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Toxicology Letters

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ABSTRACTS of the 55th Congress of the European Societies of Toxicology (EUROTOX 2019)
TOXICOLOGY – SCIENCE PROVIDING SOLUTIONS
Helsinki, Finland, 8th – 11th of September, 2019

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Toxicology Letters serves as a multidisciplinary forum for research in all areas of toxicology. The prime aim is rapid publication of research letters with suffi cient importance, novelty and breadth of interest. In addition to research letters, papers presenting hypotheses and commentaries addressing current issues of immediate interest to other investigators are invited. Mini-reviews in various areas of toxicology will also be published. A new feature is the provision of a forum for the discussion and interpretation of data published in the journal. Clinical, occupational and safety evaluation, legal, risk and hazard assessment, impact on man and environment studies of suffi cient novelty to warrant rapid publication will be considered.

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Abstracts of the 55th Congress of the European Societies of Toxicology (EUROTOX 2019) TOXICOLOGY – SCIENCE PROVIDING SOLUTIONS

Helsinki, Finland, 8th–11th of September 2019

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Preface

This issue of Toxicology Letters contains the abstracts of the EUROTOX 2019 Congress. The Finnish Society of Toxicology (FST) was established in 1979 and is proud to host EUROTOX 2019, the 55th Congress of EUROTOX, in Finlandia Hall in the heart of Helsinki, the capital of Finland, on September 8th to 11th. EUROTOX, the Federation of European Societies of Toxicology, was established in 1989 by merging the European Society of Toxicology (EST) and the Federation of European Societies of Toxicology. EST dates back to 1961 when it was established in the aftermath of the thalidomide disaster. Altogether, EUROTOX has over 6000 members through its European member societies as well as a number of individual members who belong to EUROTOX directly. The Annual meeting of EUROTOX is one of the premier toxicological gatherings world-wide, and certainly the most important one in Europe. The Congress offers a unique opportunity to meet colleagues and friends, and network professionally with scientists and clinicians from Europe and beyond. The Congress is typically attended by large numbers of toxicologists from all parts of the world.

The Congress offers on its first day, Sunday September 8th, six cutting-edge continuing education courses on topics "development and assessment of adverse outcome pathways", "mechanistically-driven tools for risk assessment", "evidence-based assessment in toxicology", "real-world safety assessment for data-poor products", "dietary exposure assessment", and "safe exposure levels for occupational toxicology, application to pharmaceutical". Also on Sunday there is an opening key-note talk on atmospheric pollution by Professor Markku Kulmala. There are several further key-note presentations by eminent, internationally recognized scientists, on issues from systems toxicology in hazard assessment to "Toxicology in the era of exposure". The programme also includes the traditional, well-known SOT (Society of Toxicology, USA) – EUROTOX debate on "Classification of substances as endocrine disruptors has a public health benefit". This debate has received much attention since its beginning in 1994 because it highlights increasing international collaboration, and reflections of views between two large toxicological communities in Europe and North America. The full Congress offers a total of 31 symposia and workshops on a broad range of issues across the whole field of toxicology.

The theme of the Congress is "Toxicology – Science Providing Solutions". Here EUROTOX wants to emphasize the importance of societal innovations in addition to crucial toxicological discoveries. In today's world, for a scientific discipline such as toxicology, it is important to have an impact that enables improving the safety and prosperity in society. Hence, in addition to science, it is important that toxicology can make a contribution to chemical safety, circular economy, sustainablilty, and air quality. These issues are crucial also for having societal acceptance and justify continuous support for toxicology by society. This is especially important now, when research resources are decreasing and opportunities for research are becoming limited.

In addition to invited talks, delegates from all around the world have submitted more than 800 abstracts to be included in the programme. The poster presentations are crucial for the success of the congress, and provide an excellent platform to have vivid scientific discussions on a multitude of important and timely toxicological topics.

Careful preparation has been in a key position in organizing the congress. The International Scientific Programme Committee, chaired by the President of EUROTOX, Professor Heather Wallace from the U.K. has led the preparation of the programme of the Congress together with the Local Organizing Committee, chaired by Professor Kai Savolainen. The programme is based on a large number of excellent proposals for continuing education courses, key-note talks, debates, symposia and workshops, from Europe and beyond, of which those which were considered to be of the highest quality and most timely were chosen for the programme.

We are excited to welcome all the delegates to the EUROTOX 2019 congress in Helsinki to enjoy the Congress programme, and to join the active and fruitful scientific discussions during the Congress. In addition to the science, we also wish that the delegates to enjoy the culture and the atmosphere that the host city, Helsinki, can offer. Welcome to EUROTOX 2019.

Professor Kai Savolainen, MD, PhD, ERT,
Helsinki, Finland, 2019 EUROTOX Congress Chair

Professor Heather Wallace, PhD, ERT, FRCPath, FRSC, FRSB, FBTS, FBPhS,
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Continuing Education Courses (CECs)

CEC01 | Development and evaluation of AOPs

CEC01-01
Adverse Outcome Pathways: Background and Principles

*S.J. Munn

European Commission, DG JRC, Ispra, Italy

An AOP describes a sequential chain of causally linked events starting on the molecular level, spanning multiple levels of biological organisation, to an adverse health or eco-toxicological outcome of regulatory relevance. AOPs can provide a unifying concept or framework to capture, visualise and connect mechanistic information from all sources.

The concept was adopted by the OECD in 2012 to help member countries to make better use of increasing knowledge on how chemicals induce adverse effects in humans and wildlife [1]. The aim is to create an interdisciplinary community of practice connecting basic researchers, technology developers, regulatory risk assessors and decision makers to facilitate the sharing and synthesis of data and ideas.

AOPs are used to describe biological motifs of failure that are not chemical-specific; they are modular consisting of building blocks of key events, specialized key events (the molecular initiating event and the adverse outcome) and directed relationships between the key events (key event relationships). AOPs are linear, serving as a pragmatic functional unit of development and evaluation, with AOP networks (defined as AOPs that share at least one common element), likely to be the functional unit of prediction. Lastly, AOPs are living documents which represent the state of the science at a given point in time yet allowing constant evolution.

The background to and aims of the OECD AOP programme, along with the process and core principles of AOP development will be described in detail, illustrated with case examples.

References

[1] https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

CEC01-02
Weight of evidence/confidence evaluation for AOPs

*M.E. Meek

University of Ottawa, McLaughlin Centre, Faculty of Medicine, Ottawa, Canada

Descriptions of Modes of Action (MOAs) and Adverse Outcome Pathways (AOPs) facilitate systematic integration and assessment of mechanistic data in hazard assessment from a broad range of sources including structure activity analysis, *in vitro* assays, toxicity tests in animals and observational or clinical studies in humans. Formalized description and analysis of the extent of supporting evidence for these pathway descriptions supports their use for various applications in testing and assessment.

Bradford Hill (B/H) considerations form the basis for assessment of the extent of supporting evidence in formalized descriptions of AOPs in the public knowledge base within the OECD AOP program. These considerations, modified somewhat from their initial characterization to assess causality in epidemiological studies and adopted in international frameworks in MOA analysis include biological plausibility, essentiality and empirical support. The considerations, defined to address aspects critical in regulatory acceptance, are also rank ordered to reflect their relative importance in assessing the extent of supporting mechanistic data.

Examples illustrate the nature of datasets associated with high, moderate and low confidence for each of these considerations. Presentation of a practical example illustrates the assembly and evaluation of the weight of evidence for a documented AOP in the OECD knowledge base.

CEC01-03
AOP Wiki and live demonstration

*C. Wittwehr

European Commission, Joint Research Centre, Ispra, Italy

The Adverse Outcome Pathway (AOP) Wiki serves as the primary repository of qualitative information for the international AOP development effort coordinated by the Organisation for Economic Co-operation and Development (OECD). It describes an AOP in terms of key events (KEs), which represent measurable steps along a pathway from a molecular perturbation to an adverse outcome for an organism or population. KEs are connected via relationships (KERs), which capture the evidence supporting the AOP in a structured way. The AOP Wiki provides access to the AOP information via a web interface

that supports browsing and searching for AOPs, KEs, KERs, and stressors known to perturb the AOPs. The Adverse Outcome Pathways Knowledge Base (AOP-KB) consists of several modules, of which the AOP Wiki is the most relevant for both AOP authors and users, especially in a regulatory context.

The AOP Wiki is publicly available for browsing, with more than 200 AOPs in different stages of development available. This part of the CEC1 course will give an introduction to the main elements of the AOP Wiki, will show how to search for information, and will give an overview of the current content and what the typical life cycle of an AOP (from first entry to eventual adoption) looks like. A live demo will complement the theoretical part. In the course of the other talks during CEC1, the AOP Wiki will again be shown and used to demonstrate the hands-on, real life implementation of the scientific and regulatory concepts.

CEC01-04
Application of AOPs to consider biological plausibility of associations observed in epidemiological studies: exposure to pesticides and Parkinson's disease

*A. Terron

EFSA, PREV Unit, parma, Italy

Epidemiological studies and multiple metanalysis have observed an association between pesticide exposure and Parkinson's disease, though causality was not established. The adverse outcome pathway (AOP), OECD program, has been developed as a framework for the organization of available information linking activation of a molecular target [molecular initiating event (MIE)], via a sequence of essential biological key events (KEs), with an adverse outcome (AO). Here, we present an AOP covering the link between the binding of an inhibitor to mitochondrial complex I (i.e., the MIE) with the onset of parkinsonian motor deficits (i.e., the AO). This AOP was developed according to the OECD guidelines and uploaded to the AOP Wiki. The KEs linking complex I inhibition to parkinsonian motor deficits are mitochondrial dysfunction, impaired proteostasis, neuroinflammation, and the degeneration of dopaminergic neurons of the substantia nigra. These KEs, by convention, were linearly organized. However, additional feed-forward connections and shortcuts between the KEs were also considered and included in this AOP. The present AOP demonstrates mechanistic plausibility for epidemiological observations on a relationship between pesticide exposure and an elevated risk for Parkinson's disease development. Some principles of development and evaluation of this AOP will be illustrated through the AOP wiki.

CEC01-05
Application of AOPs for the development of Defined Approaches (DA) and Integrated Approaches to Testing and Assessment (IATA)

*G. Maxwell

Unilever, SEAC, Bedford, UK

The skin sensitisation AOP has provided a mechanism for curating and interpreting different sources of hazard information (e.g. *in silico* predictions, *in chemico* or *in vitro* data) since it was defined in 2012 (https://aopwiki.org/aops/40). Integrated approaches to testing and assessment (IATA) are AOP-driven frameworks for hazard identification, hazard characterisation and/or risk assessment of a chemical or group of chemicals, which integrate and weight all relevant existing data and guide the generation of new data to inform decision-making. As part of an IATA, a defined approach (DA) to data interpretation (e.g. statistical, mathematical models) can be used to derive a prediction from different information sources. In 2016, an OECD template (ENV/JM/MONO(2016)28) was endorsed for reporting defined approaches following the review of twelve skin sensitisation DAs for use in IATA (captured in an associated OECD guidance document: ENV/JM/MONO(2016)29). The OECD has subsequently convened a DAs for Skin Sensitisation (DASS) expert group to evaluate DAs for regulatory decision-making and this work is ongoing.

This presentation is illustrated by two different Skin Sensitisation DA examples, both developed for use in next generation risk assessment decision-making: a toxicokinetic-toxicodynamic (TKTD) mathematical model that outputs naïve $CD8^+$ T cell activation as a surrogate measure of sensitisation induction in humans and a bayesian multi-level regression model that estimates the human sensitiser population threshold under the conditions of a human repeat insult patch test (HRIPT).

CEC02
Application and integration of increasingly mechanistically driven tools for risk assessment

CEC02-01
Overview of WHO/IPCS chemical risk assessment methodology tools

*R. Brown

World Health Organization, Geneva, Switzerland

WHO/International Programme on Chemical Safety (IPCS) work in the field of methodology aims to promote the development, harmonization and use of scientifically-sound methodologies for the evaluation of risks to human health from exposure to chemicals. A number of WHO/IPCS tools and guidance documents have been developed over many years in collaboration with large numbers of scientists internationally, intended for adoption and use by countries and by international bodies across multiple sectors which use chemicals. These tools and approaches enable chemical risk assessments to be performed using transparent, internationally accepted and scientifically-sound methods. The methods available address different aspects of chemical risk assessment (i.e. assessment of exposure, hazard characterization including dose-response analysis and risk characterization), are interrelated, and can be applied to different tiers of assessment. This overview will describe the different tools and guidance documents which are available, and will describe how they can be used in an overall framework where tools are selected through consideration of tiered approaches. Based on problem formulation, increasingly data-informed approaches may be used, depending on the level of precision which is required to achieve the goal of the assessment. This application of tiered approaches will be followed in subsequent presentations, illustrating the use of particular tools from simple methods up to increasingly complex and more refined approaches.

CEC02-02
WHO/IPCS mode of action/human relevance framework: principles and application in risk assessment

*G. Fotakis

European Chemicals Agency (ECHA), Helsinki, Finland

The World Health Organization/International Programme on Chemical Safety (WHO/IPCS) has developed the Mode of Action Human Relevance Framework based on the premise that any human health effect caused by exposure to an exogenous substance can be de-

scribed by a series of causally linked biochemical or biological key events that result in a pathological or other disease outcome.

The WHO/IPCS framework on Mode of action has been evolving and being updated to reflect experience acquired in its application and extend its utility to emerging areas in toxicity testing and the application of non-test methods in hazard assessment.

The update captures a broader range of potential application in the area of chemical risk assessment by its incorporation within a Mode of action roadmap to enable its use in testing strategies as well as risk assessment applications.

The framework has been used as the basis for the development of the OECD Adverse Outcome Pathways Programme as well as for developing a Weight of Evidence Framework for chemicals' hazard assessment.

It also provides the basis for assessing the Mode of Action for endocrine disruptors according to the EC scientific criteria for the determination of endocrine-disrupting properties for Biocidal and Plant Protection Product active substances.

The training will cover the Evolution of the Framework, its key principles and concepts, recent regulatory applicability and advances. It will also address its application in specific case studies for chemical hazard assessment as well as its use for efficient testing strategies and application of read-across in hazard assessment. It will provide an insight of its iterative process and the use of the Bradford Hill considerations in weighing evidence and understanding confidence versus remaining uncertainty in a chemical assessment.

CEC02-03
Application and utility of chemical-specific adjustment factors (CSAF) in risk assessment

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Whether you develop human health risk assessments or are a regulator that reviews risk assessments submitted for regulatory consideration, chemical-specific adjustment factor(s) (CSAF) can be applied instead of more traditional, default uncertainty factors that are not based on chemical-specific data. By incorporating quantitative data on interspecies (i.e., animal to human) differences or human variability in either toxicokinetics or toxicodynamics (i.e., mode of action), CSAF methodology allows the risk assessment community and society as a whole to benefit from reduced uncertainty, increased confidence, and more accurate reflections of potential health risks or regulatory decisions resulting from environmental chemical exposures. This session will demonstrate the utility of CSAF methodology and help risk assessors and regulators determine what types of toxicokinetics or toxicodynamics (i.e. mechanistic) data can be used for CSAF derivation, how much data are adequate, and how to describe or report the data to facilitate interpretation, based on lessons learned from an analysis of more than 100 CSAF examples over the past couple decades. This session will also discuss CSAF that were considered but not adopted by regulatory agencies, for example, due to inadequate underlying toxicokinetics or mode of action information and/or insufficient confidence in, verification, or validation of PBPK models used for chemical-specific interspecies adjustments. Examples of data-derived extrapolation factors (DDEF), a broader, related and useful methodology will also be illustrated. Overall, CSAF and DDEF methodologies align with more predictive and mechanistic data-driven (i.e. bottom-up) approaches compared to traditional top-down, observation-based assessment approaches.

CEC02-04
Expressing uncertainty in hazard characterization and exposure assessment of substances: Principles and practice using APROBA-Plus

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In 2017, WHO/IPCS published a guidance document [1] on evaluating uncertainties in hazard characterization. Instead of relying on (conservative) point estimates, it was proposed to (better) quantify the level of conservatism in the final hazard characterization outcome (i.e. a health-based guidance value such as an RfD or ADI) by quantitatively evaluating the uncertainties involved in a so-called probabilistic assessment. Here, the single values are replaced by uncertainty distributions resulting in an overall uncertainty distribution of the final hazard characterization outcome. This presentation will introduce the principles described in the WHO/IPCS guidance document to arrive at a probabilistic health-based guidance value. In addition, the user-friendly Excel tool APROBA-Plus is presented, which facilitates probabilistic hazard characterization and risk assessment. The tool is developed as an addition to the WHO/IPCS tool APROBA, which can do probabilistic calculations in an approximate but quick and easy way by applying lognormal uncertainty distributions to the different aspects of the hazard characterization (such as Point of Departure, inter-, and intraspecies extrapolation). This results in a probabilistic health-based guidance value rather than the usual deterministic point estimate. In the extended APROBA-Plus tool, exposure estimates with an uncertainty range can be included to create a single plot, which visualizes the uncertainties in exposure and hazard.

APROBA-Plus can be used as a quick tool for risk assessment while making the (approximate) uncertainties in both the hazard and the exposure visible. By making the uncertainties visible, the outcome from a risk assessment becomes more transparent and informative than the more usual deterministic approaches, so that risk managers can make better-informed decisions, e.g. directly taking measures or asking for refinement of the risk assessment. If the latter, APROBA-Plus can help in showing which aspects in the risk assessment contributed most to the overall uncertainty, as an indication what type of refinement would be most effective.

In a demonstration, participants will be guided through an APROBA-Plus assessment. Participants who would like to perform the analysis in parallel are asked to bring their own laptops. APROBA-Plus requires Microsoft Excel 2010 (version 14) or higher. The APROBA-Plus tool can be downloaded from: https://www.researchgate.net/publication/326422432_APROBA_PLUS-V100_v012_TEMPLATE.

[1] https://www.who.int/ipcs/methods/harmonization/areas/hazard_assessment/en/

CEC02-05
Combined exposures to multiple chemicals – tiered integration of tools

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A World Health Organization (WHO) International Programme on Chemical Safety (IPCS) Framework outlines a pragmatic approach to the identification and consideration of priorities in the grouping and assessment of combined exposures to multiple chemicals. The Framework, illustrated by several case studies drawing from assessments internationally, includes formal problem formulation followed by stepwise consideration of both exposure and hazard in several

tiers of increasingly data-informed analyses. These analyses build on recent developments in assessment, incorporating predictive approaches in early tiers and increasingly refined, more data-informed mode of action and probabilistic analyses in later tiers. Several of these approaches and tools correspond with those presented in other lectures in the course.

Recently, the Framework has been additionally developed and applied in a number of initiatives in both research and application, including guidance of the Organization for Economic Cooperation and Development and the European Food Safety Agency and the development of tools within the European Horizon 2020 Research Project, Euromix. A recent WHO Publication on Chemical Mixtures in Source and Drinking Water also illustrates application of the Framework in prioritizing mixtures in drinking and source water for risk assessment and management in a range of additional case studies including those addressing pharmaceuticals, microcystins and synthetic oestrogens.

The framework will be illustrated through presentation of examples and case studies drawing from several of these sources for early through more complex tiers of both exposure and hazard. The case studies include application of the threshold for toxicological concern, development of semi-quantitative estimates of exposure and probabilistic characterization of both exposure and hazard. Implications of increasing experience in application of the framework will also be addressed.

CEC03 | The pre-specified protocol part of evidence-based assessment in toxicology

CEC03-01
The role of a pre-specified protocol in evidence-based assessments

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The European Food Safety Authority (EFSA) is the reference body for risk assessment of food and feed in the European Union. Its work covers the entire food chain, from field to fork, and one of its main tasks is to carry out risk assessments for chemicals in food and feed. A crucial aspect of EFSA's work is that the outcome of the scientific assessment is communicated unambiguously to decision makers, the wider scientific community and stakeholders. For this, a structured and clearly documented approach used for the risk assessment is essential. This presentation will set the scene for this CEC and introduce EFSA's experience in developing pre-specified protocols for identifying and assessing the validity of studies and using weight of evidence approaches to integrate the evidence.

CEC03-02
Systematic review pre-specified protocol – scoping, literature search strategy, inclusion and exclusion criteria

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Scoping
- Purpose – why are we doing this systematic review? What is the expected end result? Who is it for?
- Iterative – will the method be adapted depending on results?

Literature search strategy
- What are the data sources? e.g. PubMed, regulatory "grey" literature
- What is the date range?
- Can the search be automated? How is "manual" searching defined?
- Should foreign-language papers be included?
- Constructing search strings (broad/narrow...)

Inclusion and exclusion criteria
- Basically asking two questions: Is the study relevant to the review's purpose? Is the study acceptable for review?
- Perhaps the most complex and critical step; the choice of criteria can determine the scope and validity of the review output.
- Criteria must be unambiguous and sufficiently detailed to permit replication by third parties
- May require (peer review) piloting for iterative adaptation (effect of broad versus narrow inclusion/exclusion).

The process is illustrated using the EFSA 2017 BPA hazard assessment protocol.

CEC03-03
Assessing internal validity

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The evaluation of internal validity of studies, or study reliability, is one of the critical steps in regulatory hazard and risk assessment. Regulatory assessment conclusions are the basis for decisions to authorize or restrict the use of chemicals. It is therefore important that the scientific data underpinning the risk assessment is adequate, i.e., sufficiently reliable and relevant for this purpose. Reliability, is defined as the inherent quality of the study and the confidence in the findings including, for example, considerations of the scientific soundness and appropriateness of the study design and methodology used, as well as the reproducibility of findings between experiments.

To ensure an efficient, transparent and methodologically rigorous re-assessment of the safety for consumers of bisphenol A (BPA), the European Food Safety Authority (EFSA) developed a protocol detailing a priori the approach and methodology for performing BPA hazard identification and characterisation. Among other parts, the protocol states upfront and in detail the methods and the criteria for assessing internal validity to be used in the ongoing BPA re-evaluation.

