROTACs.

In conclusion, our results provide mechanistic confirmation that Ikaros degradation confers a risk of hematotox and guides our PROTAC chemical design. The development of CRBN KO HSPCs will have broad application going forward for mechanistic safety assessment of hematotoxicity for PROTAC modalities.

LP-98

Biochemical and Histopathological Effects of Acute and Chronic Pregabalin Cardiac Toxicity in Adult Albino Rats

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Background: Pregabalin (PGB) misuse and abuse are increasing problems worldwide. It is widely used in clinical practice. In Egypt, an increasing number of patients were admitted to poison control centers intoxicated with PGB. **Aim:** To identify the possible changes in heart attributable to PGB administration in both acute and chronic toxicity in adult albino rats. **Materials and Methods***:* Ninety-six adult albino rats weighing (100-150g) were randomly divided into 3 equal groups. Group I served as a control group. Group II (acute PGB toxicity) was administrated a single oral LD_{50} of PGB (5000mg/kg body weight) and group III (chronic PGB toxicity) was administrated different doses (500 and 1000 mg/kg body weight /day representing 1/10 and 1/5 $LD_{50,}$ respectively) for 6 and 12 weeks. The biochemical assay of cardiac enzymes was performed with an assessment of the histopathological changes in the heart. **Results:** Significant increase ($p<0.05$) of cardiac enzymes (Creatine kinase and lactate dehydrogenase) in group II and III compared to control one. These results were supported by the histopathological examination in groups II and III compared to control group I. Regarding histopathological changes; cardiac tissue of group II revealed myocyte degeneration and congested intra-myocardial capillaries, while in group III showed necrosis, cytoplasmic vacuolization, congested intra-myocardial capillaries and fibrosis. **Conclusion:** Pregabalin proved to be cardiotoxic at the acute and chronic levels.

LP-99

Sparse toxicological similarity with supervised learning can evolve transcriptional read-across

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Purpose

Read-across (RAx) uses toxicity information of analog chemical substances based on similarity with a target substance. In general, structural similarity is used in traditional RAx, but it sometimes overlooks the toxicity of target chemicals. Hence, transcriptional RAx using toxicogenomics data based on toxicological similarity has emerged as a novel approach to improve traditional RAx. However, both the RAx have same limitation that similarities unrelated to toxicity interferes with precise evaluation. If toxicity-related structure or genes are automatically selected in similarity evaluation, RAx will be more accurate and will provide the rationale for their evaluation. Thus, the aim of the present study was to integrate supervised learning and modeling, which select toxicity-related features, in RAx to improve accuracy and interpretability.

Methods

We studied *in vivo* and *in vitro* rat transcriptome data of 115 chemical substances in the open TG-GATEs. The relationships among 115 substances were visualized using principal component analysis (PCA), Partial Least Squares – Discriminant analysis (PLS-DA), Orthogonal PLS-DA (OPLS-DA), sparce PLS-DA (sPLS-DA). PLS-DA,

OPLS-DA, and sPLS-DA are based on supervised learning using hepato-toxicity class, and sPLS-DA automatically selects toxicity-related features by regulating the sparseness. Discriminative ability of toxic substances and interpretability of mode of actions were evaluated.

Results and Discussions*In vivo* transcriptome data could discriminate hepato-toxic substances in all analyses, indicating low interference caused by non-toxic features. *In vitro* transcriptome data could not separate the toxic substances in PCA, and supervised methods (PLS-DA, OPLS-DA, and sPLS-DA) could contribute to distinguish toxicity classes. This indicates that non-toxic gene expression interferes the separation of hepato-toxic substances, and supervised learning removes the interference. Moreover, supervised methods narrowed down the genes that were involved in the key events in the adverse outcome pathway. PCA detected multiple mechanisms in the separation, while supervised methods detected them as a merged mechanism. Important genes identified were different among the methods; OPLS-DA and sPLS-DA tended to emphasize robustness as biomarkers for identification rather than importance in toxicity expression. Therefore, similarity using selected features related to toxicity would increase the accuracy of RAx and could help us understand the key events; however, we should be aware of the characteristics of statistical methods for RAx.

LP-100

Non-clinical evaluation of COVID-19 vaccines

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This study has evaluated the choice of species selection and study design in association with the outcomes of the non-clinical safety studies of the various SARS-CoV-2 vaccines. The study has been focused on Repeated Dose Toxicity (RDT) studies and on the Developmental and Reproductive Toxicity (DART).

