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# ASBTRACT BOOK

Park Regis, Birmingham

# Regulation of Chemicals in Food

Claire Potter

Regulation of Chemicals in Food, Sky Loft, April 17, 2023, 9:35 AM - 10:00 AM

*Biography:*
*Claire graduated with a BSc (hons) in biochemistry with a year in industry from the University of York in 2003. Claire then worked for Pfizer as a cellular toxicologist. During this time, she helped set up a number of cell-based assays and set up a GLP stem cell lab. Following the closure of the Pfizer site in 2011 Claire joined the Food Standards Agency (FSA) as a toxicological risk assessor in the Chemical Risk Assessment (CRA) Team. She has remained at the FSA as a senior toxicological and radiological risk assessor working across the chemical and radiological risk assessment teams. Most recently she has become a Team Leader of the CRA Team with oversight of certain regulated products regimes and also acts as the technical lead for the Chemical Hazards Research Programme at the FSA.*

Many chemicals are present in food, some are naturally occurring, some are intentionally added and some occur through processing. Other chemicals which are not intended to be present in food occur through contamination or illegal addition. To ensure the safety of food, legislation can limit the level of some chemicals and authorise the use of others; but not all chemicals are covered by specific legislation.
The FSA Chemical Risk Assessment Team undertake risk assessments to ensure food is safe. These can be rapid responses to incidents, detailed reviews presented to a Scientific Advisory Committee, or, part of the authorisation (or not) of Regulated Products. The risk assessment process uses CODEX principles. Estimated exposures to a food chemical are compared to indicative safety values (health-based guidance value, margin of exposure or a threshold of toxicological concern). The outcome of the risk assessment is then communicated to Policy colleagues who then make a risk management decision.
Food regulation and risk assessment is changing. One of the biggest tasks currently faced by chemical risk assessors is ensuring that the regulation of chemicals in food uses all the best science and evidence and takes into account new and emerging technologies. EU exit has provided an opportunity for the UK regulatory process to be evaluated and updated to ensure that the complete evidence base is considered, new techniques can be accommodated and the principles of the 3Rs are upheld throughout the process of ensuring food safety.

# Regulation of Chemicals in Food

Ms Cath Mulholland

Regulation of Chemicals in Food, Sky Loft, April 17, 2023, 9:35 AM - 10:00 AM

*Biography:*
*Cath Mulholland attended Liverpool Polytechnic, doing a degree in Applied Biochemistry. After some adventures in malaria research and a spell in food research, she joined the Department of Health, initially working on cosmetics and consumer products. Moving into food toxicology, she has been with the Food Standards Agency since it started in 2000 and is currently a Team Leader in the Chemical Risk Assessment Unit as well as Scientific Secretary to the Committee on the Toxicology of Chemicals in Food, Consumer products and the Environment (COT)*

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# Regulation of Pesticides

Dr Susy Brescia

Regulation of Pesticides, Sky Loft, April 17, 2023, 10:00 AM - 10:25 AM

*Biography:*
Susy Brescia has a degree in Biological Sciences and Human Genetics from 1991 and a PhD in Occupational Toxicology and Epidemiology (1997), including post-graduate and post-doc experience in the field of genetics and molecular epidemiology.
She started work as a regulatory toxicologist with the UK Health & Safety Executive in 1999, being responsible for performing human health hazard and risk assessment for substances regulated under the Biocidal Product Regulation (BPR), Plant Protection Product Regulation (PPPR) and REACH. Also contributing to the technical aspects of Harmonised Classification and Labelling proposals under the CLP Regulation. She is currently the Head of the Toxicology Team with the Chemicals Regulation Division of the UK Health and Safety Executive.

She has been involved in drafting technical guidance documents for performing chemical risk assessment in the context of REACH and BPR and preparing WHO/IPCS, OECD, EFSA and ECHA technical reports on specific chemicals and various aspects of chemical hazard and risk assessment.
She is a member of several national and international committees and working groups, including the WHO/FAO JMPR (Joint Meeting on Pesticides Residues), the UK NC3Rs (National Centre for the 3Rs) NAM (New Approach Methodologies) oversight group, the OECD RCEG (Residue Chemistry Expert Group) for the preparation of the guidance on the residue definition for risk assessment for pesticides.
She is a registered toxicologist at the British Society of toxicology (BTS), chair of the risk assessment speciality section and member of the BTS Scientific SubCommittee.

HSE CRD is the regulatory authority in the UK for several chemical regimes, including pesticides. Pesticides are regulated under the retained Reg 1107/2009 as it applies in GB (Great Britain). Active ingredients are subject to an extensive assessment before they can be approved for use. An active substance can only be approved if it does not meet hazard-based exclusion criteria for certain endpoints, the dietary and non-dietary risks are acceptable and MRLs (Maximum Residue Levels) are set for all affected commodities. Once a substance is approved, it can then be formulated in different products, which also require assessment before authorisation can be granted. Hazard identification, classification and characterisation and subsequent risk assessment are performed according to standard practices using GLP (Good Laboratory Practice) and guideline studies. Evaluation work is multidisciplinary, requires expert knowledge and judgement and is supported by the advice of expert committees. This ensures robustness of decision-making which needs to be transparent and scientifically sound.
The world of regulatory toxicology however is currently undergoing dramatic transformation. This is due to the impact of Brexit and ensuing regulatory reform and to the development of a new paradigm due to the development of NAMs (New Approaches Methodologies). Traditional toxicology based on animal tests is at a crossroad, with the 21st century witnessing a gradual and steady increment in the more confident application of NAMs and alternatives to animal testing. Regulatory toxicology has never been more exciting!

# Regulation of Consumer Products

Mrs Frances Hill

Regulation of Consumer Products, Sky Loft, April 17, 2023, 10:55 AM - 11:20 AM

*Biography:*
*Frances Hill is a Regulatory Toxicologist, having worked within the UK government for 20 years on a range of topics. Frances moved to BEIS in 2021 to lead Chemical Risk Assessment Team within the Office for Product Safety and Standards (OPSS) working to ensure that chemical risks relating to consumer products are identified and regulated appropriately.*

Chemical risk is only one of the risks posed by consumer products. Traditional chemical risk assessment methodologies don't work for many of these risks. I'm going to describe some of the methodologies we use and the challenges we have in trying to maintain a consistent approach across consumer products.

# Regulation of Pharmaceuticals

Dr John Clements

Regulation of Pharmaceuticals, Sky Loft, April 17, 2023, 11:20 AM - 11:45 AM

*Biography:*
*John obtained a PhD in genetics from the University of Leicester, before further academic research at the University of Rochester, USA. On returning to the UK he held various roles in the biopharmaceutical industry focussing on drug discovery and progressing drug candidates into early clinical development. John made the move to a regulatory role as a non-clinical assessor at the MHRA 14 years ago. At the MHRA John works in the Safety and Surveillance Division and is primarily responsible for evaluating non-clinical data in the assessment of the benefit-risk profile of new and marketed medicines.*

The talk will review the current regulatory requirements for pharmaceuticals and provide perspectives on the evolution of toxicity testing to strengthen regulatory risk assessment. Nonclinical testing of human pharmaceuticals is conducted to assess the safety of compounds to be studied in human clinical trials, for marketing of new drugs and during the post-marketing phase. Although there is no single guide on the type of studies required the general approach is outlined in various MHRA, EMA and ICH guidance documents and involves a combination of in vitro assays and whole animal testing methods. The current landscape for new medicines involves a number of different presentations from traditional small molecule medicines to more complex biological molecules, and innovative advanced therapy medicines which present particular challenges. There is inherent flexibility in medicines regulation and for different classes of drug the testing approach can vary if scientifically justified whilst following the principles of the guidance. Looking to the future, advances in biology such as omics technology, stem cells, micro-physiological systems and in silico modelling are creating new opportunities to better predict human outcomes.

# Plenary Lecture: Toxicological aspects of nano- and micro-plastic particles

Dr Todd Gouin

Plenary Lecture: Toxicological aspects of nano- and micro-plastic particles, Sky Gallery, April 17, 2023, 12:45 PM - 1:30 PM

*Biography:*
*Dr. Todd Gouin received his PhD specializing in the field of environmental chemistry from Trent University, in Canada, through the Watersheds Ecosystems Graduate Programme in June 2006. Following his graduate studies, Dr. Gouin, has obtained both experimental and modelling experience in assessing diverse chemical exposures including current use pesticides in Costa Rica and polycyclic aromatic hydrocarbons in the Arctic regions of Alaska. More recently, he was employed for eight years by Unilever, where he was involved in the development and application of tools aimed at both screening and prioritization of chemicals and high-tier risk assessment methods. He now provides research consultancy work on a range of topics, where his current activities include the development and application of risk assessment methods for particulates, such as microplastic particles, nanomaterials, and UVCBs, as well as the development and application of models to better assess chemical exposure for both humans and the environment.*

Concern regarding the human health implications that exposure to nano- and microplastic particles (NMPs) potentially represents continues to increase. The relative level of concern is influenced by a combination of factors, including an exponential increase in the number of studies reporting on the detection and potential adverse effects associated with NMPs over the last 20 years in both the scientific literature and popular media. While the majority of research has focused on evaluating the concentrations of MNPs in the environment relative to ecotoxicological effect levels, human health toxicity studies have only recently emerged. The available human health hazard data are thus limited, and have been accompanied by various concerns regarding the relevance and reliability towards understanding the potential human health implications that exposure to NMPs might represent. This presentation aims at summarizing the issue of NMPs, with a particular emphasis in the context of human health, including what is known and unknown in the context of both exposure and effects. An important element aims at considering how well the data available in the scientific literature represent information that is 'fit-for-purpose' in the context of human health, and which thus requires a need to critically evaluate the relevance and reliability of information obtained from numerous data sources. Evaluating 'fit-for-purpose' can be achieved through the use of screening tools, capable of evaluating both exposure and human health effects studies against a suite of quality assurance and quality control (QA/QC) criteria. By assigning criteria against specific assessment categories, such as particle characterization, experimental design, and applicability for risk assessment, it can be demonstrated how data might be screened and prioritized. The overall approach results in an observation that the majority of studies evaluated report effects data typically for a suite of monodisperse particles, predominately spheres (≈60%), consisting of polystyrene (≈46%). Furthermore, the majority of studies have tested particles < 5 µm, with a minimal particle size of 10 nm and a maximum particle size of about 200 µm. These data, however, are observed to be inconsistent with exposure data, which typically report the presence of a heterogeneous mixture of particles of varying polymeric composition, shapes and sizes, typically >10 µm, resulting in an obvious mismatch between effects data and NMPs that might be consistent with an environmentally relevant exposure. Insight gained through this critical evaluation, nonetheless, provides valuable insight towards helping to identify important research priorities to be addressed if data obtained from future studies are to be identified as 'fit-for-purpose' in the context of

assessing the implications to human health. These include a need for the generation and access to standard reference materials representative of human exposure to NMPs for use in toxicity test systems and/or the improved analytical characterization and verification of NMPs present in the environment, as well as an overwhelming need for researchers to more routinely adopt standardized study design guidance, such as recommended by OECD, when conducting either in vivo inhalation or oral ingestion toxicity tests and/or when sampling and analysing exposure in any environmental matrix.

# New application of dose-response analysis: deriving relative potencies for mixture risk assessment

Dr Bas Bokkers

New application of dose-response analysis: deriving relative potencies for mixture risk assessment, Sky Gallery, April 17, 2023, 3:00 PM - 3:30 PM

*Biography:*
*Bas GH Bokkers (PhD, ERT) is a human health risk assessor and modeller at the Centre for Safety of Substances and Products at the Dutch National Institute for Public Health and the Environment (RIVM). He started working on benchmark dose analysis in 2002, as a major component of his PhD research on probabilistic risk assessment of chemicals. His current work is primarily associated with benchmark dose analysis, and probabilistic (human) exposure assessment, hazard characterisation, risk assessment. He applies benchmark dose analysis to derive a point of departure for risk assessment, but it is also utilised for other goals, for example to derive data based extrapolation factors or relative potency factors (e.g. of PFASs).*

In toxicology and risk assessment dose-response analysis is generally used to derive a point of departure for further risk assessment, such as a BMD(L) or ED50. Another application of dose-response analysis is the derivation of the relative potency of substances. So called relative potency factors (RPFs) provide valuable information to perform quantitative risk assessment of exposure to mixtures. The RPF methodology has previously been applied to several groups of compounds, such as dioxins, organophosphorus pesticides, carbamate pesticides and per- and polyfluoroalkyl substances (PFAS). In this approach one compound of the group is considered as the reference (or index) compound. Using RPFs the exposure to each compound in a mixture is expressed in equivalents of the reference compound. In the mixture risk assessment, the sum of the equivalents can be compared to a guidance value of the reference compound.
RPFs can be derived using dose-response analysis, which should account for possible differences between underlying experiments. In this presentation the approach for deriving RPFs will be discussed, including the general prerequisites for deriving RPFs. Depending on the available toxicological data, two methods were developed and implemented in RIVM's dose-response modelling software, PROAST, to derive relative potency factors. Both methods will be discussed and illustrated using several experimental datasets.

# Learnings from when preclinical drug development failed to predict adverse events in clinical trials

Lap Hing (Leo) Chi

Learnings from when preclinical drug development failed to predict adverse events in clinical trials, Sky Loft, April 17, 2023, 3:00 PM - 3:30 PM

*Biography:*
*Leo is a junior postdoctoral researcher at the Peter MacCallum Cancer Centre in Melbourne, Australia. He completed his PhD in the study of breast cancer metastasis at the Olivia Newton-John Cancer Research Institute in 2022, and is experienced in the use of pre-clinical cell line, organoid and mouse models to investigate molecular and immune signalling pathways that are altered during cancer progression.*

The development and testing of novel therapeutics are essential to provide effective therapies that improve patient outcomes. However, this process is associated with high rates of attrition. For therapeutics entering phase I clinical trials, the probability of clinical success is approximately 13.8%. One of the frequent causes of therapeutic attrition is the onset of adverse events that were not predicted in preclinical studies. In several serious cases, they have led to organ failure, cancer development and deaths in clinical trial participants that were not expected outcomes in clinical trials. In this presentation, we examine a number of unexpected adverse events, such as on-target toxicities of TGN1412, navitoclax and CAR-T cell therapies, off-target toxicities of thalidomide, BIA10-2474 and gene therapies, and metabolism- or microbiome-mediated drug toxification, for which the mechanisms of toxicity have been revealed recently. We note that many conventional technologies and animal models used for preclinical safety testing suffer from the inability to faithfully represent human physiology, leading to failure in prediction of serious adverse events. A number of in vitro, ex vivo and in vivo models, including high-throughput screening technologies, organ-on-chips, microbiome-containing drug-testing platforms and humanised mouse models, have been developed to improve preclinical testing. We highlight the successful application of these technologies in the mechanistic studies and prediction of toxicity, and propose the incorporation of similar preclinical tests into future drug development to reduce the likelihood of hazardous therapeutics entering later stages of clinical trials.

# Non-monotonic dose-responses: state of the art

Prof Alan Boobis OBE

Non-monotonic dose-responses: state of the art, Sky Gallery, April 17, 2023, 3:30 PM - 4:00 PM

*Biography:*
*Alan Boobis is emeritus Professor of Toxicology, Imperial College London where he was a member for over 40 years. His main research interests lie in mechanistic toxicology, mode of action and chemical risk assessment. He has published over 250 original research papers (H-factor 80). He is or has been a member of several national and international advisory committees, including current chair of the UK Committee on Toxicity, member of the WHO Study Group on Tobacco Product Regulation (TobReg), FAO/WHO JECFA (veterinary residues) and FAO/WHO JMPR (pesticide residues). He is a member of the Board of Trustees of the International Life Sciences Institute (ILSI) and of the Board of Directors of ILSI Europe. Prof Boobis is a fellow of several learned societies and has received a number of prestigious awards, including Officer of the British Empire (OBE).*

Much has been written about non-monotonic dose response, yet there is still debate on whether it is of toxicological significance, how often it occurs, what are the underlying mechanisms, and should our approach to risk assessment change. Identifying whether a dose-response is non-monotonic is itself problematic. When reported, they are often over only one or two doses, which poses major statistical challenges. Random fluctuations in response in biological systems, particularly in intact organisms, are not uncommon. Is there confidence that even a statistically significant deviation from monotonicity in a study is biologically meaningful? In most cases where non-monotonic dose-response has been observed, no mechanistic basis is apparent. As in toxicology more generally, it is important to consider weight-of-evidence, including reproducibility, consistency and supporting evidence, for example in related key events. Toxicological significance means that a response is adverse, or at least potentially adverse. Even if non-monotonic changes occur in the dose-response for a molecular initiating event, this would have to be translated into a non-monotonic dose-response for an adverse outcome for it to be toxicologically significant. However, homeostatic and adaptive processes mean that this is very unlikely. Indeed, there are extremely few examples of where this has been reliably established. Where this is the case, it is due to the superimposition of opposing dose-response curves for different biological phenomena. With increasing mechanistic insight, for example through the analysis of adverse outcome pathways, it would seem more productive to pursue this rather than the concept on non-monotonicity per se.

# Thresholds for genotoxicity

Professor Gareth Jenkins

Thresholds for genotoxicity, Sky Gallery, April 17, 2023, 4:00 PM - 4:30 PM

*Biography:*
Gareth is Professor of Molecular Carcinogenesis at Swansea University's Medical School and an expert in DNA mutation research. Gareth has degrees from Kings College London (BSc Biophysics, 1990), the University of the West of England (MSc Biotechnology, 1991) and a PhD from University of Wales in 1997 in "Molecular Mutagenesis".

His research group at Swansea investigates the mechanisms underlying DNA mutagenesis and carcinogenesis. He mainly works in the "Genetic Toxicology "field studying hazards and risks posed by exposure to new products (drugs, foodstuffs, chemicals, nanomaterials etc). His group have been designing new cell-based testing approaches for mutagenic agents to reduce the numbers of animals used in safety testing. For 20 years he has also researched the Barrett's oesophagus/oesophageal adenocarcinoma model to better understand the initiation of cancer and the role of blood cell mutations as biomarkers of disease.

Gareth is the Chair of the UK Government's Committee on Mutagenicity (COM) and sat on COM as a member from 2009 till 2019. He is a Senior Editor for the journal "Mutagenesis" and President of the UK Environmental Mutagen Society (UKEMS) and the International Association of Environmental and Genomics Societies (IAEMGS). Gareth is also the Associate Dean of Research for the Faculty of Health and Life Science at Swansea University.

It was assumed in the past that DNA damage and DNA mutation was induced in a linear dose manner. Thus, it was implied that genotoxic agents induced DNA damage at even the lowest of exposure concentrations and that no "safe" dose range existed for carcinogenic risk. This was interpreted as a "one hit" scenario where a single DNA damaging event in a cell had the capability to induce a single point mutation in that cell that could (if positioned in a key growth controlling gene) lead to increased proliferation leading ultimately to the formation of a tumour. Previous research performed at high doses and extrapolated back to zero, appeared to support the linear concept for a wide range of mutagens including alkylating agents, radiation and chemicals inducing bulkier forms of DNA damage.
However, recent evidence accumulated over the past decade or so, has supported the concept of dose response thresholds for DNA mutation. This more recent data has produced a paradigm change in the field of genotoxicology, away from the linear-no-threshold (LNT) model that had previously been assumed for DNA reactive agents. The new threshold model accepts that low dose exposures to genotoxic carcinogens may not induce irreversible DNA mutation and may therefore be tolerated by cells and organisms. However, the threshold dose response data is still only available for a relatively small number of chemicals and so it is not clear how generalisable the threshold concept is. Mechanistic data supporting the threshold argument on a case-by-case basis (role of DNA repair, or detoxifaction, or role of antioxidant effects) is vital to fully accept the threshold argument. Using in vitro models in more quantitative ways to describe dose responses and identify thresholds (rather than binary genotoxic yes or no approaches) may also reduce the need to move to in vivo studies by default which has important 3Rs considerations.

Convincing data from many in vitro studies on genotoxic dose responses and in vivo data from the “viracept” incident in 2007/2008 and more recent dose response data on low dose nitrosamine exposures have provided key data to persuade regulatory agencies about the existence of genotoxic thresholds. Regulatory agencies now accept threshold arguments when reliable data is presented. Taking one step further, there is now data to suggest that very low dose genotoxic exposures can induce “hormetic” effects and show beneficial responses (reduced background mutation levels) in studies in vitro.