Internal validity relates as to whether a study answers its research question 'correctly', that is, in a manner free from bias. Risk of bias relates to the propensity of a study to be affected by systematic error. In the EFSA BPA protocol risk of bias considers two aspects: (i) those that introduce a systematic difference between the control and the exposed group only and (ii) those potentially affecting to the same extent control and exposed study groups.

A structured approach is described for appraising the internal validity of human epidemiological and experimental animal studies. Internal validity of human and animal studies is evaluated by study design and by endpoint. The approach is based on the NTP Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration (NTP-

OHAT) and the SciRAP tool (Science in Risk Assessment and Policy) and has been adapted to aspects that have been judged relevant, appropriate and important for the specific case of bisphenol A.

The separate sets of questions for human and animal studies, respectively, are rated based on the Risk of Bias. The ratings of the key and non-key questions are integrated to classify the studies in tiers corresponding to levels of internal validity. All the studies are considered jointly to evaluate the confidence in the overall body of evidence.

CEC03-04
Summarising and synthesising the evidence

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After the step in which the weight of evidence is assessed for one specific endpoint (for example: What is the weight of evidence for mammary cancer in animal models? What is the weight of evidence for mammary cancer in human studies?) the next step in the assessment process is to summarise and to synthesise the evidence.

In a systematic approach, which is different from the usual narrative approach, this step has to be pre-specified in the protocol which requests decision making on the weight of results from animal models and from human studies respecting the degree of evidence for the results. It is important to include the evidence from two sides: whether the effect is present and to which level of confidence or whether the effect is absent and to which level of confidence. This process therefore is multidimensional (animal – man; effect present: level of confidence high/moderate/low/insufficient; effect not present: level of confidence high/moderate/low/insufficient).

The multidimensional approach to synthesise the evidence from human and from animal studies described in the protocol for the systematic review for BPA prefers human study data before animal study data. However, in case the evidence from human studies is inadequate the evidence from animal data becomes decisive.

The approach will be presented and discussed.

Summarizing and synthezising: Likelihood that effect is present

Human Studies evidence: effect not present	High	Unlikely	Unlikely	Unlikely	Unlikely
	Moderate	As Likely as Not	As Likely as Not	As Likely as Not	Unlikely
	Low	Not classifiable	As Likely as Not	As Likely as Not	As Likely as Not
	Inadequate	Not classifiable	Not classifiable	As Likely as Not	As Likely as Not
	Level of Evidence	Inadequate	Low	Moderate	High
		Animal Studies evidence: effect not present			

Summarizing and synthesizing: Likelihood that effect is present

Human Studies evidence: effect present	High	Very likely	Very likely	Very likely	Very likely
	Moderate	Likely	Likely	Likely	Very likely
	Low	As Likely as Not	As Likely as Not	Likely	Very Likely
	Inadequate	Not classifiable	As Likely as Not	Likely	Very Likely
	Level of evidence	Inadequate	Low	Moderate	High
		Animal Studies evidence: effect present			

CEC03-05
Aspects of weight of evidence

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Reliability, relevance and consistency are generally considered as basic elements of the weight of evidence assessment. They should be reported in a systematic and transparent way to allow the reader to understand how the answers to relevant questions in the assessment were developed. According to the EFSA Scientific Committee guidance for assessing the weight of evidence (2017), considerations on/assessment of relevance and reliability contribute to the first two basic steps in the weight of evidence assessment, i.e. assembling and weighing of the evidence while the third step, i.e. the integration of evidence is based on the assessment of consistency. In the EFSA bisphenol A (BPA) hazard assessment protocol the appraisal of internal validity evaluates accuracy, i.e. systematic errors (e.g. biased allocation of animals to study groups, inappropriate test methods). This approach is mainly in line with the consideration of reliability but the latter also includes the concept of precision (degree of random error; EFSA guidance). For experimental animal studies the BPA protocol requests also the evaluation of external validity which indicates whether the specific endpoint measured in animals would be relevant to humans. To compare inter-study consistency for a specific endpoint (apical or intermediate) the results of epidemiological and animal studies (based on the human equivalent dose) will be graphically represented indicating the quality and quantity of the effects such as the directive and the magnitude along with the internal and external validity evaluation. In addition to consistency considerations some other elements are used for upgrading the confidence in the body of evidence, e.g. a reliable dose-response relationship and a large magnitude of the observed effect. The evaluation of limitations in reliability, relevance and consistency of the evidence contributes to the uncertainty analysis in hazard assessment.

CEC04 | Real world safety assessments for data-poor products: How to approach data gaps

Supported by ILSI Europe

CEC04-01
Properties of typical products requiring safety assessments: Focus on non-intentionally added substances (NIAS)

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All food contact materials are regulated via the so-called framework Regulation (1935/2004), this means no substance resp. no material is not regulated. However for specific materials a clear positive listing on allowed substances is completely missing on EU level and for other materials like plastic (Regulation 10/2011) a non-conclusive positive list exist. It is obvious that this concept bears some gaps. For non-listed, but widely used substances, a risk assessment might be based on national EU member state resp. on non-EU states like Switzerland for printing inks assessment. For listed substances, specific migration values may exist. However when running migration simulation experiments not only the known (non-listed) and even listed substances show-up, but also many substance, which are not know as they may be formed as reaction and/or breakdown product or they can represent some impurities in starting compounds. This "new" substances are called NIAS – non-intentionally added substance. Currently burning questions on the safety of such NIAS are coming more and more in the focus of the whole food packaging material value chain. ILSI published in 2015 a Guidance on Best Practices on the Risk Assessment of Non Intentionally Added Substances (NIAS) in Food Contact Materials and Articles. This was a very good starting point, but nevertheless there are many topics not yet solved, starting with how to deal with missing information, with insufficient analytical tools and finally how to assess substances only known by structure and even more worse unknown structures. A substance class something in between known and unknown structures are oligomers. As they represent mixtures, the assessment of such compounds deviates from the classical approaches. In the current talk all this topics are tackled on the base of examples and will be discussed in terms of possible solutions. NIAS and especially their assessment will be a never-ending story if at the end no kind of scientific compromise will be agreed. This starts with some more transparency up to developing more robust analytical methods and tools for assessment like the existing tools TTC, Matrix, FACET etc. For unknown structures, the bioassay approach to be discussed later may give a good add-on in assessment of NIAS.

CEC04-02
The use of quantitative structure-activity relationships (QSARs) and grouping approaches including read-across and category formation to fill data gaps

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Computational, or *in silico*, toxicological approaches offer a wide variety of techniques to predict toxicity, or fill data gaps, to assist in safety assessment. These approaches are used broadly across a number of industrial sectors and are finding increasing use in new areas such as for food contact materials. Quantitative Structure-Activity Relationships (QSARs) are statistical models relating the activity of substances to one or more physico-chemical properties or structural descriptors. As such, they provide a means to predict toxicity rapidly from chemical structure. QSARs are available for many toxicity endpoints and are particularly well developed for endpoints such as mutagenicity. Various software packages are available that allow for predictions to be made easily. The predictions from QSARs need to be used carefully and appropriately taking account of uncertainties and, where possible, utilising expertise gained from their use for regulatory purposes i.e. ensuring the QSAR is valid and the target compound is within the applicability domain of the model. Implementation of the OECD Principles for the Validation of QSARs for Regulatory Purposes has been valuable in assisting in their successful use. Grouping of similar compounds is a related *in silico* approach which has gained much popularity in the past decade, especially to provide assessments of the hazard of difficult or complex endpoints (such as organ level toxicity). If a similar molecule(s) with high quality toxicity can be identified to that with the data gap, the principle is that the data may be read across potentially negating the need for testing. Freely available tools such as the OECD QSAR Toolbox facilitate this process, however, from the outset, expert judgement must be exercised to ensure that the similarity is appropriate and justifiable, and that as much supporting evidence is available as possible. There is much guidance to assist the use of appropriate frameworks for read-across assessments e.g. from OECD, ECHA, US EPA, ECETOC etc. Uncertainties can be defined for read-across to fill data gaps and approaches such as the Read-Across Assessment Framework (RAAF) are valuable to assess the quality of predictions. In summary, QSARs and read-across provide a means to make predictions of hazard from chemical structure as part of the safety assessment process. Whilst QSARs may be rapid to use, all *in silico* approaches require expert evaluation and consideration of the available evidence to support a prediction.

CEC04-03
Thresholds of toxicological concern (TTC)

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Analytical methods are able to detect large numbers of man-made and natural chemicals at increasingly lower concentrations, so that risk assessors and risk managers have to take decisions about priorities. Where chemical-specific data are lacking, Thresholds of Toxicological Concern (TTC) are one of the tools for hazard and potency estimation – along with (Q)SAR and read-across. TTC thresholds are based on large datasets of oral cancer and non-cancer repeated dose *in vivo* toxicity data and describe *de minimis* exposures below which there is a low risk of any appreciable risk to human health. At this point in time, the TTC concept and read-across are the only options to perform repeated dose systemic risk assessments when there is a lack of time, resources and /or legal acceptance to run animal studies on specific natural or man-made chemicals. Therefore, it is important to understand its scientific basis, where it can be applied with confidence, where improvements are being developed and for which cases it should not be applied currently.

CEC04-04
Role of bioassays to support the application of the threshold of toxicological concern to prioritize unidentified chemicals in food contact materials

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Chemical Substances may leach from packaging materials into food. Many of these substances are unidentified (Non Intentionally Added Substances – NIAS) or toxicologically not characterized. Safety assessment of food contact materials therefore poses quite a challenge. Chemical identification and toxicologicas characterization of each migrating substance is not feasible. Instead, it has been proposed to use the Cramer class III threshold of toxicological concern (TTC) to prioritize the unknown NIAS on which further safety investigations should focus. This approach may be appropriate if sufficient evidence is available that the unknown chemicals under investigation are not direct DNA-reactive mutagens. *In vitro* bioassays capable of detecting DNA-reactive mutagens at very low concentrations are important tools. Investigations on the Ames test show that this assay is capable of specifically detecting DNA-reactive mutagens in migrates at quite low concentrations. The limit of biological detection of highly potent genotoxic carcinogens may be lower than 10 ppb for some DNA-reactive mutagens. 10 ppb is a pragmatic cut-off limit applied in the EU regulation 10/2011 to manage unknown substances in packaging migrates. The ILSI expert group on the applicatibility of bioassays for the risk assessment of food contact material proposes, that for a specific migrate the evidence of an absence of mutagenic substances based on Ames test, together with chemical analysis and information on packaging manufacturing could allow applying the Cramer class III TTC to prioritize unknown NIAS. Recommendations have been developed on sample preparation and bioassay improvement with the ultimate aim to improve limits of biological detection of mutagens.

CEC04-05
Data sources for exposure assessment

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Chemicals are the building blocks of our lives. There are more than 148 million known unique organic and inorganic chemical substances (CAS registry, 2019), with the vast majority of them identified over the last few decades. Therefore, the sustainable and safe use of chemicals became a priority for policy makers worldwide, resulting in over 348,000 chemicals being regulated across the globe (CHEMILIST, 2019). However, only few percent of those have yet undergone safety/risk assessments (ILO, 2014).

The ultimate goal of any chemical management system is to improve the protection of human health and the environment from potential risks from possible chemical exposure. The latter is being the function of many variable parameters, such as physicochemical properties of a substance, chemical product use conditions, human population characteristics and behavioral patterns. An insightful chemical safety assessment will, thus, hinge on the quality and robustness of data sources for those parameters.

The objective of this training session is to familiarize the course participants with freely accessible databases on human exposure determinants, different exposure assessment approaches that vary depending on the complexity of hazard characterization and the ultimate goal of the risk assessment, as well as modern exposure evaluation methodologies/tools tailored specifically for safety assessment of data poor chemicals (e.g. exposure data read-across), characterization of exposure data quality and accompanied uncertainties. After completion of this interactive training, the participants will be able to confidently navigate themselves in the space of chemical exposure data, will acquire a clear understanding of tiered exposure assessment strategy, and the importance of exposure uncertainty characterization. Moreover, the participants will learn how exposure data can help better inform hazard testing and more efficiently prioritize chemicals for further regulatory actions.

CEC05
Dietary exposure assessment

CEC05-01
Dietary exposure assessment: an overview

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The health impact of chemical hazards in food is estimated by comparing dietary exposure to toxicological levels of concern. The accuracy of any dietary exposure assessment will ultimately depend on the precision in the two calculation inputs – chemical concentration and food consumption.

Data from individual dietary surveys are understood to more closely reflect actual consumption and are therefore preferred for the assessment of dietary exposure within the risk assessment process. Depending on the purpose of the exposure assessment chemical concentration data can originate from different sources, e.g. analytical determinations from monitoring and surveillance programs, legislated limits, usage levels as reported by manufacturers, etc. The representativeness of the data will vary according to the measurement method, whether it is based on estimated levels or actual analytical results, the sampling strategy and the market coverage. The links between food consumption information on the one hand and chemical concentration data on the other are rarely direct. The use of a standardised system for classifying and describing food is a preliminary condition to combine this data and facilitates the assessment of exposure.

Guidance documents have been produced over the last years at international level that describe the current state-of-the-art of methodologies for dietary exposure assessment. A number of different methods exist ranging from quick worst-case estimations to refined methods aimed at assessing actual exposure. As the accuracy of dietary exposure assessments increases, the cost of undertaking the assessments also increases.

Some of the methodological differences across disciplines are not fully justified by the specific requirements of the class of substances under evaluation. There is potential to further harmonise the way exposure is estimated with the availability of more refined and accurate information.

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CEC05-02
Unravelling the chemical information hiding in our food

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Dietary exposure and risk assessment are based on mathematic models and calculations. These come with a (sometimes high) uncertainty, as models themselves make use of simplifications and assumptions. For the risk assessor (and hopefully end-user), this uncertainty is, to some extent, known and should be reported together with the study conclusions. What is often (if not always) forgotten by the end-user, is that even the best model is only as good as the input data that was used. Concentration and mass fraction data are a cornerstone in exposure modelling alongside the consumption data (which is modelled on its own). The quality of the input data (i.e. results of chemical measurements) is whereupon this lecture will shed some light.

Concentration and mass fraction data, i.e. how much of a certain chemical is present in a food, is sometimes not easily determined. A series of operations, in combination with expensive and high-complexity instrumentation is necessary. You will learn how this information is extracted from foods and what quality control measures should be in place to ensure the data is fit-for-purpose. You will also learn that chemical measurements are not always as easy as they seem. In some cases the analyte might be unstable once separated from the food, while in other instances the analyte is not even well defined. Apart from these challenges, it will also be made clear to you that each measurement is not more than an estimate of the true value of the analyte under study. The quality of this estimate (read: the uncertainty) is not only driven by science and technical performance, but also by economics. Fact remains: each value is wrong, to a certain extent. All these aspects will be explained using case studies of actual dietary intake studies.

In summary, you will walk away from this short course with new insights on where the numbers actually come from, how data quality can affected and influenced by many factors and finally that the numbers obtained might not be even close to the true value. But then again, how close is close …

CEC05-03
Food consumption data

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Food consumption data is a fundamental part of food safety related risk assessment. Best data to calculate individual long-term exposure come from individual food surveys and are also used for acute exposure assessments. For about ten years the European Food Safety Authority (EFSA) has collected and published food consumption data based on national data collections as well as principles for harmonizing the European data collections.

The collecting of food consumption data is a time and resource demanding effort and needs to be planned and carried out using high quality standards at each level of the process, i.e. the planning of the sampling frame, sampling methods, sample size, the compilation of the study protocols, piloting data collection tools and methods to be used, food list, food description and quantification methods used and background information to be included in the data collection. Fundamental parts of food consumption surveys are also the training of the staff and quality assurance throughout the survey.

The National Institute for Health and Welfare has monitored the dietary habits and nutrient intake of the adult population in Finland since 1982. The most recent food consumption data collection, the FinDiet 2017 Survey was carried out in collaboration with the FinHealth 2017 Survey in 50 study locations around Finland between January and May 2017 using the EFSA EU Menu methodology. For the FinHealth 2017 Survey, an eligible sample of adults aged 18 years and above was randomly drawn from the Population Register (n=10 247). A 30% random sub-sample with age 18–74 years old (n=3 099) of the FinHealth 2017 Survey sample were invited to participate in the FinDiet 2017 Survey. Diet was assessed by two non-consecutive 24-hour dietary recalls. The 24-hour dietary recalls were recorded by dietary interviewers using the in-house dietary software Finessi, which included the food list and descriptors of the national food composition database Fineli® and additional food descriptors of the EFSA FoodEx2 system. A picture booklet of food portions was used to estimate portion sizes. The use of food supplements was also studied. The final food consumption data included the accepted, non-consecutive 24-hour recalls from 1 655 participants (53% of the original subsample). The data collected is the basis for dietary monitoring and risk assessment both at the national level as well as by EFSA.

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CEC05-04
Total diet studies: benefits and challenges

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The protection of consumers from potential hazards in the food supply is one of the most important public health functions for any government. In this regard, total diet studies (TDS) are national studies recognized as one the most cost-effective tools for assessing intakes of essential nutrients and dietary exposures to a range of potentially hazardous chemicals, including heavy metals, environmental contaminants, food additives, pesticide residues... Complementary to monitoring programs and biomonitoring surveys, TDSs consider exposure from whole diets and are based on food contamination as consumed rather than contamination from raw commodities, thus ensuring a realistic exposure measure.

A TDS consists of three main steps: (i) a food sampling covering at least 80% of the whole diet and preparation of samples “as consumed” by the targeted population to be representative of reality (ii) analysis of the samples with analytical limits as low as possible, and (iii) dietary exposure assessment by combining occurrence data with consumption datasets, and risk assessment by comparison of exposure levels with health-based guidance values.Foods that contribute most to total exposure can then be identified, and recommendations can be set for risk managers and decision makers to monitor food contamination, for agricultural sector and food and beverage industry to reduce contaminant levels, and to consumer to reduce its exposure. Research recommendations can also be proposed to refine the conclusions relating to the risk associated with exposure to certain compounds.

Recommended by WHO, FAO, and EFSA, TDSs have been conducted in several countries worldwide including USA, Canada, Australia and New Zealand, China, Cameroon, UK, Italy, Spain, France... Various approaches and methods are used, but challenges remain on sampling plan design based on consumption surveys, food collection method, analytical issues, exposure calculation and risk assessment, while taking account of budget limitations.

The importance of TDSs in assuring the safety of the food supply and identifying possible health risks will be described. The presentation will explore the scientific and technical knowledge underpinning TDSs for exposure assessment, and provide insight into methods and approaches, and the quality of data.

CEC05-05
Dietary exposure modelling to chemicals

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A dietary exposure assessment is performed to determine whether the presence of an adverse chemical in food is a health risk for a population. Depending on the toxicity of the chemical, an acute or chronic exposure assessment is performed. Acute exposure relates to the exposure to a chemical during a period of 24 hours or less. This exposure is relevant for chemicals that may cause acute effects on ingestion. Chronic exposure relates to exposures over a longer period of time, and is relevant for chemicals exerting an adverse effect after a longer period of ingestion (months, years). Considering the concentrations in which chemicals are present in food and the amounts in which foods are typically consumed, acute exposure is mainly relevant for pesticide residues and chronic exposure for contaminants and additives. A tiered approach is often used to determine the exposure, starting with simple approaches and moving up to more refined approaches if needed. This presentation will highlight the differences between acute and chronic exposure assessments, focussing on the input data needed and models available. It addresses the exposure to single chemicals, but also developments on exposure to multiple chemicals sharing the same toxicological effect (cumulative exposure).

CEC05-06
Statistical modelling: BIKE model

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Dietary exposure modelling crucially depends on quality and quantity of data. Small sample sizes and missing data make the uncertainty assessment pivotal for the task. Concentration data can largely contain values below detection or reporting limits with different limits for each subset of data (even combinations with below/between/above limit values), from more or less detailed categories of food items/ingredients. Likewise, individual consumption data can represent one or several days records of consumption frequencies and amounts for comparable food/ingredient categories. Considerable uncertainties need to be assessed, particularly with rare occurrences or with tails of the distributions, and with population subgroups. Not only are averages uncertain, but also the variances and correlations which determine the population intake distribution of interest. There is variability in non-zero consumption amounts between days and between individual mean (of non-zero) amounts, and variability of consumption frequencies between individuals. Likewise, the variation of concentrations between food servings and of mean concentrations between food types. Detailed data allows more refined direct modelling of the features in intake exposure whereas sparse data with missing values calls for advanced model structures, such as hierarchical modelling and possibly evidence synthesis from multiple data sources. Bayesian modelling can be used for combining evidence into a simultaneous uncertainty assessment of all model parameters needed for the intake assessment. To allow context dependent modifications and extensions of the models, BIKE was developed for a flexible model building approach as a combination of R and OpenBUGS software. It was intended for both microbiological and chemical exposure assessment, for acute and usual intakes which could be extended with dose-response models in an integrated manner.

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CEC06 | Determining safe exposure limits in occupational toxicology, application to pharmaceuticals

CEC06-01
Key elements of reliable risk assessment of chemicals

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Risk analysis is a complex process that includes three key steps: risk assessment, risk management and risk communication under specified conditions of exposure (FAO/WHO 1985). The procedure is well documented in the European Directive 93/67/EEC of 20.07.1993 developed within the World Health Organization, the United Nations Environment Programme and the International Labour Organization's (WHO/UNEP/ILO) International Program on Chemical.

1.) The **hazard identification** consists of initially identifying the adverse effects produced by the test substance. Although hazard identification it is a crucial step in the risk characterisation process, it is important to underline that it would be inappropriate to evaluate the risk posed by a chemicals solely on the results of hazard identification that is based merely on the intrinsic toxicity of the molecule.
2.) The **hazard characterization** should be able to identify a NOAEL "no observed adverse effect level" (i.e. the highest dose that does not cause an adverse effect) derived from validated experiments.