Rat and rabbit were the most commonly used species in preclinical toxicity testing. The toxicity studies showed no serious adverse effects. Minor effects were observed as expected, i.e. local reactogenicity, immune response and macroscopic findings at the injection site. Some COVID-19 vaccine candidates show a DART study design suboptimal for antibody transfer during lactation.
In addition, comments received during the EMA assessment of the vaccines have been evaluated and consisted most frequently of commentary on study design, species selection and missing data regardless of the utilized vaccine concept. Use of supportive platform studies often substantiated the commentary on these main three categories.

Animal model-based toxicity testing has shown limited value in establishing safety of the vaccines, and, more importantly, low translational value in supporting clinical development. From a 3R perspective sponsors are encouraged to focus on products from the supportive platform, both with respect to RDT and DART studies. Regulatory emphasis on data obtained from vaccines with the same platform technology data can be used to support marketing approval of new vaccines.

LP-101

Novel read-across approach predicting *in vivo* transcriptome data using a virtual DNA microarray integrating machine learning -RAID™-

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Purpose

Since the EU enforced a marketing ban on cosmetic products and ingredients assessed by animal testing, non-animal methods to evaluate the safety of chemical substances have been a critical topic in toxicology. Read-across using toxicity profile of analog chemicals based on structural similarity is a potential non-animal method; however, chemical structures are not necessarily related to toxicity, and the mechanism of action is challenging to evaluate. Hence, a variety of *in vitro* examinations are necessary to assess biological similarities and refine read-across reliability, however, the data gap between *in vitro* and *in vivo* conditions has emerged as a new concern. Thus, we aimed to develop a novel read-across strategy using predicted *in vivo* gene expression profiles as similarity measures, which may concur with the weaknesses of the current read-across.

Methods

A virtual DNA microarray, regression analysis-based inductive DNA microarray (RAID™) that quantitatively predicts *in vivo* transcriptome data was developed using the chemical structure and *in vitro* transcriptome data. Rat *in vivo* and *in vitro* transcriptome data of 115 chemical substances were extracted from the open TG-GATEs, and chemical descriptors were calculated by alvaDesc. The elastic-net model, which uses machine learning, was repeatedly constructed to predict each *in vivo* gene expression level. Principal component analysis (PCA) was conducted to visualize predicted transcriptome data. Genes with high loading values were applied to gene ontology and pathway analyses to interpret the biological meaning for read-across. Moreover, as external validation, 21 chemicals (potential CYP inducible substances) derived from Ingenuity Knowledge Base were subjected to RAID™.

Results and Discussions

PCA of RAID™ predicted that *in vivo* transcriptome data discriminated toxic compounds from non-toxic compounds, whereas *in vitro* and chemical descriptor data did not separate them. Gene ontology and pathway analysis revealed that hepato-toxic substances were separatable using gene expression on xenobiotic metabolic activation via aryl hydrocarbon receptor (AHR) and peroxisome proliferator-activated receptor-alpha (PPARa). Moreover, visualization analysis of downstream pathways revealed toxicity-related adverse outcome pathways (AOP) and some important key events (KE), including peroxisome proliferation and oxidative stress. Validation studies revealed that chemical substances associated with these AOP and KE could be detected as toxic substances. These results indicated that the RAID™ could predict *in vivo* transcriptome space to search for adequate analogs for read-across that were not identified by *in vitro* studies and structural similarity. Thus, RAID™ could be helpful as an alternative to animal testing for a repeated-dose toxicity test with toxicogenomics.

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LP-102

Reproductive and developmental toxicity study of abamectin on male and female Wistar Hannover rats

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Abamectin is a widely used insecticide in agriculture and also used as a veterinary anthelmintic. Abamectin, 97% technical, was administered orally by gavage to four groups of animals, 20 males and 20 females each, at doses of 0, 0.1, 1.0 and 2.0 mg/kg body weight for 11 weeks for males and 10 weeks for females before mating, during mating and pregnancy. Experimental animals were mated with untreated (intact) animals. At the end of the exposure, the functional indicators of the gonad state and the animals' reproduction ability were studied. The indices of mating, conception, fertility, and pregnancy were determined. Based on the results, it can be concluded that under the conditions of the experiment, the test substance Abamectin 97% technical, when administered by gavage at doses of 1.0 and 2.0 mg/kg of body weight, has pronounced reproductive toxicity and fetotoxic effect. In males, antiandrogenic activity is manifested: a decrease in the percentage of motile spermatozoa, conception and fertility indices, the number of live fetuses, litter weight, fetal body weight, and an increase in post-implantation fatal death in intact females mated with experimental males. In females, a decrease in the average weight of fetuses and impairment of the oestrous cycle were established. Changes in fetal development at a dose of 2.0 mg/kg (slowdown in the skeleton ossification processes and increase in the number of fetuses with the presence of 14 pairs of ribs and shortening of the 13th pair of ribs) were revealed. At a dose of 0.1 mg/kg, the test substance Abamectin, 97% technical, showed no systemic toxic effect and did not harm reproductive function when exposed to male and female Wistar Han rats. The no-observed--adverse-effect level (NOAEL) of Abamectin technical for reproductive toxicity and fetal development was established at the level of 0.1 mg/kg body weight, based on the observed effects.