# Benefits of humanised mouse models in drug discovery

Prof Roland Wolf

Benefits of humanised mouse models in drug discovery, Sky Loft, April 17, 2023, 4:00 PM - 4:30 PM

*Biography:*

*Professor Wolf's research work has focused on molecular, genetic, biochemical and pharmacological studies into the pathways that have evolved to combat our exposure to environmental chemicals and oxidative stress. These pathways play a pivotal role in disease aetiology and in defining the outcome of drug treatment. His research work has encompassed a wide range of experimental approaches, including defining genetic variation in the expression of these genes and their association with disease susceptibility and therapeutic outcome, defining the structures of the proteins involved, particularly the cytochrome P450-dependent mono-oxygenase system, to molecular, genetic and pharmacological studies using transgenic models. In recent times the development and application of transgenic approaches has been the research focus of his group, where a wide range of genes involved in drug metabolism and pathways of chemical toxicity have been either deleted or humanised. In addition, a range of reporter systems which allow in vivo toxicological and environmental responses to be measured . These models are currently being used in academia and pharma in the drug discovery programmes and to predict drug/drug interactions.*

In order to circumvent the limitations of animal models to study drug efficacy and drug disposition several humanized mouse models for cytochrome P450s, CAR, and PXR have been described . However, the utility of these models has been compromised by the functional redundancy in P450 reactions across gene families, whereby the remaining murine P450s can metabolize the compounds being tested. To eliminate this confounding factor and create a mouse model that closely reflects human pathways of drug disposition, we have substituted 33 murine P450s from the major gene families involved in drug disposition, together with Car and Pxr, for human CAR, PXR, CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP3A4, and CYP3A7 (the 8HUM model). We have also created an immune deprived version of this line and mouse line in which 34 P450s have been deleted. (CypC4KO). Using these models, the role of the P450 system in the disposition of a particular compound can be determined and also drug exposure for NCEs can be significantly extended. We have therefore generated mouse models which replicates a major pathway of drug disposition in man and have carried out studies to demonstrate their utility for a range of applications in drug discovery and development and in the testing for and prediction of potential drug/drug interactions (DDIs). We also describe how the models can be used to optimize the design of clinical trials, particularly where the use of drug combinations are involved ie in polypharmacy.

# Endocrine disruptors: is a threshold approach justified?

Dr. Christopher Borgert

Endocrine disruptors: is a threshold approach justified?, Sky Gallery, April 17, 2023, 4:30 PM - 5:00 PM

*Biography:*

*Christopher J. Borgert, Ph.D. is President of APPLIED PHARMACOLOGY AND TOXICOLOGY, INC. (APT), a consulting firm that specializes in applied research in the areas of mechanistic pharmacology and toxicology, safety assessment, and study design. Dr. Borgert also holds a courtesy faculty appointment in the Department of Physiological Sciences, University of Florida College of Veterinary Medicine. He received an Artium Baccalaurei from Kenyon College, Gambier, Ohio, a doctorate in Pharmacology and Therapeutics from the University of Florida College of Medicine and completed a postdoctoral fellowship in toxicology at the University of Florida Center for Environmental and Human Toxicology. He served on the U.S.EPA Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as the general representative for Small Business stakeholders, testified before Congress regarding the Endocrine Disruptor Screening Program, and served on numerous national and international expert and peer-review panels such as the Society of Toxicology Expert Panel on Chemical Mixtures and the OECD Peer-Review Panel for Validation of the Uterotrophic Assay. His publications address methods for evaluating chemical mixtures and cumulative risk assessments for human exposure to drugs, dietary supplements and chemicals, Weight of Evidence approaches and mechanistic methods for evaluating Endocrine Disruptors and carcinogens, and use of kinetics in dose-setting and interpretation of toxicology studies.*

A threshold dose is one that is “just sufficient,” and an Endocrine Disruptive Chemical (EDC) is one that produces an adverse effect via an endocrine mode of action (MoA). Therefore, the appropriate approach for identifying EDCs depends on whether endocrine MoAs have threshold dose-response characteristics, not whether a lowest-observable or no-observable toxicological effect level can be identified or at what dose an adverse effect might be produced. In other words, the question of thresholds is about mechanistic properties, not about exposure and observable toxicity. Since the endocrine system functions via the interaction of small molecules (hormones) with biological macromolecules (receptors, enzymes, transporters, DNA-response elements, etc.), the dose-response relationship for EDCs is governed by fundamental principles of receptor, enzyme and transport kinetics. These kinetic principles comprise 1) potency, which is the product of affinity and intrinsic efficacy, and 2) mass action, which is a function of affinity and concentration. The understanding of potency and mass action has enabled unprecedented advancements in endocrine pharmacology over the past century. We have used these principles to evaluate whether a threshold approach should be used to identify EDCs by approaching the question from opposite ends of the mechanistic continuum. Using the human oestrogen receptor-alpha subtype (ERα) and its endogenous and exogenous ligands as a test system, we identified minimum levels of ligand potency – not administered dose or blood levels – associated with clinically observable oestrogenic effects. This approach demonstrated that ERα ligands with potency lower than one one-thousandth that of the endogenous hormone 17β-oestradiol do not produce clinically observable oestrogenic effects, which allowed us to propose a conservative Human-Relevant Potency Threshold (HRPT) for ERα ligands of 1 x 10-4 relative to 17β-oestradiol. We have since corroborated this HRPT using mass action calculations to identify minimum levels of ligand affinity required to occupy meaningful fractions of oestrogen receptors amidst the normal milieu of endogenous ERα ligands, comprised of precursors to hormones, metabolites of hormones, and other normal products of metabolism. Based on these analyses, we can now explain the physiological and

biochemical basis of potency thresholds for EDCs, which are established by the ligand potencies and background concentrations of the normal endogenous metabolomic milieu. Thus, the fundamental principles of endocrine pharmacology establish that a threshold approach to the identification of EDCs is not only justified but obligate.

# Unravelling species differences in hepatic stress response capacity to inform the selection of animals for use in preclinical drug safety assessmento

Ms Hannah Coghlan[1], Dr Ian Copple[1]
[1]*MRC Centre for Drug Safety Science, Department of Pharmacology & Therapeutics, University Of Liverpool, Liverpool, United Kingdom*

Oral Communication: Unravelling species differences in hepatic stress response capacity to inform the selection of animals for use in preclinical drug safety assessment, Sky Loft, April 17, 2023, 4:30 PM - 4:45 PM

Stress response pathways can influence the sensitivity of a species to drug toxicity affecting the liver and other organs. This study builds on our recent work[1] and investigates differences in inducible hepatic NRF2-mediated antioxidant response and unfolded protein response (UPR) capacity between humans, mice and rats that may influence sensitivity to hepatotoxicity during preclinical drug safety assessment.

Using publicly-available basal hepatic gene expression data[2] for humans, CD-1 mice and Sprague-Dawley rats, stress response-associated gene expression was compared. NRF2-associated genes had a rank order of expression of rat>mouse>human; UPR-associated genes had an expression rank order of human>rat>mouse (Figure 1). Primary hepatocytes isolated from the same species were exposed to the NRF2 activator CDDO-Me, the UPR inducer thapsigargin, or 0.5% DMSO as vehicle control, for 8 or 24h. qPCR analysis revealed that mouse and rat display greater upregulation of NRF2- and UPR-associated genes than human (Figures 2 & 3). Further, upregulation of UPR-associated genes was generally greater in mouse than rat; e.g., spliced:unspliced-XBP1 ratio of 20.3±11.0 (mouse) versus 3.3±2.0 (rat) following 8h 100nM thapsigargin exposure ($p=0.042$).

This work demonstrates that basal gene expression trends may influence a species' sensitivity to hepatotoxicity but cannot predict species-specific patterns of inducible stress response activity. Mouse and rat have greater transcriptional responses to pathway activation than human, which may underly increased tolerance to chemical insult or hepatotoxicity in rodents. Ongoing work is investigating the extent of these differences in non-rodent preclinical species including macaque, beagle and minipig, and encompasses additional stress response pathways and hepatotoxic drugs.

# Malcolm Blackwell Award & NEST Lecture: A cheap unlimited supply of donor-free functional hepatocytes for Toxicology, a NAM hiding in plain sight

Prof Matt Wright

Malcolm Blackwell Award & NEST Lecture: A cheap unlimited supply of donor-free functional hepatocytes for Toxicology, a NAM hiding in plain sight, Sky Gallery, April 17, 2023, 5:20 PM - 5:50 PM

*Biography:*
*Prof Matthew Wright has over 30 years research experience with liver-related drug /chemical metabolism and toxicity. He was the first to demonstrate that stimulating liver myofibroblast apoptosis reversed fibrosis; developed a human single chain antibody to target these cells (licensed to Pharma) and demonstrated an anti-fibrogenic, and anti-inflammatory role for the PXR in the liver. Current research is focussed on in vitro systems to model toxicity in the liver and the potential role of xenobiotics found in the environment as a trigger for the chronic liver disease primary biliary cholangitis (PBC). He has published in excess of 240 full research papers and scientific opinions related to toxicology; was an expert for the European Food Safety Authority ANS Panel (2011- 2018) and Chair for the Working Group pertaining to Article 8 – rapid response opinions on member state/EC concerns). He currently acts as an Associate Editor for the journal - Toxicology - and is a member of the European Food Safety Authority FAF Panel (since 2020), the UK Committee on Toxicity (COT) and the UK Expert Committee on Pesticides (ECP).*

Hepatocytes are valuable in Toxicology but they are not readily expandable in vitro and undergo de-differentiation, which limits and complicates their use. I will present the data from students that have worked with me on understanding how a progenitor cell line has repeatedly provided us with hepatocytes for over 20 years (with no donor required!). We initially showed that the hepatocytes express liver-levels of many liver-specific proteins (including drug metabolising enzymes). We then moved on to trying to understand why the cell line is stable in its expandable progenitor phenotype and what mechanism(s) regulate its change to a non-replicate (as expected!) hepatocyte. all this by using a single cheap hormone. Extraordinarily, the hepatocytes remain stable for many weeks in culture, in contrast to primary cultures of hepatocytes isolated from livers. These studies lead us to characterise the role of the SGK1 and Wnt pathways and their cross-talk. Along the way, we have used both the progenitor and hepatocyte phenotype as models in toxicological projects. Some of these will be described.

# The RTgill-W1 cell line assay replacing the use of fish in support of OECD Test guideline 249

Prof Kristin Schirmer

The RTgill-W1 cell line assay replacing the use of fish in support of OECD Test guideline 249, Sky Loft, April 18, 2023, 9:00 AM - 9:30 AM

*Biography:*
*I am a cell biologist by training, advancing cellular models to understand the impact of chemicals and other stressors on fish as important part of aquatic ecosystems. I learned the trade of fish cell culture in the laboratory of Professor Niels Bols at the University of Waterloo in Ontario, Canada, amidst an inspiring group of distinguished environmental toxicologists. Using such cells to develop experimental models and build strategies that aid in the reduction and replacement of fish in chemical screening and environmental risk assessment has become my passion. One of the greatest successes that I have been able to accomplish with my team, after years of intensive research, are the adoption of the so-called RTgill-W1 cell line assay for acute fish toxicity prediction as ISO standard 21115 and OECD Test Guideline 249, the very first such globally accepted protocols using fish cells. Moreover, our work has resulted in a flourishing enterprise: aQuaTox-Solutions GmbH is the first company offering animal-free alternative testing methods for fish, focusing on fish cells and fish embryos to test chemicals, environmental samples, fish feed additives etc. in a resource-efficient and ethical manner. In my presentation I will share insights about the road toward global acceptance of the RTgill-W1 cell line assay and our vision of where to take fish cell lines as alternatives to conventional tests with fish next.*

Millions of fish are used every year in the environmental safety testing of chemicals and water samples. Alternatives are urgently sought for ethical reasons but as well because these tests are little informative and so resource-intensive (time, infrastructure, personnel) that they cannot keep pace with testing demands. We therefore work on strategies that provide for testing without the need of fish, by using fish cell lines instead.
A prime example is the establishment, standardization and international validation of the RTgill-W1 cell line assay to predict fish acute toxicity based on a cell line originating from the gill of a rainbow trout (Oncorhynchus mykiss). We chose this cell line based on the assumption that it is the gill of fish that is mostly affected in acute exposure scenarios, leading to fish death. Viability of these gill cells is measured as proxy of fish death, showing an excellent correlation. The RTgill-W1 cell line assay has been adopted in 2019 by ISO (ISO standard 21115) and in 2021 by OECD (OECD TG249), marking the first in vitro ecotoxicology test guideline.
Although a 1:1 replacement of the acute fish toxicity test (OECD TG203) has not been expressly accepted by regulatory agencies, chemical industry from a variety of branches as well as environmental protection agencies trust in the value of the assay. Indeed, based on the services provided by aQuaTox-Solutions in 2022 alone, thousands of fish were spared, in addition to other important benefits, namely high testing throughput and little amount of test material needed.

# Liver safety at the transition between pre-clinical and clinical trials

Dr Dominic Williams

Liver safety at the transition between pre-clinical and clinical trials, Sky Gallery, April 18, 2023, 9:30 AM - 10:00 AM

*Biography:*
*DW spent 15 years in the Dept of Pharmacology at Liverpool University leveraging +£16M grant funding then joined AstraZeneca in 2014 completely redesigning AZ's Hepatic Safety Strategy. Industrial firsts include using 3D hepatic spheroids, Bayesian modelling and liver-on-a-chip applied to +140 projects across the AZ pipeline using statistically weighted assays in a Bayesian machine learning format, incorporating new visualisations & communicating data uncertainty from in vitro models. The Bayesian model has been used for all CDID/ESPC since 2017, without any Phase 1 drug attrition due to hepatic safety, is the first example of predictive hepatic safety using machine learning models. DW was the industrial lead for the 16 partner, £32M IMI1 consortium – MIP-DILI the findings were published in Nature Reviews Drug Discovery.*

Drug induced liver injury (DILI) is a major cause of drug attrition, leading to the withdrawal of potentially valuable therapies. It is thus clear that current preclinical testing paradigms, based on in vitro models, are poorly predictive of the potential of a new drug candidate to cause DILI. Furthermore, it is estimated that between 38% and 51% of compounds showing liver injury in man do not show similar effects in animal studies. In vitro test systems based on human cells or tissues are required, which reflect the in vivo situation with respect to drug metabolism, cellular adaptation and toxicological response. No single in vitro assays is fit for purpose as a universal test for the patient-specific, temporal, multifactorial pathophysiological process of DILI in humans. Broadly, the Pharmaceutical Industry has a portfolio or 'toolbox' of validated, well-characterized in vitro assays which are routinely utilized to predict DILI likelihood and rank series and compounds across all chemistry entering non-clinical safety and that these assays are acceptable in a theoretical and practical sense to academic, industry and regulatory agencies. However, should candidate molecules show elevated liver stress/injury clinical chemistry parameters in early clinical trials (FTIH; First Time in Human studies), a translational approach may help clarify the mechanism underlying these signals. A case report of a live Phase 2 drug molecule will be described, where serious hepatic signals (Hy's Law) were observed FTIH, which led to an ongoing translational program of clinical and pre-clinical work ultimately de-risking the molecule for Hy's Law and significantly advancing mechanistic understanding of the adverse signals.

# Environmental pollution and cardiovascular disease: why is it all up in the air?

Dr Mark Miller

Environmental pollution and cardiovascular disease: why is it all up in the air?, Sky Gallery, April 18, 2023, 10:00 AM - 10:30 AM

*Biography:*
Mark Miller is a Reader (Senior Research Scientist/Associate Professor) working in the Centre for Cardiovascular Science at the University of Edinburgh (United Kingdom) funded by the British Heart Foundation.

Mark obtained his degree in Pharmacology at the University of Edinburgh. He then pursued a PhD in the cardiovascular effects of novel nitric oxide donor drugs at the same Institute, continuing his interest in this topic in postdoctoral positions at the University of Strathclyde.

Over the last 15 years, Mark's research has addressed the health effects of air pollution. A notable focus has been the biological pathways by which the particles in vehicle exhaust cause adverse effects in the cardiovascular system. He also has an interest in the potential for manufactured nanoparticles to cause harm to the cardiovascular system. His work encompasses a broad range of approaches from in vitro assays, in vivo models of disease and controlled exposure to pollutants in human subjects.

Mark has published more than 90 articles in peer-reviewed journals and contributed to the acquisition of £~20 million GBP in research funding. He has given over 50 invited presentations across the scientific community, the third sector and public engagement.

Mark is an Editor of the journal Particle & Fibre Toxicology. He is a member of the World Heart Federation Air Pollution Expert Group and the Environmental Protection Scotland Air Quality Expert Advisory Group, as well as a Special Adviser to the UK Clean Air Champions Knowledge Exchange Group. He is also an Expert Member of the Committee on the Medical Effects of Air Pollution (COMEAP), which advises the UK governmental Department of Health on this subject.

Mark's research findings have received extensive coverage in the national and international media, and featured in documentaries on UK television. His work contributed to the Department of Cardiology's Queens Anniversary Award 2014-16 for outstanding contribution to scientific research. His work was highlighted as a case study in the DEFRA (UK Government) Clean Air Strategy 2019. He has recently been acknowledged for his contributions to the 2021 World Health Organisation Global Air Quality Guidelines and the Chief Medical Officer for England's Annual Report: Air Pollution (2022).

The Lancet Commission recently reported that pollution, in total, causes 9 million premature deaths.1 Pollution, together with climate change, are the dominant environmental risk factors, with air, water, soil, noise and light pollution being associated with many forms of non-communicable disease, especially cardiovascular conditions. This is exemplified by air pollution, which is responsible for more than 20% of cardiovascular deaths worldwide and imposes an alarming level of morbidity.2

Air pollutants such as particulate matter, nitrogen dioxide, and ozone, are linked to cardiovascular conditions ranging from coronary artery disease, ischaemic heart disease, cardiac arrhythmia and arrest, heart failure, thromboembolism, hypertension and stroke. The associations are strongest for the particulate matter in air pollution, especially the smallest particles deriving from combustion.3
This presentation will provide an overview of our research investigating the cardiovascular effects of diesel exhaust particles, using controlled exposures in human subjects and mechanistic studies in cell cultures and animal models. The work demonstrates that diesel exhaust particles have multiple detrimental effects on different facets of the cardiovascular system through mechanisms such as inflammation, oxidative stress, autonomic re-regulation and particle accumulation at sites of vascular disease. These findings not only support the causality of epidemiological associations, but, importantly, highlight the need for strategies to reduce the most harmful emissions such as those from traffic.

The presentation will conclude with a brief discussion of some of the emerging areas research into the health effects of air pollution, the interactions with other environmental risk factors and potential co-benefits of action to protect public health through environmental change.

1Fuller et al. (2022). Pollution and health: a progress update. Lancet Planetary Health 6:E535-47.
2World Heart Federation (2021). Clean air, smart cities, healthy hearts: Action on air pollution for cardiovascular health. A World Heart Federation policy brief. https://world-heart-federation.org/wp-content/uploads/2021/09/WHF-Policy-Brief-Air-Pollution.pdf
3Miller & Newby (2020). Air pollution and cardiovascular disease: car sick. Cardiovascular Research 116:279-94.

# Case Report of the Chemical Investigation of Incidents of Hepatitis of Unknown Origin in Children in 2022

Dr Jinkang Zhang[1], Dr Atallah El Zein[1], Dr Robie Kamanyire[1], Professor David Russell[1], Professor Timothy Gant[1], Dr Timothy Marczylo[1]
*[1]UKHSA, Harwell Science and Innovation Campus, UK*

Oral Communication: Case Report of the Chemical Investigation of Incidents of Hepatitis of Unknown Origin in Children in 2022, Sky Gallery, April 18, 2023, 10:30 AM - 10:45 AM

In April 2022 reports of an increased level of non-HBV associated hepatitis in children under ten occurred in the UK [1]. By 4th July 2022 UKHSA had been informed of 274 confirmed and 11 possible UK cases with 15 liver transplants and no deaths [2]. SARS-COVID2 was found in 9.7% of cases and eliminated as causative. Adenovirus in particular adenovirus 41F (HAdV-F41) was detected in 70.5% of cases and was a leading hypothesis for causation via an adverse immunological reaction [3]. While a credible hypothesis the evidence is not definitive [4] and led to further investigation including untargeted chemicals analysis. Analyses were conducted on blood and urine by GC/MS, Orbitrap LC/HRMS and ICPMS for metals. Quantitation was performed for some known hepatotoxins. Many substances administered therapeutically or associated with the pathophysiology were detected. Paracetamol was found in a small number of cases but not at levels considered to be causative for the disease. Phenol, caffeine and aspirin were detected but not at levels considered adverse. Nothing abnormal was detected with metals. Quantitative analysis for mycotoxins has to date not found any abnormal levels. While the toxicological analysis has not revealed a chemical cause, it has led to some lessons learnt, and highlighted the clear need for toxicology in these incidents.

Equal contribution of first two authors to laboratory work.