The observed NOAEL value is then used to calculate the Health Base Guidance Values, which can be taken daily by for a lifetime without causing any appreciable health risks.

The Benchmark Dose (BMD) is proposed as an alternative for the classical NOAEL. The BMD is based on a mathematical model being

fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable response (the benchmark response BMR) typically chosen at a 5 or 10% incidence above the control".

1.) The **exposure assessment** is part of the risk assessment and is defined as a quantitative evaluation of the likely exposure to a chemical substance, taking into account all relevant sources.
2.) The **risk characterization** integrates the information derived from the identification and characterization of the hazard associated with the exposure assessment to provide useful information for decision-making and managing risk.

This is a simplified overview of the risk assessment process, which integrates several related elements such as the toxicokinetic and toxicodynamic properties of the test substance to help ascertain qualitative and quantitative aspects of systemic absorption, bioaccumulation potential, tissue distribution, metabolism, mechanism of action, inter- species differences, local tolerance concerns (skin and eye irritation as well as skin sensitization etc. It is a rigorous and globally accepted scientific approach to assess the severity and probability of a possible adverse effect in humans or the environment following exposure to a chemical.

CEC06-02
Regulatory perspective on application of health based exposure limits (HBEL) in drug manufacturing

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The implementation of the EMA "Guideline on setting health based exposure limits (HBELs) for use in risk identification in manufacture of different medicinal products" in 2015 has been a challenge for the pharmaceutical industry. This guideline addresses the accidental cross-contamination during the manufacturing of medicinal products in shared facilities.

From a regulatory point of view, it is expected that manufacturers perform a toxicological evaluation for products manufactured in such facilities to assess the risk for workers and patients. These assessments rely on all the data available for a drug substance, i.e. pharmacological and toxicological data but also results from clinical trials. They have to be done by toxicology professionals or experts.

The calculation of the Acceptable/Permitted Daily Exposure (ADE/PDE) or Occupational Exposure Limit (OEL) allows a compound specific assessment which assists manufacturers to differentiate and categorize between individual products. Any risks encountered during production in shared facilities caused by cross-contamination should be identified and characterized. Hence, adequate cross-contamination risk mitigation measures can be defined and implemented.

Overall, the goal of this guideline is to recommend a scientific approach for the exposure control limits to protect workers and patients who may be incidentally in contact with a drug.

CEC06-03
Derivation of acceptable daily exposures (ADE) or occupational exposure limits (OEL) – an industry approach

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The standard approach for deriving a health-based exposure limit (HBEL) uses a formula that converts a dose from a toxicity study in animals, typically the NOAEL, into the corresponding human dose. Then, several adjustment factors are applied to lower the human dose down to a level that can be regarded as safe. While this method is well established for chemicals and solvents, it does not always represent the best practice for active pharmaceutical ingredients (APIs) for several reasons. The dose range in toxicity studies is typically above the therapeutic dose rage and endpoints of pharmacodynamics (PD) are often not measured in toxicity studies. Therefore, the NOAEL may not represent an ineffective dose and PD data have to be taken into consideration. PD effects are typically mediated by receptors which may differ between animals and humans, particularly in the case of therapeutic peptides and proteins. As soon as pharmacokinetics in animals and humans are available, they have to be taken into count for the dose transformation from animals to humans. In addition, some APIs may be carcinogenic, mutagenic, or teratogenic (CMR substances) which requires specific analyses of dose-response-relationships and mode of action. When clinical data are available and regarded as more relevant, HBELs should preferably be derived from human data rather than animal data. In summary, setting HBELs for APIs can be very complex and the use of the standard formula may be insufficient or even inappropriate.

CEC06-04
Overcoming data gaps: generic versus substance-specific approaches in health based exposure limit (HBEL) setting

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Calculation of HBELs is a based on defined process. The first step that lays the ground for further expert evaluation is data acquisition. This step is followed by selection of Points of Departure (PoD) and adjustment of variability and uncertainty that are specific for a given chemical and based on the entire available dataset, considering consistency and interdependence of effects. While marketed pharmaceutical substances typically have abundant dataset, which allows robust interpretation of information for the target population and defined exposure of patients, drugs in development and pharmaceutical intermediates have very limited data sets which may not be sufficient to calculate a substance specific HBEL. The large data gap is also evident for new types of therapies that are based on new therapeutic modalities or applied with non-standard routes of administration. In this presentation we will address the types of data gaps that can be observed and address situations when the HBEL calculation may be considered unreliable, leading to a generic HBEL determination strategy.

CEC06-05
Case studies of Health-Base Exposure Limit (HBEL) calculation – Route-to-Route extrapolation and specific case of skin sensitizer using the Quantitative Risk Assessment (QRA) methodology

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The calculation of Health-Based Exposure Limits (HBEL) follows a well-defined scientific process that is common to different industries (dataset compilation, selection of a Point-of-Departure, application of safety factors to cover the uncertainties and when necessary, application of a pharmacokinetic factor for route-to-route extrapolation) (Sussman et al, 2016).

In some cases, route-to-route extrapolation is necessary when the POD comes from another route of exposure than the HBEL (e.g., for the setting of Occupational Exposure Limits [OEL]). Case studies will

be presented for oral-to-inhalation extrapolation and for oral-to-dermal extrapolation (Jandard et al, 2018).

For the specific case of topical drugs, when skin sensitization is feared in case of cross-contamination, an HBEL can be set following the QRA (Quantitative Risk Assessment) methodology used by the cosmetic industry for fragrances (Api et al, 2008). The QRA methodology will be presented and its potential use for pharmaceuticals will be discussed following a case study.

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CEC06-06
ECHA's experiences with OELs

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Since January 2019, the European Chemicals Agency (ECHA) has been supporting the European Commission in establishing occupational exposure limits (OELs) for selected chemicals to improve the protection of workers' health and safety.

The Commission and ECHA agreed in January 2019 that the Agency will start providing recommendations for priority OELs under occupational safety and health (OSH) legislation. The agreement followed a pilot project run from 2017 to 2018 concerning five carcinogenic substances. ECHA's Committee for Risk Assessment (RAC) was able to recommend OELs for three of these substances using a threshold approach which took into consideration a mode of action with a threshold. This approach has been reported in the recent findings of the RAC-SCOEL Joint Task Force. These five substances are currently passing through the decision-making process of the Commission.

Based on the experience from the pilot, ECHA aims for an open and transparent process through public consultations. The work on OELs will bring employers and trade unions even closer to ECHA's operations. The decision for setting OELs is based on extensive consultations, including employers, workers and Member State authorities.

In 2019, the Commission asked ECHA to assess lead and its compounds and diisocyanates: ECHA was asked to assess lead because it is a major reprotoxic substance for which stakeholders have long since asked for a scientific reassessment of the 20-year-old limit value. Diisocyanates are substances to which a high number of workers are exposed and which causes many cases of pulmonary asthma each year.

OELs are at the core of occupational safety, as key tools in helping protect the health of workers exposed to chemicals in the workplace. An OEL is a regulatory value setting a safe concentration level of a chemical substance in the air of a workplace. They are set at EU and national level by regulatory authorities and help employers protect the health of workers from possible risks when using chemicals at work. The OSH Framework Directive, the Chemical Agents Directive (CAD) and the Carcinogens and Mutagens Directive (CMD) lay out the main principles of worker protection from the risks posed by dangerous substances at the workplace at EU level.

OELs can either be indicative or binding. Indicative OELs are adopted directly by the Commission. For binding OELs, the Commission adopts a legislative proposal based on the opinion of the Committee for Risk Assessment (RAC) and discussion between the Member State authorities and social partners. The proposal is then sent to the Council and the European Parliament for the final adoption. The scientific evaluations carried out by ECHA/RAC underpin the legislative proposal for EU OELs for specific chemicals or substances, whether they are indicative or binding. Having a sound scientific basis is crucial to any occupational safety and health action, particularly in relation to dangerous chemicals.

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Invited Sessions: Keynotes – EUROTOX/SOT Debate – Bo Holmstedt Memorial Fund Lecture – SOT Merit Award – HESI CITE Lecture

Keynote Lectures

Keynote Lecture 01

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K01 – Atmospheric aerosols: from molecular clustering to regional air quality and global climate

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The atmosphere forms a major part of the environment to which life on Earth is sensitively responsive. The atmosphere closely interacts with the biosphere, hydrosphere, cryosphere and lithosphere as well as with urban surfaces on time scales from seconds to millennia. Changes in one of these components are directly or indirectly communicated to the others via intricately-linked processes and feedbacks resulting in local, regional and global scale effects on climate and air quality, as well as for water and food supply. Human and societal actions, such as emissions-control policies, urbanization, forest management and land-use change, as well as various natural feedback mechanisms involving the biosphere and atmosphere, have substantial impacts. To be able to meet challenges related to our Earth system we need to have enough deep understanding including proper comprehensive observational data.

The production of molecular clusters and their growth to larger sizes, is a world-wide phenomenon, with a significant contribution to aerosol particle number load and indirect radiative effects as well as human health via urban air pollution. Understanding the very initial steps of atmospheric aerosol formation requires detailed knowledge of interlinked physics and chemistry in sub 3 nm size range.

There is always more or less intensive clustering in the atmosphere but only some fraction of those clusters are able to growth to 3–4 nm and further to cloud condensation nuclei and haze particle sizes. However, NPF is a major aerosol source affecting significantly to global aerosol and CCN load as well as regional/local air quality and global climate.

Since we typically spend more than 90% of our time indoors, all these processes need to be understood also in indoors point of view.

In the presentation I will focus on:

- Environmental grand challenges
- Continuous, comprehensive observations, SMEAR (Stations for Measuring Earth surface -Atmosphere Relationships) stations
- COBACC (*COntinental Biosphere-Aerosol-Cloud-Climate*) feedback loop
- Gas-to-Particle conversion / New particle formation (NPF); the contribution of NPF on haze formation
- Global and regional aerosol load – air quality and climate effects

Keynote Lecture 02

K02 – Systems toxicology: A Key Towards Reliable Hazard Prediction

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Classical toxicological testing paradigms still rely heavily on animal testing, despite societal pressures to switch to alternative in vitro test methods. The 21st century toxicology paradigm calls for a shift away from descriptive toxicology, based on a large extent on animal testing of toxicants one-by-one and with a multitude of functionally disconnected assays, towards a predictive toxicology grounded in a more solid understanding of the relevant toxicity and modes of action in humans, or the environment. Systems biology combines advanced analytical and computational tools providing quantitative information on systems-level molecular changes leading to information on how biological networks are perturbed by toxicants. Systems toxicology aims to change the way in which adverse effects of chemicals or other toxicants are characterized, from isolated empirical end-points to integrated pathways of toxicity.

Huge amounts of information are generated in omics experiments. In order to identify the hazard-relevant molecular features (signatures), one needs to be able to isolate the relevant information while taking into account the statistical dependency between the variables. In such a context, the group of features that best predicts the safety of chemicals/toxicants might not be composed of components derived from only one data layer, but also by features with a combinatorial effect derived from multiple layers of data. Thus, by systematically integrating multiple layers of experimental data together with information extrapolated from the relevant literature, a more robust hazard predictions can be achieved.

In the context of nanosafety, systems toxicology promises to shed new light on the interactions of engineered nanomaterials (ENMs) with biological systems, and reveal the causal connection between changes in the expression of genes, proteins or metabolites and the biological pathways that underlie the toxicity phenotypes. Key chal-

lenges are also how to link reliably identified gene profiles/networks to toxicological phenotypes (for example, immunotoxicity, genotoxicity and so on), and how to demonstrate reliability and prediction accuracy of computational models in real life.

The NANOSOLUTIONS project aimed to generate a computer algorithm capable of predicting the safety of ENMs based on a minimal but the most informative set of features selected across multiple data layers. Based on integration of data from several different omics layers (e.g. transcriptomics, proteomics, epigenomics), physicochemical properties and proteomics-based biocorona profiling, along with in vitro and in vivo toxicity data for a panel of more than 30 ENMs, a classifier algorithm designated the ENM safety classifier, composed only of approximately one dozen most informative hazard associated features, was generated that is capable of predicting ENM toxicity with high accuracy.

Computation predictive tool approach is a major leap forward and may enable progressing towards faster predictive hazard classification based on relatively small amount of toxicity studies to be carried out for hazard evaluation.

EUROTOX-SOT Debate

D – Classification of substances as endocrine disruptors has a public health benefit

Martin van den Berg (EUROTOX) and Paul Foster (SOT)

Each year, the SOT Annual Meeting includes a debate in which leading toxicologists advocate opposing sides of an issue of significant toxicological importance. The debate continues a tradition that originated in the early 1990s. This year, the debaters will address the proposition "Classification of Substances as Endocrine Disruptors Has a Public Health Benefit."

Endocrine disruptors are compounds that produce adverse responses in various organ systems, but particularly the reproductive system, by interfering with normal hormonal signaling. There has been considerable public concern about endocrine disruptors, particularly in how much of a role they may play in causing certain cancers, infertility, and birth defects, as well as population declines in wildlife. This public concern has led to the passage of various laws in the United States and Europe to identify and regulate compounds that have endocrine-active properties. This includes action by the European Commission to develop a classification system for endocrine disruptors. It is unclear, however, whether classification as an endocrine disruptor conveys any public health benefit; endocrine disruption is a collection of modes of action, not an adverse response, and if the adverse responses are already the subject of regulation, does classification provide any additional protection? The debaters will discuss whether there is value in an additional classification scheme.

Regardless of their personal convictions, each scientific debater will present relevant evidence and compelling scientific arguments to persuade and appeal to the audience in order to obtain the approval or refusal of the motion. The first debate took place in Baltimore, Maryland, during the 58th Annual Meeting and ToxExpo, March 10–14, 2019. In Helsinki, Finland, during the 55th Congress of the European Societies of Toxicology, the debaters will take reverse motion positions.

Bo Holmstedt Memorial Fund Lecture

B – Understanding fundamental quantitative principles is a prerequisite for improving toxicological science and risk assessment

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Advances in science often follow upon the invention of more sophisticated measurement tools and laboratory techniques. The recent progress in molecular biology clearly demonstrates this. Accordingly, toxicologists tend to focus on modern techniques that are able to measure the deeper biological and biochemical processes underlying toxic properties of chemicals. It is generally believed that understanding the mechanisms of toxicity will provide the answer to any scientific or risk assessment question. However, acquiring new knowledge is, in the end, based on experimental data, and understanding quantitative principles is crucial for making progress just as well. While toxicologists excel in examining toxicological mechanisms and defining AOPs, they are much less educated in quantitative principles and methods. Here lies an important weakness in toxicology and risk assessment, with rather drastic consequences. Some examples of practical concepts about which toxicologists employ inadequate quantitative ideas are: the dose threshold, classification of chemicals, false positives/negatives, shape of dose-response, (non) linear relationships, NOAEL, non-monotone dose-response, and the KMD (kinetically derived maximum dose). The inadequate thinking about all these concepts can be explained by a general misunderstanding of three fundamental principles. I will discuss these three fundamental principles, and illustrate how a better understanding of them will change your view on the concepts just exemplified. Furthermore, I will make the point that misunderstanding or ignoring the three fundamental principles hampers further development of both toxicological science and risk assessment.

SOT Merit Award Lecture

SOT – Bis-Indoles as receptor ligands and novel anticancer agents

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The aryl hydrocarbon receptor (AhR) was initially identified as the intracellular protein that binds and mediates the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and structurally related compounds. Subsequent studies with AhR knockout animal models have demonstrated that this receptor plays an important role in maintaining cellular homeostasis in multiple tissues and in pathophysiology. The AhR binds TCDD but also structurally diverse ligands which include pharmaceuticals and health promoting phytochemicals such as bis-indole compounds. These molecules are selective AhR modulators (SAhRMs) and exhibit tissue/cell-specific AhR agonist and antagonist activities and can be used as drugs to target the AhR and its functions in multiple diseases including cancer. In our studies on SAhRMs derived from 1,1-bis (3'-indolyl) methane (DIM), a series of synthetic analogs typified by 1,1-bis (3'-indolyl) -1- (p-hydroxyphenyl) methane (DIM-C-pPhOH) were characterized as AhR-inactive

and results of receptor screening showed that they target the orphan nuclear receptor 4A1 (NR4A1, Nur77, TR3) and other analogs interact with NR4A2 (Nurr1). Endogenous ligands for NR4A1 and NR4A2 have not been identified and ongoing studies show that both NR4A receptors are important in maintaining cellular homeostasis and in pathophysiology. Selective NR4A1 modulators such as DIM-C-pPhOH and related compounds are being investigated as potential drugs for treating metabolic diseases, arthritis, immune dysfunction, neurological and cardiovascular problems, and cancer. DIM-C-pPhOH and a series of more potent second generation analogs exhibit cell/tissue-specific NR4A1 antagonist and agonist activities and this presentation will highlight some of the potential clinical applications of bis-indole derived NR4A1 ligands and focus primarily on their inhibition of NR4A1-dependent pro-oncogenic pathways and genes in solid tumors.

HESI CITE Lecture

H – Toxicology in the era of the exposome

*R. Barouki

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Toxicology studies the hazards related to the interaction of a stressor with a living organism. Assessment of environmental chemicals toxicity has traditionally focused on the properties and toxic effects of each substance taken individually. During the last decades, more consideration was given to the properties of the target (molecular, cellular and the organism itself) and to the context of the exposure (other environmental, nutritional or behavioral conditions).

Based on these recent developments and on the framework given by the exposome concept, a new vision of toxicology has emerged highlighting the importance of integrating different stressors, taking into consideration the target, the context in a broad sense, the time and pattern of exposure and relying on a variety of new technologies.

The knowledge gained from decades of research on dioxin like compounds and on the Arylhydrocarbon Receptor (AhR) perfectly illustrates those evolving perspectives. Initially, it was thought that the AhR function was to detect and to transduce the adaptive and toxic effects of dioxin and related chemicals. Then it was found that the receptor has critical endogenous functions and that the panel of its ligands is extremely large and includes, in addition to toxic pollutants, dietary substances, microbial compounds and endogenous metabolites. Interestingly, those categories of compounds constitute the so-called "internal chemical exposome". Thus, understanding the effects of AhR ligands actually requires an integrated approach provided by the exposome framework.

Other examples also illustrate the relevance of the exposome framework for toxicity assessment. Among them, the integration of toxicity studies with environmental, epidemiological and modeling studies, the analysis of mixture effects, the importance of long-term effects particularly those related to developmental programming disruption and epigenetic mechanisms and the advance in analytical approaches allowing the combined characterization of the chemical exposome and the endogenous metabolome.

These developments in toxicology are expected to have regulatory implications and impacts in public health.

S01 | Metabolic capacity and functionality of the gut microbiome

Supported by ECETOC

S01-01
Determining the role of the gut microbiota in the toxicity of foodborne chemicals *in vitro*

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The human organism is host to a huge number and variety of microorganisms that are considered to play a significant role in the health of the host, among others through metabolism of indigestible food components, production of essential vitamins, and protection against opportunistic pathogens. Increasing evidence shows that despite its non-mammalian nature the intestinal microbiome can also play an important role in toxicology, as it can affect the bioactivity of (foodborne) xenobiotics through a wide range of biochemical and metabolic activities, leading to the formation of metabolites with often uncharacterized toxicokinetics and toxicodynamics. Especially in modern *in vitro-in silico* based testing strategies used for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) this important function of the intestinal microbiome is generally overlooked. To predict intestinal microbial metabolism of xenobiotics, an *in vitro* method was developed based on anaerobic incubation of fecal samples that allows definition of maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) of these reactions. This method was applied to study metabolism of various foodborne xenobiotics, and results will be presented showing the interspecies differences in the intestinal microbial metabolism of the mycotoxin zearalenone for different host species. To put these into perspective, the resulting catalytic efficiencies of the intestinal microbiome are compared to the liver. Further results will be presented on the intestinal microbial metabolism of the phytoestrogen daidzein. An existing physiologically based kinetic (PBK) model for daidzein was fitted with an intestinal microbial compartment to predict plasma concentrations of the microbially produced metabolites (S)-equol. The developed testing strategy allows the prediction of *in vivo* consequences of intestinal microbial metabolism of xenobiotics, thereby contributing to the *replacement*, *reduction* and *refinement* (3Rs) principles and 21st century toxicity testing strategies.

S01-02
Human metabolism and interactions with the gut microbiome in health and disease

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There is increasing awareness that the study of how the body responds and adapts to environmental challenges – termed metabolic flexibility – is key to decipher the metabolic determinants of human health. Systems biology approaches are increasing employed in clinical studies as a research driver to enhance our understanding of the role of genetics, environmental factors and gut microbiota as metabolic determinants of metabolic flexibility. Such applications have contributed to the study of the metabolic inflexibility of obesity and type 2 diabetes, and mechanisms governing fuel selection between glucose and fatty acids. Such approaches are essential to explore the effect of nutrition on metabolic health by understanding how macro-

and micronutrient composition of the food influences metabolic outcomes and the metabolic state of individuals. Applications in human clinical intervention studies eventually will lead to new personalized nutritional approaches to improve or maintain metabolic health.

Amongst the new omics technologies, metabolic and nutritional phenotyping approaches, have emerged as robust platforms to capture metabolic and nutritional requirements by enabling, in a minimally invasive fashion, the monitoring of a wide range of biochemical compounds [1,2]. Their variations reflect comprehensively the various molecular regulatory processes, which are tightly controlled and under the influence of genetics, diet, and gut microbiota. They are providing key insights into complex metabolic phenomena as well as into differences and specificities at individual and population level. From a nutritional perspective, we are genuinely interested in the human gut microbiome [1-3]. A particular focus of our research lies on how dietary macro- and micronutrients are co-metabolized by the human body and its gut microbial population, and sub-subsequently contribute to nutritional status and disease etiology.