LP-103

Adaptogenic activity of 2,6-Dimethylpyridine-N-Oxide

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Introduction. Combined use of pesticides and plant growth regulators (PGR) based on pyridine-N-oxide derivatives reduce acute and sub-chronic toxicity of xenobiotics for mammals.

Results. In the Morris Water Maze, each group treated by Ivin showed reduced latency to reach the hidden platform on the 1st day after exposure and the testing day (after the training period) on 26% and 24%, respectively, compared to intact rats. The escape latency time was also significantly lower in the positive control group. The obtained results suggest links between Ivin administration and improvement of visual short-term memory and learning ability. Improvement of rats' cognitive function may be due to the antistress properties of Ivin, the same as Eleutherococcus exhibits. Exploratory behaviour of rats was positively affected by Ivin at a dose level of 0.013 mg/kg/bw. Rearing frequency in closed arms of Elevated Plus Maze significantly increased on 14 and 28 exposure days compared to intact rats and to initial data. Based on these results, can assume that improvement in exploratory behaviour may be due to the anxiolytic-like effects of Ivin. Rotarod test showed a positive correlation between Ivin exposure and test

performance of rats. Forced motor activity after a single exposure to Ivin in both doses was increased more than 10 times. Obtained similar results at a dose level of 0.013 mg/kg/bw on the 14th and 28th exposure days. The riding time of rats significantly increased compared to the intact and control groups. Eleutherococcus caused a positive effect only after 28 days of exposure. In conclusion, it can be assumed that Ivin is not inferior in its adaptogenic activity to Eleutherococcus.

LP-104

Pharmacokinetics and the dermal absorption of bromochlorophene, a cosmetic preservative ingredient, in rats

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Recently, the concern for the safety of cosmetic ingredients is increasing. Risk assessment is a process to identify the possible hazards of using specific products. In the risk assessment of cosmetic ingredients, dermal absorption is considered an important factor in calculating the systemic exposure dosage (SED) of the application of the product. Bromochlorophene (BCP) is a commonly used cosmetic preservative. To evaluate the effects of BCP exposure, in vitro dermal absorption and in vivo pharmacokinetic (PK) studies were conducted using gel and cream formulations. The Franz diffusion cell system and rat dorsal skin were used for tests according to the Korea Ministry of Food and Drug Safety guidelines for in vitro skin absorption methods. After the dermal application (1.13 mg/cm^2) of BCP in the gel and cream formulations, liquid chromatography–mass spectrometry (LC–MS/MS) was used to evaluate the amount of BCP that remained unabsorbed on the skin (WASH), and that was present in the receptor fluid (RF), stratum corneum (SC), and (epi)dermis (SKIN). The total dermal absorption rate of BCP was 7.42 ± 0.74% for the gel formulation and 1.5 ± 0.9% for the cream formulation. Total recovery in an in vitro dermal absorption study was 109.12 ± 8.79% and 105.43 ± 11.07% for the gel and cream formulations, respectively. In vivo PK and dermal absorption studies of BCP were performed following the Organization for Economic Cooperation and Development guidelines 417 and 427, respectively. When intravenous (i.v.) pharmacokinetics was performed, BCP was dissolved in glycerol formal and injected into the tail vein (*n* =3) of the rats at doses of 1 and 0.2 mg/kg. Dermal PK parameters were estimated by applying the gel and cream formulations (2.34 mg/kg of BCP as an active ingredient) to the dorsal skin of the rats. Intravenous and dermal PK parameters were analyzed using a non-compartmental method. The dermal bioavailability of BCP was determined as 12.20 ± 2.63% and 4.65 ± 0.60% for the gel and cream formulations, respectively. The representative dermal absorption of BCP was evaluated to be 12.20 ± 2.63% based on the results of the in vivo PK study.

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LP-105

Determination of pyrrolizidine alkaloids in honey and accompanying safety assessment for human consumption

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Pyrrolizidine alkaloids (PAs) are naturally occurring heterocyclic phytotoxins that are widely distributed in nature and present in more than 6,000 flowering plant species. PAs may be present as contaminants in honey, when bees forage on flowers of PA-containing plants. Human exposure may also result from use of PA producing plants as herbal teas and food supplements. In this study, PAs were determined in honey samples collected from 60 locations from different parts of Indonesia. Samples were quantitatively analysed for 65 PAs by using an ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method. In total, 24 honey samples contained PAs with concentrations ranging from 0.5 to 545.1 µg/kg. In these samples several slightly different PA profiles were seen, which were dominated by the monoesters intermedine and rinderine. In addition, a risk assessment was performed to estimate the risk of the detected PA concentrations to humans. Based on the levels of PAs present and directions for use given by the producers, an exposure and safety assessment of consumption of these honeys was performed

using the estimated daily intake (EDI) and margin of exposure (MOE) approach, as used by EFSA for evaluating chronic exposure. Evaluation of shorter-than-lifetime exposure was also considered using Habers' rule. The results of the study can support risk management in formulating regulatory actions with respect to the presence of PAs in honey.