# Are non-animal systemic safety assessments protective? A toolbox and evaluation strategy

Doctor Alistair Middleton[1], Doctor Joe Reynolds, Sophie Cable, Maria Teresa Baltazar, Hequn Li, Samantha Beven, Paul Carmichael, Matthew Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Mark Liddell, Sophie Malcomber, Beate Nicol, Benjamin Park, Hiral Patel, Sharon Scott, Chris Sparham, Paul Walker, Andrew White

[1]*Unilever, ,*

Oral Communication: Are non-animal systemic safety assessments protective? A toolbox and evaluation strategy, Sky Loft, April 18, 2023, 10:30 AM - 10:45 AM

An important question in toxicological risk assessment is whether non-animal new approach methodologies (NAMs) can be used to make safety decisions that are protective of human health, without being overly conservative. In this work we propose a core systemic toxicity NAM toolbox and workflow for conducting consumer safety assessments, together with a strategy for evaluating how protective and useful it is. The strategy is based on the principle of benchmarking toolbox data against historical safety decisions. The toolbox includes physiologically-based kinetic (PBK) models to estimate systemic Cmax levels in humans, and three bioactivity platforms, comprising high-throughput transcriptomics, a cell stress panel and in vitro pharmacological profiling, from which points of departure are estimated. A Bayesian model was developed to quantify the uncertainty in the Cmax estimates depending on how the PBK models were parameterised. The feasibility of the evaluation strategy was tested using 25 benchmark exposure scenarios from 11 different chemicals, some of which would be considered high risk from a consumer goods perspective and some low risk. Using novel protectiveness and utility metrics it was shown that up to 62% of the low-risk benchmark exposure scenarios could be identified as such using the toolbox, whilst being protective against all the high-risk ones. The approach demonstrated how robust safety decisions could be made without using any animal data. Current progress on implementing a full evaluation of the toolbox, to test whether it is protective and useful across a broader range of chemical exposure scenarios, will also be discussed.

# VM7Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists (OECD TG 455, ANNEX 3) – Method set-up and validation

Dr Poornima Paramasivan

BTS Study Director Workshop, Sky Loft, April 18, 2023, 11:30 AM - 1:00 PM

*Biography:*
Poornima Paramasivan is a senior scientist and study director at the toxicology service line at Concept Life Sciences, Dundee. In addition to directing the client studies in toxicology, Poornima is actively involved in the implementation of various OECD tier-2 and bespoke assays for chemical testing under endocrine disruption platform at Concept Life Sciences. Poornima has a PhD in biotechnology from India and she has developed her professional and technical skills through postdoctoral positions from University of Liverpool, Bangor University and Abertay University, UK, and lectureship at Bharathiar University India. Previously, she was a senior research fellow of council for scientific and industrial *research, India.*

Concept Life Sciences (CLS) offers validated assays within a Good Laboratory Practice (GLP)-accredited facility, to assess endocrine disruptors, including various OECD tier-2 assays. The VM7Luc4E2 estrogen receptor (ER) transactivation test method for identifying estrogen receptor agonists and antagonists is one of the performance-based test guidelines (TG) under OECD (OECD TG 455, ANNEX 3) that uses VM7Luc4E2 cells as test system1
. Before testing unknown chemicals, it is obligatory for each laboratory
to demonstrate proficiency in the method by testing and correctly classifying the 24 proficiency items for agonist and antagonist assay provided in the guideline.
During the implementation of the assay at CLS, both positive and negative proficiency items for the antagonist assay, and the positive proficiency items for the agonist assay were correctly classified according to the guideline. However, the negative proficiency items (atrazine, ketoconazole, spironolactone, and reserpine) for the agonist assay were classified as inadequate on several independent occasions (including different operators) while following the decision criteria set by the TG455. Troubleshooting of the assay was undertaken, including changes to the plate map, cell number, cell conditioning time, removal of antibiotics from cell culture media and testing other substances listed in TG455; none of these changes improved the performance of the assay.
A comparison of the decision criteria in TG455 (ANNEX 3) and in the initial validation report from ICCVAM2
revealed inconsistencies between the two publications. When the data generated at CLS was evaluated following the decision criteria contained in the ICCVAM validation report, all negative proficiency items for agonism were correctly classified as such. A review of the data package used in the ICCVAM validation report revealed that negative proficiency items for agonism would not have been correctly classified had the decision criteria contained in TG455 been applied for this purpose. This suggests that the TG455 classification criteria should be aligned with those present in the ICCVAM validation report. Moreover, the VM7Luc4E2 cell line should be made available in public cell culture collections to improve consistency across testing laboratories.
References
1. OECD (2021), Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264265295-en.

2. ICCVAM. (2011). ICCVAM Test Method Evaluation Report, The LUMI-CELL® ER (BG1Luc ER TA) Test Method:
An In Vitro Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals, NIH Publication No. 11-7850.

# Rodent Intracranial Surgery – Gene/Cell Therapy Safety Testing

Mrs Hannah Kilbee

BTS Study Director Workshop, Sky Loft, April 18, 2023, 11:30 AM - 1:00 PM

Gene and cell therapies aim to treat genetic diseases by either using cells or genes as a vector or carrier of the therapy or they alter the cells/genes within the body with the goal of curing or reducing the effects of a genetic disease, such as Parkinson's disease and epilepsy. Here at Labcorp Huntingdon we have conducted three gene or cell therapy studies, using both dogs and rodents as the animal model. This presentation will discuss the nude rat as the animal model and intracranial surgery as the dose route for stem cell therapy preclinical safety testing. In addition, the challenges faced within both study design and conduct will be discussed and how these were resolved.

# Understanding mechanism of TAK-875 drug induced liver injury

Mrs Julie Eakins

BTS Study Director Workshop, Sky Loft, April 18, 2023, 11:30 AM - 1:00 PM

*Biography:*
Julie is an Associate Principal Scientist at Cyprotex where she is responsible for developing in vitro toxicity assays, along with this she is also a Toxicology Project Manager.
Julie has extensive experience in the field with over 35 years in the industry. She joined Cyprotex in 2014, having worked at AstraZeneca previously. Her main area of interest is mitochondrial toxicity, where she has developed numerous assays especially around the use of the Agilent Flux Analyser (Seahorse).

TAK-875, a GPR40 agonist developed for diabetes, was terminated in Phase 3 due to liver signals. There had been no pre-clinical findings in the rat and only evidence at high doses in the dog. Furthermore in early in-vitro toxicity assays no toxicity was indicated, although some inhibition of hepatobiliary transporters was seen. It is highly protein bound (99.84%) with a Cmax value of 10µM.
A collaboration with DILIsym was established to investigate the Drug Induced Liver Injury (DILI) signal. The mitochondrial stress test (Seahorse assay) using the Agilent XFe96 Flux analyser, was established to determine effects on mitochondrial respiration by measuring oxygen consumption rate (OCR) and reserve capacity (RC)
Using this approach, HepG2 cells exposed to TAK-875 over 1 or 24 hours showed a decrease in both OCR (AC50 of 11.9 and 7.06µM respectively) and RC (AC50 5.0 & 1.37µM) indicative of a mitochondrial toxicant.
Using the acute seahorse assay, we identified TAK-875 as a potential inhibitor of the electron transport chain (ETC). Further investigations using permeabilised HepG2 cells and the XFe96 analyser identified TAK-875 as a Complex I inhibitor.
We conducted the Glu/Gal assay in serum versus serum-free conditions, whereupon TAK-875 was identified as a mitochondrial toxicant in serum-free but not serum containing conditions. Further assays also established changes in mitochondrial Reactive Oxygen Species and membrane potential confirming effects on mitochondrial function. Furthermore effects were also demonstrated in both human and rat hepatocytes.
This demonstrates that using the correct approach can improve the predictivity of in vitro assays.

# Physicochemical Characterisation and Potential Health Effects of Tyre Wear Particles

Mr David O'Loughlin[1], Dr. Liza Emirali[1], Dr. Molly Haugen[2], Prof. Anne Willis[1], Prof. Adam Boies[2], Prof. Marion MacFarlane[1]

*[1]MRC Toxicology Unit, Cambridge, United Kingdom, [2]Department of Engineering, University of Cambridge, Cambridge, United Kingdom*

BTS Selected Short Oral Communications, Sky Gallery, April 18, 2023, 2:00 PM - 2:45 PM

*Biography:*

*David O'Loughlin is a PhD student in the MacFarlane group at the MRC Toxicology Unit, University of Cambridge. His research explores the potential health effects of non-exhaust (break and tyre) wear particles. He holds a BSc (Hons) in Pharmaceutical and Biomedical Chemistry from Maynooth University, Ireland, graduating in 2020. As part of this degree, he spent six months working in forensic toxicology with the State Laboratory, the national scientific analysis laboratory for the Government of Ireland. In 2021 he graduated from University College Dublin with a MSc in Toxicology and Regulatory Affairs. This included a three-month placement as a regulatory toxicologist for a start-up company founded by Prof. Wenxin Wang, developing non-viral polymer based CRISPR-Cas9 gene editing medicines for rare skin diseases. His master's thesis focused on ways to evaluate and improve the EMA's Orphan Designation process.*

Tyre wear is an increasing source of particulate matter pollution in the UK and globally. Currently, tyre wear accounts for 11% of total PM2.5 in the UK, and over the next 10 years that figure is projected to increase, with brake and tyre emissions projected to reach 6 kilotonnes by 2030. Being able to identify and monitor tyre wear PM in ambient sources is of increasing importance due to its potential health effects.

Here we present a series of characterisation experiments on tyre particles. Chemical and elemental composition has been determined by inductively coupled plasma mass spectrometry (ICP-MS) and two dimensional gas chromatography time of flight mass spectrometry (GCxGC-TOF-MS), with particle morphology studied using a scanning electron microscope and energy dispersive spectroscopy (SEM-EDS) and an electrical low pressure impactor (ELPI).

The potential health effects of these particles have then been assessed in vitro using human bronchial epithelial cells for 24 hours following the IARC characteristics of carcinogenicity, looking at (pro)inflammatory cytokines, cytotoxicity and mitochondrial function.

Tyre wear particles have the ability to reduce mitochondrial function significantly from doses as low as 5µg/ml after 24 hours, with the induction of cytokines and chemokines currently being profiled.

Additionally, a representative elemental and chemical fingerprint of tyre rubber has now been established; zinc contributing 1% to the mass of the tyre, aluminium and iron at 374 and 252 mg/kg respectively.

By understanding the composition and morphology of these particles, we can begin to detect them and understand their potential health effects

# Linking metabolic and reproductive disruption induced by organophosphate flame retardants using an in vitro human 3D cell culture models

Mr. Chander Kant Negi[1], Lola Bajard[1], Ludek Blaha[1]
[1]*RECETOX, Masaryk University, Faculty of Science, Brno, Czech Republic*

BTS Selected Short Oral Communications, Sky Gallery, April 18, 2023, 2:00 PM - 2:45 PM

*Biography:*
*Chander K. Negi is a Ph.D. candidate at RECETOX, Masaryk University, Czech Republic. His research focuses on the characterization of toxicological effects and mechanism of action of novel flame retardants using in silico, in vitro, and in vivo models. He employs high throughput screening and toxicogenomic approach to characterize molecular initiating events and key events associated with flame retardants-mediated metabolic and reproductive dysfunction. He received his MS degree in regulatory toxicology from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India. His master's thesis was focused on investigating the effects of dietary activation of the master regulator of endogenous antioxidants, Nrf2, in diabetes and related complications in laboratory animals. His current research aims to contribute to developing adverse outcome pathways and mechanistic understanding of toxicants-mediated adverse effects.*

Organophosphate flame retardants (OPFRs) have been consistently detected in increasing concentrations in the environmental and human matrices indicating possible human exposure. Therefore the present study aims to evaluate the effects of OPFRs on human cell culture (monolayer and 3D spheroids) to characterize the toxicological effects and potential mechanisms through morphological, transcriptional, and biochemical assays.

Our findings suggest that the OPFRs, including tricresyl phosphate, triphenyl phosphate, tris(1,3-dichloropropan-2-yl) phosphate, and 2-ethylhexyl diphenyl phosphate (EHDPP) induced the lipid accumulation in human liver (HepG2) cell culture by altering the expression of genes encoding for hepatic lipogenesis and mitochondrial dysfunction. In silico analyses identified PXR and PPARγ as potential molecular initiating events. Moreover, EHDPP-mediated dysregulation of hepatic lipidome was observed in human HepG2 3D hepatospheroids along with alteration in several genes involved in lipid homeostasis, including ACAT, ABCA1, CYP27A1, GPAT2, PNPLA2, PGC1α, and Nrf2. The human adrenal (H295R) cells exposed to EHDPP showed altered secretion of hormones, including progesterone, androstenedione, and cortisol. Consistently enhanced expression of corticosteroidogenic genes, encoding for cytochrome P450 (CYP11B2, CYP21A1) and hydroxysteroid dehydrogenases (3β-HSD2, 17β-HSD1) was observed. Intracellular lipidomics identified EHDPP-mediated disruption of intracellular lipid profile in 3D hepatospheroids and H295R cells indicated by reduced cholesterol esters, sphingolipids, fatty acyls, and increased phospholipids and triglycerides species, indicating connections between EHDPP-induced metabolic and reproductive pathologies.

In summary, our study identifies several OPFRs as potential risk factors for endocrine-related metabolic and reproductive pathologies that are of increasing importance due to the risk of occupational or cumulative environmental exposure to humans.

# Translation byproducts from therapeutic messenger RNAs and off-target immune responses

Dr Thomas Mulroney[1], James Thaventhiran[1], Anne Willis[1]
[1]*MRC Toxicology Unit, University Of Cambridge, , United Kingdom*

BTS Selected Short Oral Communications, Sky Gallery, April 18, 2023, 2:00 PM - 2:45 PM

*Biography:*
*Thomas Mulroney graduated from the University of Birmingham with a BSc in Biochemistry in 2016, where he started his research at the School of Biosciences investigating nonsense-mediated mRNA decay mechanisms in the lab of Dr Saverio Brogna. He then joined Prof. Anne Willis' lab at the MRC Toxicology Unit and Dr Ann Doherty's team at the IMED Biotech Unit, AstraZeneca, to investigate the toxicity of modified nucleosides and in vitro transcribed messenger RNAs, obtaining his PhD from Jesus College in 2021. Dr Mulroney received a postdoctoral research fellowship from the Integrated Toxicology Training Partnership to continue his research at the MRC Toxicology Unit in the labs of Prof Willis and Dr James Thaventhiran, where he investigated translation fidelity of in vitro transcribed mRNA therapeutics. Dr Mulroney is currently working on the development of next-generation mRNA-based drugs and technologies with support from Welcome Leap RNA Readiness and Response.*

Messenger RNA therapeutics are effective drug modalities for preventing or treating human disease. However, synthetic mRNAs (transcribed in vitro) contain several features that are required for sufficient bioavailability and limited innate immunogenicity, but which are also unfavourable for protein synthesis – namely modified ribonucleotides. We investigated whether modifications to synthetic mRNA coding regions affect protein synthesis using in vitro transcribed reporter mRNAs. We demonstrate that modified mRNA is prone to errors in protein synthesis. Using mouse and human models of mRNA vaccination, we show that byproducts of mRNA mistranslation can prime T cell responses in vivo. We suggest that mistranslation of synthetic mRNA represents a source of potential off-target toxicity to mRNA therapeutic application, which is important for preclinical development of mRNA-based drugs.

# Novel microRNA Biomarkers for Monitoring Distinct Profiles of Chronic and Acute Methotrexate-Induced Liver Toxicity Represented in a Pre-Clinical in vivo Model

Mr Joseph Brown[1], Dr Shiva Seyed Forootan[1], Prof Chris Goldring[1]
[1]*University Of Liverpool, Liverpool, United Kingdom*

BTS Selected Short Oral Communications (2), Sky Gallery, April 18, 2023, 2:45 PM - 3:30 PM

Drug induced liver injury remains an issue clinically and in drug development, in part due to limitations of current biomarkers (1). microRNAs have been proposed as novel toxicity markers due to their sensitivity, specificity and stability in circulation (2).

We isolated basal liver tissue RNA from n=8 Wistar rats, alongside plasma, hepatocytes, cholangiocytes and liver sinusoidal endothelial cells (LSECs), then performed Next Generation RNASeq. This allowed unbiased discovery of liver cell-selective microRNA candidate biomarker panels (Fig1.) which were evaluated using an in vivo model of acute/chronic methotrexate toxicity, with common clinical methotrexate administration shown to cause hepatotoxicity (3).

Acute methotrexate was administered 20mg/kg single-dose i.p. to n=5 rats, whilst chronic methotrexate was given single p.o. dose daily at 0.125mg/kg (n=5) and 0.25mg/kg (n=5) for 28 days. Both arms had a time-matched saline vehicle-control cohort (n=5).

Methotrexate caused minimal ALP/ALT serum biomarker changes. Histopathology revealed some sinusoidal dilation and hepatic atrophy in acute-treated animals, whilst 0.25mg/kg daily administration caused some mild micro-vesicular steatosis. In liver tissue chronic treatment caused elevations of VEGFR2, VEGFR3, NFKB, TNFa, iNOS and CD31, whilst acute treatment saw increased oxidative stress response markers and decreased VEGFR2/VEGFR3 (Fig2.).

Plasma of acute treated animals showed increases of hepatocyte-selective miR-122 and LSEC-selective miR-335, with matched decreases in whole liver. Chronic treatment led to dose-dependent plasma increases of miR-126 and decreases of miR-335 and miR-362-3p (Fig.3).

These results indicate changes in circulating miRs have potential to act as sensitive markers of methotrexate-related liver insult in the absence of noticeable aminotransferase increases.

# Identification of aripiprazole-binding proteins using thermal proteomics

Yizhou Yu[1], Bini Ramachandran[1], Nuno Santos Leal[1], Catarina Franco[1], L. Miguel Martins[1]
[1]*University Of Cambridge, ,*

BTS Selected Short Oral Communications (2), Sky Gallery, April 18, 2023, 2:45 PM - 3:30 PM

*Biography:*
*Alzheimer's disease is the most common age-related neurodegenerative disorder. My research focuses on identifying potential toxic compounds that increases the risk of developing Alzheimer's disease, using cell and fly models in combination with human genetic, biochemical, behavioural and brain imaging data. I recently combined experimental results from flies with insights from human medical records and showed that increasing the bioavailability of the coenzyme nicotinamide adenine dinucleotide to promote mitochondrial health could reduce pathologies related to Alzheimer's disease. I am now investigating drugs that can increase AD pathologies through the same pathway..*

Third-generation antipsychotic agents are marketed as safe drugs with few side effects. One of these drugs, aripiprazole, is a dopamine receptor 2 antagonist used to treat conditions including schizophrenia, major depression and autism. However, aripiprazole has been linked to constipation, sleepiness, and movement disorders, suggesting that aripiprazole might exhibit off-target effects. Thermal proteome profiling (TPP) detects drug-protein interactions by calculating the melting temperatures of the proteome. Small molecules like aripiprazole will change the melting curve of a candidate protein, showing that this protein is likely to interact with aripiprazole.
Here, we exposed SH-SY5Y neuroblastoma cells to aripiprazole and performed proteomics analysis. Using unsupervised learning to select proteins that distinguish the proteome of the aripiprazole-treated cells compared to controls, we identified pathways linked to mitochondrial toxicity. We thus investigated markers of mitochondrial toxicity and observed that aripiprazole treatment increased levels of reactive oxygen species in cultured cells and Drosophila melanogaster. Next, we sought to unveil the mechanisms of aripiprazole-induced toxicity and screened for proteins that interact with aripiprazole using TPP. We exposed cells treated with aripiprazole to a temperature gradient ranging from 37 to 67 °C followed by quantitative proteomics. Using a non-parametric analysis of the thermal proteome profiles, we discovered that aripiprazole could bind to subunits of mitochondrial complex I. To gain further mechanistic insights, we performed molecular dynamics simulations on the binding mechanisms and interactions of aripiprazole in the quinone-binding site of mitochondrial complex I. We conclude that aripiprazole causes mitochondrial toxicity through complex I inhibition.

# Pioneering platform enables rapid translational data review

Dr Brenda Finney[1]
[1]*Instem, Stone, UK*

BTS Selected Short Oral Communications (2), Sky Gallery, April 18, 2023, 2:45 PM - 3:30 PM

*Biography:*
*Brenda obtained her PhD from Cardiff University in 2008 working on lung development. After a post-doc working on transgenic platelet-receptor models she moved into the CRO space as a Principal Scientist in the Biosciences department at Sequani. While there she helped to establish immunohenotyping and in vivo micronucleus assays by flow cytometry. She then moved to Propath where she lead the Molecular Pathology team, establishing their capabilities in Nanostring gene expression and spatial biology. She joined Instem in January 2022 where she worked on SEND exploitation and most recently has taken over management of the new Centrus platform as the Director of Translational Science Solutions.*

With the FDA announcement that it is no longer a requirement to test on animals prior to human trials; consequently, there is potential for a paradigm shift in the conduct of regulatory investigations. Translational evaluation of historical data can streamline, support and enhance program planning. However, it can be resource and time intensive to aggregate the data from disparate sources, integrate findings and translate between species. ToxHub, a translational science tool developed by eTRANSAFE, allows use of integrated databases containing public clinical and legacy preclinical data with visualization and modelling tools that could aid in planning testing programs.
For example, a search for zolmitriptan (selective 5-HT receptor agonist) resulted in 20 preclinical studies and 335 clinical studies in three separate databases. Event counts within MedDRA system organ class (SOC) terms, quickly revealed overlap in 12 SOC across the databases. The nature of these potentially translationally relevant findings was investigated by reviewing the results filtered by eTOX finding and MedDRA preferred term. Findings like somnolence and diarrhoea were seen across humans(H), rats(R) and dogs(D). Terms which could be grouped such as decreased activity(D)/fatigue(H) or nausea(H)/vomiting(D)/hypersalivation(D,R) were also noted. Additional findings such as pain, discomfort, dry mouth, dizziness and paraesthesia were unique to human reporting. Understanding the relative importance of findings such as these to the testing program could allow refinement of the strategy for similar compounds. Use of the ToxHub integrated platform with a unifying ontology for terminology enabled a optimised review of the data.