Yet, due to the higher complexity of metabolic phenotypes, and their variability in space and time, plus their subtle response to environmental stimuli such as diet, it is very challenging to generate holistic insights into a gut microbiome at protein and/or metabolite level. Here we will discuss how we study human metabolic phenotypes by quantifying specific molecular species over time, across conditions, before and after interventions, and between individuals [4]. We will also discuss analytical approaches to enable data integration, with an emphasis on the longitudinal component [5]. We will illustrate current examples, challenges and perspectives in the applications of metabolic monitoring and modelling approaches in the context of clinical research in pediatric populations with metabolic and gastrointestinal conditions related to (pre)-diabetes and inflammatory bowel diseases [5-11].

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S01-03
Influence of the microbiome on metabolite patterns – an inter-omic approach

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In the last years, research has demonstrated the impact of the gut microbiome on the host, especially on the state of the host health. As emerges from the gut microbial research a key aspect is the gut microbial functionality. Microbiome-derived metabolites arisen in the presence of gut microflora can affect the host, for example *via* nuclear receptors, leading to changes in various signaling pathways. Since the metabolite profile in plasma combines both the microbiome-derived or -associated metabolites as well as the endogenous metabolites, the scope of our project is to find out which metabolites are derived specifically from the gut microbiome and what kind of impact they have on the metabolite profile, using the metabolomics approach and the existing knowledge and data availability of the MetaMap®Tox database, in which the metabolome and toxicity data of more than 800 compounds are stored.

To identify microbiome-related metabolites in rat plasma, Wistar rats were treated with antibiotics which are known to induce a shift of the microbial community. After 28-day oral administration, metabolomics of plasma, feces, and cecum-content was done. Additionally, DNA was extracted from rat feces and the 16S subunit was sequenced to perform a core diversity analysis.

Specific plasma metabolome patterns were established, and microbiome-related metabolites identified as key metabolites, e.g. hippuric acid and indole derivatives, in MetaMap®Tox. In general, most changes were observed in metabolites belonging to the class of bile acids, complex lipids, fatty acids and related metabolites, as well as amino acids and related metabolites. Both the community and metabolome analyses in feces and cecum-content showed a treatment-related effect, as well as only minimal, if any, differences between samples of male and female animals as well as between different vehicle controls.

With these results, the first stage of investigations assessing the functionality of the microbiome using metabolomics in the field of microbiome and toxicology were performed. The results suggest that plasma based metabolic profiling is a suitable tool to investigate the functionality of the gut microbiome, and laid the basis for the development of a promising method to elucidate the underlying mechanisms leading to adverse effects in the host system.

S01-04
How drugs interact with our bugs

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Bacteria in our gut can modulate the availability and the efficacy of therapeutic drugs and these drugs can in turn change our microbiome. Yet, interactions at the level of specific drugs and bugs are only recently beginning to emerge. I will present latest results from my lab and our colleagues at EMBL providing new insights into drug-microbiome interactions and discuss their implications for drug design, toxicity and personalized medicine.

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S02 | Fetus – the most sensitive individual

S02-01
Fetal exposure to toxic compounds

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Except for a short time at the beginning of pregnancy, placenta is the only route from the mother to the fetus. Thus, practically all fetal exposures occur through the placenta. Placenta develops throughout the pregnancy and goes through major changes during the development. At the beginning of the second week of pregnancy trophoblastic cell layer differentiates into syncytiotrophoblast without clear cell borders and a layer of cytotrophoblasts. Maternal blood enters the trophoblastic blood lacunae at around day 12 of pregnancy. Later in pregnancy the exchange between the maternal and fetal circulations takes place in chorionic villi, which are functional units of human placenta. Term human placental barrier consists of syncytiotrophoblast with some remaining individual cytotrophoblasts facing maternal blood space, fetal capillary endothelium and some connective tissue between them [Benirschke *et al*., 2006].

In addition to physiologically important exchange of gases and nutrients, most exogenous compounds cross placenta, including drugs, environmental hormonal compounds and carcinogens. Some toxic compounds may accumulate in placenta, e.g. cadmium [Kippler *et al*., 2010] and Bisphenol A [Cao *et al.* 2012] with a wide interindividual variation. Passive diffusion, the most common mechanism of transfer, depends on chemical characteristics of compounds, but both trophoblastic and endothelial cells contain also a variety of transporter proteins. Especially important for exogenous compounds are the ABC (ATP binding cassette) efflux transporters, e.g. p-glycoprotein (ABCB1) and BCRP (breast cancer resistance protein or ABCG2) [Karttunen *et al*. 2017]. These have been shown to protect placental cells and/or the fetus in some settings [Behravan and Piquette-Miller, 2007; Vähäkangas and Myllynen, 2009]. Functionally significant polymorphisms in human placental ABC transporters have been described.

Selection and activity of the enzymes metabolizing xenobiotics is very restricted in the placenta [Myllynen *et al*., 2009]. A wide inter-individual variation occurs in the activity of these placental enzymes. There are differences in the expression of transporters and xenobiotic metabolizing enzymes at different stages of the placental development, but the full toxicological significance of these changes is not known. Transplacental transfer as well as the role of transporters and xenobiotic metabolizing enzymes in the transfer can be studied after birth in the born placenta by ex vivo placental perfusion [Karttunen *et al*. 2017]. Comparison of concentrations of compounds in cord and maternal blood gives an idea of the *in vivo* transfer. In general, the results gained by these two methods correlate quite well. In this context, *in vitro* trophoblastic cell models are mainly used to study expression and regulation of transporter and enzymes proteins.

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S02-02
Environment and male reproductive health: testicular dysgenesis syndrome and germ cell cancer

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During the past 50 years there has been a worldwide increase in incidence of testicular germ cell cancer (TGCC) among young men. The incidence is still increasing, particularly in previously low-incidence countries. Although several susceptibility genes have been reported the rapid increase in incidence of TGCC can only be explained by environmental factors.

Epidemiological studies, which have shown strong birth cohort effects are in line with cellular studies demonstrating embryonic characteristics of the precursor cells of germ cell cancer, *Germ cell Neoplasia In Situ (GCNIS*). Risk conditions for GCNIS are undescended testis, infertility and disorders of sex differentiation. We have suggested that these conditions may be linked through a testicular dysgenesis syndrome (TDS) which seems to be more common among men in industrialized countries. Recent reports on falling sperm counts should be seen in context with increasing rates of TGCC, high rates of male infertility and increasing need for assisted reproductive techniques (ART). Last year almost 10% of all Danish children were born after ART, including IVF, ICSI and insemination with sperm from donor or partner.

Many of these fertility problems may be due to 'late onset' of developmental problems with origin from exposures of fetal gonads, although germ cells are susceptible for endocrine disruption at all stages including the mature sperm.

During the past half century where we have witnessed increasing male reproductive problems, birth rates have plummeted below replacement levels in many countries, e.g. Japan, Germany, South Korea and Singapore. We urgently need to explore the possible role of environmental exposures for these changes, which undoubted will result in fewer young and relatively more old people, and – eventually- falling populations [1,2].

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S02-03
Epigenetics in fetal susceptibility to toxicity

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The global incidence of obesity and related metabolic disorders represent the major public health challenges of our time. It is increasingly clear that environmental factors early in development, such as exposure to chemicals, play a role in the aetiology of obesity. Using an integrated toxicological and epidemiological approach to research the role of chemicals in obesity, recent research has shown that that prenatal exposure to endocrine disrupting chemicals affects processes involved in obesity development, including increased differentiation of adipocytes and altered function of adipocytes, changes in energy metabolism and elevated weight in childhood. A major challenge for the future is to better understand how fetal chemical exposure programs an organism to be more susceptible to disease later in life and even across generations. Environmental perturbations to the epigenome during development can affect gene expression patterns resulting in a phenotypic change, a process known as altered epigenetic programming. Alterations in epigenetic programming are increasingly associated with common human diseases, such as cancer, cardiovascular diseases, type 2 diabetes, and obesity. However, while the field of epigenetic research in human health is advancing at an astonishing rate together with technologies that allow high-resolution genome-wide characterization of multiple epigenetic marks, our understanding of how chemical exposure during development alters the programming of lifelong health is still in its infancy. This presentation will present the state of the art of the role of chemical exposures in metabolic disorders. It will also describe translational research to determine the molecular epigenomic mechanisms underlying the effects of developmental exposures on obesogenic phenotypes that persist into adulthood and in subsequent generations.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825489 (GOLIATH)".

S02-04
Towards a mechanistic approach in toxicology: Retinoic acid balance disturbance leading to neural tube closure defects

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Retinoid signaling plays an important role in vertebrate embryo-fetal development and its disruption is teratogenic. The retinoic acid (RA) pathway controls retinoid homeostasis and regulates embryonic cell fate via nuclear receptor (RAR, RXR) activation. The RA pathway thus serves as an excellent prototype for adverse outcome pathway (AOP) elucidation associated with developmental defects. The disruption of the RA pathway, leading to defects in neural tube closure, was the basis for the construction of a developmental toxicity ontology. The prototype ontology describes retinoid homeostasis and putative molecular initiating events in chemical teratogenesis. Basic elements in the ontology are subjects (enzymes, receptors, cell types) and their quantitative relationships (response-response relationships), together forming a network of biological interactions that can be mapped to a vulnerable window for teratogen-induced neural tube defects such as spina bifida. We have searched literature using text-mining tools that allowed rapid identification of relevant information. We collected known molecular interactions, genetic signals and responses that: (a) play a crucial role in neural tube cellular differentiation; (b) establish anterior-posterior gradients (FGF and RA signaling) and dorsal-ventral gradients (zinc factors (Zic) and BMP signaling) for regional specification. Molecular initiating events important for RA balance (like CYP26 enzymes and RALDH2) potentially affected by xenobiotic compounds (using high-through-put screening data), were connected with toxicological data on the development of posterior neural tube defects. Ultimately, this network can be dynamically modeled *in silico*, providing an integrated computational systems model with which toxicity predictions can be made at the level of adverse outcomes in the intact individual. *This work does not reflect EPA policy.*

S03 | The exposome – understanding the role of environmental exposure in human health and disease

S03-01
EXPOsOMICS: Novel approach to the assessment of exposure to high priority environmental pollutants

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EXPOsOMICS is a European Union funded project that aims to develop a novel approach to the assessment of exposure to high priority environmental pollutants, by characterizing the external and the internal components of the exposome. It focuses on air and water contaminants during critical periods of life. To this end, the project centres on 1) exposure assessment at the personal and population levels within existing European short and long-term population studies, exploiting available tools and methods which have been developed for personal exposure monitoring (PEM); and 2) multiple "omic" technologies for the analysis of biological samples (internal markers of external exposures). The search for the relationships between external exposures and global profiles of molecular features in the same individuals constitutes a novel advancement towards the development of "next generation exposure assessment" for environmental chemicals and their mixtures. The linkage with disease risks opens the way to what are defined here as 'exposome-wide association studies' (EWAS).

S03-02
Chemical exposure metabolomics

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During our lifetime we are exposed to an assembly of food and environmental chemicals. These exposures broadly impact the etiology of a large share of human disease, but occurrence and mechanisms often remain elusive and toxicological interactions are poorly understood. To address this key issue in current public health research, the concept of the exposome, i.e. investigating the sum of lifespan exposures and their biological effect, was proposed but analytical technology and bioinformatic data processing remain a major limitation to the field [1].

In this contribution the role of metabolomics in addressing the current issues in exposome research will be discussed. Specifically, a global metabolomics/exposomics workflow based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) will be presented which allows for the simultaneous readout of exposure and effect in biological samples [2]. Moreover, metabolomics-guided pathway analysis for deciphering the toxicological impact of a specific toxin or toxin mixture in cell-based models will be a focus [3]. Proof-of-principle experiments will highlight the advantages and limitations of this approach. Furthermore, the expansion of METLIN, an important resource for metabolite identification, will be presented. This metabolite and mass spectrometry spectral repository recently became a major exposome database with more than one million unique metabolites. This includes a large share of environmental toxicants, food bio-actives and contaminants, drugs, and other xenobiotics. Finally, the potential of global metabolomics and metabolomics activity screening [4] for the comprehensive investigation of drug-exposome interactions at the systems biology level will be presented. As an example the interplay between dietary xenoestrogens and a breast cancer combination therapy in a cell model will be discussed [5].

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S03-03
Challenges and promises of the Exposome concept for environmental health research

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The Exposome encompasses all environmental exposures humans undergo from conception to death. This concept, which calls for a holistic view of exposures, is in line with the (mostly) observational nature of epidemiology and with the recognition that, following the industrial revolution, humans are exposed to thousands of chemical factors, several of which could influence health on the short or long-terms.

The promises of the Exposome include first a descriptive component, corresponding to a thorough characterization of the distribution of exposure levels to numerous factors in human populations [5], of their mutual correlations [9] and of of "environmental inequalities", understood as the variations of exposure levels across socio-demographic categories [7]. A second ("exposure-specific etiologic") promise relates to a better understanding of the possible health and biological effects of each exposure considered separately [e.g. 1,7,11]; here, exposome-wide studies offer the opportunity to limit publication bias and selective reporting of results, which plague epidemiological research. A third and possibly longer-term ("exposome-wide etiologic") promise lies in the establishment of a ranking of the health effects and possibly population impacts of all exposures, as an input for "burden of disease" studies, a quantification of the overall share of the environment in disease aetiology, and in the identification of synergistic effects between exposures.

Huge challenges face those tempted by the Exposome adventure, both in terms of exposure assessment (e.g., do we have tools to successfully increase the number of exposures assessed without simultaneously increasing exposure misclassification? Can the temporal component of the Exposome be efficiently characterized? Do some 'omics layers offer opportunities to improve exposure assessment?) and statistical modelling (e.g., can the rate of false positive efficiently be controlled? Is there enough power to identify synergy between exposures?). We discuss these issues in light of the first results of HELIX early-life exposome project [6,10], in which a series of biostatistical simulations [2,3] as well as analyses on real data [1,7,11] have been undertaken. The latter are based on a unique cohort of 1300 European mother-child pairs in whom over 100 exposures (based on biomarkers and environmental models) were assessed, and health outcomes, DNA-methylation, transcriptomics as well as metabolomics were characterized.

Without immense efforts and creativity from the research community and financial support equivalent to that provided to establish the sequence of the human genome, the cruise towards the Exposome might end in the cliffs of the Lorelei, with a collection of underpowered studies with strong measurement error. A source of creativity would come from toxicologists and epidemiologists progressing towards a common language and stronger collaborations for improved knowledge in environmental health.

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S03-04
Developing the regulatory utility of the exposome

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The Exposome offers new risk assessment opportunities that build from our previous "one chemical at a time approaches" for risk assessment. Although risk assessment guidelines exist for dealing with mixtures these have been largely guided by predicted mixtures from environmental assessments. In many cases these measured environmental assessments are then summed to identify the potential for multiple and potentially interacting exposures. Various risk models have been available to take these summed exposures or distributions to predict health risks. In some cases, physiologically based pharmacokinetic modeling is linked and these estimates reflect probability of exposure with resultant impacts on health. Because the exposome can allow for a comprehensive assessment of exposure profiles over our life stages it provides key biomonitoring data from which to improve these health assessments. The challenge to these multi-faceted exposure profiles is then how to predict risk. Techniques that are chemical, mechanism, and disease based will be presented. The strengths and weaknesses to each of these methods will be first provided in the context of addressing problem formulation for risk assessment and secondly in the context of answering a broader question of wellbeing.

S04 | How innate immune cells recognize toxicants

S04-01
Innate cells sense toxicants as microorganisms – introduction

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S04-02
Inflammasomes in inflammatory pathology

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Inflammasomes are a set of multi-protein complexes that drive production of the inflammatory cytokines interleukin (IL)-1β and IL-18, and induction of lytic cell death. These innate immune pathways respond to a broad suite of pathological agents and contribute to a broad range of diseases with an inflammatory component. Here I will review and discuss the roles and mechanisms by which inflammasomes drive inflammatory pathology.

S04-03
Metal-induced immunotoxicity: ionic metals, innate immune receptors and skin allergy

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Allergic contact dermatitis (ACD) is a common inflammatory skin disease caused by an unnecessary immune response to topically applied allergens. According to a large epidemiological study, 27% of the general population in 5 European countries suffer from ACD to at least one of the common contact allergens, and nickel was among the most prevalent ones. In fact, metal allergy is the most frequent type of contact allergy, with nickel allergy affecting 14.5% of the European population. The other well-described metal allergens are cobalt, chromium and palladium. People are mostly exposed to metals by contact with jewelry, clothing ornamentation and coins.

From an immunological point of view, ACD is the result of T-cell activation mediated by T cells recognizing low-molecular weight chemicals or metals ions presented by antigen-presenting cells such as dendritic cells (DC). However, the presence of the contact sensitizer per se is usually insufficient to promote a T-cell response. A second signal, noted "danger signal", is mandatory for effective DC activation and subsequent T-cell priming. Nickel is the perfect example of a "complete" contact sensitizer that follows this "two-signal" hypothesis. Alongside its ability to act as an antigen, nickel ions can directly trigger activation of human Toll-like receptor 4 (TLR4) on DC. During the sensitization phase of ACD, mature DCs play an important role in driving T-cells polarization into different T helper (Th) cell type which influence the clinical outcome. Recently, nickel-specific Th17 cells were found in the blood and the skin of nickel allergic patients. The outcome of T-cell polarization (Th1, Th17) is probably an important factor in the pathophysiology of ACD to metals.

Our hypothesis is that metals are acting directly on the DC to promote phenotypes altering T-cell polarization. Indeed, NiSO4-treated human DCs produced a higher IL-23/IL-12p70 ratio compared with untreated DCs or NiSO4- and IFN-gamma-treated MoDCs, thus promoting Th17 polarization. In this presentation, results will be presented showing the interplay between different signaling pathways and immune receptors activated in the DC by metals that could explain the mechanism of metal skin allergy.

S04-04
Characterization of inflammatory responses and redistribution of MWCNT following aerosol exposure in B6C3F1 mice

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The majority of research on the potential adverse effects of nanomaterials has focused on lung inflammation. However, little is known regarding systemic effects of inhaled MWCNT. Therefore, we examined lung and systemic effects in B6C3F1 mice that were exposed to a relatively pure MWCNT (L-MWCNT-1020, Sun Innovation). *In vitro* studies suggested that this material had very little toxicity or ability to stimulate the NLRP3 inflammasome using THP-1 macrophage-like cells. B6C3F1 mice were exposed by inhalation at concentrations of 0.06, 0.2, 0.6 mg/m³ with humidified air. Mice were housed in Hazelton 2000 exposure chambers and exposed for a total of 22 days over a period of one month. The mice (10 mice per group along with a filtered air exposed group) were received 2 weeks post exposure and tissues collected within 24 hr of arrival. Cytokines in lung lavage fluid and plasma were analyzed using Meso Scale Discovery. Laser Scanning microscopy, Stimulated Raman Scattering and CytoViva

(Spectral Feature Fitting) was used to analyze MWCNT in macrophages and distribution in tissues. Flow Cytometry was used to examine changes in various immune cells in the lung and spleen. We observed minor increases in IL-1b, TNF-a, and IL-6 in lavage fluid. In contrast, we noted significant dose dependent decreases in TNF-a, IL-6, IL-10 and IL-33 in plasma and an increase in IL-1b. Macrophage numbers in the lung interstitium substantially decreased. The content of MWCNT in lung macrophages increased dose-dependently. Stimulated Raman scattering and CytoViva techniques demonstrated that MWCNT redistributed from the lung through the lymph nodes to the spleen where there were distinct changes in lymphoid cell populations including increases in cytotoxic T cells and B cells. Histological examination of spleens demonstrated reduction in white pulp and presence of apoptotic bodies. Therefore, an apparently benign nanomaterial caused significant systemic effects. Consequently, reliance on *in vitro* screening needs to be re-evaluated for triaging nanomaterials for *in vivo* studies.

S04-05
Revisiting the paradigm of silica pathogenicity: molecular description of the toxicity-relevant surface features

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Occupational exposure to respirable crystalline silica (RCS) dust may cause severe pathologies, including silicosis and lung cancer [1]. RCS is also a trigger for systemic autoimmune diseases [2]. The mechanism by which RCS elicits adverse effects on human health includes persistent inflammation and tissue remodelling. RCS-induced inflammogenic activity is elicited by the activation of the NALP3 inflammasome after lysosomal membrane perturbation is being sensed [3,4]. Only crystalline silica particles with specific surface features, generated during fracturing, were shown to destabilize cell membrane and induce inflammatory responses, whereas intact crystals in respirable size elicited negligible responses [5]. Silica surface is populated by families of silanols (≡Si-OH), week monoprotic acids that impart, at physiological pH, a negative surface charge (≡Si-OH ⇔ ≡Si-O^- + H^+). According to their surface chemical arrangements, silanol families are characterized by different chemical properties [6] and only few specific silanol families can establish strong interactions with biomolecules, including polar heads of membrane phospholipids. To investigate how surface chemistry modulates the inflammatory response of quartz, model silica samples were prepared and surface tailored. Recent advances in RCS synthesis and an innovative surface-specific spectroscopic approach allowed us to correlate the quantitative description of the silanol families with cell membrane destabilization, cytotoxicity, and inflammation *in vivo*. Current results deliver a novel understanding of the molecular initiating event of silica-induced inflammation and suggest molecular recognition phenomena to take place between silica surface silanols at a specific distance apart and biological supramolecular structures, including lysosome membranes.