LP-106

Moving from detection of cardiovascular liabilities to quantitative mechanistic translational understanding: challenges and opportunities

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Cardiovascular safety findings encompass a range of perturbations covering ECG changes, haemodynamics and cardiac pathology. These changes can occur independently or concomitantly, either directly or indirectly related to PK parameters. Within cardiovascular safety, molecular understanding is key to developing quantitative translational insights and ultimately to predicting quantitative outcomes in patients. The concepts and techniques used depend on whether the molecular mechanism is known or unknown. Molecular understanding of different cardiovascular effects varies, currently the key ion channels responsible for changes in QT, QRS and PR intervals are well established. In addition to some mechanisms for haemodynamic perturbations are known, for example inhibition of VEGFR2 and blood pressure increases. Integrated mathematical models have been developed that apply this mechanistic knowledge to predict the changes in ECG parameters or blood pressure based on *in vitro* inhibition at key molecular targets, predicted PK and determined transduction factors. This approach relies on both mechanistic insight and a single cellular perturbation underlying the physiological response.

However, cardiovascular effects are often multifactorial, and the mechanisms are largely unknown, presenting bigger challenges. Technological developments in terms of 'omics' technologies, off-target profiling and data mining/bioinformatics have the potential to begin to fill this void enabling novel insights over different biological scales. Using an in vitro model three dimensional (3D) human heterocellular cardiac microtissue with three constitutional cardiac cell types, we have successfully developed a combined experimental and computational workflow from cultivating cardiac microtissue to *in situ* single cell signalling pathway analysis (TOB*is* IMC). This integrates imaging mass cytometry (IMC) with a customised barcoding strategy of thiol-reactive organoid barcoding in situ (TOB*is*). It can simultaneously detect *in situ* signalling of interest with single cell resolution across multiple treatment conditions. Using validated cell-type markers and post translational modification signalling (PTMs) panel, this TOB*is* IMC pipeline successfully generated single cell data from three cardiac populations. The *in situ* single cell signalling data allows us to identify different patterns of cardiac cell-type specific responses to cardiotoxin treatments, for example, pAKT from PI3K pathway is preferably activated in cardiomyocyte population whereas pMEK1/2 from MAPK pathway is dominantly expressed in cardiac endothelial cells. This approach could be used as the basis for further mechanistic understanding. Success will facilitate quantitative predictive outcomes in patients and informed drug design.

LP-107

Repeated Dose 28-Day Oral Toxicity of Granite Diorite

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Granite diorite is a rock distributed in Mungyeong, Gyeongbuk and is also called Mungyeong Yakdol in South Korea. It is provided to livestock farmers in the Mungyeong area as a feed additive in the form of powdered raw stones collected from granite diorite outcrops, manufactured as a compound feed, and fed to Korean cattle and pigs.
In this study, 7-week-old male Spraque-Dawley(SD) rats were administered by oral gavage, once per day for 28 days. Those were separated into 4 groups (5 female and 5 male for each group) and given three dose of 500, 1000, 2000 mg/kg test article or vehicle. The test was conducted by observing mortality, clinical sign, body weight change, gross findings, measurement of feed intake and organ weight. The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs. Moreover, statistically significant changes were not observed in organ weight, hematological tests, and blood biochemical tests. Together, the no-observed adverse effects level (NOAEL) is considered to be 2000mg/kg/day in animals.

LP-108

UV light based digestion as an alternative method in high-throughput thyroid hormone uptake assays for monocarboxylate transporter 8