# Characterisation of morphological and molecular changes in macrophages following exposure to brake wear particles

Dr Liza Emirali[1], Dr Catarina Franco[1], Dr Nobuhiro Morone[1], Ms Maria Guerra Martin[1], Professor Anne Willis[1], Professor Marion MacFarlane[1]
[1]*MRC Toxicology Unit, University of Cambridge,, Cambridge, United Kingdom*

Frank Sullivan Award and Early-Career Prize Lecture: Characterisation of morphological and molecular changes in macrophages following exposure to brake wear particles, Sky Gallery, April 18, 2023, 3:45 PM - 4:30 PM

Brake wear (BW) is a metal-rich pollutant, emitted by combustion and electric vehicles. Targeted assays show that BW induces pro-inflammatory cytokine secretion, impairs mitochondrial integrity and inhibits bacterial phagocytosis in macrophages to the same degree as diesel exhaust particles (DEP). However, as BW is compositionally distinct from DEP, the mechanisms that underlie its toxicity may differ. As such, untargeted characterisations of morphological and molecular responses to BW exposure were characterised.

RAW 264.7 monocyte-derived macrophages were exposed to an Fe-rich (78% w/w) mixed-source brake wear sample (0-16 µg/ml) for 24h. Post-exposure, 2D-TEM confirmed that the cells ingested BW and that it appeared to localise solely within endosomes. Despite this, the mitochondria of exposed cells were visibly smaller than those of control cells and exhibited disrupted cristae. In accompaniment, intracellular ATP concentrations were significantly reduced (60-84%, $p \leq 0.01$), and comparable to reductions caused by Fe particles alone (64%).

Proteomic profiling via TMT-labelled mass spectroscopy determined that, despite losing mitochondrial function, the macrophages responded adaptively to BW exposure. Intracellular concentrations of ferritin light and heavy chain proteins plus glutathione-S-transferase, increased by 256-805%, 206-498% and 44-134% respectively, as compared with control ($p \leq 0.05$-$0.001$). Demonstrating the functionality of these changes, intracellular concentrations of chelated $Fe^{3+}$ (but not free $Fe^{2+}$) increased by 385-1040% in exposed cells while ROS concentrations remained comparable to control.

Together, these data highlight iron homeostasis and antioxidant activity as mechanisms of adaptation to brake wear exposure in macrophages. Importantly, they suggest that these adaptations can persist following considerable decreases in ATP availability.

# BTS: Barnes Prize Lecture: Carcinogenesis: DNA Reactivity, Cell Proliferation, and Human Relevance

Professor Samuel Cohen

BTS: Barnes Prize Lecture: Carcinogenesis: DNA Reactivity, Cell Proliferation, and Human Relevance, Sky Gallery, April 18, 2023, 5:30 PM - 6:30 PM

*Biography:*

*Samuel M. Cohen obtained his MD and PhD (experimental oncology) degrees from the University of Wisconsin-Madison (1972), did his residency training in anatomic and clinical pathology at St. Vincent Hospital in Worcester, MA (1972-5), and was a visiting professor at Nagoya City University, Japan, (1976-7), returning to St. Vincent Hospital as a staff pathologist and the University of Massachusetts Medical School as an Associate Professor (1977-1981). He has been Professor at the University of Nebraska Medical Center since 1981 in the Department of Pathology and Microbiology and the Buffett Cancer Center (formerly Eppley), including as vice chair (1981-1992) and chair (1992-2007). His research has focused on chemical carcinogenesis, extrapolation from animal models to humans, and overall risk assessment processes with an emphasis on mode of action. He has served on numerous national and international committees, editorial boards, and grant review organizations, and has published more than 460 peer-reviewed manuscripts and more than 50 book chapters. He has received several honors and awards, including the Merit Award and the Lehman Award from the Society of Toxicology, the Distinguished Scientist Award from the American College of Toxicology, the Lifetime Achievement Award from the Society of Toxicologic Pathology, and was elected fellow of the American Association for the Advancement of Science (AAAS). He has also been elected as a fellow of the Academy of Toxicological Sciences. In addition to his active research career, he has been a practicing surgical pathologist.*

Carcinogenesis has provided a stimulating, challenging, and varied career in research and medicine, beginning with investigations on DNA reactive carcinogens, primarily focused on nitrofurans and primarily on the urinary tract. This eventually led to demonstration of the 2-stage model for carcinogenesis in the rat urinary bladder, utilizing one of the nitrofurans as the genotoxic initiating stimulus followed by high doses of the non-genotoxic sodium saccharin in the diet as the promoting agent. Given the controversy at the time related to saccharin, this led to numerous investigations into the mechanism by which it produced the bladder effects in rats but not in mice, hamsters, or non-human primates. High doses of the sodium salt of any moderate to strong acid, such as saccharin or ascorbate, alters the rat urinary composition leading to the formation of a cytotoxic, calcium phosphate-containing amorphous precipitate in the urine. This was rat specific, did not occur in other species, and was shown not to occur in humans. We eventually mathematically modelled chemical carcinogenesis, utilizing a Monte Carlo probabilistic approach and based on data from urinary carcinogenesis studies in rats and mice. The data were incompatible with the initiation promotion model, which led to the development of a more generalized multistage model incorporating the role of increased cell proliferation and genotoxicity. This has been applied to numerous chemicals in the evaluation of mode of action (MOA). More recently, I have focused on inorganic arsenic, showing that it is a threshold carcinogen acting by inducing cytotoxicity with consequent regenerative cell proliferation. The research on chemical carcinogenesis led to participation in the development of a MOA/human relevance framework which was adopted by the International Programme on Chemical safety (IPCS). The model and the MOA/human relevance framework provide the basis for an alternative approach to screening for carcinogens in place of the 2-year bioassay. The focus is on human cancer risk, rather than worrying about producing cancer in rodent models. Carcinogenesis in humans is due to genotoxicity, immunosuppression, estrogenic activity, or cytotoxicity with regenerative cell proliferation. These effects can readily be screened for by in vitro (human cells) or short term in vivo studies, taking into account human relevance and dose response.

# Development of a TG/GD for nanoparticle toxicokinetics - A joint NL/UK initiative

Dr Rachel Smith

Development of a TG/GD for nanoparticle toxicokinetics - A joint NL/UK initiative, Sky Gallery, April 19, 2023, 9:45 AM - 10:15 AM

*Biography:*
*Dr Rachel Smith leads the UKHSA Toxicology Department's Experimental Toxicology Programme. She has a particular interest in inhalation and (nano)particle toxicology and aerosol deposition in the lung.*

The current OECD Test Guideline (TG) on Toxicokinetics (Test No. 417) is not applicable to nanomaterials, which are being produced in ever increasing quantities, and therefore a project to develop a TG on nanomaterial toxicokinetics was initiated at the OECD by The Netherlands (RIVM) and is co-led by the UK (UKHSA). This project aims to provide a new TG to harmonize the generation of toxicokinetic data on nanoparticles for regulatory purposes. Information on the toxicokinetics of nanoparticles is necessary to establish the behaviour (uptake, distribution, transformation, excretion and accumulation) of nanoparticles in the body that may lead to toxic effects. The TG will define the minimum requirements of in vivo nanoparticle toxicokinetic studies, including: dose levels/ranges, exposure duration, post-exposure periods, time points for determining organ burdens, and key organs/tissues to be analysed. As an initial step, a gap analysis was undertaken to identify requirements for new experimental data. To address these, inhalation and oral toxicokinetic studies have been undertaken by partners in The Netherlands, Denmark, Korea, Germany and Australia, with a focus on CeO2 and TiO2 nanoparticles (representing very poorly soluble nanomaterials) and silica nanoparticles (representative of moderately soluble nanomaterials). The results are being used in supporting modelling studies. Focused literature reviews to address issues such as the significance of particle size, analytical technique and solubility to study design are also progressing. An update of the current project status will be provided.
This project is supported by the NanoHarmony project under grant agreement No. 885931 of the EU's Horizon 2020 programme.

# Preliminary OECD guidance document for in vitro genotoxicity testing of nanomaterials

Professor Shareen Doak

Preliminary OECD guidance document for in vitro genotoxicity testing of nanomaterials, Sky Gallery, April 19, 2023, 10:15 AM - 10:45 AM

*Biography:*
Shareen Doak is Professor of Genotoxicology and Cancer in Swansea University Medical School where she is co-lead of the In Vitro Toxicology Group. Shareen is a UK and EUROTOX Registered Toxicologist, an invited Fellow of the Royal Society of Biology (FRSB) and an elected Fellow of the Learned Society of Wales (FLSW). Shareen's research interests focus on the genotoxic profiles of chemicals and engineered nanomaterials, the mechanisms underlying their DNA damaging potential and subsequent consequences upon human health, including carcinogenesis. Her interests extend to the development of advanced 3D culture models and mechanism-based bioassays for safety assessment to reduce the need for animal testing.
Shareen sits on the UK Government Committee on Mutagenicity (COM) and the Scientific Advisory Group on Chemical Safety in Consumer Products (SAG-CS) within the Office for Product Safety and Standards (OPSS). Shareen is also an independent member of the Health & Safety Executive (HSE), Science Quality Assurance Group (SQAG), and is the Editor-in-Chief for the journal Mutagenesis.

Standard in vitro genotoxicity testing approaches have limitations for testing nanomaterials (NMs) and in 2013, an OECD expert panel concluded it was necessary to adapt the in vitro mammalian cell micronucleus test (OECD TG487) to facilitate evaluation of manufactured NMs. An OECD project was initiated, aimed at developing a new OECD Guidance Document detailing the necessary steps to adapt OECD TG487 for NMs. An extensive programme of work was undertaken to design a set of standardised NMs that were well characterised in dry state and under experimental conditions. Several cells relevant for OECD TG487 were evaluated for their capacity to internalise the NMs. Finally, a harmonised protocol for the in vitro micronucleus assay was established and trialled across two laboratories. The study outcome was detailed guidance on how to conduct the test with NMs, including recommendations on treatment regimens, cell line selection, positive controls and top dose. The OECD Guidance Document was subsequently published in Sept 2022 (Series on Testing and Assessment No. 359; ENV/CBC/MONO(2022)15). Whilst ongoing efforts have focused on adapting existing OECD TGs for NMs testing, limitations remain as existing in vitro approaches lack physiological relevance, often do not consider long-term exposure effects, and do not cover all mechanisms of action underpinning genotoxicity. New approach methodologies (NAMs) provide the opportunity to overcome these issues and there have been substantial developments in this area over recent years. The broad field of NAMs to better support regulatory risk decision making is gaining momentum, with a variety of promising technologies emerging.

# Towards multi-omic predictors of aquatic acute-to-chronic toxicity ratios

Professor Viant Mark

Towards multi-omic predictors of aquatic acute-to-chronic toxicity ratios, Sky Loft, April 19, 2023, 10:45 AM - 11:15 AM

*Biography:*
*Mark Viant is co-Founder and CEO of Michabo Health Science, Professor of Metabolomics at the University of Birmingham, and Executive Director of Phenome Centre Birmingham – a centre specialising in toxicometabolomics. His research focuses on developing and applying metabolomics in the field of human and environmental toxicology, with the goal to find novel molecular mechanistic solutions for industry and regulators in chemical safety science. He co-led the Ecetoc MEtabolomics standaRds Initiative in Toxicology (MERIT) project, currently co-leads the omics activities within the OECD's chemical safety programme, and leads the Cefic MATCHING international ring-trial in toxicometabolomics. Mark has co-authored over 200 publications and his work has been recognised by the award of a 2015 Lifetime Honorary Fellowship of the International Metabolomics Society.*

Chemical risk assessment relies heavily on chronic toxicity tests that set safety thresholds based on animal pathology or fitness. Chronic tests are resource expensive and lack mechanistic insight. Since molecular perturbations precede adversity, early-response molecular biomarkers may enable shorter, more resource efficient testing that can predict chronic animal fitness. In ecotoxicology, the Daphnia magna acute-to-chronic ratio (ACR) can be used to estimate chronic toxicity from experimental Daphnia acute immobilisation studies. However, the ACR can range from below 10 to greater than one thousand, making these predictions of chronic toxicity imprecise and uncertain. This presentation will describe progress in the application of 'omics technologies to attempt to discover early-response molecular biomarkers that can predict chronic toxicity in D. magna.

# Advanced in vitro approach to investigate nanoparticle behaviour in the oro-gastrointestinal tract

MD Isabella De Angelis

Advanced in vitro approach to investigate nanoparticle behaviour in the oro-gastrointestinal tract, Sky Gallery, April 19, 2023, 10:45 AM - 11:15 AM

*Biography:*
Isabella De Angelis, graduated in Biological Sciences, is researcher at the Department of Environment and Health of Italian National Institute of Health.
Her research interests are focused on application of cell culture in toxicology with particular attention to absorption processes of chemicals and nanoparticles through epithelial barriers. She has been involved in several National and European projects, also as PI, on in vitro toxicology and nanotoxicology.
IDA is Head of Delegation at the OECD Working Party of Manufactured Nanomaterials and EFSA expert. She carries out an intensive dissemination activity on nanosafety and application of in vitro *methods in institutional courses.*

Oral ingestion is considered highly relevant for hazard evaluation of engineered nanomaterials (ENMs) so European regulatory agencies and international organizations emphasize the importance to determine the dissolution profile of ENMs in the digestive tract as well as their (eventual) internalization/translocation through the intestinal barrier.
Different protocols for in vitro digestion assay were developed for simulating physiological conditions in the human oro-gastro intestinal (OGI) tract after food consumption and many of them have been successfully applied to ENMs digestion. Usually, they cover the first steps of the digestion process, i.e., ENMs behavior in mouth, stomach and intestine.
In parallel, several research groups, highlighting interesting application possibilities, explored the use of advanced in vitro models of intestinal barrier for studying ENMs internalization and translocation.
Present study is aimed to set up a two-step in vitro approach simulating the intestinal digestion of ENMs in the
OGI tract and their interactions with the intestinal mucosa. Experimental procedures for both endpoints were
explored with a view to selecting the best test conditions in terms of reproducibility and robustness of the tests.
The information gathered in the study will be collapsed in a new OECD Guidance Document (GD) specifically addressed to determine the intestinal fate of orally ingested ENMs.
The proposal of this new GD was approved by the OECD-WNT in April 2022 and included in its programme of work.
This project was funded by the EU Horizon 2020 research and innovation programme – GA No 885931, NanoHarmony and by the Italian Ministry of Health, section 4145 REACH

# Assessing the relevance of an OECD Adverse Outcome Pathway (AOP) to the mammalian toxicity of fenazaquin

Mr Kim Travis[1], Dr Christian Strupp[2]
[1]*Regulatory Science Associates, Inverkip,, Scotland,* [2]*Gowan Crop Protection Ltd, Reading,, England*

Oral Communication: Assessing the relevance of an OECD Adverse Outcome Pathway (AOP) to the mammalian toxicity of fenazaquin, Sky Loft, April 19, 2023, 11:15 AM - 11:30 AM

OECD AOP #3 starts with binding to mitochondrial Complex I in nigro-striatal neurones as the Molecular Initiating Event, and ends with parkinsonian motor deficits as the Adverse Outcome. Fenazaquin acts as a pesticide by inhibiting Complex I, so this work reviews all the evidence concerning the relevance of the AOP to the mammalian toxicity of fenazaquin. A targeted literature review was performed and the US-EPA CompTox Chemicals Dashboard was consulted.

Fenazaquin inhibits Complex I at low nanomolar concentrations in subcellular systems. Respiration can be inhibited in mammalian cells exposed to fenazaquin, but at higher concentrations than are needed in subcellular systems, and evidence for increased production of reactive oxygen species is lacking. The metabolism and excretion of fenazaquin in mammals is rapid. After rats were given a fifth of a lethal dose of fenazaquin daily for 30 days, the maximum plasma concentration was less than that shown to affect almost all the cell systems studied in vitro. Based on the analysis of in vitro and in vivo databases, Complex I inhibition in the brain is unlikely to be possible following oral dosing of fenazaquin.

In acute and subchronic rat neurotoxicity studies, fenazaquin did not cause the degeneration of dopaminergic neurons of the nigro-striatal pathway, did not cause neuroinflammation and did not result in parkinsonian motor deficits. This is consistent with the in vitro and kinetic evidence, and indicates that systemic exposure of the brain in vivo to fenazaquin is too low for the processes in the AOP to occur.

# Understanding how CeO2 nanoparticles modulate bleomycin-induced inflammatory and fibrotic events in both in vivo and in vitro models

Dr Chang Guo[1], Dr Martin Leonard[1], Dr Alison Buckley[1], Dr Sarah Robertson[1], James Warren[1], Alan Hodgson[1], Prof Tim Gant[1], Prof Eugenia Valsami-Jones[2], Dr Rachel Smith[1]
*[1]UKHSA, , United Kingdom, [2]School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, , Birmingham, United Kingdom*

Oral Communication: Understanding how CeO2 nanoparticles modulate bleomycin-induced inflammatory and fibrotic events in both in vivo and in vitro models, Sky Gallery, April 19, 2023, 11:15 AM - 11:30 AM

Cerium oxide nanoparticles (CeO2NPs) from some diesel fuel additives and other applications have been detected in ambient air. Concerns have been raised over their potential human health impact in situations of inadvertent exposure. Oxidative mechanisms have been suggested as a common feature for pulmonary injury in response to airborne particulate matter, including engineered nanomaterials. To understand in depth how CeO2NPs may influence oxidative stress induced pulmonary inflammation and fibrotic events, we used both in vivo and in vitro bleomycin-induced lung injury models. Male Sprague-Dawley rats were intratracheally instilled with bleomycin or saline (control) followed by nose-only inhalation exposure to nano-sized CeO2NP aerosols (mass concentration 1.8 mg/m3) or water (controls) for 3 hours per day for 4 days per week for one or two weeks. At 3 days post exposure, animals were sacrificed and bronchoalveolar lavage (BAL) fluid, lung histopathology and global mRNA expression analysed. Bleomycin exposure resulted in an increase in total BAL cells, fibrotic staining and significant induction of inflammatory and oxidative stress on mRNA sequencing analysis. Modifications of these responses by one-week exposure to CeO2NPs included attenuation of fibrotic staining and gene expression markers of lung function, inflammation and epithelial-mesenchymal transition (EMT). CeO2NP alone resulted in increased inflammatory responses but did not appear to cause fibrotic changes. Interpretation of these responses at a cellular level was further explored using 3D human small airway epithelium cultures (SmallAirTM) in an aerosol exposure air-liquid-interface system. This also indicated that some bleomycin-induced cellular responses could be interfered by exposure to CeO2NP aerosols.

# Systematic review of PBK models to facilitate a read across approach

Alicia Paini

Systematic review of PBK models to facilitate a read across approach, Sky Gallery, April 19, 2023, 1:30 PM - 2:00 PM

*Biography:*
*Alicia Paini, ERT, holds a MSc degree in Food Science, Technology and Food Safety. She performed her PhD in Toxicology at the Nestlé Research Centre (Switzerland), developing physiologically based kinetic (PBK) models for genotoxic chemicals awarded by Wageningen University, The Netherlands. For 9 years she worked on developing, implementing and promoting in silico tools for next generation risk assessment at the EC-Joint Research Centre. She contributes to more than 70 peer-reviewed articles in the area of alternative to animal testing. In order to translate the science to policy and make the policymakers trustful of model simulations, she led efforts at the OECD to draft guidance on how to Characterize, Validate, and Report PBK Models. As of October 2021 she joined esqLABs GmbH, as a Principal Scientist, lead Systems Toxicology, where she will continue her work in developing, applying and promoting in silico approaches in science and for regulatory purposes within the EU-funded ONTOX project and in EFSA-funded projects.*

Across multiple sectors, including food, cosmetics and pharmaceutical industries, there is a need to predict chemical distribution and effects. These effects are determined by the chemical interaction with the biological system considering its concentration-time profile at the target site. Physiologically-based kinetic (PBK) models can predict organ-level concentration-time profiles. These models are, however, data intensive. Read-across is an approach that can be used, where information from a data-rich chemical is used to fill data gaps for a data-poor chemical. With the continuing growth of published PBK models, this presents the opportunity to use a read-across approach for PBK modelling parametrisation; using PBK model information from one chemical to inform the development or evaluation of a PBK model for a similar chemical. Essentially, this process identifies the chemicals for which a PBK model already exists. Herein, the results of a systematic review of existing PBK models will be presented. Model information, including species, sex, life stage, route of administration, the software platform used and the availability of model equations, was captured for 7541 PBK models (published up to 2020). Chemical information (identifiers and physico-chemical properties) has also been recorded for 1150 unique chemicals associated with these models. This PBK model data set has been made readily accessible as a Microsoft Excel® spreadsheet, providing a valuable resource for those developing, using or evaluating PBK models in industry, academia and the regulatory sectors. In this presentation, we will show how this resource can inform model development and demonstrate the results of a case study using alkenyl benzene, wherein PBK model development for a data-poor chemical was informed using information from a data-rich chemical.