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S05 | New tools and application in reg. risk assessment – moving toward mechanistic risk assessment

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S05-01
Development of *in vitro* tests – quality assurance and cross system testing

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In the EU-ToxRisk project several *in vitro* test methods from different laboratories were developed and combined. These *in vitro* test methods cover liver, lung, kidney and the nervous system and were combined with further new approach methods (NAM), such as toxicokinetics, PBPK modeling to a comprehensive organ IATA. This was accompanied by several challenges including the implementation of a prediction model and graphical representation of the data of the whole battery. Further, a unified, ideally universal, procedure on how to document the methods, and the data obtained therefrom is a precondition for the use of the test information for academic prediction models, strategic decisions and/or for regulatory purposes. The EU-ToxRisk project developed such a generic scheme, consisting of three blocks, to provide regulatory valid data, using an exemplary panel of >20 assays. Besides the classic standard operating procedure (SOP) documents, we have implemented a new database of in depth methods descriptions that is accessible by any interested user. These test method descriptions focus on the readiness of the different test methods, which includes the toxicological needs such as baseline variations, positive and negative controls, sensitivity and specificity. The IATA comprises different *in vitro* models, which provide multiple pieces of evidence and differ with regard to their uncertainty. This talk will present the newly established data documentation pipeline and the outcome of a cross system testing with 19 compounds within the different *in vitro* models.

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S05-02
Modelling the impact of several *in vitro* systems in a read-across approach – applicability of the Dempster-Shafer Theory

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Purpose: Dempster-Shafer Theory is presented as an unbiased alternative for merging information from several sources into a final outcome/decision incorporating the underlying uncertainty of the sources.

Method: Dempster–Shafer theory (DST), a generalization of the Bayesian theory of subjective probability, is used for merging the

outcome from several *in vitro* and *in silico* models in order obtain an unbiased overall prediction taking the varying uncertainties of the models into account. DST is applied to read-across examples from the EU-ToxRisk Project case studies as well as genotoxicity. The input data to DST, in addition to the model predictions for the target(s), consist of reliability as well as positive and negative prediction accuracy of the model obtained through internal cross-validation of the sources.

Results: The results demonstrate that DST produces, for the most part, well defined overall predictions with relatively small uncertainties when using a sufficient portion of sources of good quality, determined by the internal cross-validation. The opposite result, i.e. overall predictions of poor quality and large uncertainties, is obtained if, deliberately, using sources with lower reliability as well as prediction accuracies.

S05-03
Incorporating QIVIVE and PBTK into toxicity testing and assessment

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The vision for toxicity testing and risk assessment in the 21st century aims to increase the use of human relevant *in vitro* model systems and reduce, refine and, ultimately, replace the use of animal models through quantiative *in vitro* to *in vivo* extrapolation (QIVIVE). An important component of successfully implementing this strategy is the use of modelling and simulation to translate the toxic effects and associated concentrations at which these effects are observed from the *in vitro* to *in vivo* situation. Biokinetic modelling of the distribution of test compounds in *in vitro* cell assay systems allows the prediction of the free culture medium and intracellular concentrations. Predictions are made based on the physicochemical properties (e.g. $\log P_{ow}$, pKa, solubility) of test compounds and the set-up of the *in vitro* assay (e.g. cell type, cell number, composition of culture medium). These concentrations can be considered as being the more relevant driving concentrations in translating toxicological endpoints quantified *in vitro* to *in vivo*. Whole-body physiologically based toxicokinetic (PBTK) modelling and simulation enables the prediction of systemic and tissue exposure to compounds in both human and non-clinical animal models. Thus, PBTK models can not only inform the translation of *in vitro* toxicodynamics to *in vivo*, but also facilitate the translation from animal to human. Integrating these two modelling approaches, the effective concentrations identified *in vitro* can be corrected to more *in vivo* relevant driving concentrations, and then, through a reverse-dosimetry approach, translated to an equivalent human dose resulting in plasma or target tissue concentrations identified as hazardous *in vitro* can be predicted through simulation with PBTK models. Drawing on experiences from within the EU-ToxRisk project, as well as the wider literature, the advantages and current challenges in using biokinetic and PBTK models as part of an integrated approach to chemical testing and risk assessment will be discussed.

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S05-04
Development of qualitative and quantitative AOPs and their integration into risk assessment

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Chemical hazard assessment can directly use qualitative adverse outcome pathways (AOPs) to integrate data generated by alternative methods or *in vivo* testing. Risk assessment requires quantitative relationships from exposure to effect timing and magnitude: quantitative AOPs (qAOPs) should be able to provide such dose-time-response predictions. There is also an intermediate level of quantification, in which qAOPs are able to make predictions about the probability of a chemical to belong to a category such as toxic/nontoxic, or low/medium/high toxicity. Bayesian networks have typically been used in the latter case, and are suitable for refined hazard assessment. We will first briefly review the various methods and their main applications so far.

In EU-ToxRisk, we have extended the Bayesian network (BN) approach to encompass continuous dose-time-outcome qAOPs. We compared BN to empirical dose-response modeling and to systems biology (SB) modeling. This was done for an oxidative stress induced chronic kidney disease AOP, using *in vitro* data obtained on RPTEC/TERT1 cells exposed to potassium bromate. We showed that, despite the fact that dose-response models give adequate fits to the data they should be accompanied by mechanistic modeling to gain a proper understanding of domain of applicability of the quantification. BNs can be both more precise than dose-response models and simpler than SB models, but more experience with their use is needed.

We have since extended our work to qAOPs of mitochondrial disruption induced toxic effects in HepG2 (liver), RPTEC/TERT1 (kidney) and LUHMES (neuronal) cells, after exposure to several chemicals, and present those new results in this session. Comparison of the results across cell types and chemicals will be discussed, together with the assumption of chemical independence of the qAOPs developed.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 as well as from the Innovative Medicines Initiative 2 Joint Undertaking (IMI2/JU) under grant agreement No 777365.

S06 | Developments in the use of systematic review in chemical risk assessment

S06-01
Principles of systematic approaches for chemical risk assessment

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Health risk assessment is a complex process that often relies on case-by-case expert judgment. The basic risk assessment principles are similar regardless of the legislative framework, but the amount, quality and type of data available, as well as how expert judgment is applied, vary. In addition, there are often considerable uncertainties,

for example due to lack of appropriate data or due to conflicting data. Thus, case-by-case expert judgment requires extensive expertise and long experience. In addition, risk assessment is getting increasingly complex due to new regulatory demands and scientific possibilities, e.g. the ambition for using non-animal methods and making use of all available information (of varying relevance and reliability), as well as inclusion of mechanism/mode of action evidence into the risk assessment. Moreover, society struggles with particularly complex issues, such as endocrine disrupters and mixtures.

The demand for transparency in risk assessment is increasing, both from the public and from stakeholders involved. People want to know and understand the scientific basis for the specific conclusion and the reasoning behind it. Systematic approaches (based on systematic review methodology) are now starting to be used by several organizations and authorities. Such methodology is also required in legislations, such as the EU legislations for identification of endocrine disrupters in plant protection products and biocides.

Systematic approaches usually include the key steps of a systematic review. These steps are: preparing the review (protocol development), data identification (search strategy, selection of studies), extracting data from studies, appraisal of studies for methodological quality, data synthesis, presentation of results, interpretation of results and drawing conclusions. In a risk assessment thorough integration of data, often from different types of study designs, is often a critical step.

A number of tools have been developed to aid the systematic process of risk assessment. For example, tools are available for extraction of data as well as for assessment of the relevance and/or reliability of individual studies. Also, systematic methods for integration of data and weight of evidence assessment are being developed.

In order to perform sustainable risk assessments for all the numerous chemicals used in society, in accordance with both new legislative demands and scientific development, there is a need for further development and implementation of systematic approaches, tools and frameworks that also increase transparency and are useful for training of future risk assessors.

S06-02
Getting the balance right between objectives and resources for a systematic review – the importance of problem formulation

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Problem formulation has the goal to explicitly define the question(s) or statement(s) to be evaluated and to develop the process of how the systematic review evaluation will be conductedin order to maximize its utility for human health risk assessment. It identifies all factors critical to a systematic review and considers the purpose of the assessment, scope and depth of the necessary analysis, analytical approach, available resources and outcomes, and overall risk management goal. Problem formulation is an iterative process, starting in the planning and scoping phase with defining the problem, gaining a sense of the literature, considering depth, breadth and boundaries of the analysis. Framing the specific review question(s) is a critical step and often challenging in that it needs to be done in a way that makes the overall problem amenable to systematic reviews. This is helped by using the PECO concept (Population, Exposure, Comparator, Outcome) as the framework to formulate specific questions, e.g. does chronic oral exposure to a given chemical (E) induce a specific health effect (O) in children (P) compared to unexposed children of the same age (C)? An example will be shown to illustrate how pathway-oriented thinking can help develop a conceptual model of how different evidence streams can be integrated to address specific subsets of the overall problem. Systematic reviews are hugely time- and labour-intensive and require the expertise of various disciplines. It is therefore important to decide as part of the problem formulation whether a full systematic review is required or whether other, less-resource-intensive tools such as evidence mapping, scoping reviews or rapid evidence assessments may serve the intended purpose. This will depend on the context of how the output of the review process is going to be used, e.g. to generate general awareness of the evidence base, or make a risk management or even a policy decision. The importance of outreach to technical experts, stakeholders and the public during the process of scoping and focusing an evaluation question is emphasized.

S06-03
Systematic review in the regulatory food safety area – experiences from a JECFA evaluation

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A systematic review is a way of collecting and evaluating scientific literature in a transparent and reproducible manner using prespecified and standardized methods to answer a specific research question. Systematic reviews have been widely used in all kind of areas related to human health, most often in intervention studies in clinical and non-clinical settings. Nonetheless, its application in the area of food safety has been rather scarce. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was requested by the Codex Committee on Contaminants in Food (CCCF) to perform a full risk assessment on pyrrolizidine alkaloids (PAs). PAs are plant toxins that can be found in about 6000 plant species. PAs are genotoxic carcinogens and can cause apart from various tumors other serious health effects like hepatic veno-occlusive disease. A systematic review on PAs was initiated to identify all relevant information with respect to their biochemistry, toxicity and human health effects as input for the risk assessment. In this way, experience for JECFA was gained with the usefulness of applying the systematic review methodology in a broad risk assessment question.

For development of the systematic review protocol, the methodology as outlined in guidance published by the European Food Safety Authority (EFSA), World Health Organization (WHO) and the National Toxicology Program (NTP) was followed. The SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) provided support via a hands-on training and coaching during the systematic review. Six research questions were formulated to capture all publications related to the biochemical aspects and toxicological effects of PAs found *in vitro*, in animals and in humans. Based on the search strategies developed for these six research questions, scientific peer-reviewed articles were searched in several databases, such as MEDLINE, Embase, Toxcenter, CAB abstracts etc. In addition, grey literature was searched at websites of known risk assessment bodies using PA names or the names of PA-containing plants. The systematic review methodology was followed up to and including the phase of selecting articles based on title and abstract. Due to the large number of publications retrieved, it was resource-prohibitive to continue to follow the systematic review protocol and thereafter, the regular JECFA approach of appraisal of studies for risk assessment was followed. Experiences obtained from conducting this systematic review and lessons learned will be shared. Ideas on the applicability of a systematic review for answering food safety-related questions will be discussed as well.

S06-04
Use of systematic review methods by national programs – example from the USA

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Systematic review is a predefined, multistep process that fosters a transparent, rigorous, objective, and reproducible identification and evaluation of scientific evidence to reach conclusions on specific research questions. Because of their transparency and objectivity, systematic review frameworks are increasingly utilized and, in some cases, mandated by law for developing risk assessments in United States federal programs. Hazard conclusions and risk assessments on environmental chemicals need to consider a broader evidence base than the narrow clinical datasets that systematic review methods were originally developed to address. The National Toxicology Program's Office of Health Assessment and Translation (OHAT) established the OHAT Approach for Systematic Review and Evidence Integration to reach hazard conclusions by integrating relevant data from epidemiological studies, animal toxicology studies, and mechanistic information. The potential association between exposure to traffic-related air pollution and hypertensive disorders of pregnancy was recently evaluated using the OHAT approach. This evaluation will serve as a case example to demonstrate key steps in systematic review of environmental questions including: 1) summarization of the problem formulation efforts and the role of stakeholders in defining and refining the research question; 2) development of a protocol to outline the approach for conducting the review; 3) use of specific tools to identify and extract data from relevant studies; 4) application of a risk of bias tool to assess internal validity of individual studies; 5) rating confidence in available studies using the GRADE framework to consider the strengths and weaknesses of the bodies of evidence; and 6) incorporation of qualitative and quantitative approaches for integrating evidence to reach hazard conclusions. In addition, this case example will demonstrate an approach as well as key considerations for developing hazard conclusions across multiple exposures. Finally, the use of mechanistic data by the National Toxicology Program will be discussed within the context of ongoing discussions by multiple environmental health groups to consider mechanistic studies in human health assessments for decision-making.

S07 | Speeding up hazard assessment of nanomaterials

S07-01
Hazard assessment of engineered nanomaterials: setting the scene

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Engineered nanomaterials (ENMs) are being developed at an increasing rate with numerous new materials being placed on the market every year. For this reason, it is important to speed up the hazard assessment of ENMs, in support of risk assessment and regulation [Fadeel B, et al. Advanced tools for the safety assessment of nanomaterials. Nat Nanotechnol. 2018;13(7):537-543]. The implementation of new test paradigms that make use of mechanism-based *in vitro* assays promises to speed up hazard assessment and may also provide a basis for assigning structure-activity relationships of ENMs. Systems biology/systems toxicology appproaches, combining so-called omics methodologies with detailed computational analysis of the data, may shed light on the underlying toxicity pathways and the mode-of-action of nanomaterials. Moreover, omics data may inform the development of adverse outcome pathways (AOP) that enable the representation of mechanistic toxicity data in support of risk assessment. New approaches for hazard assessment of ENMs have been developed in recent years not least in the frame of the EU-funded projects, FP7-NANOMILE and FP7-NANOSOLUTIONS. The present EUROTOX session on "Speeding up Hazard Assessment of Nanomaterials" provides an overview of the state-of-the-art of new and emerging approaches in hazard assessment of nanomaterials including high-content/high-throughput screening, systems toxicology approaches, and development of adverse outcome pathways. The present lecture will set the scene and will also provide a few examples of omics-based approaches using *in vitro* and *in vivo* models to explore and predict the biological impact of different ENMs including graphene-based materials.

S07-02
High – throughput /-content – screening of nanomaterials as a versatile tool for hazard assessment

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For effective safety screening and hazard ranking of manufactured nanomaterials (MNMs), it is necessary to speed up the testing by *in vitro* test systems which include microscopy-based high-throughput/-content (HT/C) methods. Microscopy-based screening approaches not only reduce time and costs, but also circumvent misinterpretation of results obtained by some conventional toxicity tests, with which MNMs often interfere. They can also provide the basis for quantitative structure (property)–activity relationships (QS(P)ARs), allow identification of no-observed-adverseeffect levels (NOAELs), support the development of MNMs with improved safety, help to rapidly unravel toxicity or adverse outcome pathways (AOPs) of MNMs and refine, reduce or replace (3 Rs) animal experiments, all of which could have an impact on the decisions and approaches of regulatory authorities. Nanomaterials selected from a library of over 120 different MNMs with varied compositions, sizes, and surface coatings were tested within the European project NanoMILE by four different laboratories for toxicity by high-throughput/-content (HT/C) techniques. The selected particles comprise 14 MNMs composed of CeO_2, Ag, TiO_2, ZnO and SiO_2 with different coatings and surface characteristics at varying concentrations. The MNMs were tested in different mammalian cell lines and zebrafish embryos to link physical–chemical properties to multiple adverse effects. Some of the key findings will be presented including also the pros and cons of HT/C assays. The broader applicability of the HT/C technology will be exemplified in more detailed follow-up studies for silica MNMs to address mechanisms of toxicity and the role of surface coverage for biocompatibility.

S07-03
Systems biology approaches for nanomaterial hazard classification

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In classical toxicology, the phenotypic effects are analysed to evaluate the hazardous potential of the tested compounds, but virtually

nothing is known about exposure-dependent molecular alterations. Therefore, while this approach allows the development of predictive models, where relevant intrinsic properties of the exposures are related to phenotypic effects, it gives no information on their mechanism of action (MOA).

Omics technologies are increasingly used in (nano)toxicology to clarify the exposures MOA mediating their phenotypic effects. Although some efforts have been already made to promote the use of omics for regulatory purposes, there is still not a complete integration of omics-based evidence in these decisions for multiple reasons. First, omics experiments are laborious, expensive and the analysis of their readout is complicated. Second, rapidly evolving technologies and analytical methods often prevent their standardization. Third, there is a general lack of knowledge concerning the best practices, consequently creating a divergence between the expectations and the realistic possibilities of omics technologies, especially among industrial and legislative bodies.

Our team's ultimate goal is to provide robust and highly accessible methods to facilitate the implementation of toxicogenomics studies for hazard and risk assessment. By integrating multiple data layers, we build comprehensive models that explain the exposure MOA and predict their hazard potential. In this talk, I will present examples of our activities, which include:

- The development of eUTOPIA, an integrated software for the standardized preprocessing of toxicogenomics data.
- The development of INfORM, a software for the robust inference of molecular networks, and its application to assess similarities between *in vitro*and *in vivo* ENMs MOA.
- The definition of multi-omics approaches to define ENMs MOA.
- The development of computational solutions for the dose-dependent and dose/time dependent analysis of omics data.
- The development of INSIdE NANO, an integrated network-based computational approach for MOA-based ENMs prioritization and read-across.
- The development of integrated and Systems toxicology-based predictive models.

Our results contribute to a full integration of omics-derived evidence to the safety assessment of ENMs and further promote a systems toxicology approach in nanotoxicology.

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S07-04
Systems toxicology to support development of adverse outcome pathways

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"Systems toxicology" represents a recent and rapidly developing "Big Data analysis era" in the environmental health sciences field, including the nanotoxicology discipline. Steadily increasing amounts of safety testing results need to be comprehensively interpreted related to accumulated public data and applied to risk characterization of manufactured nanomaterials. Regulatory "systems toxicology" embraces the network character of systems biology as well as the complementary linear analysis scheme characteristic of the adverse outcome pathway (AOP) concept. Focused to addressing these areas, our laboratory collaboration utilizes a tiered testing approach built on the analysis of high-throughput screening-derived cytotoxicity and omics results in cell culture models, allowing libraries of nanomaterials and other agents to be cost effectively hazard ranked to the mode-of-action (MoA) level. We furthermore generated a 14 gene component-based "predictive toxicogenomics space (PTGS)" concept that provides toxicity estimates intrinsic to omics-data via broad dose-dependent coverage of toxicity reactions and mechanisms. Recent validations show that PTGS enables application of *in vitro* data to predict tissue injury in multiple organs of experimental animals subjected to long-term repeated-dose toxicity bioassays. A further successful dimension of the concept is prediction of human drug-induced liver injury. The omics data-based PTGS component modeling concept may well be the first high-throughput analysis tool that effectively bridges systems toxicology and AOP-directed linear data integration, as it serves excellently to define toxic MoA coupled also with identification of key events in AOP schemas. We consider overall our high throughput tiered testing and PTGS concept to be directly applicable to an evolving *in vitro* and *in silico*-based safety testing market, including taking a precautionary safety-informed approach to the manufacturing of nanomaterials.

S08 | Human adaptation to environmental pollution: dose-response relationship revisited

S08-01
Hormesis and hormetins for healthy ageing and longevity

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A contemporary understanding of the biological basis of ageing rules out the existence of any gerontogenes which cause ageing. Genes determine our ability to live and maintain health for a limited period, known as the essential lifespan (ELS), required by nature for the con-

tinuation of the species. We are surely able to live much longer than our species' ELS, but our natural survival abilities then need a helping hand for living longer, for maintaining health and beauty, and for protecting ourselves from diseases in old age. A promising and wholistic approach for achieving this is the phenomenon of mild stress-induced hormesis. Physical, nutritional and mental hormetins, which induce hormesis, lead to the stimulation and strengthening of the maintenance and repair systems in cells and tissues. Exercise, heat and irradiation are examples of physical hormetins, which activate heat shock-, DNA repair- and anti-oxidative-stress responses. Several non-nutritional chemical components in the food, such as flavonoids and polyphenols present in spices, herbs and other sources, are examples of nutritional hormetins, which induce anti-oxidative and anti-inflammatory stress responses. Calorie restriction and intermittent fasting are also hormetins, which activate the autophagic and sirtuin-mediated stress responses. Intense brain activity and focussed attention comprise mental hormetins, which also induce various stress responses. A combination of different hormetins can therefore be the drugs for maintaining, improving and recovering health during ageing.

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S08-02
Biomarkers of adaptive responses in human health

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Since 1948, the WHO defined health as a state of complete physical, mental and social well-being. Currently, a more dynamic definition is used, *viz*., the ability to adapt. Adaptation is an important feature of evolution. Development of resilience to the evolutionary toxicity of oxygen is a major example of adaptation.

The design of drugs employs a form of selective toxicity in order to reach health according to the 1948 definition. Drugs act specifically and preferably via one target, thus preventing side effects. Food however displays a more multi-target action and is in line with the adaptive health response. In fact, the health promoting effect of many bio-actives in fruit and vegetables can be seen as the effect of mildly toxic compounds triggering this adaptive stimulus. The challenge is to measure these frequently mild physiological or hormetic responses. One way is to integrate the various mildly changing health parameters. Another way is to challenge the physiological system to a stressor and investigate the developed resilience. In case of quantifying the health effect of food, both methodologies have been applied. Adaptive responses largely explain the health benefit of fruits and vegetables [1].

We increasingly recognize that environmental toxicants frequently also display a hormetic response [2]. This has immense consequences in risk assessment [3]. We now understand the molecular mechanisms of this hormetic response. This enables us to define specific biomarkers to follow this process [4].