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Adequate amount of thyroid hormones (TH), specifically the prehormone thyroxine (T4) and the biologically active 3,5,3'-tri-iodothyronine (T3), are essential for early neurodevelopmental processes. Thyroid hormone transmembrane transporters (THTMT) are critical for regulating the passage of maternal TH over the placenta, as well as their passage from fetal circulation into the brain. Monocarboxylate transporter 8 (MCT8) is a highly specific TH transporter and essential for regulating the transport of T3 into the brain. Mutations in MCT8 are known to cause severe neurodevelopmental and cognitive effects known as the Allan-Herndon-Dudley syndrome (AHDS). Therefore, MCT8 inhibition may be an important molecular target for endocrine disrupting compounds (EDCs). Despite this no validated test assays exists yet to screen potential EDCs for reducing T3 uptake via MCT8. Recently a non-radioactive high-throughput screenings assay was developed for MCT8 using overexpressing cell models. A crucial part in this assay is the digestion of TH into free iodide by ammonium persulfate (APS) digestion, but possible better alternatives have never been tested. In this study we developed a new method to separate iodide from TH using UV light and incorporated this digestion method into the non-radioactive TH uptake assay. We used this modified TH uptake assay to screen a total of 31 potential EDCs, including seven positive and three negative control compounds. Furthermore we compared the recovery of iodide from TH between the UV light based digestion and the APS digestion.
We tested our new digestion method with the previously established non-radioactive assay using MDCK cells overexpressing MCT8. We measured T3 uptake in MCT8-MDCK cells in combination with a single high concentration

of 100 µM or 10 µM of the chemical. Only chemicals that significant reduction of T3 uptake in the initial screening were tested in a full dose response curve.
Of all 31 compounds tested, all known MCT8 inhibitors tested positive in our test system, while all negative control compounds tested negative. Additionally we have identified methylmercury (BMC20: 2 µM), bisphenol-Z (BMC20:11.2 µM), bisphenol-AF (BMC20:18.1 µM) and verapamil (BMC20: 26.9 µM) as weak MCT8 inhibitiors. Our results demonstrate that iodide recovery from the UV light digestion increased 2.5 fold for T4 and 1.5 fold for T3 compared to APS digestion. Moreover with UV light digestion differentiation between the iodide content of T3 and T4 was observed, something that is not present with APS digestion. These data demonstrate that UV light digestion would be a better alternative for the non-radioactive TH uptake assays compared to APS digestion.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant number 825161 (ATHENA)

LP-109

Developing TXP Immunoaffinity- Assay for Glomerular Injury Protein Biomarker Candidates in Rat and Human Urine

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Kidney biomarkers specific for glomerular injury are lacking. Albuminuria can reflect renal dysfunction but it fails to distinguish impaired tubular albumin reabsorption from glomerular leakage. Thus, the goal of this study was to identify urinary glomerular injury protein biomarker candidates in rat urine and then apply the novel biomarker candidates to detect glomerular injury in humans.
For glomerular biomarker discovery, a rat model of glomerulopathy was developed with an MSD compound that causes glomerular injury in rat in long term studies. For the discovery analysis, we applied a novel proteomics platform integrating antibodies specific for c-terminal 4 amino acid motifs (TXP-Abs) with liquid chromatography-mass spectrometry (IA-LC-MS/MS). This method reduces the plasma proteome background effect and allows for semi-targeting of podocyte specific proteins. Here, we used 58 TXP-Abs to fractionate rat urine samples collected from 42 rats with induced glomerular injury and performed label-free IA-LC-MS/MS quantitative proteomics analysis. As a result, three urinary proteins, Dedicator of Cytokinesis 1 (DOCK1), Podocin (NPHS2) and Microtubule Actin Crosslinking Factor 1 (Macf1), were identified as glomerular injury biomarker candidates and were used for further verification analysis in rats treated with doxorubicin or puromycin (compounds known to cause glomerular injury in rat). All three protein candidates were shown to be elevated in urine of doxorubicin and puromycin treated rats by 5 to 4 to 3-fold, respectively, when normalized to urine creatinine.

Utilizing our short epitope motif enrichment strategy (TXP), we further validated the glomerular injury IA-LC-MS/MS assay (assay linearity, digestion kinetics, intra- and inter variation) to quantify the glomerular injury biomarker candidates in human urine. For the latter human assay validation, we have included Nephrin (NPHS1) and Synaptopodin (SYNPO) in addition.
This 5-plex assay was fit-for-purpose validated and showed sensitivity in the upper pg/mL range for all analytes. Reproducibility was within 100 +/- 20 % and analytes showed stability up to 3 freeze/thaw cycles, 24 h at room temperature, and 72 h in the autosampler. Performance of the multiplex assay was initially evaluated in urine samples

from healthy volunteers and patients with glomerular injury. Within the Innovative Medicine Initiative project TransBioLine we plan to confirm the utility of these candidate biomarkers in patients' urine samples with glomerular injury as manifested by progressive proteinuria and impairment of kidney function. These data represent an initial assessment in identifying glomerular-specific toxicity biomarkers in rat and human.

LP-110

Cannabis extract Nanoemulsion has significant anti-tumor activity against Glioblastoma in an in-vivo study

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Introduction:
The majority of the brain and other CNS tumors are glioblastomas. Recently, the antitumor effects of Cannabis against a variety of cancers has attracted researchers' attention. Here, we reported a nanoemulsion (NE) preparation for improved drug delivery of cannabis extract into glioblastoma animal models to compare the efficacy of the NE with the bulk form of the extract.