# The potential of PBK modelling to inform agrochemical safety and reduce toxicity testing

Dr Joseph Leedale

The potential of PBK modelling to inform agrochemical safety and reduce toxicity testing, Sky Gallery, April 19, 2023, 2:00 PM - 2:30 PM

*Biography:*
*Joe is an experienced mathematical modeller specialising in the development and application of toxicokinetic, toxicodynamic, and PBK models to better understand the impact of crop protection products on human safety. Before joining agrochemical company Syngenta in 2021, Joe obtained a PhD in mathematical biology at the University of Liverpool and went on to apply mathematical modelling in a variety of research-based positions across a range of interdisciplinary projects in academia from environmental sciences to drug safety. Joe also has a diploma in advanced pharmacology from the British Pharmacological Society and was awarded a research fellowship from the Medical Research Council in 2019.*

Demonstrating agrochemical safety currently requires multiple animal studies for both acute and chronic exposures. The utility of these studies is limited due to the low-throughput nature of the data, biophysical/biochemical complexity, inter-species variability, and a lack of mechanistic understanding. Physiologically-Based Kinetic (PBK) models offer a high-throughput, mechanistic, in-silico approach to simulate and predict exposure, based on organism physiology and physicochemical properties. Examples of our use of PBK-modelling during product safety testing include: predicting long-term internal exposure for repeat-dose studies, dose optimisation, and mechanistically explaining different behaviours and potential health risks of similar compounds. This in-silico approach has guided subsequent in-vivo studies and minimised animal use.
Mechanistic mathematical modelling, with in-vitro-guided parameterisation, offers the most ethical and effective way to reduce animal testing currently and even more so in the future as these technologies develop. In the near-term, we believe that these approaches can contribute to the removal and replacement of long-term animal studies for assessing risk of toxicity related to chemical exposure. However, many challenges remain before we can completely remove the use of in-vivo data for PBK model calibration. In this talk, we outline some of these key challenges including anticipating non-linear mechanisms in the model (particularly at higher doses) and improving the quality and relevance of in-vitro/in-silico inputs. Overcoming such challenges will improve confidence and uptake of PBK modelling in research and development in agrochemicals, reserving animal studies for more confirmatory rather than exploratory purposes.

# ERGO: Breaking Down the Wall between Human Health and Environmental Testing of Endocrine Disrupters

Lisa Baumann

ERGO: Breaking Down the Wall between Human Health and Environmental Testing of Endocrine, Sky Loft, April 19, 2023, 2:00 PM - 2:30 PM

*Biography:*
*Lisa Baumann is an assistant professor at the Vrije Universiteit Amsterdam, Amsterdam Institute for Life and Environment (A-LIFE), Section Environmental Health & Toxicology. She is specialized in environmental toxicology with focus on endocrine effects, hepatotoxicity and immunotoxicity in fish. Special focuses of her work are developmental studies with zebrafish (embryos), as well as histopathological analyses. She is part of the EFSA Working Group for Endocrine Disruptors and the OECD Thyroid Disruption Expert Group.*

The EU H2020 project ERGO project aims at breaking down the wall between mammalian and non-mammalian vertebrate regulatory testing by using a cross-species extrapolation approach between vertebrate classes. The highly conserved thyroid hormone system (THS) is being used as the "proof of concept" for this approach by identifying, developing and aligning THS-sensitive biomarkers and endpoints for linkage of effects between different vertebrate classes. As an applied outcome ERGO partners, in collaboration with several other research projects and OECD member countries are working together to include THS sensitive endpoints into OECD fish test guidelines (TGs). Here we present an update of these efforts and a proposal for a forthcoming OECD validation of selected endpoints, preferably into already existing TGs, for example, OECD TG 210 the Fish Early Life-stage Toxicity Test (FELS) or OECD TG 236 the Fish Embryo Toxicity Test. Such refined TGs will improve the evaluation of THSDCs and will be included in approaches for extrapolation of THSD effects across mammalian and amphibian species. ERGO-generated fish data support project 2.64 (inclusion of THS sensitive endpoints in OECD fish Test Guidelines) which is on the OECD Work Program under the Validation Management Group for Ecotoxicity Testing (VMG-Eco) and co-lead by Denmark, Belgium and Germany, as well as project 1.35 of the OECD Adverse Outcome Pathways development programme workplan which is co-lead by the same countries and the US. Based on the results of ERGO and previous projects, four endpoints have been selected for further evaluation. These endpoints are: 1) swim bladder inflation, 2) thyroid hormone (T3, T4) levels, 3) thyroid histopathology, 4) eye development and they are all supported by OECD VMG-Eco to bring to validation. Additionally, multiple adverse outcome pathways (AOPs) linking thyroid hormone system disruption (THSD) to adverse effects on swim bladder inflation, eye development and survival in fish were developed. Most of the AOPs have now been endorsed by the Working Group of the National Coordinators of the Test Guidelines program (WNT)/ Working Party on Hazard Assessment (WPHA) and will soon be published in the OECD Series on Adverse Outcome Pathways. We are now working on a broader network of THS AOPs to support cross-vertebrate extrapolation of mechanisms and effects of THSDCs for informing both on environmental and human health.

# Next Generation Risk Assessment of the Anti-Androgen Flutamide Including the Contribution of Its Active Metabolite Hydroxyflutamide

Tessa Van Tongeren

Next Generation Risk Assessment of the Anti-Androgen Flutamide Including the Contribution of Its Active Metabolite Hydroxyflutamide, Sky Gallery, April 19, 2023, 2:30 PM - 3:00 PM

*Biography:*

*Tessa van Tongeren is a PhD candidate at the Division of Toxicology at Wageningen University & Research and Unilever's Safety and Environmental Assurance Centre (SEAC). Her research focusses on Next Generation Risk Assessment to inform human-relevant safe levels of chemical exposure, integrating in vitro and in silico approaches for chemicals with putative (anti)androgenic and estrogenic effects. PBK modelling is core of her work to translate in vitro toxicodynamic responses to in vivo dose levels to derive new approach methodologies-based PoDs to set safe human exposures.*

In next generation risk assessment (NGRA), non-animal approaches are used to quantify the chemical concentrations required to trigger bioactivity responses, in order to assure safe levels of human exposure. A limitation of many in vitro bioactivity assays, which are used in an NGRA context as new approach methodologies (NAMs), is that toxicokinetics, including biotransformation, are not adequately captured. The present study aimed to include, as a proof of principle, the bioactivity of the metabolite hydroxyflutamide (HF) in an NGRA approach to evaluate the safety of the anti-androgen flutamide (FLU), using the AR-CALUX assay to derive the NAM point of departure (PoD). The NGRA approach applied also included PBK modelling-facilitated quantitative in vitro to in vivo extrapolation (QIVIVE). The PBK model describing FLU and HF kinetics in humans was developed using GastroPlus™ and validated against human pharmacokinetic data. PBK model-facilitated QIVIVE was performed to translate the in vitro AR-CALUX derived concentration-response data to a corresponding in vivo dose-response curve for the anti-androgenicity of FLU, excluding and including the activity of HF (-HF and +HF, respectively). The in vivo benchmark dose 5% lower confidence limits (BMDL05) derived from the predicted in vivo dose-response curves for FLU, revealed a 440-fold lower BMDL05 when taking the bioactivity of HF into account. Subsequent comparison of the predicted BMDL05 values to the human therapeutic doses and historical animal derived PoDs, revealed that PBK modelling-facilitated QIVIVE that includes the bioactivity of the active metabolite is protective and provides a more appropriate PoD to assure human safety via NGRA, whereas excluding this would potentially result in an underestimation of the risk of FLU exposure in humans.

# Replacing the need for in vivo BCF studies - the use of in vitro biotransformation data in a WoE approach for the REACH registration of fragrance chemicals

Dr Karen Jenner[1], Heike Laue[2], Lu Hostettler[2], Andreas Natsch[2], Gordon Sanders[3], Georg Kreutzer[3]
*[1]Givaudan UK Ltd, Ashford, UK, [2]Givaudan Schweiz AG, Kemptthal, Switzerland, [3]Givaudan Suisse SA, Vernier, Switzerland*

Oral Communication: Replacing the need for in vivo BCF studies - the use of in vitro biotransformation data in a WoE approach for the REACH registration of fragrance chemicals, Sky Loft, April 19, 2023, 3:00 PM - 3:15 PM

The REACH Regulation promotes and supports the 3Rs principles (reduction, refinement, replacement) with obligations to reduce animal use via data sharing, read-across and the implementation of alternative methods. For chemicals manufactured or imported at ≥ 100 t/y information on bioaccumulation in aquatic species is required, unless the substance has a low potential for bioaccumulation (log Kow ≤ 3). Usually, the bioconcentration factor (BCF) is measured in fish according to OECD TG 305. The number of fish used is approximately 100-150 per study.
In vitro assays to determine biotransformation rates in primary trout hepatocytes or liver S9 subcellular fractions (RT-S9) have been validated and adopted as OECD TG 319A/B. The measured biotransformation rates can be used to improve in silico predictions of BCF. To demonstrate the applicability of this approach, we have previously performed and published benchmarking studies on up to 30 chemicals for which in vivo BCF data was already available. In this presentation, we will use four case studies to show how in vitro biotransformation data used in a weight-of-evidence approach can lead to reliable BCF estimates for regulatory purposes. The case studies include the use of in vitro data to support read-across; the application of in vitro-in vivo extrapolation to predict BCFs; identification of metabolites to support rapid excretion in vivo; and in vitro RT-S9 testing of primary biodegradation products to address PBT assessment. We have applied these approaches to at least 13 REACH registrations avoiding the need for in vivo BCF studies and saving approximately 1500 fish.

# The journey towards confidence— ADME characterizations and development of a human PBK model for making safety decisions: a case study of an UV-filter benzophenone-4

Dr. Hegun Li[1], Dr. Beate Nicol[1], Dr. Matt Dent[1], Miss Sophie Cable[1], Dr. Joe Reynolds[1], Dr. Maria Baltazar[1]
[1]*Unilever Safety and Environmental Assurance Center, Colworth Science Park, Sharnbrook, United Kingdom*

Oral Communication: The journey towards confidence— ADME characterizations and development of a human PBK model for making safety decisions: a case study of an UV-filter benzophenone-4, Sky Gallery, April 19, 2023, 3:00 PM - 3:15 PM

Combined with in vitro bioactivity data, physiologically-based-kinetic (PBK) modelling has increasing applications in animal-free safety decision making. The purpose of this work was to see if new approach methodologies could be used to estimate systemic exposure of an UV filter, benzophenone-4 (5% in sunscreen products and other formulation), via PBK modelling.

A tiered approach was applied for ADME data generation to understand the kinetic behaviour of benzophenone-4 for PBK model parameterisation. The skin penetration assay showed only a small amount of benzophenone-4 enters systemic circulation via dermal absorption. The metabolism assays showed benzophenone-4 has negligible liver clearance. Active transporter assays using over-transfected cells showed it is a substrate of several transporters in kidney. Then, the human isolated kidney proximal tubule cell monolayer on overall permeability confirmed that benzophenone-4 undergoes filtration, secretion, and reabsorption.

Upon model construction, sensitivity and uncertainty analyses were performed to identify influential parameters on PBK model output. Probabilistic PBK modelling through Monte Carlo simulation and Bayesian statistical analysis was then conducted to obtain plausible Cmax distributions by accounting for population variability, parameter uncertainty and model uncertainty. For the described exposure scenario, the median plasma level of benzophenone-4 was predicted to be 1.3 µM, with a 95th percentile of 9.8 µM.

Although human clinical data are not available for validation, the pragmatic approach of parameterisation provided a sound biological basis for the PBK model. The probabilistic modelling assured that the predictions are fit for the purpose and provided conservative estimates of human systemic exposure.

P2

# Heavy metals analysis of different varieties of legumes grown in different area of zangon kataf local government area, kaduna state.

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Legumes are considered vital crops for achieving food and nutritional security for both high and low income earners. Pre and Post harvest methods attracted the use of chemicals to control pests while improving crop yield. Toxic contaminants from these chemicals maybe introduced in addition to contaminants from urban and industrial effluents, wastewater, fertilizers and pesticides. Hence, the need to determine the trace elements contents of this class of food crops. The concentration of heavy metals in selected dried Beans (Pigeon peas, Brown Beans, Soy beans, and Black eye pea) grown and collected from two area of Zango kataf L.G.A (Samaru Kataf and Kurmin Masara villages) of Kaduna State Nigeria were analyzed. Samples were ground to powder, and analyzed for Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb) and Mercury (Hg) using Atomic Absorption Spectrometer (AAS). The results showed that all legume samples grown in Samaru kataf had the highest concentration of the heavy metals analyzed compared to those grown in Kurmin masara. Although the results from this study indicates that the concentration of heavy metals in all the samples analyzed were below the intake limit for heavy metal set by the World Health Organization 2019, caution should be taken when consuming legumes grown or stored with unknown chemicals.

P3

# 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 bearing strong structural and physicochemical similarities to certain food dyes approved in Europe. An evaluation of the available evidence for the low bioavailability of Atrorosin E using physiologically-based-pharmacokinetic (PBPK) modelling of experimental studies and read-across from colourants with similar structures that are accepted for use in foods in Europe was undertaken. At the request of Chromologics, Inc. Ramboll US Consulting, Inc. (Ramboll) scientists developed a 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 colourants with structures and properties similar to Atrorosin E was 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. 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 colourants with similar chemical structures and properties.

P4

# In silico pharmacokinetics and toxicity study on the active anti-polycystic ovarian syndrome constituent in Parquetina nigrescens leaves

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Polycystic ovary syndrome (PCOS), a leading cause of infertility in pre-menopausal women is characterized by hyperandrogenism, polycystic ovaries, obesity and insulin resistance. Parquetina nigrescens have been used for treatment of various reproductive diseases including PCOS. Therefore, the study was aimed at evaluating the potential toxicity of active constituent identified in the plant. Gas Chromatography-Mass Spectrophotometry (GCMS) analysis was carried out to identify the compounds present in P. nigrescens. Thereafter, the compounds were screened via molecular docking against four important proteins associated with PCOS for multiple inhibitory activity. The compound identified was subsequently subjected to pharmacokinetic and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) test. Result obtained indicated glutaric acid, 2-ethylbutyl heptyl ester (CID91705405) as a multiple inhibitor of androgen receptor, estrogen receptor, fructose bi-phosphate and phosphoenol pyruvate carboxykinase. Pharmacokinetic studies showed that CID91705405 had the properties of orally druggable compounds with a molecular weight of 356.46 g/mol, zero hydrogen bond donor and 4 hydrogen bond acceptors. ADMET study indicated 93.68% absorption rate for CID91705405, while the steady state volume of distribution (VDss) is 0.173 log L/kg. CID91705405 effectively inhibited cytochrome P450 (CYP450) isoforms (CYP3A4, CYP1A2, CYP2C19). CID91705405 had a total clearance of 1.40 log ml/min/kg, and does not identify as an organic cation transporter 2 substrate. CID91705405 exhibited a good pharmacokinetic property and showed no potential toxicity, however, there may be risk of drug interaction due to its inhibitory effect on CYP450 isoforms. This compound may be considered in rational PCOS drug design subject to further experimental validation.

P5

# Toxicological evaluation of ethanolic extract of Parquetina nigrescens leaves (afzel.) on letrozole-induced polycystic ovarian sydrome: an in vivo study

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The toxicological effect of ethanol extract of Parquetina nigrescens leaves (EEPNL) on letrozole-induced polycystic ovarian syndrome (PCOS) in Wistar rats were evaluated. Twenty female Wistar rats (170.81 ± 5.25g) were randomly assigned into 5 groups (A - E) of four animals each. Animals in group A received 1 ml of distilled water and group B-E received 1mg/kg body weight of letrozole daily basis for a period of 21 days orally. The letrozole-treated groups, B-E, were then administered 1 ml of distilled water, co-administration of 7.14mg/kg of metformin and 2mg/kg clomiphene citrate (reference drug), 50 mg/kg b.wt of EEPNL and 100 mg/kg b.wt of EEPNL respectively for a period of 28 days. The animals were sacrificed 24 hours after the last treatment dose and the blood obtained via jugular puncturing was used in determining some toxicological indices such as liver function indices (albumin, bilirubin, globulin and total protein), kidney function indices (Urea, uric acid and creatinine), and enzyme assay (Alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase). The result reveals that the serum AST and ALT of EEPNL at the doses investigated compared favorably ($P>0.05$) with the control animals. There was a significant increase ($p<0.05$) and a significant decrease ($p<0.05$) at 50mg/kg and 100mg/kg of EEPNL on kidney and liver function indices respectively compared to the control. Therefore, the mild alterations in the parameters under-studied in this work suggests that the ethanol leaf extract of Parquetina nigrescens at doses investigated may not be completely safe when used in the treatment of PCOS.

P6

# Potential antidotes for 2,4-Dinitrophenol toxicity: a systematic review

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2,4-Dinitrophenol (2,4-DNP) was first proposed as a weight loss agent in 1933. (1) It is a potent, dose-dependent, oxidative phosphorylation uncoupler responsible for increased thermogenesis and multi-system dysfunction in vivo (2) The United Kingdom (UK) National Poisons Information Service (NPIS) has reported an increase in cases of 2,4-DNP exposures over the last two decades. (3) However, to date, no definite treatment for acute 2,4-DNP toxicity has been identified. Therefore, we aimed to explore the efficacy of potential antidotes for acute 2,4-DNP toxicity, in non-dermal exposure cases, through a literature search.

We performed a database search on MEDLINE OVID between March 1935 and June 2022. The percentage of survived was the primary outcome. Secondary outcomes included temperature reduction and improvement in clinical symptoms. Results are summarised in table 1. Treatment adjuncts, such as cooling, were included in many studies. Dantrolene, which is recommended by TOXBASE for 2,4-DNP toxicity, was effective in four out of seven reported cases. The average 2,4-DNP dose ingested associated with survival after dantrolene treatment was 5-10 mg/kg. Efficacy of magnesium in 2,4-DNP-induced hyperthermia was due to temperature reduction in a single rodent study. (4) There was insufficient data to carry out in-depth analysis of each potential treatment route.

Further research in both animal models and humans are necessary to determine the optimum management of 2,4-DNP toxicity. Combining potential treatment modalities and studying compounds similar to 2,4-DNP may provide further insight.

P7

# Replacing the need for Acute Toxicity Studies in Fish: The RTgill-W1 Assay

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Testing for acute fish toxicity is an integral part of the environmental safety assessment of chemicals. The Fish Gill Cell Line (RTgill-W1) assay provides a non-animal alternative that has been validated and adopted as an OECD Guideline (TG 249). To demonstrate the applicability of this test, we have previously performed and published a benchmarking study on 38 fragrance chemicals for which we had high quality historical in vivo data on fish toxicity covering a broad range of physicochemical properties and diverse chemistries. This study showed a very strong correlation ($R^2 = 0.94$) between the logarithmic in vivo LC50 values, based on fish mortality, and the logarithmic in vitro EC50 values based on cell viability.

To promote regulatory acceptance and further demonstrate the high in vitro–in vivo correlation for fragrance chemicals, we present here our latest results for 7 new chemical products or expanding product registrations where we were required to perform an in vivo acute fish study for European or global notification purposes. The challenge of assessing the toxicity of poorly soluble chemicals that are expected to have no toxic effect up to their solubility limit is also explored. Further we provide 3 case studies, for example products used predominantly or exclusively in cosmetic applications, where we have submitted registrations without in vivo testing. We demonstrate how the RTgill-W1 assay can be used as a bridging tool between in silico predictions and full in vivo testing and how it can be used to support hazard and risk assessment.