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S08-03
Clues to adaptation of the human population to the environment: lessons from Czech biomonitoring studies

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Living organisms, including humans, are continually exposed to numerous harmful environmental factors, causing negative biological effects and/or deregulation of biomarker levels. However, studies reporting no or even positive impacts of some stressors on biological systems are also reported. Such observations are in conflict with the concept of the linear dose-response relationship that is generally accepted in toxicology. This concept assumes that negative effects associated with exposure to toxic compounds or radiation increase linearly with increasing dose. Despite of that, results of numerous studies suggest that organisms may adapt to adverse effects of the environment. In this process, repeated exposure to low levels of toxicants induces protection of the organism against negative effects of higher doses. This phenomenon is called adaptive response. Although most of the data on adaptation was received in model systems, results of some studies indicate that this response appears also in humans exposed to radiation or environmental pollutants suggesting the adaptation to be a general biological phenomenon. Nevertheless, the concept of human adaptation is considered controversial by many scientists and mechanisms of its induction are not well understood.

Here, a comprehensive overview of the last decade of Czech biomonitoring research, concerning the effect of various levels of air pollution and radiation on the differently exposed population groups is presented. Because some results obtained from cytogenetic studies were in conflict with hypotheses, we have searched for a meaningful interpretation in genomic/epigenetic studies. A detailed analysis of our data supported by the studies of others and current epigenetic knowledge, leads to a hypothesis of the versatile mechanism of adaptation to environmental stressors via DNA methylation settings which may even originate in prenatal development, and help to reduce the resulting DNA damage levels. This hypothesis is fully in agreement with conflicting data from our studies. It is also supported by differences in DNA methylation patterns in groups from regions with various levels of pollution. In light of the adaptation hypothesis, the following points are suggested for future research: (i) the chronic and acute exposure of study subjects should be distinguished; (ii) the exposure history should be mapped including place of residence during the life and prenatal development; (iii) changes of epigenetic markers should be monitored over time. In summary, investigation of human adaptation to the environment, one of the most important processes of survival, is a new challenge for future research in the field of human biomonitoring that may change our view on the results of biomarker analyses and potential negative health impacts of the environment.

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S08-04
The role of microRNA in adaptive response to environmental carcinogens

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MicroRNA are pivotal regulators of gene expression at postgenomic level. This regulations deeply affect early response of organism to the counteract the damages induced by exposures to environmental carcinogens. In the few hors following the start of the exposure, the microRNA machinery promptly react changing its expression mainly in the sense of selective downregulation thus allowing the full expression of genes encoding for phase I/II detoxification activities as well as DNA/protein repair (Izzotti et al, 2009). Indeed, in the unexposed lung there is not any correlation between the expression messenger RNA and related proteins of these defensive activities; however, when the lung is exposed to hexavalent chromium, the correlation is promptly restored due to the downregulation of microRNA that do not block any more the translation of these messenger RNAs into proteins (Izzotti et al., 2004). This adaptive response is based on molecular mechanisms making the microRNA machinery highly sensitive to the environmental exposures to toxic agents. Genotoxic electrophilic metabolites of toxic agents binds covalently to the terminal G rich loop of stress response microRNA in cell cytoplasm as demonstrated by the formation of microRNA-xenobiotic adducts; these modified molecule cannot be further processed by the microRNA maturation machinery thus resulting in microRNA downregulation and triggering of adaptive response. In case of long term exposures, electrophilic metabolites of toxic pogressively accumulate in the neclophilic areas near to the catalityc pocket of DICER, the main enzyme catalyzing microRNA maturation from pre-microRNA to mature microRNA in the cytoplasm. On a long term basis, this event results in DICER blockage thus inducing a poorly reversible microRNA downregulation (Izzotti et al., 2014). This event can be interpreted as a damage that irreversibly block the microRNA machinery, being DICER, due to its molecular complexity, not reparable or renewable. Accordingly, long term exposure results in the irreversible alteration of the whole microRNA machinery also targeting microRNA involved in oncogene silencing (. Under these circumstances, long term exposures to environmental carcinogens results in both induction of oncogene mutation as well as parallel disruption of microRNA usually blocking, in unexposed organisms, the phenotypic expression of these mutations. This situation results in the blown of the carcinogenesis process.

Accordingly, the length of the exposure is much more important than the dose in determining the adverse health consequences of toxic agents.

These molecular adaptive mechanisms explains why population exposed at low doses of toxic agents well adapt to the adverse environmental situation having only minimal alteration in their molecular damage biomarkers (Rossnerova et al., 2017). However, whenever exposures persists for many decades, it represents a main risk factor for cancer appearance.

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S09 | Application of new approach methods and development of integrated approaches to testing and assessment – moving toward mechanistic risk assessment

Supported by EU-ToxRisk Project

S09-01
Read-across concept in EU-ToxRisk and integration of new approach methods into risk assessment – example branched carboxylic acids

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The replacement of *in vivo* studies by of new approach methodologies (NAMs), like *in silico* and *in vitro* methods, in risk assessment is a challenge. In EU-ToxRisk, scientists with different expertises develop a read-across framework, which uses NAMs to better illustrate shared toxicokinetic and toxikodynamic properties within the grouped compounds.

In this talk, the EU-ToxRisk read-across concept using NAMs is presented and illustrated with the results of a case studies on branched carboxylic acids. In this case study, we tested 10 structurally related compounds, of which two compounds had *in vivo* animal studies. The *in vivo* animal studies were used to derive a read-across hypothesis and to design the *in vitro* testing battery.

We report on the recent results and limitations of integrating the results of 2D and 3D *in vitro* assays systems, together with high-content technologies (omics, HCI) using computational modelling to uncover the causal relationships with apical findings arising from traditional *in vivo* animal data.

Further we will report on *in vitro* to *in vivo* extrapolation using state of the art PBPK modelling to derive oral human equivalent doses for the grouped compounds.

The read-across will be illustrated for two analogues, one being as active as the two analogues with *in vivo* data, one being less active.

S09-02
Integration of new approach methods in a structure based read-across for DART effects

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Read-across is one of the most often applied alternative tools for hazard assessment, in particular for complex endpoints such as toxicity after repeated exposure or developmental and reproductive toxicity. We have applied this approach to a series of six aliphatic carboxylic acids that have developmental toxicity data, some being positive, some negative. For one of these compounds, 2-Methylhexanoic acid (MHA), we have specifically blinded this toxicity data, and we have applied new approach methodologies (NAM) to substantiate the read across of the other compounds (as source compounds) to MHA, and to explore whether these NAM correctly predict the *in vivo* developmental toxicity of MHA. Thus, we have tested MHA and the five analogues in a battery of *in vitro* tests with clear relevance to DART, i.e. the Zebrafish Embryo Test (ZET), mouse Embryonic Stem cell Test (mEST), iPSC-based neurodevelopmental model (UKN1), and a series of CALUX Reporter assays, and combined this with toxicokinetic models to calculate effective cellular concentrations and associated *in vivo* exposure doses. We also included two positive, and one negative control compound in this test. As the histone deacetylase enzyme is postulated to be the molecular initiating target leading to neural tube defects with these compounds, we have also investigated the potential of these six analogues to inhibit this enzyme in ZET, mEST, and UKN1 models. The NAM quite well predicted the *in vivo* developmental outcome of these six aliphatic carboxylic acids. This presentation will discuss the combining of results from multiple NAMs for predicting the teratogenic properties and potency of this series of structurally related chemicals and how this information can be used to establish a framework of testing for regulatory applications.

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S09-03
Learnings from EU-ToxRisk read-across case studies: application of new approach methods

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The classical read across generally evaluates chemical structural similarity without taking into account detailed information on similarity of mode-of-action. The development of adverse outcome pathways with the detailed description of molecular initiation events and key events based on a systematic literature review and weight of evidence analysis, has facilitated the uptake of new approach methods that represent these MIE and KEs. There is an anticipated hazard for agrochemicals that inhibit complex I of the mitochondrial respiratory chain to cause toxicity to the nigra-striattal neurons leading to symptoms that reflect Parkinson disease. This effect has been described in an AOP (Terron *et al.* 2018) that has been accepted and published by the OECD (https://www.oecd-ilibrary.org/environment/adverse-outcome-pathway-on-inhibition-of-the-mitochondrial-complex-i-of-nigro-striatal-neurons-leading-to-parkinsonian-motor-deficits_b46c3c00-en). Our aim was to assess the application of an AOP approach in a read across safety assessment setting of structurally closely related mitochondrial complex I inhibitors, rotenone and deguelin. We have used various *in silico* and *in vitro* approaches including structural modelling, mitochondrial respiration measurements, high content imaging assays, and high throughput transcriptomics approaches to evaluate the biological similarity of these two analogues. Subsequently, PBPK and QVIVE was applied to translate the *in vitro* findings to an *in vivo* situation. The methodologies were also translated to other complex I inhibitors that are used in the agrochemical sector. Our findings demonstrate that an AOP-based testing strategy is highly valuable for read across. The uncertainties in such an assessment and the generality of applying AOP-based testing in read across will be discussed.

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S09-04
Ab initio- prediction of liver toxicity by *in vitro* systems and spatio-temporal modelling

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Drug-induced liver injury (DILI) cannot be accurately predicted by animal models. Also, currently available *in vitro* methods do not allow for the estimation of hepatotoxic doses or the determination of an acceptable daily intake (ADI). To overcome this limitation, an *in vitro*/*in silico* method was established that predicts the risk of human DILI in relation to oral doses and blood concentrations. This method can be used to estimate DILI risk if the maximal blood concentration (C_{max}) of the test compound is known. Moreover, an ADI can be estimated even for compounds without information on blood concentrations. To systematically optimize the *in vitro* system, two novel test performance metrics were introduced, the toxicity separation index (TSI) which quantifies how well a test differentiates between hepatotoxic and non-hepatotoxic compounds, and the toxicity estimation index (TEI) which measures how well hepatotoxic blood concentrations *in vivo* can be estimated. *In vitro* test performance was optimized for a training set of 28 compounds, based on TSI and TEI, demonstrating that (1) concentrations where cytotoxicity first becomes evident *in vitro* (EC_{10}) yielded better metrics than higher toxicity thresholds (EC_{50}); (2) compound incubation for 48 h was better than 24 h, with no further improvement of TSI after 7 days incubation; (3) metrics were moderately improved by adding gene expression to the test battery; (4) evaluation of pharmacokinetic parameters demonstrated that total blood compound concentrations and the 95%-population based percentile of Cmax were best suited to estimate human toxicity. With a support vector machine-based classifier, using EC_{10} and Cmax as variables, the cross-validated sensitivity, specificity and accuracy for hepatotoxicity prediction were 100, 88 and 93%, respectively. Concentrations in the culture medium allowed extrapolation to blood concentrations *in vivo* that are associated with a specific probability of hepatotoxicity and the corresponding oral doses were obtained by reverse modeling. Application of this *in vitro*/*in silico* method to the rat hepatotoxicant pulegone resulted in an ADI that was similar to values previously established based on animal experiments. In conclusion, the proposed method links oral doses and blood concentrations of test compounds to the probability of hepatotoxicity.

S10 | The process of ageing and its modulation: telomeres as biomarkers in *in vitro* and *in vivo* studies

S10-01
Clinical aspects of precision medicine using as biomarkers telomere length, fatty acids and organic acids

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Chronic diseases are responsible for 70% of global deaths and for almost half (47%) of the global burden of disease, and are mainly caused by modifiable risk factors. Because 80% of the risk factors for chronic diseases are related to lifestyle, nutrition, and environmental factors, the application of precision medicine to chronic diseases gains increasing attention recently.

Precision medicine integrates data from genome, microbiome, dietary and lifestyle habits, to identify the causes that led to disease. Among others, metabolomics is a powerful tool of precision medicine and provides a detailed overview of the phenotype. That is the outcome of genetic expression in the regulation of environment, represented by metabolites. Quantification of metabolites can identify underlying conditions even before symptoms appear and help health professionals monitor the response to treatment. A complementary approach to track aging and the onset of chronic diseases related to aging is the analysis of telomeres, the protective caps of chromosomes. Telomeres shorten every time cells divide and the pace of telomere attrition is a robust marker of aging and aging-related diseases.

We have developed a semi-automated worksheet, BIOTEL, to generate individual and group telomere length statistics and provide a crude estimation of biological age. Activation of telomerase has been shown to contribute telomere length maintenance and stability, thus, modulators stabilizing telomeres and increasing telomerase expression/activity has been proposed as potent in anti-aging.

Conclusively, precision medicine offers the possibility to address all the causes that led to the disease, and together with standard methodology can lead to the effective management of chronic diseases.

S10-02
Low grade chronic inflammation and telomere shortening: immunosenescence process in human

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Aging is a complex process that involves a gradual decline in critical cellular processes and signaling pathways. While vast number of studies have investigated telomere length and telomerase activity as prognostic biomarkers, there is scanty information available in the literature regarding chronic low-grade inflammation related pathologies such as obesity and breast cancer. Telomerase activity is strongly influenced by the ongoing inflammation, and production of reactive oxygen species, but the molecular basis of these effects is not yet fully understood. In this presentation, whether the obesity related chronic low-grade inflammation and leptin resistance dependent alteration in telomere length are risk factors for breast cancer will be discussed.

S10-03
Live fast, die young mode: influence of substance abuse on telomeres and telomerase

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Aging is a complex senescence process that follows maturation, and is characterized by time-related functional decline due to genetic, biochemical, physiological and anatomical degeneration in tissues and organ systems. Loss of genome integrity is a key feature in senescence and the consequent development of aging-related diseases and cancer. Telomeres are repetitive tandem DNA sequences that cap chromosomal ends, protecting the integrity of information-carrying DNA. However, telomere length decreases with aging (and therefore its protective activity), as a result of repeated cell replication or environmental factors, namely those involving inflammation and oxidative stress. Consequently, shorter telomeres have been linked with shorter lifespan, and telomere length has been suggested as a biomarker of aging. It has been shown that substance abuse, namely of alcohol, tobacco, cocaine and heroin, has been independently associated with telomere shortening, both at periphery and in the brain. This presentation aims to provide an update on the current knowledge on the influence of substance abuse on telomeres and telomerase, exploring the mechanisms involved, the related biomarkers of exposure, and health consequences.

S10-04
Telomeres biology involvement in thyroid neoplasia: from aging clock to aggressive cancers

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Cell clock mechanisms involve proteins and clock genes interrelated in a very precise manner. Every cell has a specific lifetime. The number of cell divisions is monitored through small fragments of DNA ending the chromosomes, telomeres. Individual somatic cell mitoses are marked by progressive shortening of telomeres which are an important cell clock aging mechanism, preventing unlimited cell proliferation. Molecules involved in this process, transcription factors, enzymes (telomerases), as well as corresponding genes are more and more investigated in physiology and molecular oncology. Telomerase reverse transcriptase (TERT) is responsible for telomere maintenance and its expression is normally suppressed. Some cells exhibit indefinite proliferation, as are embryonic, stem cells but also cancer cells, therefore investigating the TERT system is rewarding in deciphering the way cancer cells are immortal.

Thyroid cancer (TC) is the most prevalent endocrine neoplasia. Despite being indolent in most cases, some express aggressive metastatic biological behaviour. TERT promoter mutations are a key hallmark of aggressiveness in less differentiated TC, poorly differentiated papillary (PTC), follicular (FTC) and anaplastic (ATC), as well as of their metastatic capacity. From a clinical evidence of a thyroid nodule, to detailed ultrasound and fine needle biopsy (FNAB), diagnosis of TC is well established through guidelines and protocols. Detailed genetic analysis was developed in the last years, using samples from FNAB, in order to select surgical cases in Bethesda categories 3 and 4. Commercially available systems (*Afirma, Thyroseq, ThyGenX, Reveal*), reached now the third generation in sensitivity and specificity, and are including TERT as an important component of risk stratification.

More than 150 papers emerged in the last 3 years showing the importance of telomerase system in TC in predicting a worse prognosis, a potential prognostic tool for identifying aggressive forms of TC at diagnosis, and therefore acting in advance for a more aggressive follow-up and treatment. TERT mutation was proven only in follicular cell – derived TC and not in MTC, i.e in FTC, PTC . In decreasing frequency, TERT was involved in more than 50% ATC, 25% PTC and 20% FTC. Presence of TERT mutation is associated with higher age at diagnosis, presence of metastases, and a shorter overall survival. Data from Chernobyl cohort in paediatric TC failed to find TERT mutations, therefore radioactive fallout seems to impact on other processes apart TERT. While the most detectable genetic abnormality in DTC is $BRAF^{V600E}$, it seems that it is not associated with TERT. On the other hand, TERT mutation is highly associated with short telomeres and is not age-related. Altogether, the involvement of telomerase system into molecular oncology was described as *telomere crisis*. Aging impact upon telomere length and telomerase activity in normal thyroid cells. Young patients are telomerase proficient with longer telomeres, while older are telomerase deficient with shorter telomeres. Oncogenic events trigger active thyroid cell proliferation with further erosion of their telomere. Telomere dysfunction or even telomere crisis occurs in later ages because of their initial shorter telomere and lack of telomerase activity. Telomere crisis triggers both genomic instability and telomerase activation: the TERT promoter mutation is thus the consequence of genomic instability, whereas in turn contributes to derepressing TERT transcription and telomerase activation.

In conclusion, detailed characterisation of telomere biology contributes to a risk stratification of TC and better treatment strategy.

S11 | Challenges of non-animal approaches for food safety: from inception to application

Supported by ILSI Europe

S11-01
High throughput screening in the risk and benefit assessment of food ingredients

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The Tox21 and ToxCast programs are high-throughput *in vitro* screening (HTS) programmes ran by the U.S. National Toxicology Program with the goal to forecast biological effects *in vivo*, especially toxicity, based on bioactivity profiling. Whereas much effort is being devoted to the applicability of such high-throughput screening in many chemical sectors, little has been done to relate these approaches to the assessment of foods and food ingredients. HTS approaches are designed to measure biological perturbations, rather than toxicity per se. This makes the interpretation of Tox21/ToxCast HTS data for food-relevant chemicals difficult, as for various compounds in foods the biological activities can also reflect desirable effects, depending on the concentration of the chemical present. The ILSI Europe Tox21/ToxCast Expert Group aims to explore how the data from the Tox21/ToxCast programs on food relevant compounds can be exploited and to assess the utility of the data in the risk and benefit assessments of food chemicals. Starting point are the 556 direct additives that have been identified in the Tox21/ToxCast database. These different chemicals were subdivided into structurally related chemical groups and functional use classes according to EU regulation (e.g. E-numbers, nutrients, flavourings, regulatory-restricted chemicals). Different approaches were taken to derive the critical biological targets of the functional and chemical groups. Most informative insights were obtained when focussing on the biological targets that are induced by multiple chemicals within a chemical group. An overview of the possibilities and challenges in the use of non-animal Tox21/ToxCast data in food safety evaluations will be provided.

S11-02
Adverse outcome pathways and beyond

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The field of human toxicology is currently transitioning from classical toxicology, focusing on measuring apical endpoints for toxicity in animal models, to predictive *in vitro* toxicology, relying on information on toxic mechanisms. This paradigm shift has been reinforced by the introduction of a number of pathway-based approaches, including the adverse outcome pathway (AOP) concept, which is gaining momentum worldwide. AOPs share a common structure consisting of a molecular initiating event, a series of key events connected by key event relationships, and an adverse outcome. Development and evaluation of AOPs ideally complies with guidelines issued by the Organization for Economic Cooperation and Development. AOP frameworks have yet been proposed for major types of human toxicity. AOPs can serve a number of purposes pertinent to the fields of human toxicology and risk assessment, in particular the establishment of quantitative structure-activity relationships, the development of novel *in vitro* toxicity screening tests and the elaboration of prioritization strategies. This presentation will focus on the relevance and potential use of AOPs for hazard identification of food additives.

S11-03
Strategies for avoiding animal testing in food safety and efficacy evaluation: challenges and opportunities

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The assessment of the anticipated efficacy and undesired side-effects of chemicals, drugs and food ingredients on the function of target organs demands animal experimentation from a regulatory perspective. Technological innovations have uncovered the various cell signaling pathways that can be qualitatively and quantitatively assessed by high throughput omics technologies. In parallel stem cell biology now allows the recapitulation of (diseased) organ models in 3D cell cultures. These technologies have been applied in early drug discovery and development and are also thoroughly evaluated for safety testing of cosmetic ingredients. The overall progress in non-animal testing strategies now opens a window of opportunities for the evaluation of the food safety and efficacy using non-animal approaches. Within the EU-ToxRisk project we assess various *in silico* and *in vitro*

methods for integration and application in chemical safety testing. This involves both high throughput phenotypic screening of cell signaling and cell behaviour, high throughput transcriptomics to assess biological perturbation programs, and anchoring of these responses to relevant *in vitro* apical endpoints of toxicity for different target organs. The application of these approaches for efficacy testing will be exemplified based on high throughput screening of Nrf2 activity using automated live cell imaging. Uncovering the modulation of cell signaling pathways will be illustrated based on high throughput targeted RNA sequencing and anchoring to the *in vivo* situation. Also the application of induced-pluripotent stem cell technologies in combination with fluorescent reporter imaging will be described. The future challenges of the integration of such new approach methods for safety and efficacy will be discussed.

S11-04
Regulatory perspective on non-animal approaches to assess foods and food ingredients

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Animal-free testing or research strategies are increasingly becoming available. ILSI Europe's Task Force on Alternatives to Animal Testing in the Food Sector is reviewing in a forthcoming publication how these methodologies can be implemented to replace animal studies in the area of Food Safety/Toxicology & Nutrition.

For the different areas of food improvement agents, novel foods, foods for specific groups, genetically modified foods and health claims, the acceptability of non-animal approaches is evaluated in comparison to legislative requirements in Europe. The approaches considered cover *in-silico* and *in-vitro* methods, organoid models and organs-on-chip, system biology approaches and high-throughput methods for mode-of-action assessment.

The publication will highlight that different food sectors/categories are moving at different paces regarding acceptance of new approaches and Three Rs (Replacement, Refinement and Reduction) methodologies.

One conclusion will be that it would be desirable to update EU legislation and guidance as soon as new methods become available. The incorporation of non-animal methods for food safety assessment and nutrition is a challenging issue to be solved by multidisciplinary expert groups working hand in hand with regulators, ideally at global level.