Methods:
The NEs were successfully prepared using the titration method. DLS, Zeta potential, and TEM were used for finding optimal NE and characterization. In-vivo studies were done using the C6 tumor model on rats with cerebral glioblastoma.

Results:
The required HLB 12.71 had the most stable Cannabis extract NE (NE drug) with the smallest particle size. This combination remained stable for more than 2 years. The stress tests, such as heating-cooling-cycling, centrifugation studies, and freeze-thaw cycles, demonstrated the physical stability of the NE formulations and showed no signs of degradation. Also DLS, Zeta potential, and TEM confirmed the size, stability, and morphology of NEs.
Animal studies confirmed that the tumor growth rate in the NEs group containing Cannabis extract was slower than in the free drug-treated and control groups.

Discussion:
Our platform showed that these NEs which contain drug could be beneficial for lowering the progress of tumor growth in cerebral glioblastoma, although there is more room to test the challenges with the platform.

LP-111

Thimerosal effects on microglia[1]

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Introduction: Microglia is a crucial glial component that has important functions in inflammatory diseases particularly in Central Nervous System (CNS), being considered central for toxicity. Furthermore, microglia are responsible for the activation of immune responses and play essential role in the maintenance of brain homeostasis. The activation of microglia is important for the removal of unnecessary cells and pathogens and thus, it also has a neuroprotective role against CNS diseases including malignant brain tumours.
Thimerosal (TM) and its metabolite ethylmercury (EtHg) are very controversial compounds in terms of neurotoxicity in relation to the administration of thimerosal-containing vaccines (TCV), although to our knowledge there are no studies evaluating their effects of on microglia.

Objectives: This study aims to determine the effects of TM and EtHg, on N9 microglia cell line.

Materials and Methods: The effects of TM and EtHg, on N9 cell line viability were determinethrough MTT assay. To determine the activation of microglia by TM and EtHg, IL-1β; iNOS and TNF-α genes were analyzed by using RT-PCR. Beclin-1 expression was determined through Western Blot analysis.

Results: The viability results obtained through the MTT method, indicate that TM is the most toxic compound for N9 cells (GI_{50} 1.4 μM) in comparison to EtHg (GI_{50} 2.3 μM) at 24h as well at others time points studied (48 and 72h).
By using PCR, it was verified a higher expression of interleukin IL-1β specially at 3h, which indicated that microglia are activated by TM and its metabolite. In the case of iNOS, another inflammatory cytokine, there is also an increase of expression at 3h.
TNF-α appears as a consequence of a more critical inflammation state and its concentration was very low at all timepoints tested. Therefore, TM and EtHg activate microglia, but this does not necessarily imply cell death.
The results obtained from Beclin-1 expression at 3h and 24h, showed that TM at 1 μM and 2μM leads to an increase of Beclin-1, which is responsible for the beginning of autophagosome formation.

Conclusions: Our study demonstrates that, TM and its metabolite EtHg, activate microglia by eliciting inflammation and this new data should be considered on risk assessment of neurotoxicity by TM exposure, although these compounds have been restrained in vaccines and other pharmaceutical formulations.
On the other hand, the results obtained are also of outmost importance for the development of new therapies for glioma tumours as hard-repurposing of thimerosal through microglia activation may constitute a second line of treatment.

Acknowledgments: This study was funded by FCT - Portugal through projects Target_Cancer (PTDC/MED-FAR/31136/2017) and by iMed.ULisboa (UIB04138/2020) also from FCT.

LP-112

Toxicity assessment of flavoured e-liquids and their aerosols using cigarette smoke as context

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The safety of e-cigarette vaping and its potential as a harm reduction alternative to smoking is the subject of much scientific discussion. E-liquid flavours play a vital role in providing choice to cigarette consumers switching to vaping. It is essential that the safety of flavours is assessed to ensure that their inclusion does not diminish the harm reduction potential of e-cigarette use relative to cigarette smoking. Here we detail a two-tiered *in vitro* approach for the assessment of e-cigarette aerosols whereby Vuse ePod e-liquids were initially screened for cytotoxicity potential using a lung cell line. The aerosols generated from a selected subset of these flavoured e-liquids were then further assessed in an optimised, physiologically relevant organotypic whole aerosol exposure model. 3D reconstituted airway tissues (MucilAir) were coupled with an LM4E Borgwaldt Aerosol generator, which delivered undiluted aerosol to the tissues at the air liquid interface (ALI).