P8

# Abrin induces apoptosis via inhibition of Akt phosphorylation in human lung epithelial A549 cell

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Abrin, a lethal phytotoxin, has similar properties to the ricin toxin, which is a schedule 1 (OPCW) and category B (BWC) agent. Explication of the mechanisms of abrin-induced cellular toxicity is crucial for the toxin treatments. In the present study, we report abrin-induced apoptosis in A549 cells through downregulating Akt pathway. Protein (ELISA and immunoblot) and gene expression (RT-PCR) of Akt and its six downstream effectors, i.e., phospho-p38, phospho-SAPK for stress, NF-κβ as a pro-survival factor, γ-H2AX for DNA damage, PARP and caspase-3 as an executor of apoptosis, are studied. Treatment of cells with 1ng/ml abrin induced cytotoxicity in a time-dependent manner. Phospho-Akt markedly upregulated at shorter times (0.5h, 4h) and subsequently decreased at 16h and 24h. Expressions of phospho-p38, phospho-SAPK, NF-κβ, γ-H2AX, PARP, and caspase-3 were increased in later times and inversely correlated with phospho-Akt levels. Inhibition of Akt using wortmannin significantly decreased cell viability and the survival fraction from 0.67 to 0.19, even at early time points. Further, Akt inhibition increased phospho-p38, phospho-SAPK, γ-H2AX, and caspase-3 levels and increased apoptosis. Additionally, an increase in DNA damage was observed via immunofluorescence. Induction of apoptosis is accompanied by the downregulation of NF-κβ expression. Inhibition of Akt not only accelerated apoptosis at early time points but enhanced the magnitude at later stages. It indicates that abrin induces apoptosis by direct downregulation of Akt and inhibiting survival signals. Further, cross-talk between Akt and other signaling molecules may regulate cytoprotection and the degree of apoptosis. We propose this mechanism for explaining abrin toxicity.

P9

# What is the association between smoking or vaping and COVID-19 susceptibility?

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The association between smoking and COVID-19 susceptibility is conflicted as there is limited research especially on the impact of vaping. This systematic literature review identified genes and signalling pathways that are affected by smoking or vaping and may play a role in altering the susceptibility of an individual to COVID-19.

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, OVID and Web of Science databases, were searched. Abstracts and subsequent full texts were screened for eligibility by RB, with EM and AB screening 5% each. Discrepancies were discussed and agreed between all. Papers were excluded for reasons such as focussing on comorbidities, immune cells or those with no exposure. Data from included papers detailing the exposure (type, duration and dose if applicable), pathway or gene of interest, sample type (species and cell type) and the overall findings, were extracted. Pathway analysis was undertaken to identify those pathways in common between smoking, vaping, nicotine and COVID-19.

We identified genes within the respiratory tract involved in both the initial viral response and those affected by smoking, vaping or nicotine exposure. This enabled us to determine signalling pathways of interest and therefore areas for further study. This systematic review will inform further research to identify if smokers or vapers are likely to have any altered risk to SARS-CoV-2 infection and /or potential susceptibility to severe COVID-19. This will inform Public Health Policies, highlighting vulnerable groups and provide guidance of relevance to both COVID-19 and other respiratory viruses.

P11

# In Vitro High-Throughput Toxicological Assessment of E-Cigarette Flavors in Human Bronchial Epithelial Cells and the role of TRPA1 in Cinnamon Flavor-Induced Toxicity

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Background: Electronic cigarettes (ECs) are considered a less hazardous alternative to tobacco smoking but are not harmless. Growing concerns about the safety profiles of flavors in e-liquids underpin the need for this study.
Methods: Here, we screened 53 nicotine-free flavored e-liquids (across 15 flavor categories) across a 3-point concentration range (0.25%, 0.5%, and 1% v/v) in a high-throughput fashion in human bronchial epithelial (HBEC-3KT) submerged cell cultures to identify 'toxic hits' using in vitro endpoint assays comprising cell count, cell viability, and lactate dehydrogenase (LDH).
Results: We observed significant, dose-dependent adverse effects only with cinnamon, vanilla tobacco, and hazelnut e-liquids compared to media-only control and PG/VG vehicle controls. Hence, we further analyzed these three flavors for their effects on HBEC-3KT proliferation, mitochondrial health, and oxidative stress. A significant decrease in cell proliferation after 36h was observed for each e-liquid toxic hit compared to media-only and PG/VG controls. Hazelnut (at all concentrations) and vanilla tobacco (1%) increased cytoplasmic reactive oxygen species generation compared to media-only and PG/VG controls. Conversely, all three flavors at 0.5% and 1% significantly decreased mitochondrial membrane potential compared to PG/VG and media-only controls. We hypothesized that the cytotoxic effects of cinnamon flavor in e-liquids might be mediated via TRPA1; however, TRPA1 antagonist AP-18 (10 µM) did not mitigate these effects, and cinnamon significantly increased TRPA1 transcript levels. Therefore, pathways that mediate cinnamon's cytotoxicity warrant further investigations.
Conclusion: This study could inform public health authorities on the relative health risks assessment following exposure to EC flavor ingredients.

P12

# The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT): review of chemicals in the maternal diet.

Ms Sabrina Thomas[1], Gail Drummond[1], Cleanncy Hoppie[1], Emily Hudson[1], Jocelyn Frimpong-Manso[1], David Kovaic[1], Joseph Shavila[1], Rhoda Aminu[1], Emma French[1], David Gott[1], Claire Potter[1], Cath Mullholland[1]
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The Committee on Toxicity of chemicals in Food, Consumer Products and the Environment (COT) was asked by the Scientific Advisory Committee on Nutrition (SACN) to conduct toxicological risk assessments on chemicals in the diet as part of a project on nutrition and maternal health, focusing on maternal adverse outcomes during pregnancy, childbirth and up to 24 months after delivery. Two prioritisation papers were presented to COT in 2021, on biological origin and contaminants, formalising the process of deciding which compounds, based on available data, should be considered.

Papers on cadmium, iodine, vitamin A, vitamin D have been published in 2022. The following papers are currently under discussion by the COT: lead, ginger, ergot alkaloids and raspberry leaf.

The toxicity of these chemicals was reviewed along with the basis of published health-based guidance values (HBGVs) or tolerable upper level (TUL). Exposures from food were estimated using the mean and 97.5th percentile of UK consumption data. Calculated dietary exposures were compared with the respective HBGVs or TUL for risk.

The estimated dietary exposures for iodine were below their respective HBGVs (i.e., upper level and provisional maximum tolerable daily intake). Vitamin A and D were within their TULs, and cadmium was within its tolerable weekly intake (TWI). These chemicals are therefore not of toxicological concern.

Future work currently includes arsenic, mercury, selenium, phytoestrogens and mycotoxins. However, the list of potential chemicals to be included are subject to change dependent on discussions within the COT and SACN.

P13

# Methylene blue as an adjunctive treatment of shock secondary to severe metformin poisoning reported to the National Poisons Information Service from 2010 to 2022.

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Methylene blue is suggested for the treatment of severe metformin poisoning with refractory drug induced shock. However, currently there is insufficient evidence to recommend the treatment routinely. We report cases of severe metformin poisoning discussed with the National Poisons Information Service between 1 January 2010 and 31 December 2022, where patients were given methylene blue as a treatment.

Methylene blue was reportedly given to 11 patients treated for a severe intentional metformin overdose. All cases involved adults between 25-64 years old. Eight patients were male, 3 were female. Nine cases were multi-drug cases involving metformin and at least two other agents, all including a calcium channel blocker drug, 5 cases also included an ACE inhibitor drug. Four cases reportedly gave only one bolus dose of methylene blue, one case administered 3 x 2 mg/kg bolus doses, and two cases started a methylene blue infusion.

Four multi-drug cases and one metformin-only case reported benefit or short-term improvement in clinical condition specifically following methylene blue administration. Other supportive treatments included blood pressure and cardiac support, ECMO, intralipid, sedation and renal replacement therapy. Four patients died following their overdose despite treatment. Seven patients have an unknown outcome due to incomplete follow up of the case, which is our main limitation.

Our case series demonstrates that alongside other supportive treatments, methylene blue can have some benefit for patients with severe overdoses involving metformin. Overall, patients who responded to the methylene blue treatment had a lower ingested dose and a less severe lactic acidosis.

P15

# Switching to high genetic-barrier integrase inhibitors reduces drug-drug interactions in people living with HIV

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Background
Boosted protease inhibitors(PI/b) have decreased mortality in people living with HIV(PLWH). Co-administration with cytochrome P4503A4(CYP3A4) inhibitors increases toxicity risk and harmful drug-drug interactions(DDI), especially in ageing populations with polypharmacy. Second generation integrase strand inhibitors, such as Bictegravir(BIC) and Dolutegravir(DTG), proven to be efficacious replacements for PI/bs, reduce DDI potential and polypharmacy risks. This analysis aimed to assess benefits of proactively switching people living with HIV from PI/b to INSTIs between March 2021-2022.

Methods
Data were normality tested and subjected to univariate and multivariate analysis regarding demographic and HIV-related independent variables impacting likelihood of a successful switch. $p<0.05$ was deemed statistically significant. Frequency distributions identified the most common drug interactions before and after changes in medication. The University of Liverpool HIV Drug interactions website helped analyse effectiveness of switching to assess the number and significance of interactions.

Results
155 individuals were referred to the treatment optimization MDT; 148 (95)% successfully switched to two NRTIs(TDF or TAF plus FTC) and either BIC or DTG. 7(5%) were assessed to exemplify excessive resistance, requiring continuation of the PI/b regimen. 205 red and amber DDIs were identified before and 52 after the switch, illustrating a statistically significant decrease($-1.135\pm1.45$, $p<0.0001$) in mean DDIs. The most prevalent interactions were between PI/bs and atorvastatin(n=48), citalopram(n=13) and amlodipine(n=9).

Conclusion
PLWH on PI/b can switch to INSTI regimens despite harbouring NRTI resistance. HIV is a known independent cardiovascular risk factor, therefore, switching PLWH to INSTI-regimens represents potential to optimise long-term health, by reducing burden of drug-related adverse effects.

P16

# Use of transcriptomics and metabolomics in assessment of liver tumour mode of action

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Transcriptomic and metabolomic analyses during chemical evaluation for new crop protection chemicals enable the prediction and exclusion of modes of toxicity and carcinogenicity, reducing the need for additional mode of action and carcinogenicity studies.
Plasma and/or liver were analysed for metabolomic or transcriptional profiles after 2, 7 or 28 days of exposure of male mice to cyclobutrifluram (TYMIRIUM® technology), a novel nematicide which inhibits mitochondrial complex II succinate dehydrogenase (SDHI), or phenobarbital (PB). In addition, liver enzyme activity, histopathology, and hepatocellular proliferation were assessed.
The analyses support a threshold-based constitutive androstane receptor (CAR)-mediated mode of action for mouse liver tumours, demonstrating direct activation and altered target gene expression of CAR, increased Cyp2B and 3A4 activity, and an early, transient increase in hepatocellular proliferation. Transcriptomic assessment showed induction of several CAR-activated genes, including Cyp2b10 and phase 2 enzymes, by cyclobutrifluram and PB, and a significant correlation with a CAR transcriptional signature. Activation of peroxisome proliferator activated receptor alpha (PPARα) and the aryl hydrocarbon receptor (AHR) were excluded. Metabolomic assessment confirmed the absence of alternative tumour pathways and any unique toxicity associated with cyclobutrifluram. These data support the weight of evidence for nuclear receptor-mediated liver tumours and exclusion of other modes of toxicity or liver tumourigenicity in chronic carcinogenicity studies.
Transcriptomic and metabolomic analyses, as part of a traditional tumour mode of action study, confirmed the proposed mode of action, excluded alternative modes of action, and demonstrate how this could be used to reduce the need for animal testing.

P17

# A Framework for Grouping Multi-Component Nanomaterials to streamline hazard assessment (including mixture effects)

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The GRACIOUS Framework (www.h2020gracious.eu) supports the grouping of nanomaterials to streamline their hazard testing, and to allow the read-across of hazard data. The Framework includes a template to generate grouping hypotheses based upon:

(i) the nanomaterial use and life cycle stage (to inform exposure route),
(ii) physicochemical properties (what they are),
(iii) environmental fate or toxicokinetics (where they go) and
(iv) hazard (what they do).

The gathering of evidence to test these hypotheses was supported by tailored Integrated Approaches to Testing and Assessment (IATAs).

Subsequent NMBP-16 projects have developed the hypothesis template for multicomponent nanomaterials. Modifications include additional information on:

(i) The complexity of composition (what they are)
(ii) Enhanced properties,
(iii) The relationship between composition, enhanced properties and the mechanism of hazard.
(iv) The potential for the components to dissociate, disintegrate or dissolve, with different kinetics, leading to a complex exposure scenarios.
(v) Using the information in (iv) to inform the hazard assessment
(vi) Potential interactions between the components leading to antagonism, synergism or potentiation of response.
(vii) Formulation of clear questions to test all aspects of the hypothesis, with these questions being used to formulate the hazard IATA.

The hazard IATA outcome is designed to inform Safe-by-Design modifications.

Case studies of different multicomponent nanomaterials are being used to inform the design process, and demonstrate its usefulness.

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P18

# Investigating mitochondrial complex I inhibition as a potential mechanism in drug-induced liver injury (DILI)

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Mitochondrial complex I is an entry point of electrons from NADH into the electron transport chain (ETC), with vital roles in regenerating NAD+ to sustain the tricarboxylic acid (TCA) cycle, and establishing the proton gradient that drives ATP synthesis via ATP-synthase. Complex I activity is associated with the production of reactive oxygen species (ROS) which have been implicated in physiological cell signalling as well as numerous pathologies. Inhibition of complex I therefore has detrimental cellular effects, exemplified by rotenone: a canonical complex I inhibitor linked to neurotoxicity and parkinsonism. It is now theorised that drugs known to cause hepatotoxicity can exhibit these adverse effects via complex I inhibition.

To assess the role of complex I in DILI, 150 drugs from a DILI library were screened for complex I inhibition in a series of biochemical assays using bovine mitochondrial membranes and complex I proteoliposomes. 10 potent complex I inhibitors were identified, showing ≥80% inhibition. These compounds were then tested on the human HepG2 C3A cell line via mitochondrial stress tests using Seahorse XF analysis. $IC_{50}$ values of their effect on cellular oxygen consumption rate (OCR) were found and compared to clinical Cmax values. At least 6 drugs showed clinically relevant $IC_{50}$ values in both the mitochondrial stress tests and the biochemical assays.

These results indicate a potential link between complex I inhibition, mitochondrial toxicity and DILI. While further studies on primary hepatocytes are now required, a promising toxicity mechanism is demonstrated which will help inform future drug design and hepatotoxocity screening.

P19

# Impact of secondary polyethylene terephthalate (PET) microplastics test materials on cell proliferation using Caco-2 and HT29-MTX monocultures.

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Humans are exposed to environmental microplastics (MP) primarily through inhalation and ingestion. It has been estimated that adults are exposed to 86-168,000 particles per day. However, the risk associated with MP exposure is not well understood for either oral or inhalational exposure. Some studies have observed down regulation of cell proliferation post MP exposure in GIT models. These studies are limited though in that they have only used primary MP and limited polymer types, not wholly representative of environmental MP. This study used MP made from 4 different sources of polyethylene terephthalate (PET); virgin and 3 different water bottle brands (A-C). MP were created using a solvent-free cryogenic abrasion method. The median diameter of MP produced ranged from 31-37 µm. As a primary measure of effect growth curves of Caco-2 and HT29-MTX monocultures with a concentration range (0.01-1 mg/ml ) of MPs were undertaken over 72 hours. Images and analysis were conducted using Molecular Devices Pico and Cell ImageXpress. It showed that higher concentrations of MP exposure resulted in reduced cell growth compared to lower exposures which had little to no effect against both cell lines. This seems to suggest that MP interaction with cells can impact cell proliferation. Further analysis is underway using transcriptomics and Q-PCR to assess mode of action of the particles. These data will help to fill knowledge gaps of hazards and allow an understanding of MP exposure to human risk.

P20

# Xenobiotic endocrine disruptors – investigating the modulatory effect(s) of methylimidazolium ionic liquids (MILs) on the human ER alpha in vitro and in vivo

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Methylimidazolium ionic liquids (MILs) are man-made solvents used in a variety of industrial processes (e.g., biofuel processing). Given their low volatility, they are proposed to be environmentally-friendly solvents. Recent work has identified both contamination of soil around a landfill site with an 8C alkyl chain MIL (M8OI) and its presence in the human population.

This study aimed to screen a range of MILs with different alkyl chain lengths for their ability to activate the human ERα using the HeLa 9903 cell line (OECD TG455) and in vivo in an oral uterotrophic study in rats (OECD TG440). Ethical approval was granted by both Cairo University Ethics Committee (PT 3228) and the Animal Welfare and Ethical Review Body, Newcastle University (#664). Metabolism in human hepatocyte-like cells (differentiated HepaRG cultures) and their stability in Tyne River water were also examined.

The results showed that short-chain MILs (e.g the 2C alkyl chain EMI) activated the ERα upon treating the HeLa 9903 cells with concentrations ≥ 100 nM and induced a 2-fold increase in uterus wet weight in vivo upon oral administration of 80 mg EMI/kg bw. Short-chain MILs also resisted metabolism in HepaRG-derived hepatocyte-like cultures. In contrast, the longer-chain MIL - M8OI- was metabolised to monooxygenated and carboxylic acid metabolites. Short-chain MILs also resisted degradation in river water samples while the longer-chain MILs were partially mineralised after 8 weeks of incubation.

In conclusion, short-chain MILs are ERα activators, show oestrogenic activity in vivo and resist both hepatic and environmental degradation.

P21

# Reproductive Toxicology in Men: Drugs in Semen- Toxicodynamics, Toxicogenomics and Teratogenicity

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## Introduction

The incidence of congenital malformations in the overall population is 7-10%, of which chemical exposures account for 2-3%, however the source of exposure is often unclear. We investigated the potential for chemical transmission via the semen in males exposed to known teratogenic pharmaceuticals and lead compounds.

## Methodology

A literature review of PUBMED and EMBASE identified a list of 10 teratogenic pharmaceutics with established thresholds for teratogenicity. Pharmacokinetic data on serum and semen concentrations was identified from the literature and SmPC.

## Results

The teratogens include: fluconazole, lead, isotretinoin, methotrexate, lithium carbonate, sodium valproate, carbamazepine, lamotrigine, topiramate and phenytoin.
Chemicals of concern are those where the predicted therapeutic: teratogenic concentration ratios in semen and teratogenic threshold concentration approximates to or exceeds 1.0.
This was observed in isotretinoin, methotrexate, lithium carbonate, sodium valproate, carbamazepine, lamotrigine, topiramate, methotrexate and phenytoin (Table 1).

## Conclusion

Teratogenic chemical concentrations in semen may exceed concentrations associated with teratogenesis. This potential source of teratogenic exposure merits further investigation.

P22

# Assessing exposure to fungal bioaerosols in transport environments: Analysing fungal composition of passive dust samples collected in UK railway stations

Dr Emma Marczylo

Bioaerosols consist of a complex mixture of airborne microorganisms including fungi, bacteria, pollen, particulates and by-products of cells. While exposure to diverse microorganisms is essential for normal immune system development, bioaerosol inhalation has been associated with respiratory allergy and inflammation. Bioaerosols are ubiquitous, yet their composition within different environments is not well understood. Such information is essential for assessing exposure and associated health impacts. Here we focus on transport environments, namely railway stations (RSs), which pose a potential source of occupational and community exposure.

Over 1200 passive dust samples were taken from 17 RSs across the UK from 03/2014-05/2015. Geographic information system (GIS) methods were used to provide information on population characteristics of the immediate surrounding area of the RSs. Together with information on RS layout and passenger numbers, this informed the selection of 250 samples, representing 9 RSs of varied geographical location, layout and footfall, for further analysis. High throughput sequencing (HTS) with a metabarcoding approach targeting the internal transcribed spacer2 (ITS2) region of the ribosomal RNA genes was used to analyse the fungal composition of the sample subset. Such HTS techniques provide an opportunity to measure a wider range of microorganisms than traditional culture or microscopy techniques.

RS characteristics were varied and included different layouts and annual footfall. Fungal taxa across all RS and seasons were dominated by yeasts, many of which are known pathogens and/or allergens. There were significant ($p<0.01$) differences in the diversity/composition of fungal taxa and guilds between the stations and seasons, likely driven by specific RS characteristics or meteorological factors, respectively. Such work is key to better understanding fungal exposures within public spaces and their associated health impacts.

P23

# In vitro human hepatocyte proliferation assays: analysis of responses to reference compounds in studies from CropLife Europe member companies

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Liver cancer in rodents is a common finding in response to lifetime chemical exposure and frequently occurs via a non-genotoxic mode of action (MOA), with increased cell proliferation via activation of nuclear receptors (such as the constitutive androstane receptor, CAR). Exemplification of chemical specific MoAs and assessment of human relevance involve bespoke mechanistic studies examining key events in the MoA and to examine human relevance, studies looking at DNA synthesis as a surrogate for proliferation in isolated human hepatocytes.

CropLife Europe member companies retrospectively collated the available human hepatocyte data to understand the demographics of human hepatocyte donors used and respective proliferative responses to control compounds. Data were collected for studies run between 2011-2020. 40 individual human hepatocyte donors were identified as have being tested with the following demographics, 18/40 male; 22/40 female; age range of donors covered 11-73 years for males and 10 months to 80 years for females.