S12 | Implications of biodistribution of inhaled nanoparticles: effects in organs other than the lung

S12-01
The lung as a barrier to inhaled particles: dosimetry and biodistribution

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Emerging hybrid, experimental/computational approaches to cellular dosimetry can be used by particle toxicologists to accurately calculate the delivered dose to cells for various particles and under different *in vitro* experimental conditions as a function of exposure time. Likewise, *in vivo* lung dosimetry models allow researchers to estimate the delivered particle dose in any region of the respiratory system, as well as study the implications of particle properties and breathing parameters for diverse animal species. Moreover, knowing the deposited dose will also facilitate the extrapolation from experimental animals (rat, mouse, rabbit, pig and monkey) to humans of all ages. Most importantly, incorporating such dosimetric methodologies in the study design enables particle toxicologists to bring *in vitro* and *in vivo* doses to the same scale, an important step towards the development and validation of *in vitro* cellular screening assays. Dosimetric modeling of deposition requires input of several aerosol characteristics, including density, and modeling retention involves knowledge about particle bio-dissolution. For both *in vivo* (inhalation) and *in vitro* (cell cultures) studies knowledge about Exposure-Dose-Response relationships is key for comparing *in vitro* and *in vivo* results on an equal dosimetric basis, and at the same time it provides an opportunity to validate *in vitro* assays. Ultimately, a careful attention to dosimetric details allows a scientifically justified risk extrapolation of toxicological results from animal studies to humans.

S12-02
Effects of particles on the central nervous system

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Evidence is mounting from epidemiological and toxicological studies that exposure to increased concentrations of ambient particulate air pollution contributes to the development of neurological and neurodegenerative disorders. Rodent inhalation studies have revealed that markers of oxidative stress, neuroinflammation and neurotoxicity are induced upon exposure to concentrated particulate matter or diesel engine exhaust particles. Two principle pathways have been discussed whereby particulate matter may induce adverse effects in the central nervous system, namely, a direct pathway whereby particles physically enter the brain parenchyma, and an indirect pathway whereby peripheral systemic effects contribute to neurotoxicity. Indeed, inhalation studies have provided evidence for the translocation and deposition of ultrafine particles (UFP) in different brain regions. However, inhaled particles are also known to trigger pulmonary and systemic oxidative stress and inflammation and thus possibly affect the blood-brain barrier integrity and brain homeostasis in an indirect manner. Current advancements in the development of alternative *in vitro* models for neurotoxicity testing are highly promising regarding the testing of chemicals. However, current paucity of particle composition-specific translocation kinetics limits the applicability of such experimental systems for mechanistic studies of ambient UFP and for the hazard assessment of novel manufactured nanoparticles. Future research into the effects of particles on the brain should consider that direct and indirect mechanisms could act together in an additive or even synergistic manner. It should also acknowledge that many of the established adverse health effects of particulate air pollution are observed in association with long-term exposures.

S12-03
Effects of Particles on the Placenta: studies on *in vivo* and *in vitro* models

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The relevance to investigate the effects of maternal exposure to engineered nanomaterials (ENPs) has recently emerged. The placenta represents the interface between maternal and foetal circulation, regulating the exchange of nutrients, gases, and waste material, as well as translocation of xenobiotics. Over the last years we have demonstrated the placenta as a site of accumulation of ENPs, and have studied the physic-chemical properties driving translocation across the placental barrier. We recently showed that different amorphous silica nanoparticles (SiO_2NPs, I.V. administered) do not induce maternal toxicity, nor affect placental/foetal development. Biodistribution studies demonstrated that particles distributed to placentas and foetuses, although size, surface charge and gestational stage influenced biodistribution. Similarly, silver NPs accumulated in placentas and foetuses after inhalation exposure during the first 15 days of pregnancy, clearly indicating that once NPs access the circulatory system they likely arrive to the placenta, being this a highly vascularized organ. Due to ethical reasons, studies on placental translocation and toxicity of ENPs are meanly performed in rodents; however transposition to humans of results obtained in rodents should be done with caution, as species-specific differences in placental organization exist, which may result in differences in permeability and effects. Currently, the only alternative to study placental translocation in humans is the *ex vivo* human perfusion model, which however allows short term studies and give no information on the potential toxicity to foetal tissues. Alternative models resembling the human placental barrier are greatly needed. We are currently developing a novel *in vitro* model based on 2 types of stem cells derived from the pre-implantation embryo: Embryonic Stem cells (ESC), which can be induced to differentiate into all foetal tissues; Trophoblast Stem Cells (TSC) from which all trophoblast lineages can be derived. These cells, available from rodents and humans, can be easily maintained in culture. TSC, cultured on transwell (TW) inserts in the absence of growth factors, resemble the syncytiotrophoblast layer of the placenta. Administration of TiO2 NP in the upper chamber of TW impairs differentiation of ESC cultured in the lower chamber. Presence of syncytial TSC on the TW re-establishes proper differentiation, suggesting that translocation of particles is reduced. Similar results were obtained for the expression of the mesodermal differentiation marker Brachyury, which is highly expressed by ESC after 10 days of differentiation. Culture in the presence of TiO2 NP interferes with Brachyury expression in the absence of syncytial TSC, while the presence of the syncytial layer re-establishes normal expression. Our results indicate that the simulated barrier is able to counteract the adverse effect of TiO2 NP on differentiation of foetal tissues.

S12-04
Effects of nanoparticles on male and female fertility

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As engineering of nanomaterials have emerged, so has concern that these might interfere with reproductive function. Human exposure to particles has been studied to a very limited extent relative to reproductive function. In occupationally exposed men, welding particles may decrease male fecundity, but the association is not consistently described. Air pollution is also proposed as a risk factor, but diversity in exposure and outcome study parameters hampers firm conclusions. Rodent studies more consistently show that nanosized particles are able to interfere adversely with male reproduction, but the airway route of exposure, i.e. the most relevant route in occupational settings, is rarely applied, and effects probably depend on particle type [Skovmand et al., 2018]. Interestingly, particle exposure has in some studies also shown able to interfere with the male reproductive organs during embryonic development but, again, findings are not completely consistent [Skovmand et al., 2019]. Female reproductive function has received much less attention, but some support for an association is available from epidemiological studies. The few available rodent studies indicate that particle exposure may pose a risk also to females, at least when exposure occurs acutely [Johansson et al., 2017].

The underlying mechanisms are as yet unknown, but both male and female reproduction may be sensitive to inflammation and oxidative stress. Upon inhalation, many particle types, engineered and anthropogenic, may induce oxidative stress and lung inflammation. Inflammatory mediators may leak to the systemic circulation and subsequently reach reproductive organs or interfere with the hypothalamic-gonadal-axis. Alternatively, small amounts of particles or particle constituents may translocate from the lungs to the lung capillaries, the systemic circulation and the testes. Nanoparticles may possibly, due to their small size, traverse the blood testis barrier and enter the seminiferous tubules from the interstitially located capillaries as well as the female reproductive organs. In conclusion, pulmonary exposure to nanoparticles, engineered as well as process-generated may potentially affect both male and female fertility and more research is warranted to identify the relevant mechanisms of action.

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S13 | Knowledge-based computational approaches in predictive toxicology

Supported by EU-ToxRisk Project

S13-01
The power of workflows – toxicological read across using integrated life science data

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Unforeseen toxicity comprises one of the main reasons for failures in drug development. One of the options to mitigate this risk is to pursue extensive read across studies. This means to query databases and the available literature for compounds which are structurally similar to the respective development candidate in order to retrieve information on potential hazards. Obviously, the results depend heavily on the similarity algorithms used. Furthermore, due to lack of integration of the underlying data sources, this process is time consuming and prone to errors.

With the public availability of large life science data sources such as ChEMBL, and their integration in semantically enriched platforms like the Open PHACTS Discovery Platform, retrieval of data sets for e.g. ligand-protein interactions is no longer a time consuming process. Furthermore, the use of workflow engines such as KNIME allows for queries across multiple data sources and enables complex post-processing tasks. Deployment of these workflows in web-applications enables broader use without advanced knowledge on workflow implementation.

In this presentation we will outline the use of KNIME workflows for selected tasks related to toxicological read across. This includes an assessment of different similarity algorithms, which shows that especially in the 3D-space, similarity rankings might change dramatically. Furthermore, searching across multiple data sources also allows for an exhaustive assessment of candidates, including target profiles and pathway enrichment. The latter was used to identify pathways which are enriched for compounds which have been withdrawn from the market.

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S13-02
Different KNIME workflows for read-across and successive use for weight-of-evidence strategy

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The evaluation of the toxic effects of substances is a complex task, due to the huge amount of factors involved in the biological processes at the basis of the effect. This requires taking advantage of all elements that can be used in the assessment of the property values. The read-across approach and the *in silico* methods, collectively called non-testing methods, can be integrated within a weight-of-evidence strategy. This integration is typically performed manually.

Furthermore, also the read-across process in most of the cases relies on expert decisions, which may be subjective, and based on some initial choices. In this approach, there is a risk of making poorly reproducible results besides losing important pieces of information. In addition, a main shortcoming in read-across is that the process may not identify some of the relevant source compounds.

In order to cope with these problems, we explored software tools able to assist the expert. The factors related to similarity which we used to select source compounds were: structural, physico-chemical, toxicological and pharmacokinetic features. These tools analyse the similarities of the compounds in "full or partial" way, i.e. merging all the features or selecting only those more relevant. Furthermore, the steps of the process can be done in a parallel or sequential way.

Finally, we combined the results of the read-across procedure with those from *in silico* models.

We will describe the added value of these programs, implemented in KNIME.

We acknowledge the project EU-ToxRisk (a project funded by the European Union's Horizon 2020 research and innovation program under grant agreement No 681002).

S13-03
Predicting with confidence: Toxicological *in silico* model building and prediction using conformal prediction

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Purpose: To demonstrate the utility of confidence predictors such as Conformal Prediction as an *in silico* modelling framework for obtaining predictions with known, and mathematically proven, error rates set by the user as well as the graceful handling of highly imbalanced datasets, typical in toxicology, without the need for balancing measures such as under- and/or oversampling.

Method: Mondrian Conformal Prediction (MCP) was used as a framework for building highly predictive *in silico* models for toxicological end points of severely imbalanced datasets from PubChem (0.8% toxic compounds on average). The method generates models with a guaranteed error rate (% errors) for each class (toxic or non-toxic) set by the user given that the investigated data is exchangeable. MCP is constructed on top of commonly used algorithms, e.g. Random Forest or Support Vector Machines, for *in silico* model building by internal calibration of the prediction outcomes.

Results: The results obtained for highly imbalanced toxicity datasets show that *in silico* models with good predictive performance for both the toxic minority as well as the non-toxic majority class can be derived using MCP.

S13-04
Small is beautiful: application of local models in toxicology

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With the growing availability of toxicological information in digital sources and the advances in "big data" methodologies an increasing effort is being devoted to the development of models aimed to predict

complex (apical, organ toxicity) endpoints using massive data. However, the complexity of the biological phenomena behind these endpoints, the disparity of the mechanisms involved and the interference of toxicokinetics hampers the predictive ability of such models. Despite "big data" models have demonstrated their suitability and performance in other fields, there is a risk that an improper use of such methodologies to address toxicological problems produces results not meeting the user expectations and discourage potential users of applying them, particularly in industrial or regulatory settings.

To face this challenge, we propose the use of a strategy which prioritizes the use of highly relevant and consistent data for each prediction over the use of massive datasets. Our method starts with the definition of a local space around the query compound using a relevant similarity metric. Depending on how this local space is populated and the properties of the compounds therein they can be used to infer directly the biological properties of the query compound or can be used to build *ad-hoc* local models (either quantitative models or classifiers). In other cases, when the local space is identified as not suitable for obtaining good predictions, it will be expanded to incorporate additional data points.

We will start presenting the rationale supporting this approach and how it can mitigate common problems present in general models. Then we will show a few examples illustrating the kind of results obtained with our proposed strategy for predicting toxicity endpoints (e.g. liver toxicity or oxidative stress responses). Finally, we will discuss the difficulties of carrying out a fair comparison between local and general computational models, explaining in part why the former are much less used nowadays.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 (EU-ToxRisk) as well as from the Innovative Medicines Initiative 2 Joint Undertaking (IMI2/JU) under grant agreement No 777365 (eTRANSAFE).

S14 | Understanding the interindividual variability in toxicity involving the psychotropic drugs

S14-01
Psychotropic drug poisonings admitted to the emergency department: epidemiology and morbidities

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Psychotropic drugs such as benzodiazepines and antidepressants are frequently involved in patients admitted to the emergency department (ED). Accidental poisoning is rare in contrast with intentional poisoning (self-harm). Intentional poisoning with psychotropic drugs may account for one third of ED admissions and these are often accompanied by co-ingestion of ethanol or multiple other agents that may have therapeutic implications. Intentional poisoning with self-harm is an important aspect requiring adequate care because of the considerable psychological morbidity and also the threat of mortality by suicide. Increased susceptibility to psychotropic drugs in therapeutic doses e.g. in the young and older age groups and in patients with specific genetic patterns of metabolizing enzymes or during drug interactions must also be kept in mind.

It is important for the emergency physician to be aware of the clinical picture of poisoning with psychotropic agents in view of the important implications for treatment.

Symptoms of an overdose with *antipsychotics* appear to be an extension of the adverse effects at therapeutic doses like e.g. orthostatic hypotension, (reflex) tachycardia, cardiac conduction disturbances, central nervous system depression, anticholinergic symptoms, sialorrhea, extrapyramidal symptoms, thermoregulatory problems, seizures. The profile of symptoms differs according to the class of the antipsychotic agent. As far as the cardiac effects are concerned a negative inotropic action and quinidine-like (Type IA) antiarrhythmic effect with sodium and potassium channel blockade contribute to the toxicity and this has important therapeutic implications. One should also be aware of the Post Injection Delirium Sedation Syndrome (PIDSS) due to the accelerated release of the long acting olanzapine pamoate into the general circulation after intramuscular injection.

Benzodiazepine overdoses usually present with a mildly depressed sensorium to coma, disturbances of motor skills and dysarthria. However respiratory depression may occur for instance with coingestion of other central nervous system depressant drugs. One should also keep in mind that the decreased consciousness level may hamper the history taking and lead to overlooking the coingestion of other dangerous drugs like e.g. paracetamol. The specific benzodiazepine antagonist flumazenil is very rarely indicated and has proven to be dangerous as it will antagonize the protective effect of the benzodiazepine in cases of co-poisoning with e.g. cyclic antidepressants and lead to life threatening convulsions and cardiovascular instability. Flumazenil can also induce abstinence with seizures in patients who take benzodiazepines chronically.

Overdoses of the psychotropic drug *bupropion*, used as an antidepressant and in the treatment of nicotine addiction seem to be on the rise in the USA. These poisonings lead to important effects like conduction disturbances (not due to sodium channel blockade) and seizures and deserve special attention.

Major toxicity of *cyclic antidepressants* consists of altered mental status, cardiac dysrhythmias or conduction defects with widening of the QRS complex and right axis deviation of the terminal 40msec of QRS in limb leads (based on sodium channel blockade), hypotension and seizures.

Monoamine oxidase inhibitor poisonings are not frequently reported as these drugs are only used as third or fourth line therapy in refractory depression. However these can be very severe and toxicity may also be due to simultaneous use with other drugs like e.g. other antidepressants or to an interaction with tyramine in food.

There are different classes of *serotonergic antidepressants* and their toxicity is in general lower than with tricyclic antidepressants. Toxic effects consist of symptoms related to the central nervous and cardiovascular system and there are differences among the classes. The serotonin syndrome is most commonly reported and may also be due to interaction with other serotonergic drugs during therapeutic use.

In conclusion, psychotropic drugs are frequently involved in patients admitted with acute poisoning to the ED and can pose life threatening somatic problems. These should be anticipated, correctly diagnosed and adequately dealt with by the admitting emergency physician. Great care should also be given to the underlying psychiatric problems since poisoning with psychotropic drugs in the ED is frequently of intentional nature.

S14-02
Drug-induced toxicity at therapeutic doses versus acute overdose – physiopathological differences

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Objective: To provide examples and their pathophysiological background for differences between drug-induced toxicity at therapeutic versus supratherapeutic levels.

Methods: Literature review on PubMed, common textbooks and chart review of patients

Results: Reasons for drug toxicity despite therapeutic doses frequently include the following: i) increased absorption (e.g. dumping, protein-rich or fatty cost) ii) missed therapeutic drug monitoring (TDM) iii) narrow therapeutic index of drugs (e.g. colchicine, digoxin, lithium); iv) inappropriate dose for patients medical condition; v) inappropriate dose at given metabolism (fast and poor metabolizer; variability of cytochrome P450 metabolism; vi) impaired drug-clearance vii) impaired intestinal efflux: inhibition of MDRP (P-gp) or BCRP and viii) drug-drug (e.g. lithium, digitalis) or drug-food interaction.

In the case of acute poisoning, the onset is typically fast at a normal clearance, symptoms are typical for the given toxin, plasma drug concentration are usually – but not always – elevated, and duration of symptoms short. If toxicity at therapeutic doses appears, the onset is usually subacute to chronic, clearance of the drug often impaired, symptoms may be atypical or subtle, diagnosis frequently made late due to normal TDM, and the duration of symptoms and treatment often prolonged. Typical pharmaceuticals of the latter are e.g. lithium, phenytoin and digoxin. Timely identification of patients at risk for preventable adverse drug reactions (ADRs) is of utmost importance and should particularly include patients with organ failure, advanced age, chronic illness and under polypharmacy. According to data in literature, ADRs occur in about 60% of patients with psychiatric medications with lithium, phenytoin, second-generation and antipsychotics.

Conclusion: ADRs are frequent, can be severe, are in many but not all cases preventable and should particularly be considered in patients under polypharmacy.

S14-03
Inter-individual ethanol toxicokinetic differences and effect variations

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The effect of ethanol in the central nervous system is dependent on ethanol brain concentration dependent, at the same time, on blood ethanol concentration.

The blood alcohol concentration should be correlated with the ingested doses, but the clinical practice shows, not only effect differences but also individual BAC differences between subjects under similar doses. These differences come, on the one hand, from different circumstances related to alcoholic beverages consumption, and on the other hand, to individual characteristics of metabolic parameters and distribution.

We will review the main circumstances affecting the absorption, distribution, metabolism and elimination rates.

EtOH absorption through the gastric and intestinal mucosa is produced by a passive diffusion mechanism. Its velocity is determined by several circumstantial factors such as time of the day, length of ingestion, type and grade of beverage and quantity and type of food in the gastrointestinal system. Sex-related differences in the speed of absorption and gastric metabolism have been postulated but not fully verified. Lung and skin absorption, although anecdotic, has also been studied.

Total body water plays a crucial role in ethanol distribution through variation of its volume of distribution. Main interindividual variations of distribution are caused by the amount of body fat linked to sex, age, and obesity.

During 70 years ethanol kinetics has been interpreted with the zero-order model drawn by Widmark in 1932. This model assumes the organism as a uniform compartment and a constant rate of clearance of ethanol and doesn't furnish any explanation for inter-individual variability. A Michaelis Menten model showed a more accurate approach at low doses and is incorporated to the Norberg's Model of alcohol dynamics limited by its restriction to ethanol intravenous infusion. A Minimal Model of Ethanol Dynamics has been proposed to be applied to oral alcohol intake.

The rate of ethanol metabolism depends on genetic and environmental factors determining the efficacy of the metabolizing systems. Polymorphisms of the ADH/ALDH encoding genes account for ethnic differences. Induction of CYP2E1, promoting the action of MEOS metabolic pathway, causes tolerance in heavy drinkers. CYP2E1 polymorphisms also seem to be associated with alcoholism due to the enhancement of the protein inducibility.

In silico models on ethanol kinetics can be useful for understanding these inter and intra-individual kinetics variations, much more complex than expected.

S14-04
Lithium-induced toxicity: determinants of inter-individual variability and decision of extracorporeal toxin removal in poisoning

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Lithium (Li) is the cornerstone of bipolar disorder treatment, despite to its narrow therapeutic index. When considering Li overdose, three patterns are described depending on the ingested dose, the duration of exposure and the renal function, i.e. acute, acute-on-chronic and chronic poisoning characterized by discrepancies between toxicity features and the plasma Li concentration. In mild poisonings, significant neurocognitive and behavioral adverse effects occur, resulting in psychomotor slowing, apraxia, dysarthria and impaired memory. In life-threatening poisonings, coma, seizures, pyramidal syndrome and multiorgan failure occur. Neurotoxicity is usually reversible but syndromes of irreversible lithium-effectuated neurotoxicity (SILENT) have been reported. We investigated the reasons for interindividual variability in Li-induced neurotoxicity using rat models mimicking human poisoning patterns. Brain lithium distribution was shown to be rapid, inhomogeneous and with delayed elimination. Brain lithium accumulation was more marked in acute-on-chronically than acutely poisoning. Brain lithium distribution was increased in chronically compared with acute-on-chronically poisoning. Differences between patterns regarding Li-induced hypolocomotion were better explained by Li exposure duration than by brain accumulation. Severity of Li-induced encephalopathy, effectively scored using electroencephalography, was dependent on the poisoning pattern, shown to determine Li accumulation in the brain. Regarding Li poisoning management and given its pharmacokinetics (i.e. no protein binding, limited volume of distribution, absence of metabolism, and exclusive renal elimination), extracorporeal toxin removal (ECTR) by hemodialysis represents the method of choice for enhancing Li elimination in addition to optimal supportive care to rapidly reverse Li-attributed neurotoxicity. However, ECTR indications and benefits remain controversial. Recently, recommendations were published by the international EXtracorporeal Treatments In Poisoning (EXTRIP) workgroup based on a systematic literature review. Variability of ECTR results will also be discussed.