The optimised approach yields greater potential for differentiating between e-cigarette products by employing an increased number of functional endpoints relevant to lung disease. The use of undiluted aerosol enables higher throughput (due to decreased exposure times), while the exposures used allows human relevance of the dose-response to established (when comparing to average daily consumption). To contextualise vapour aerosol responses, we compared the vapour responses to those induced by cigarette smoke (1R6F research cigarette) and calculated the percentage reduction in toxicity using a point of departure (PoD) approach. The measurement of nicotine in basal cell culture medium following exposure to aerosols allowed direct comparison of cigarette and e-cigarette responses.
Taking the IC_{80} (exposure dose that results in 20% reduction in cell viability) as the comparative reference point, aerosols from all flavoured Vuse ePod e-liquids tested showed a >95% reduction in cytotoxicity compared with cigarette smoke exposure in the MucilAir model. Using this two-tiered approach, we demonstrate that the toxicology of the most complex flavours remains significantly reduced when compared to cigarette smoke toxicity.

LP-113

Challenges in safety assessments of delivery systems in the context of vaccine and gene therapy

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Advances in viral and non-viral delivery systems are bringing a wave of hope to many patients in the gene therapy and vaccine spaces. Whether it is through the development of innovative and highly effective delivery nanoparticles and/or new nucleotide modalities, sponsors have a growing arsenal to deliver the promise of these technologies. An ever-expanding toolkits indeed represent a huge opportunity to save lives, however, the combinations of complex new technologies comes with their own risks.

The safety of these systems remains one of the key challenges to overcome in the context of clinical translation. Immune-related risks have been associated to these technologies and are acknowledged to be the cause of adverse events in the clinic. For DNA-based modalities, a risk of DNA integration in host cells genome exist. Traditionally, pre-clinical animal work has been used to test the safety liabilities associated with delivery systems and nucleotide modalities. Could *in vitro* models do more?In this poster, we will review some of the risks associated with these technologies, influencing factors and discuss *in vitro* strategies to evaluate and mitigate safety concerns.

LP-114

Mice kidney Wildly-Target metabolomics after the exposure to cantharidin through oxidative stress combined with network pharmacology

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Cantharidin (CTD) is principal bioactive component of traditional Chinese medicine Mylabris for clinical treatment of tumors. But CTD clinical application is limited due to nephrotoxicity and the mechanism remains obscure. In this study, an accurately quantification based widely□targeted metabolomics combined with network pharmacology and cell experiments were utilized to investigate the nephrotoxicity mechanism after exposed to CTD. Serum creatinine and urea nitrogen levels were increased with renal injury in mice exposed to CTD. A total of 76 differential metabolites including free fatty acid (18:1), ubiquinone-1, and ADP were detected in mice. Four metabolic pathways including tyrosine and pyrimidine metabolism were disturbed. Furtherly, network pharmacology indicated that CTD could activate oxidative phosphorylation and oxidative stress (OS), of which NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12, nuclear factor erythroid 2-related factor 2, and Kelch-like ECH-associated protein 1 may be therapy targets of CTD-induced nephrotoxicity. Subsequently, HK-2 cell experiments showed that CTD could reduce superoxide dismutase and increase malondialdehyde levels. In conclusion, tyrosine and pyrimidine metabolism could be disturbed by CTD, leading to oxidative phosphorylation and OS of nephrotoxicity.

LP-115

Challenging the proposed CLP classifications of the contact irritants Captan and Folpet

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Captan and Folpet have an EU harmonized classification based on an evaluation from the early 2000's. Due to the re-registration processes regarding their use as active substances in plant protection products, the harmonized classification is proposed to be revised.
The datasets for Captan and Folpet clearly establish local acute irritation as their only toxic mode of action. There is no convincing evidence for any systemic, non-local or non-acute effects; all effects are of primary acute aetiology. Such a clear hazard profile is rare for fungicides and should be appropriately captured in the hazard classification and consequent labelling of products.

We discuss whether the proposed, revised hazard classification for five hazard categories for the same underlying toxicity (irritation) represents an extreme case of "double classification". Based on the data, Captan and Folpet can be robustly classified for acute inhalation toxicity, eye irritation and skin sensitization. Other classifications are inappropriate (STOT RE 1 and skin irritation), redundant (STOT RE 1) or irrelevant for humans (STOT RE 1, skin irritation, carcinogenicity) and inappropriately communicate the substances' hazards for humans.

LP-117

Could herbal soup be a potentially unrecognized cause of hepatotoxicity at autopsy?