All donors tested induced increases in S-phase DNA labelling index versus control when exposed to the reference controls, epidermal growth factor (EGF, 7.7±5.8-fold versus control) or hepatocyte growth factor (HGF, 2.6±1.7-fold versus control) in a dose dependent manner. No influence of age or sex on S-phase DNA labelling index was observed in response to EGF or HGF. Treatment with the CAR activator phenobarbital did not affect S-phase DNA labelling index in human hepatocytes.

This data indicates that human hepatocytes are a robust model for assessing the human relevance of key events in the CAR-mediated mechanism for liver carcinogenesis in rodents.

P24

# Establishing a patient-derived model of cholangiocarcinoma using precision cut tumour slices (PCTS).

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Cholangiocarcinoma (CCA) is an aggressive hepatobiliary malignancy with increasing incidence and a persistently poor prognosis. Accurate models that faithfully recapitulate in-vivo tumour biology are urgently required to better understand therapeutic response/resistance, whilst serving as a platform for novel drug discovery.

Precision-cut tumour slices (PCTS) are patient-derived tumour explants cultured ex-vivo, which retain the tumour microenvironment and recapitulate critical aspects of cancer biology, providing an advantage over 3D organoid culture. Our aim is to establish and validate the use of the PCTS as a patient-derived model of cholangiocarcinoma.

Tumour slices (250µm, 5mm diameter) were prepared using a Krumdieck tissue slicer from fully consented CCA patients (5 = perihilar CCA, 3=intrahepatic CCA). PCTS were randomly incubated (5% CO2, 37°C) in supplemented Williams E media and cultured for 0-15 days in 24 well plates (n=3/timepoint). Tissue perfusion and air-liquid interface was maintained using Millipore organotypic inserts. Following protocol optimisation, tissue viability was maintained ex-vivo at various timepoints and confirmed using in-situ MTS viability assays, alongside histological assessment of tumour morphology, immunohistochemical markers of proliferation (Ki-67) and absence of cleaved-caspase 3 apoptotic markers. Preliminary studies have shown CCA PCTS to respond in a dose-dependent manner to treatment with standard of care chemotherapy (capecitabine or cisplatin/gemcitabine). Further characterisation of the model is ongoing using SWATH-MS proteomics to ensure it remains a robust recapitulation of the in-vivo tumour. Our initial results provide encouragement for the use of PCTS as a patient derived model platform to assess therapeutic response and as potential platform for novel drug discovery.

P25

# Use of Toxicogenomics to Assess Biological Relevance of a Putative Proliferative Response

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The potential biological relevance of a small increase in replicative DNA-synthesis (RDS) observed in hepatocytes from one human donor, NLW, at one exposure concentration of a new pesticide, TYMIRIUM® technology (cyclobutrifluram) was explored using RNA-sequencing. Samples from three donors were sequenced. Expression and enrichment analyses, and a targeted examination of cell-cycle/proliferation genes and pathways were conducted.

Congruent with previous studies, genes in the cytochrome-P450 families were upregulated across all donors. A small number of genes associated with pro-proliferation signaling pathways were detected in multiple donors; however, these were not uniformly expressed, suggesting that this was not indicative of a proliferative response. No other DEGs, or pathways associated with proliferation/cell-cycle hierarchies were observed in NLW that were unique to cyclobutrifluram treatment. Furthermore, proliferation marker genes (e.g., MKI67), were not differentially expressed in any donor. Results from gene set analysis of six Hallmark proliferation signatures was also inconsistent across all donors following treatment. For example, only two gene sets were more positively enriched in NLW samples compared to the other donors, however these were not unique to the test item.

Although there was modest positive enrichment of some proliferation-related gene sets in donor NLW, overall, there was little evidence for overexpression of genes/pathways associated with proliferation in NLW, or the other donors. Consequently, an apparent small increase in RDS in NLW was inconsistent with the underlying biochemical changes and therefore was not considered biologically relevant, demonstrating how toxicogenomics can be used as a robust tool to investigate unusual in-vitro findings.

P26

# Interaction of environmental pollutants and food contaminants with gut bacteria

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The human gut microbiota modulate the toxicity of xenobiotics via multiple routes, including biotransformation and bioaccumulation. Concordantly, changes in the gut microbiome composition have been associated with diseases as well as response to medications. Hence, it is crucial to understand how different lifestyle and environmental factors shape the gut microbiome composition. In contrast to pharmaceutical drugs, other xenobiotics, such as environmental or chemical pollutants, are less well studied and their effect on gut microbiota remains underappreciated. While xenobiotics can influence bacterial growth and metabolism, bacteria in turn can bioaccumulate or chemically modify these compounds. We here screened xenobiotic-bacteria interactions spanning all major classes of xenobiotics that are likely to enter food systems. We find that a considerable fraction of compounds inhibited the growth of at least one of the tested abundant gut bacterial species. In addition, several xenobiotic compound concentrations were reduced by gut bacterial strains showing bioaccumulation or biotransformation. Our results highlight the necessity to consider the impact of environmental pollutants and food contaminants/additives on gut microbiome composition and human health.

P27

# In vitro assessment of pod-based e-cigarettes reveals that their aerosol have marked reductions in cytotoxicity, mutagenicity and genotoxicity compared to cigarette smoke

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The fourth generation of e-cigarette devices utilise pod-based atomisation technology. In addition to conventional cotton-based wick atomisers, ceramic based wicks have been developed and are now increasingly available. In the current study we assessed the aerosol from two pod-based e-cigarettes (myblu 1.6% nicotine with a cotton wick and blu 2.0 1.6% nicotine with a ceramic wick) and compared any measurable biological responses of the aerosol against 1R6F reference cigarette smoke in the Ames, In Vitro Micronucleus (IVM) and Neutral Red Uptake (NRU) assays. Three different e-liquid flavours (Tobacco, Menthol and Blue Ice) were assessed in each device. For IVM and NRU, cells were exposed to smoke or aerosol at the air liquid interface using a 'smoke aerosol exposure in vitro system' (Burghart Tabaktechnik). For the treatment of bacteria suspensions with fresh smoke/ aerosol, a smoking machine VC10® S-Type (Vitrocell Systems GmbH) was used. The Ames test was performed in compliance with OECD471 (using TA98 & TA100±S9) with the IVM performed in compliance with OECD487 (V79 cells ±S9). The NRU cytotoxicity assay used BEAS-2B cells and followed standard assay protocols in accordance with ISO17025. For all pod-based products there were marked reductions in cytotoxic responses from their aerosols when compared to cigarette smoke ranging from 99.5-99.9% less cytotoxic. Both devices showed no mutagenicity or genotoxicity under the conditions of the test, whereas significant dose responses were observed for 1R6F smoke. The data presented here adds to the growing body of evidence for pod-based e-cigarettes, as a potential tool for harm reduction.

P28

# Carbon nanomaterials (CNMs) in medical devices, where innovation meets risk. Are the human health risks sufficiently understood to justify the benefits? A systematic review.

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Carbon nanomaterials (CNMs) in medical devices offer performance innovation. Resultantly, their application in medical devices is increasing despite unknown risks. A systematic review was conducted which aimed to summarise CNM hazard identification and hazard characterisation data from empirical rodent studies, limited to non-stochastic, dose-dependent toxicity. A relationship was established in mice between inflammatory cell infiltration (ICI) of the lung and the size, in diameter (nm), P= 0.0002 and length (µm), P= 0.0001 of the multi-walled carbon nanotubes (MWCNTs) inhaled. Local and systemic effects were observed in mice exposed to MWCNTs, with lung ICI occurring at 77.33µg (CI95 49.36, 105.3), and increases in liver enzymes occurring at 50µg. Mice exposed to single-walled carbon nanotubes (SWCNTs) showed significant increases in white blood cell count (WBC) and blood urea nitrogen (BUN) at 2.18µg and 5.2µg respectively. Data suggests that rats are more sensitive to the effects of CNMs than mice with increases in non-neoplastic lung lesions following inhalation of fullerene $C_{60}$ at 97.5 mghr/m³ and 243.75 mghr/m³ (CI95 41.06, 446.44) respectively. Evidence suggested that fibrous CNMs can elicit a sustained response with raised ALT concentrations in the rat up to 90 days. This review highlighted the potential of CNMs to cause local and systemic dose-dependent effects in rodents. However, to understand the risk to humans from exposure to CNM containing/ generating medical devices, a better understanding of the exposure scenarios is required.

P29

# Biomonitoring metals using native mosses in Leicestershire, UK

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Mosses can provide information about atmospheric contamination by metals. Forty native mosses (31 urban, 9 rural) were sampled from green spaces across rural/urban environments in Leicestershire (England) in 2017. Species identification was performed by DNA barcoding using bryophyte-specific primers on DNA extracted from 100 mg of frozen, homogenised, ground moss using Isolate II Plant DNA Kit®. Copper (Cu) and manganese (Mn) were determined by atomic absorption spectroscopy following appropriate mineralisation with $HNO_3/H_2O_2$. Data was processed in the 'NADA' statistical package. Ceratodon purpureus, Brachythecium spp., Hypnum cupressiforme, Kindbergia praelonga moss species were identified. Cu was not detected in any of the samples monitored (LoD=0.0114 µg/g), meanwhile Mn was detected (LoD=0.0267 µg/g) in one moss sample collected in Bradgate Park (0.0351 µg/g), a very popular 350-hectare public park located on the outskirts of the city of Leicester, which is home to wildlife including numerous groups of Cervus elaphus and Dama dama. The levels of both elements were lower than the medians described in larger biomonitoring studies performed in Austria (5.4, non-reported), Czech Republic (6.5, 470) and Germany (9.4, 331) for both elements (data presented in µg/g for Cu and Mn, respectively), suggesting lower emission of these metals in Leicestershire. The low concentrations detected suggests that atmospheric contamination with Cu and Mn would be minimal in Leicester city and surroundings. However, analysis of a larger number and diversity of samples would be needed to confirm these preliminary observations, as both metals have been detected in Leicester's environment, including topsoil and wild mushroom samples.

P30

# Cadmium in wild mushroom species from Leicestershire, UK.

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Consumption of wild mushrooms may facilitate exposure to inorganic pollutants. 155 wild mushrooms were collected from different green areas in Leicestershire (UK) in Autumn 2018. Mushrooms were appropriately cleaned, processed (dried) and homogenised for analysis. Species identification was confirmed by DNA barcoding using internal transcribed spacer 1/4 primers after extracting DNA from 100 mg of frozen homogenised ground mushroom material using DNeasy Plant Mini Kit®. Cadmium (Cd) was monitored by ICP-MS following mineralisation with $HNO_3/H_2O_2$ [LoD=0.081 mg/kg dry weight (dw)]. Different mushroom species were identified including Panaeolus foenisecii, Mycena citromarginata and Agaricus bitorquis. The median level of Cd was higher in A. bitorquis than in P. foenisecii and M. citromarginata (3.878 mg/kg, 2.281 mg/kg and 0.556 mg/kg; all in dw). Maximum allowed concentration (MAC) for Cd (0.2 mg/kg mushroom) was exceeded in all mushroom species collected, irrespective of area sampled, which may highlight a potential public health risk, especially for the edible species of A. bitorquis. Moreover, the toxicity of poisonous species monitored such as P. foenisecii may be enhanced by the high levels of Cd found in their tissues. Cd concentrations varied between locations in P. foenisecii mushroom species, which could indicate a different distribution of the contamination by this element in topsoils across urban/rural areas monitored. Thus, P. foenisecii mushrooms sampled in Abbey park (urban) contained lower concentrations of Cd than same species picked up in Braunstone park (suburban/ rural). Consumption of wild mushrooms foraged in Leicestershire should be avoided and limited to cultivated species.

P31

# MONITORING IRON IN HAIR OF SPANISH ADOLESCENTS RESIDING IN ALCALÁ DE HENARES (SPAIN)

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Higher levels of iron (Fe) in hair have been associated with lower regional gray matter volume in young adults. We have studied Fe content in scalp hair from 97 adolescents (13-16 years-old; 68 girls) from Alcalá de Henares (Spain), as hair Fe analysis would not be affected by rapid fluctuations due to dietary intake. The Fe was analysed by ICP-MS after appropriate removal of exogenous contamination. Data was processed using statistical methods available in the 'NADA' statistical package. The limit of detection was 1.813 µg/g. Fe hair concentrations were as follows (median and percentiles; µg/g): overall 7.486 (5.993, 9.069), males 6.571 (5.204, 8.498), females 7.769 (6.686, 9.540). The presence of Fe in hair showed sex-dependency, significantly higher in females' hair (p=0.0165). In contrast, levels of this metal did not show differences due to area of residence, which might suggest minimal environmental exposure to Fe, the primary source being diet. Thus, the significantly higher content of Fe observed in females' hair might be attributed to differences in the dietary intake, although this has not been explored in this project. Overall, the presence of Fe would be lower than that described in young Spanish individuals living in Madrid city (14.8 µg/g in scalp hair collected in 11-15 years-old) and the median/reference interval suggested for Italians' hair age 3-15 years-old (12.7; 5.9-36.8 5th-95th percentile interval; all in µg/g), which might suggest a lower Fe status in the Alcalá adolescents monitored that should be further investigated.

P32

# Human health risks of gadolinium contamination in urban and industrial soils across Alcalá de Henares (Spain)

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Gadolinium (Gd) is widely used in industrial and household applications, including microwaves, fibre optics and flat screen displays. The aim was to determine the presence, distribution and risks of Gd in Alcalá de Henares (Madrid region, Spain), and its risks for the population. Ninety-four topsoil samples were collected in July 2017: 66 urban, 24 industrial and 4 public gardens. Gd was analysed by ICP-MS after acid digestion in a microwave system. Data was processed using the 'NADA' statistical package. Noncarcinogenic risks were characterised following US EPA methodologies. Significantly higher levels of Gd were found in the topsoils collected in the industrial and garden areas rather than in the urban parks (data presented as median and interquartile ranges, in mg/kg, respectively; $p<0.001$): 3.999 (3.469, 4.705), 3.615 (3.490, 3.694) vs. 2.902 (1.908, 3.366). Fertilisers, a major diffuse source of rare earth elements (REEs) in topsoils, including Gd, might explain the distribution between both main areas. Noncarcinogenic risks quotients for ingestion (1.87E-04, 1.36E-04) and dermal contact (5.13E-11, 4.83E-06) were lower than the threshold in both industrial and urban areas, respectively. Our results would suggest a minimal risk through the ingestion/dermal contact of Gd present in topsoils in Alcalá city and adjacent industrial areas. However, the risk characterisation is incomplete as the specific risks derived through the inhalation of resuspended soils would require further investigation in light of unknown toxicological data. Further studies would be needed as the demand for REEs, including Gd, is expected to increase fivefold by 2030 in the European Union.

P33

# Evaluation of emergency skin decontamination protocols in response to an "acid attack" (vitreolage).

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The continuing weaponization of corrosive substances has resulted in an increase in the incidence of “acid attacks” (vitreolage), the consequences of which can leave victims with lifelong physical and psychological disorders. The purpose of this study was to evaluate the effectiveness of different emergency skin decontamination protocols against concentrated sulphuric acid (20 µL) to identify an effective treatment window and protocol.

The study was performed using dermatomed porcine skin mounted in standard diffusion cells. Treatment groups comprised a negative control (not exposed), positive control (exposed, no decontamination), dry decontamination (absorbent tissue paper), wet decontamination (90 s water irrigation), dry & wet decontamination and decontamination using a layer cotton. Decontamination was performed at 10, 30 and 1800s post exposure. Skin damage was primarily quantified using tritiated water permeability, photometric stereo imaging (PSI) and SEM-XDS. The pH of water effluent (from water irrigation) and receptor fluid chambers was also measured.

Significant damage to skin barrier function occurred from 10 seconds and no decontamination method was significantly effective in reducing damage to skin barrier function. All forms of decontamination significantly reduced the penetration of acid through the skin when performed at 10 seconds (but not thereafter), with water irrigation being the most effective treatment. The pH of water effluent indicated complete removal of acid from the skin surface after 45 seconds.

These data clearly demonstrate the deleterious consequences of dermal exposure to concentrated sulphuric acid. There is no effective window of opportunity for acid decontamination, but water irrigation(>45 seconds) may limit burn severity.

P34

# Development and validation of a robotic system for the accurate and reproducible dermal application of candidate skin decontamination products.

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The skin surface has a complex structure, with features such as ridges, sulci and hair follicles that can physically impede the efficacy of decontamination procedures. Yet, there are very few reports investigating the impact of different decontamination techniques, and these studies tend to rely on the manual application of the decontamination products, using motions such as blotting or rubbing. This reliance may lead to variation in the measured decontamination efficacy, since there will be differences in applied pressure, application time, consistency of technique, etc. Therefore, the purpose of this study was to develop and validate an automated system for the accurate and reproducible application of decontamination products to excised skin.

A robotic arm was purchased with a reported accuracy and precision of ± 1 mm and ± 0.5 mm, respectively. An effector was 3D printed in-house to attach a decontamination product ('M295 mitt') to the robotic arm, accommodating a load cell that allowed the measurement and recording of pressure (10N force, output sensitivity of 1.0 ± 10%). The downward pressure and 2-dimensional vector coordinates on sections of excised porcine skin (20cm² diameter) were recorded during wiping, blotting and circular motions.

All motions were validated with a pressure of 0.82 ± 0.13 PSI, which is in line with laboratory-scale standard contact test pressures, with an accuracy and precision of the decontamination positions corresponding with the manufacturer's reported specifications.

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P35

# A real-time molecular epidemiological investigation into the contribution of fungal spores to seasonal asthma spikes

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Exposure to airborne fungal spores are associated with allergic sensitisation and exacerbation of asthma. Typically, microscopy-based methods have been used to analyse fungal seasonality. The recent affordability of high-throughput sequencing (HTS) methods allows deeper characterisation of fungal spore composition in outdoor air. Analysis of air samples using HTS methods will improve understanding of fungal spore seasonality in the UK, and the associations these may have with asthma exacerbations.

Outdoor air samples were collected across 2021 using Burkard rooftop air samplers in Leicester and Harwell, Oxfordshire. Extracted DNA from an even proportion (33%) of these air samples was amplified using PCR for ribosomal DNA regions. An ion torrent sequencer was used to analyse fungal composition. Exposure data will be linked to syndromic data for asthma and other relevant conditions.

The mean amount of fungal DNA per sample was higher for the more rural, Oxfordshire site. Diversity analyses of the metagenomic sequencing data suggests fungal diversity is also higher in Oxfordshire, with a greater value of fungal richness (total number of distinct fungal taxa). Multivariate analysis also reveals positive statistical associations between air temperature and fungal diversity. Comparisons between fungal composition and clinical consultation data will be presented.

This analysis will enhance our understanding of fungal spore seasonality across the UK and its relationship with episodic asthma. This may lead to identification of fungal taxa that may have an association with asthma. Further work could expand upon this through the development of real-time PCR assays for these taxa of interest.

P36

# Exploring the role of senescence and associated secreted factors in anthracycline-induced cardiotoxicity

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Anthracycline chemotherapies continue to underpin treatment strategies for countless cancer patients globally, but they have long been associated with delayed-onset cardiotoxicity, which is emerging as an unmet healthcare need in the ever-increasing cancer survivor population. The processes underpinning anthracycline-induced cardiotoxicity (AIC) remain unclear but cellular senescence has arisen as a possible contributing mechanism to the subclinical, structural changes which precede functional disturbances in the myocardium: AIC patients often display cardiac phenotypes associated with ageing, e.g. fibrosis, hypertrophy and maladaptive remodelling. We exposed human AC16 cardiomyocytes to 500 nM doxorubicin (DOX), a clinically-utilised anthracycline, for 3 hours only (representing a transient, sublethal and clinically-centred dose) or exposed cells to equivalent vehicle control. Cardiomyocytes were allowed to recover for 10 days before analyses were conducted. qPCR and immunocytochemistry analyses showed induction of classical and novel senescence markers 10 days post-DOX vs control (e.g. p21 transcript 4-fold increase, PURPL transcript 9-fold increase). In DOX-treated cardiomyocytes, morphological disturbances were observed in the treatment recovery phase, including hypertrophy. Conditioned media was collected from cardiomyocytes at days 8-10 post-treatment, and cytokine array analysis showed a classical senescence-associated secretory phenotype (SASP) in DOX-treated cardiomyocytes vs control, comprising immune-recruiting (MCP-1: 470 vs 2 pg/mL), pro-inflammatory (IL-6: 22 vs 0 pg/mL) and pro-remodelling (FGF-2: 41 vs 7 pg/mL) factors. The capacity of this SASP to both reinforce and spread the senescent phenotype through the cardiac microenvironment and beyond remains underappreciated in the cardiotoxicity of anthracyclines, and will form the basis of future work.

P37

# In vitro screening of mitochondrial toxicity: Active machine learning to determine compound selection

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Pre-clinical toxicology accounts for over 50% of drug attrition cases, further burdening the drug development process. Mitochondrial off-target toxicity, which can arise as inhibition of electron transport chain (ETC) complexes, is a major contributor to drug attrition. Novel prediction methods are required to predict mitochondrial off-target toxicity, preventing drug attrition, whilst maintaining pace with chemical development. Machine learning (ML) provides a means for cost-effective, versatile and efficient prediction of toxic endpoints. However, the development of ML predictive models of ETC complex inhibition has yet to be established.