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Unexpected hepatic failure with liver necrosis is sometimes encountered during a forensic autopsy. Determining the aetiology may sometimes be difficult, although increasingly herbal medicines are being implicated. To determine whether such effects might also be cause by foodstuffs containing herbal products, the following in vitro study was undertaken. Four formulations of traditional herbal soup advertised as *bak kut teh* were prepared and added to cultures of liver carcinoma cells (HepG2). Cell viability was assessed using an MTT colorimetric assay at 48 h demonstrating that all formulations have significant toxicity prior to dilution ($p < 0.05$). Formulation #1 showed 21% cell death ($p = 0.023$), formulation #2 30% ($p = 0.009$), and formulation #3 41% ($p < 0.0001$). Formulations #1-3 showed no significant toxicity once diluted ($p > 0.05$). Formulation #4 showed approximately 83% cell death before dilution ($p < 0.0001$) and persistent toxicity even with dilutions at 1:10 (15% ± 3.7, $p = 0.023$) and 1:1000 (14% ± 3.8, $p = 0.024$). This study has shown that herbal foodstuffs such as *bak kut teh* may be responsible for variable degrees of in vitro hepatotoxicity, thus extending the range of herbal products that may be potentially injurious to the liver. If unexpected liver damage is encountered at autopsy, information on possible recent ingestion of herbal food preparations should be sought as routine toxicology screening will not identify the active components. Liver damage may therefore be caused not only by herbal medicines but possibly by herbal products contained in food.

LP-118

Integration of Evidence Regarding the Extent of Oral Absorption of Atrorosin E

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Atrorosin E is a natural pigment derived from fungus and bears strong structural and physicochemical similarities to a number of food dyes currently approved for use by the European Food Safety Authority (EFSA). An evaluation of the available evidence for the low bioavailability of Atrorosin E using physiologically based pharmacokinetic (PBPK) modelling of the experimental studies and read-across from colorants with similar structures that are currently accepted for use in foods by EFSA was undertaken. At the request of Chromologics, Inc. Ramboll US Consulting,

Inc. (Ramboll) scientists developed a physiologically based pharmacokinetic (PBPK) model for Atrorosin E in rats to evaluate the internal consistency of plasma dosimetry data from oral gavage and intravenous (IV) dosing to investigate whether the fractional oral absorption of Atrorosin E is extremely low. Read-across from food colorants with structures and properties similar to Atrorosin E was also performed to evaluate the likelihood of such a low oral bioavailability. The PBPK model was able to replicate experimental plasma concentrations after both oral and IV administration utilizing a single set of biologically plausible parameters. The PBPK modelling results support the conclusion that the gastrointestinal absorption of Atrorosin E is negligible, as indicated by an exceptionally low absorption rate constant (Ka), in comparison to the faecal elimination rate constant (Kf). The model predicts that at 2 hours, only approximately 0.3% of the administered oral Atrorosin E will be absorbed and that ultimately only 1.4% of the entire dose will be absorbed. Read-across demonstrates that the observed low fractional uptake of Atrorosin E is consistent with experimental data for food colorants with similar chemical structures and properties. The low oral bioavailability of these colorants possibly results from their inability to diffuse through the intestinal epithelium or to be substrates for oral uptake transporters. This in turn means that the rate of uptake is much lower than the rate of faecal elimination.

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LP-119

EMA-Mutamind: Which properties determine the mutagenicity of API-derived nitrosamines?

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N-nitrosamines (NAs) are considered to be mutagenic after metabolic activation, as they can form DNA adducts, which may subsequently lead to cancer formation via mutations. For small NAs, recently differences in potency can be associated with structure-activity trends, in particular the impact of sterically hindering side chains and their electron donor or withdrawal properties (Cross et al. 2021).

As NAs are currently a topic of high concern for pharmaceuticals, due to detection of NA impurities in certain drugs, three EMA-Mutamind projects were set up to better understand the different processes involved in mutagenicity of NAs, with a special focus on bulky, drug-related compounds. The processes of interest are:

1. formation of NAs from APIs under realistic conditions in different regions of the gastrointestinal tract, dependent on e.g. the human microbiome, pH-value, nitrate/nitrite concentrations.;
2. metabolic activation of drug-derived NAs with identification of involved enzymes, compared to the known metabolism of small NAs such as diethyl- and dimethyl-nitrosamine;
3. DNA adduct formation including types and kinetics and their specific repair processes.

One of the EMA-Mutamind projects, furthermore, aims at optimizing the Ames test conditions for mutagenicity testing of NAs and to evaluate the predictive value of the *in vitro* alkaline Comet assay with different liver cell models for carcinogenicity of NAs. All generated data will finally be used to develop predictive structure activity relationships and/or to distinguish classes of less from more potent NAs.

The poster outlines the recently agreed testing strategies, as well as the study protocols for a list of over 44 selected NAs that were chosen to address the above listed biological processes.

This work is funded by the European Medicines Agency (EMA) within the framework contract EMA/2020/46/L1.02.

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