Here we built ML models to predict potential inhibitors of complex I of the ETC. We trained these models with data from the public domain (e.g. ChEMBL, Tox21). However, when attempting to predict novel complex I inhibitors, these models failed to generalise to new data from the Prestwick Chemical Library, bringing into question the chemical space coverage and reliability of the training data. To tackle this, an in vitro stepwise high-throughput screen (HTS) of an in-house xenobiotic library, orchestrated utilising active ML, was conducted. Applying this iterative method allowed for a structured approach to screening, prioritising the evaluation of compounds with poor prediction confidence.

This concept will be applied to the larger Prestwick Chemical Library, with positive hits further investigated to identify mechanisms of mitochondrial toxicity and specific ETC inhibition. These techniques establish a framework for real-world iterative HTS data generation, facilitating the building of predictive ML models for ETC complex inhibition and helping to elucidate common toxicophores for drug safety consideration.

P38

# Multifactorial impacts on early breast carcinogenesis- assessing the combined effect of endocrine disruptors and fatty acids

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Breast Cancer is the most common cancer in the world, accounting for nearly 2.1 million new cases in 2020. This high global incidence cannot be solely due to hereditary factors, as evidence has shown that lifestyle and environmental factors (e.g., endocrine disrupting chemicals, EDCs) play a role in breast cancer initiation and progression. The role of EDCs in breast carcinogenesis is still unclear. Similarly, the role of diet, especially fatty acids-rich diets on breast cancer remains inconclusive and the mechanisms responsible for this effect are not fully understood. We have investigated the impact of mixtures of EDCs and fatty acids on early breast carcinogenesis.
Non-tumourigenic human mammary epithelial cells (MCF12A) were cultured in a 3D system and treated with a mixture of 12 EDCs and fatty acids. The EDCs and fatty acids were mixed at the concentrations present in human serum. In the 3D system, MCF12A cells form structured acini that resemble normal mammary tissue architecture. Early breast carcinogenic events are recapitulated by the formation of large and deformed acini. Different parameters of the acini were measured after exposure to a mixture of EDCs, a mixture of fatty acids and as combination of the two mixtures, representative or real-life exposures.
We demonstrated that our 3D system is able to generate structures that recapitulate in vivo mammary acini, and the mixture of EDCs act together at realistic human tissue concentrations(4.6 x 10-8M) was able to disrupt acini formation, suggesting their potential involvement in breast cancer development and breast cancer risk.

P39

# Investigating the impacts of hyperglycaemia and gemcitabine co-exposure on mitochondrial genotype in human lymphoblastoid cell lines

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Mitochondrial DNA damage by the antimetabolite gemcitabine causes mitochondrial dysfunction, facilitating activation of the p53-mediated intrinsic apoptotic pathway. Hyperglycaemia is known to elevate cancer risk and affect mitochondrial dynamics through increased reactive oxygen species production inducing apoptosis. We compared the impact of hyperglycaemic conditions and gemcitabine exposure on mitochondrial-associated gene expression across a panel of 84 genes in TK6 (wild-type p53) and NH32 (p53-null) isogenic cell lines.

TK6 cells were administered gemcitabine (between 1 nM and 100 µM) for 24h. Relative population doubling (%) determined the cytotoxic gemcitabine concentration (1 nM) for co-exposure with glucose. Cultures were exposed to 'normal' glucose (NG, 11 mM), high glucose (HG, 30 mM), NG and gemcitabine, and HG and gemcitabine concentrations. Glucose was administered for 48h prior to gemcitabine for 24h, where applicable. RNA was subsequently extracted from cultures for mRNA expression analysis by reverse transcription-quantitative polymerase chain reaction revealing downregulation of CPT1B (~0.53-fold control), SLC25A16 (~0.62-fold control) and GRPEL1 (~0.69-fold control) in treatments with HG and gemcitabine, but not with NG and gemcitabine.

Our investigations indicate hyperglycaemia and gemcitabine exposure may impair the expression of genes involved in fatty acid oxidation and accumulation of coenzyme A in mitochondria, suggesting mitochondrial gene expression's potential as an alternative or supplementary endpoint for in vitro genotoxicity testing.

P40

# Identification of the molecular mechanisms of anthracycline-induced cardiotoxicity and relationship with the renin-angiotensin system.

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The effects of anthracyclines on the cardiovascular system are one of the major toxicological challenges of cancer treatment. Despite their success in the management of many cancer types, the effectiveness of these therapeutics is limited by cumulative dose-dependent cardiotoxicity, which can result in irreversible and life-threatening heart failure. In recent studies, drugs interfering with the angiotensin-signalling system have shown promise in the reduction of anthracycline-induced cardiotoxicity (AIC) in the clinic. Unfortunately, the mechanisms underpinning mitigation of AIC by these drugs remains unclear. We have previously shown both angiotensin II stimulation and exposure to the anthracycline doxorubicin induce cardiomyocyte (AC10 human cardiomyocyte cell line) cellular hypertrophy. This hypertrophic response was mitigated by pre-exposure to clinically-utilised angiotensin receptor blocker (ARB) drugs, offering an explanation for the cardioprotective effects of blocking angiotensin-signalling in AIC. However, our recent studies have demonstrated that no such morphological changes are observed in primary human cardiac fibroblasts (HCF). Furthermore, moderate angiotensin receptor (AT1R) expression changes were observed in cardiomyocytes, with a 1.5-fold increase relative to control at 100nM doxorubicin concentration. In HCF, the expression of AT1R changed in a time and concentration-dependent manner following exposure to doxorubicin. The highest level of AT1R expression was observed between 16-24 hours after exposure, with a 2-fold increase in expression at 500nM doxorubicin concentration compared to control. These findings suggest a potential interplay between these two cell types of the myocardium in AIC, involving crosstalk of the angiotensin-signalling pathway. This offers significant potential for mitigation of this toxicity in the clinic.

P41

# Use of lead isotope ratio to identify sources of exposure in Georgian children with elevated blood lead levels

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A nationally representative sample of Georgian children (n=1578) identified a widespread exposure to lead with over 40% with blood lead level (BLL) over the action level of 5 µg dL-1. The objective of this study was to document the feasibility of using lead isotope ratios (LIR) to identify and rank the relevant lead sources contributing to exposures in children across Georgia at both the household and national level. Samples of blood, spices, paint, soil, dust, flour, tea, toys, milk, and water were collected from 36 households in Georgia between Nov 2019 and Feb 2020 where a child had previously been identified with a BLL>5 µg dL-1. The fieldwork involved collection of blood and environmental (n=528 of 14 different types) samples, and completion of a demographics and behaviours questionnaire to indicate potential exposures. Total lead and LIR in blood and environmental samples were determined by inductively-coupled plasma mass spectrometry. Exceedances in Georgian reference values were observed in spices (43%), paint (55%), dust (10%) and soil (25%). At the individual level lead in spices and dust were most frequently isotopically indistinct from blood. LIR enabled the ranking of importance of environmental sources at the household and National level for direct deployment of targeted interventions in this population.

P42

# A ten-year review of intentional paracetamol ingestions in children and adolescents – is there a distinct age separating intentional and unintentional overdoses in these populations?

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Paracetamol is the main pharmaceutical involved in cases of poisoning in the UK. It is a cheap, widely available product and is often stored in household cupboards. The main objective of this study was to determine if there is a specific age separating intentional and accidental ingestions of paracetamol in children and adolescents aged between 0 and 18 years-old.

Records of all UK telephone enquiries from hospitals to the National Poisons Information Service (NPIS) relating to intentional and unintentional paracetamol ingestions between 1st January 2012 and 31st December 2022 were analysed retrospectively. Data were analysed using descriptive statistics. Student's T-test and the Chi-squared test were used for comparisons. A p-value of less than 0.05 was considered significant.

The NPIS received a total of 9969 enquires, of which 6918 (69%) involved intentional paracetamol ingestions, $p<0.001$. Enquiries concerning intentional overdoses related to children aged between 9-18 years-old, a mean age of 15 ± 1.5 years, compared with 6.9 ± 6.2 years (range 0-18 years) for accidental overdoses, $p<0.0001$. Female enquires accounted for 86% of all intentional enquiries (5915) compared to males (954), $p<0.0001$. Twelve to seventeen year-old females represented 55% of all intentional paracetamol related enquires.

Intentional paracetamol ingestions occurred more frequently in adolescent females compared to males. Rates of intentional overdoses increase with age, however intentional overdose was not reported below 9 years-of-age in females and 10 years in males. These data indicate a need for focused interventions to reduce cases of intentional paracetamol ingestions in adolescents aged 12-17 years-old.

P43

# Risk assessment for human health from exposure to arsenic based on human biomonitoring

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Exposure of Slovenian adult population to total arsenic (T-As) from the national human biomonitoring survey 2007-2014 (NHBMS), approved by the National Medical Ethics Committee, was used in risk assessment (RA).

Over 1000 volunteers of both sexes, aged 20-40 years from 12 regions participated in the NHBMS. For this RA only those having consented to further use of their data were included. Participants' personal, dietary and environmental histories were obtained by a questionnaire. T-As was determined in urine by inductively coupled plasma mass spectrometry (ICP MS, 7500ce, Agilent, Japan) using octopole collision cells with helium to reduce interferences (level of detection 0.1 µg/L). Biomonitoring equivalents for non-carcinogenic effects (nCE) of inorganic arsenic (iAs) were used as a point of departure in RA; 6.4 µg/L (8.3 µg/g creatinine) below which there is no cause for concern, and 19.3 µg/L (24.9 µg/g creatinine) above which actions to reduce risk are required.

The overall geometric mean (GM) for T-As in 471 urine samples was 6.42 µg/L (7.03 µg/g creatinine), 95th percentile (P95) was 64.98 µg/L (57.67 µg/g creatinine). Highest concentrations were found in the coastal urban area: GM 15.57 µg/L (15.47 µg/g creatinine) ascribed to consumption of fish/seafood.

T-As does not present risk for nCE at GM level nor at P95 level, where more non toxic arsenicals are expected due to fish/seafood consumption. In future HBM based RAs for arsenic, consumption of fish/seafood 72 hours prior to sample collection should be included in exclusion criteria and iAs and its metabolites should be monitored.

P44

# Development of an Integrated Approach to Testing and Assessment to support assessment of cardiovascular impacts of nanomaterials

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An Integrated Approach to Testing and Assessment (IATA) allows collation of existing information and guidance to generate missing information when addressing a specific research hypothesis. The EU project GRACIOUS used IATAs to gather evidence to support the grouping of similar nanomaterials (NMs), with the aim to read-across (share) animal hazard data from one group member to another. This project generated grouping hypotheses that 'inhalation of biopersistent NMs will induce heightened inflammatory mechanisms, chronic myocarditis and disruption of cardiac function leading to heart failure'. In addition, this project generated an IATA designed to test the grouping hypothesis. The IATA is in the form of a decision tree which consists of decision nodes (DN) that address relevant questions regarding the morphology, biopersistence, extent of translocation and inflammatory potential of the NMs under investigation. Each DN in the IATA is addressed through a tiered testing strategy, using data from simple in vitro methods in tier 1 to in vivo approaches in tier 3. For the proposed methodologies, justification is provided for the rationale and techniques used that allow grouping decisions to be made. Application of the IATA allows the user to selectively identify NMs to which the formulated cardiovascular hazard hypothesis applies. By promoting the use of alternative, non-animal approaches the IATA simplifies information gathering at each level to help inform risk assessment while reducing the ethical, time and economic issues of current testing. THIS ABSTRACT REPRESENTS THE RESULTS OF AN UNDERGRADUATE PROJECT.

P46

# Potential risks from pica during pregnancy

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Pica is the persistent consumption of non-nutritive substances, including non-food items, materials and raw food ingredients. It became a focus of interest during discussions of the Committee of Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) regarding lead in the maternal diet. The COT highlighted that due to the ubiquitous nature of lead in the environment, exposure could come from sources other than foodstuffs. While considering exposure from soil and dust, it became apparent that pica could also result in lead exposure and other potential hazards. The range of substances and the subsequent hazards that can be consumed through pica is extensive. Analysis of the literature, however, suggested that the majority of pica in pregnant women in the UK is geophagia (consumption of soil, earth and clay), which is often the result of cultural transfer in migrant populations. In some African and South Asian communities geophagia is a remedy for morning sickness, cravings and perceived mineral deficiencies. As such, baked soil products such as Sikor and Calabash are imported into the UK for consumption. Although analysis of these products has indicated highly heterogenous compositions, potential hazards identified included; silica (as the predominant mineral component), heavy metal contaminants (lead, arsenic and cadmium) and persistent organic pollutants. The prevalence of geophagia in the UK is not well understood and the practice is potentially under-reported due to perceived stigma. Better understanding of the nature of pica, and particularly geophagia, will enable more thorough risk assessments for maternal nutrition and health.

P47

# A Next Generation Risk Assessment (NGRA) case study for the bioactive food component sulforaphane

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Performing systemic toxicity assessments for chemicals without using animal data is challenging. We previously developed a decision workflow for systemic toxicity based on the integration of in vitro points of departures (PoDs) from a suite of assays with PBK predictions of human systemic exposure[1]. Evaluation of this workflow showed promise for use in a capacity that protects consumers but is not necessarily predictive of traditional toxicological outcomes. It also demonstrated that this approach may be conservative, as PoDs are based on bioactivity, which may not necessarily translate into adversity[1]. This was exemplified for sulforaphane which is formed in cruciferous vegetables. Consumption of cruciferous vegetables is associated with beneficial health effects including decreased risk of some cancers[2]. Sulforaphane is hypothesised as a plausible agent for some of this protection given, inter alia, its activation of the Nrf2 pathway, which controls the expression of cytoprotective genes. Given dietary exposure to sulforaphane may have beneficial properties, it is unsurprising that sulforaphane showed bioactivity at equivalent in vitro exposures. This illustrates a challenge for the assessment of bioactive materials under the current NGRA paradigm. In such scenarios, it is anticipated that a more refined safety assessment is performed based on e.g., improving exposure estimates and targeted testing strategies based on specific MoAs identified in the initial evaluation/ from literature. In the case of sulforaphane, future work will look to integrate additional results investigating the interplay between two key targets identified (oxidative stress and inflammation) to potentially differentiate between adaptive and adverse effects[3].

P48

# Establishing a physiologically relevant in vitro airway model to assess the health impacts from different fungal species

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Allergic airway disease (AAD) is a collective term for respiratory disorders that can be exacerbated upon exposure to airborne allergens, including fungi and other air pollutants. Fungi are of particular interest as they are present in both outdoor and indoor air and are associated with asthma and allergy. A recent literature review identified key pathways contributing to the allergic responses to fungal allergens. However, due to the heterogeneity of models and methods used, it is not clear whether there are common and/or specific pathways induced by different fungi/fungal components. Air-liquid interface (ALI) culture was used to induce differentiation of nasal (RPMI 2650), bronchial (HBEC3-KT, BEAS-2B, Calu-3) and small airways (A549) epithelial cells into a columnar stratified epithelium with cilia and goblet cells, and the formation of tight junctions. Cultures were harvested at 0, 7, 14, 21 and 28 days, and the expression of molecular markers of basal, ciliated and mucus producing cells were assessed with reverse-transcription quantitative PCR (RT-qPCR). Different cell lines produce subtly different differentiated models, with downregulation of basal (undifferentiated) cell markers and upregulation of markers of ciliated and mucus producing cells from day 7. While no ciliated cells were observed in any of the differentiated cell lines, mucus was produced in some. The differentiation of primary cell lines at ALI, and expression of genes of interest (including known fungal receptors and genes identified in driving fungal allergy) were also measured to inform selection of appropriate model(s) to better understand the allergenic response to fungi/fungal components.

P49

# Liver Injury in Children During the Acute non A-E Hepatitis Incident in 2022: Use of GC-MS to Investigate Potential Causative Toxicants

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Between January and July 2022, a spike of unexplained hepatitis cases in children under 10 has been reported in the UK and around the world. In the UK, none of the confirmed cases tested positive for hepatitis viruses A-E. and some of these children demonstrated rapid liver deterioration (5.5% of confirmed cases) and required emergency liver transplants [1]. As part of a national investigation of this incident, the analytical toxicology team at UKHSA undertook an investigation to identify possible drug overdose (e.g. paracetamol) or toxicant exposures in these children that might be responsible.
Urine and serum samples were received from children admitted to hospital at varying times after the onset of symptoms. 41 serum samples and 33 controls were processed in acetonitrile/water and analysed by liquid injection-gas chromatography (GC-MS) whereas solid-phase microextraction prior to GC-MS was employed for 37 urine samples and 5 controls.
Our analyses revealed differential levels of several compounds in serum and urine between healthy control and confirmed cases. Ketamine and Midazolam were detected in 2 serum samples. Paracetamol, phenol, caffeine, 25-hydroxycholesterol were differentially detected in urine samples. 25-Hydroxycholesterol is related to normal therapeutic interventions for hepatitis. Quantitation was performed on a triple-quadrupole GC-MS for some known hepatotoxicants, paracetamol and phenol. Paracetamol was found in a small number of cases while phenol was detected in all samples but at levels not considered adverse. No plausible causative agent was identified. Further research is needed to further investigate potential causative exposures and the pathology of these cases.

P50

# Validation: Test chemical augmentation of a new non-animal test method for assessing metabolism based on CYP450 induction

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This work supports the OECD Test Guideline Programme for use of New Approach Methods (NAM) in hazard identification. Cytochrome P450 (CYP) induction/inhibition can substantially alter in vivo exposure to CYP substrates/metabolites, therefore human-relevant CYP data is valuable. The method is designed to determine this for three CYPs (CYP1A2, 2B6, and 3A4). The human HepaRG™ cell-based test method has been developed and validated for a set of pharmaceutical proficiency chemicals (Bernasconi et al 2019). To support Test Guideline adoption, OECD member countries requested additional work to demonstrate the applicability of the test method to other chemical domains, such as industrial, pesticide and food additives (Jacobs et al, 2022).

For this NAM, cryopreserved differentiated immortalized human HepaRG™ cells are exposed to different inducer chemical concentrations and induction measured by addition of prototypical CYP probe substrates (CYP1A2: Phenacetin, CYP2B6: Bupropion HCl, CYP3A4: Midazolam). Probe substrate metabolites are quantified through LC-MS/MS and normalised to protein accounting for cell number.

Chemicals are classified as inducers based on >2-fold CYP induction with at least two consecutive concentrations observed, and then compared against expected outcome from literature (Table 1) (Jacobs et al 2022).

Preliminary data from two other laboratories demonstrate promising sensitivity through LC-MS/MS and specificity for detecting CYP inducers. This study constitutes the third laboratory, currently being established prior to the additional chemical testing. To date, CYP3A4 has been successfully established with respect to sensitivity (Figure 1) but further optimisation for CYP1A2/2B6 is taking place and will support the augmented validation of this test method.

P51

# The use of the Genomic Allergen Rapid Detection skin Dose-Response assay to assess skin sensitizing potency in developing novel fragrance ingredients

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Skin sensitization is one of the required endpoints for the development and registration of novel fragrance ingredients. Traditionally, testing has been performed using a combination of in vitro and in vivo assays, but recent developments has shifted the paradigm towards the use of NewApproachMethodologies, without the need for in vivo methods. However, none of the proposed NewApproachMethodologies are currently validated for continuous potency predictions, which is required for quantitative risk assessments of novel fragrance ingredients.

The GenomicAllergenRapidDetection®skin assay (TheOrganizationforEconomicCo-operationandDevelopment TestGuideline 442E) is a genomics-based assay for hazard identification of sensitizers. To meet the need for quantitative potency information, GenomicAllergenRapidDetection®skin Dose-Response has been developed based on the validated protocols of GenomicAllergenRapidDetection®skin and generates a dose-response curve to identify the lowest concentration of a test compound required to elicit a positive classification (cDV0 value). These values correlate significantly to LocalLymphNodeAssay EC3 and human NoExpectedSensitizationInductionLevel values.

The aim of this study was to investigate the sensitizing potency of two novel fragrance ingredients and to identify predicted non-sensitizing levels. Testing was performed in GenomicAllergenRapidDetection®skin Dose-Response, with predicted EC3 and NoExpectedSensitizationInductionLevel values of 1.93% and 27.8%, and 659µg/cm2 and 16600µg/cm2, for fragrance ingredients 1 and 2, respectively. These results in combination with data from kineticDirectPeptideReactivityAssay, KeratinoSens and in silico read- across, established the concentrations for confirmatory HumanRepeatInsultPatchTest testing (562.5µg/cm2 and 15000µg/cm2).

In conclusion, this study demonstrates how GenomicAllergenRapidDetection®skin Dose-Response combined with other NewApproachMethodologies can be used for risk assessments and to establish a concentration for confirmatory HumanRepeatInsultPatchTest testing of novel fragrance ingredients.