S15 | Investigative Toxicology Leaders Forum (ITLF): Scientific advancements and case studies for the optimization

S15-01
Olson revisited – Translational Analysis of Safety Data (IMI eTRANSAFE)

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The analysis of concordance of preclinical (*in vivo*) data with the later clinical outcome has been a topic of interest since decades not only to unveil causes of attrition due to insufficient animal predictivity but also for ethical reasons of animal protection. The seminal work of Olson (Olson et al. 2000) was a systematic comparison of preclinical safety data with adverse events observed in clinical studies for 150 compounds which triggered numerous subsequent works investigating specific adverse events in more detail with various statistical methods to assess the concordance. In order to broaden our understanding of translational safety, accessibility of large preclinical and clinical data sets remains the main obstacle. Commercial data sources such as PharmaPendium provide options for big data analyses (Clark, Steger-Hartmann, 2018), but these are limited to approved drugs, i.e. miss the data on projects which failed during clinical development. In addition, the lack of aligned ontologies between the preclinical and the clinical world (SEND-CDISC vs. MedDra/SnowMed) impedes automated statistical approaches.

In order the overcome these hurdles the IMI project eTRANSAFE (http://etransafe.eu/; Enhancing TRANslational SAFEty through integrative knowledge management) has set up an environment for data sharing of preclinical and clinical safety data. The project which started in September 2017 comprises 27 organisations, 12 of them being pharmaceutical companies which join forces to share their safety data. eTRANSAFE develops a platform for automated data sharing by using previously developed data standards (SEND), which are connected to terms used in legacy data LIM systems of the pre-SEND area. The SEND terminologies are aligned by complex ontologies to clinical terminologies such as MedDRA. The different databases and ontology components of the project will be integrated into a knowledge hub for automated translational analyses.

S15-02
Application of *in vitro* pharmacokinetic simulations using "microformulator" technology for quantified risk assessments

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Traditional cell-based assays assess concentration-dependent effects of test compounds use static drug concentrations for the course of the assay. Although useful for some applications (e.g. structure-activity-relationship assessments) the resulting data do not provide insight on potential for *in vivo* activity. To improve *in vitro - in vivo* translational understanding, a microfluidic device has been engineered to simulate drug PK profiles in microtiter plate-based cell assays. This "microformulator" creates drug exposure profiles for 24-hr cycles and can be applied for multi-day to multi-week studies, depending on the biological response being evaluated. PK parameters can be systematically modified to explore drivers of a pharmacological/toxicological response and thereby gain insight on optimal drug PK profile to maximize therapeutic index. Several case studies will be presented to demonstrate applications and utility. This system has the potential to minimize animal studies for both efficacy and safety studies and effectively guide clinical use of candidate drugs.

S15-03
Retinal-3D: Development of 3D eye models for early assessment of retinal toxicity. A CRACK-IT Challenge

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The development of drugs for eye disorders is a growing field with the market for therapies targeting retinal disorders expected to grow to $14.8 billion by 2022. Currently, there are no adequate *in vitro* models that recapitulate the complex structure of the mature human retina, therefore, the majority of efficacy and safety testing in relation to the eye during drug development is performed in animals (rodents and rabbits). This is due to the retina's complex structure, which consists mainly of glia-cells, neuron and the pigmented epithelium (RPE). It is therefore essential for drug development in general, and specifically for the evolving field of new drugs in ophthalmology, to have a human relevant retinal cell model to support compound testing *in vitro* prior to animal studies.

This "Challenge" is currently being addressed via a CRACK-IT (NC3Rs funded) project, led by NewCells Biotech Ltd. Human inducible pluripotent stem cells (hiPSC) are being used to create 3D retinal organoids that more closely recapitulate the structure and function of the human retina, and which can be used for large scale disease modelling, toxicology and pharmacology screening. 3D laminated human retinae have been shown to contain all of the major cell types and to form functional synapses. For example, initial characterization has shown electrophysiological functionality, including response to light.

These retinal organoids can be maintained for an extended time in culture, with a high degree of functionality, and are therefore compatible for future toxicity screening. However, the data show that a significant variability between different iPSC lines in the ability to generate retinal organoids exists, especially in relation to the ability to generate RPE. Organoids have been created from iPSC lines of ophthalmological diseased patients and can be potentially used for disease modelling. Preliminary toxicity studies accurately categorized two compounds known to be toxic to the human retina (Moxifloxacin and Chloroquine).

However, there are many hurdles to adress; including i) extended differentiation protocols (150-200 days); ii) line and clonal variability; iii) maturity of retina; iv) improved electrophysiological function; v) inclusion of microglia; vi) recapitulation of pharmacological and toxicological effects; vii) cryopreservation that will allow for multi lab ring trials viii) generation of organoids from other safety relevant species (i.e., pig, monkey and rat). Mayny of these aspects are now being addressed, and will be presenetd.

This paper provides an overview covering the key highlights and accomplishments so far of this CRACK-IT challenge, with the eventual goal of replacing/reducing the use of animals in drug discovery.

S15-04
Development of *in vitro* systems for characterizing ADC toxicity

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Antibody-drug conjugates (ADCs) are a novel anticancer chemotherapeutic platform designed to increase the therapeutic index of chemotherapeutics with more selective delivery of highly cytotoxic agents to cancer cells. However, non-specific uptake-mediated toxicity of ADCs in normal cells has been reported in multiple preclinical and clinical studies. Even though the mechanism(s) for non-specific uptake are not completely understood, the role of non-specific uptake related receptors such as Fc receptors and Mannose receptors are commonly implicated for toxicity of ADCs to normal cells. The expression levels of these candidate non-specific uptake-related receptors are also not well characterized in in-vitro cell models. Hence, in this study we evaluated the differential expression of Fc receptors (FcgRI, FcgRII, FcgRIIIa, FcgRIIIb) and mannose receptors (MRC 1 and MRC 2) in multiple human *in vitro* cell models such as endothelial cells (liver sinusoidal and kidney glomerular) cells, Kupffer cells, and hematopoietic cell lineages (Myeloid, Erythroid and Megakaryocytic) differentiating from CD34+ hematopoietic stem/progenitor cells. The results of RT-PCR analysis revealed a wide variation in the level of expression depending on the cell type as well as lineage and differentiation stage (hematopoietic cells). In addition, the constitutive rate of non-specific fluid phase endocytosis was characterized for each *in vitro* model as well. This study demonstrates differential expression of major receptors and dramatic differences in fluid phase endocytosis in these *in vitro* models of common ADC target tissues, which are useful in understanding and predicting toxicity associated with non-specific uptake of ADCs.

S16 | Chemical risk assessment using human *in vitro*, *ex vivo*, *in silico* and biomonitoring data

S16-01
Chemical risk assessment: How well do *in vitro* and *in silico* data predict the *in vivo* situation?

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Modern high-throughput screening technologies (HTS) (Houck et al., 2013) are enabling the use of use of new approach methodologies (NAMs) that can provide information about chemical hazard and risk assessment without using whole animals (ICCVAM, 2018). Gaining broader acceptance of NAMs in risk assessment will require comparisons between the point of departure (POD) from HTS and from traditional animal testing studies (Kavlock et al., 2018; Thomas et al., 2019). High-content imaging (HCI), a type of HTS technology, is widely used to evaluate drug safety (O'Brien et al., 2006) and to screen chemicals for toxicity (O'Brien and Edvardsson, 2017). "Sublethal" cellular alterations identified by HCI such as, mitochondrial activity, lysosomal mass, oxidative stress and apoptosis, can be important for translating *in vitro* effects to *in vivo* safety (O'Brien, 2014).

Here we describe a case study in which HCI was used to estimate *in vitro* potency values in rat hepatocytes and that were quantitatively extrapolated to *in vivo* oral administered equivalent doses (AEDs) using high-throughput toxicokinetic (HTTK modeling (Pearce et al., 2017). The AEDs were then compared with *in vivo* rat liver POD values from repeat-dose subchronic (90d) and chronic (3y) exposures. We first selected 51 chemicals from ToxRefDB v2.0 that produced hepatic effects following repeat-dose subchronic (90d) and chronic (3y) exposures. Second, we treated rat primary hepatocytes with 10 concentrations (0.2 to 100μM) of these chemicals. Third, we used high-content imaging (HCI) to measure endoplasmic reticulum stress, mitochondrial function, lysosomal mass, steatosis, apoptosis, DNA texture, nuclear size and cell number at three time points (24, 48 and 72 h). Fourth, we used the HCI data to estimate AC50 values for all *in vitro* endpoints and time points using curve-fitting (Filer et al., 2016). Finally, we extrapolated the AC50 values to administered equivalent doses (AED) by toxicokinetic modeling with multiple dose metrics and compared them with hepatic lowest observed adverse effect levels (LOAELs).

On average, AEDs derived from HCI in rat primary hepatocytes were 7 folds lower than rat liver chronic and subchronic LOAELs. While the ratio between the LOAEL and AED values varied by the HCI endpoint, *in vitro* exposure duration and choice of dose metric in toxicokinetic modeling, 60% were within 10 folds. In contrast, AED based on diverse *in vitro* assays from ToxCast were 58 folds lower than LOAELs. Our findings demonstrate the feasibility of NAMs using HTS and HTTK for screening level assessments and provide a systematic appraisal of the impact different *in vitro* factors have on the predicted margin of safety. We believe that more sophisticated computational approaches will further improve the performance of NAMs for predicting health effects.

This abstract does not reflect US EPA policy.

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S16-02
PBK modeling for chemical risk assessment: *in vitro* biomarkers for developmental toxicity and their extrapolation to the *in vivo* situation

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Use of *in vitro* assays in alternative testing strategies for risk assessment requires quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), translating the *in vitro* data to the *in vivo* situation so that points of departure can be defined. This translation of *in vitro* concentration-response data to *in vivo* dose-response curves can be achieved by physiologically based kinetic (PBK) modeling-based reverse-dosimetry. The aim of our work is to provide proofs-of-principle that this *in vitro-in silico* QIVIVE approach can provide points of departure suitable for risk assessment. Results obtained reveal that *in vitro* data for developmental toxicity, obtained in the ES-D3 differentiation assay of the embryonic stem cells test (EST), can be converted to BMDL (benchmark dose lower confidence limit) values that match points of departure derived from available *in vivo* studies on developmental toxicity. Examples will be provided for different classes of compounds including glycol ethers [1], phenol and substituted analogues [2,3] and *all*-trans retinoic acid [4]. The method also adequately predicted kidney toxicity of aristolochic acid I [5], acute liver toxicity of pyrrolizidine alkaloids [6,7], and estrogenicity of estradiol, bisphenol A and genistein [8,9]. A prerequisite for making adequate predictions for the *in vivo* situation is that the *in vitro* model selected captures the mode of action of the compound studied. This will be illustrated by results obtained for diethylstibestrol (DES). It is concluded that PBK modeling based quantitative translation of *in vitro* data to the *in vivo* situation facilitates use of *in vitro* data in risk assessment and is essential to avoid that chemical safety evaluation becomes hazard- instead of risk-based.

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S16-03
Quantitative *in vitro* to *in vivo* extrapolation (IVIVE) predict adverse male reproductive health disorders caused by pesticides

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There are currently around 350 pesticides that are approved in the EU, many of which we lack knowledge concerning sensitive endocrine effects related to male reproduction. Thus, there is an urgent need to develop new testing strategies that can predict *in vivo* exposure levels that could result in adverse effects on male reproductive health.

The development of the male reproductive system strongly depends on androgens produced by the fetal testes. Compounds capable of interfering with the synthesis of androgens or by antagonizing the androgen receptor is therefore of great concern for the developing male fetus.

Our strategy combines androgen-related activity of pesticides on human cells with physiologically-based kinetic (PBK) modeling. *In vitro* effect data on AR antagonism and androgen synthesis alert us to compounds with a potential *in vivo* activity by identifying their critical internal exposure, while the kinetic models simulate the maternal doses necessary to reach these critical levels in the fetus (reverse dosimetry). Using selected pesticides and male anogenital distance as an effect biomarker, we show, as proof-of-principle, that our IVIVE method can translate *in vitro* toxicity results to adverse *in vivo* exposures. From a pool of eleven analysed pesticides, six compounds – fludioxonil, cyprodinil, dimethomorph, procymidone, vinclozolin and linuron – were selected for an assessment of their *in vivo* kinetics and effects. Simulated exposure levels in fetal rats were within a factor of 3 from measured concentrations, and all compounds induced shorter male AGD *in vivo* at dose ranges as predicted by IVIVE.

In conclusion, we have obtained evidence that our IVIVE approach is viable and has huge potential as an efficient and economical *in vitro* safety testing method of pesticide-induced male reproductive disorders in animal and humans. Notably, the tool may have the potential in the long term to reduce unnecessary animal testing in risk assessment of chemically-induced male reproductive disorders.

S16-04
Human biomonitoring and complex serum mixture effects as biomarkers of impact on fetal growth

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Background: Studies have linked exposure to environmental persistent organic pollutants (POPs) with disturbance of foetal growth, child development, immune function and reproductive abilities.

Objectives: To investigate i. the associations between serum POP levels of pregnant women and their infant's birth weight, length, head circumference and gestational age at birth; ii. Elucidate the association between the combined xenoestrogenic activity of Perfluoroalkylated substances (PFASs) in pregnant women's serum and as indices on fetal growth effects on birth weight, length, and head circumference.

Methods: Pregnant Greenlandic (n=509) and Danish women (n=702) were enrolled during pregnancy and serum levels of the lipophilic POPs (Organochlorine pesticides, Polychlorinated biphenyls and Polybrominated diphenyl ethers), the amphiphilic POPs, the PFASs, were measured. The actual mixture of Perfluoroalkyl acids (PFAAs) from the serum of Danish pregnant women (gestational week 11-13) was obtained by solid phase extraction, HPLC, and weak anion exchange. The PFAA induced xenoestrogenic receptor transactivation (XER) was determined using the stable transfected MVLN cell line. The associations between maternal serum levels of POPs and birth weight, length, head circumference and gestational age were analysed using multivariable linear regression analysis.

Results: For the Greenlandic pregnant women we found significant inverse associations between Perfluorooctanoic Acid (PFOA) and birth weight (-119g/ng/ml), birth length (-0.37cm/ng/ml, borderline significant) and head circumference (-0.36cm /ng/ml) and a positive

association with gestational age (0.45 week/ng/ml). For the lipophilic POPs we observed an overall trend of inverse associations to foetal growth indices.

For the Danish pregnant women the association between foetal growth indices with exposure to single POPs was less clear. In contrast, we found a significant inverse association between the combined serum xenoestrogenic (XER) activity of PFAAs and foetal outcomes: An interquartile range increase of XER was associated with 48 g lower birth weight and 0.3 cm shorter birth length.

Conclusion: In Greenlandic women PFOA had a significant inversely association with foetal growth indices, whereas gestational age was positively associated. In Danish pregnant women, higher combined serum PFAA-induced xeno-estrogenic activities were significantly associated with lower birth weight and length in the offspring. Our results indicate that PFOA and PFAA mixtures can affect fetal growth suggested through disruption of the ER function. In overall, the data indicate that POPs have a negative effect on foetal growth.

S17 | Experimental comprehensive toxicological studies simulating real-life exposures: Long-term combined exposures on multi endpoints

S17-01
Experimental designs and protocols for long-term combined exposure studies from methodology to application: problems and solutions

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The real life exposure to chemical mixtures at low doses by food chain, water, consumer products and environmental media is the main gap of standard toxicological tests used by the regulatory agencies to set the safe levels of exposure. The different types of interactions between two or more chemicals that can lead to risks or even new hazard attracted the attention of the international regulatory authorities that realized the need for a cumulative risk assessment. New methodologies have been proposed by regulatory authorities for testing commercial mixtures. These studies usually adopt dosage schemes that are too high for real life risk simulation and used as endpoints only a specific type of toxicity. These types of approaches can miss the real life scenario because the use of high doses can not answer to the question if the consumers are protected by regulatory limits and can not predict non-linear dose response/hormetic effects. The use of limited endpoints only for specific types of toxicity can not identify the new hazards that can appear during mixture exposure. The new experimental methodology [1,2] proposed try to provide answer to all these questions and problems by evaluating the long term toxicity of non-commercial chemical mixtures at very-low and low doses and with the investigation of several key endpoints and systemic mechanistic pathways. This methodology can not resolve all the problems as it is not feasible to test every conceivable combination of agents, but if the hypothesis of an increased cumulative risk or even a different hazard identification is proven at doses around regulatory limits, then a new step that support the effort to pass to the era of cumulative risk assessment at low doses is done.

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S17-02
A mixture of routinely encountered xenobiotics induces both redox adaptations and perturbations in blood and tissues of rats after an 18-month exposure regimen: the dose and time issue

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Exposure of humans to mixtures of xenobiotics is a continuous situation during their everyday routine. However, the majority of the studies in the field of toxicology assess the *in vivo* effects of individual substances rather than mixtures. Therefore, the main objective of the present study, which is part of a greater experiment, was to evaluate the impact of the 18-month administration in rats of a mixture containing 13 pesticides, food preservatives and food packaging materials in three dosage levels (i.e. low, medium and high), which are well below the NOAEL (no-observed-adverse-effect level), on blood and tissue redox biomarkers. The ultimate goal was to evaluate the potential hazards of long-term low-dose exposure to chemicals and to contribute to the adoption of the rationale of the cumulative risk assessment rather than the individual-substance toxicity doctrine. Our results indicate that the mixture induces protective adaptations by enhancing the antioxidant mechanism due to mild and continuous exposure to reactive species mainly in low and medium dose levels. On the contrary, exposure to high dose level induces perturbations in the redox profile of the majority of tissues. This study simulates the real life exposure to mixtures of xenobiotics through a long-term low-dose administration regimen. The results obtained could, at least in part, provide persuasive explanations with respect to the controversial findings of toxicological approaches that promote the administration of individual chemicals and not mixtures.

S17-03
Comparative evaluation and challenges in translating endpoints from experimental studies to human epidemiological observations

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Regulatory experimental studies are usually performed according to OECD test guidelines (TG) and following good laboratory practice (GLP) principles, so that they are usually attributed higher reliability than other studies; however, this does not necessarily entail a lower risk of bias for these studies. Peer-reviewed scientific studies, although do not adhere to OECD TG or to GLP, also constitute an important part of the database used for risk assessment of chemicals. Experimental studies, however, require extrapolation from animals to humans, from high to low doses, and from single to multiple chemical exposures. Translation of animal data to human is challenging and can be affected by factors such as biological differences between species, internal validity, differences in study design between animal and epidemiological studies, insufficient reporting and publication bias. When results cannot be reproduced under similar conditions, they cannot be translated to humans. Novel technologies can add insight to data obtained from *in vivo* studies for predictive toxicity

assessment; this is the case of new *in vitro* approaches, omics-related tools, organs-on-a-chip and 3D cell culture, *in silico* methods, etc. which collectively improve the understanding of adverse outcome pathways (AOP). Validated and harmonized methods are needed to integrate the multiple lines of evidence relevant to chemical toxicity assessment. These should be generated from model systems representing different levels of biological organization, i.e. molecular, cellular, organ or individual responses. Taken together, data from these studies can be used to develop plausible hypotheses for a mode of action leading to a particular adverse outcome. The translational value of animal research can be improved using refined study designs, appropriate sample size, ethically acceptable protocols, and proper human endpoints in animal experimentation. Systematic review and meta-analyses can identify and counter the risk of bias and discrepancy from individual animal studies. On the other hand, epidemiological studies address the changes observed in heterogeneous target populations from complex exposures and identify links with specific human health outcomes difficult to detect in animal models. Although these studies can be confounded by a variety of factors, systematic reviews and/or meta-analysis of individual studies provide complementary data to analyze risks and should be contextualized together with well-designed toxicological *in vivo* studies and mechanistic studies. When both animal and human studies are available for a given outcome/endpoint, they should be assessed for reliability and strength of evidence prior to the weighting of the various sources of evidence. Such integration accounts for relevance, consistency and biological plausibility.

S17-04
The concept of RLRS for toxicology safety evaluations in our modern world

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While in our modern life we are all exposed simultaneously or sequentially to large numbers of chemicals from various sources, the chronic toxicity evaluations are still performed for single chemicals, using animal models, in order to derive reference doses and regulatory limits of presumed "safety". Epidemiological and biomonitoring studies showed that these single-chemicals' evaluations are not always relevant for real-life exposure scenarios where concurrent exposure to other chemicals usually takes place, at doses around or well below the regulatory limits. Two or more chemicals might exert a combined action leading to new hazards possibly unidentified when testing single compounds and/or increased toxicity as a result of additive, synergistic or potentiation effects. The US-EPA published Guidelines for the Health Risk Assessment of Chemical Mixtures in 1986 and was followed later by efforts of The Agency for Toxic Substances and Disease Registry, OECD, European Commission, EFSA and CLP Regulation (Regulation 1272/2008/EC, 2015) that gives the opportunity to Industry to perform animal testing in commercial mixtures as a last resort to prove a toxicological hazard. Advances in toxicological evaluation of chemical mixtures have raised deep concerns on how mixtures affect environment and human health, especially regarding low-level, long-term exposure to which humans are routinely encountered. Leading experts in the field of toxicology and related areas have developed several methodologies to study long-term toxicity of further than commercial chemical mixtures, with the aim of investigating simultaneously target organ toxicity, genotoxicity, endocrine disruption endpoints, and systemic mechanistic pathways like oxidative stress – the concept of Real Life Risks Simulations (RLRS). The results of these assays, along with those of modern omics technologies, can lead to evaluate long-term health effects of chemical mixtures. Validated and harmonized methods are needed to integrate the multiple streams of evidence relevant to mixture toxicity assessment, to predict the toxicity of mixtures consisting of chemicals of already known toxicity, and to elucidate mechanisms of possible interactions between the chemicals in the mixture.

S18 | Biomarkers in predictive toxicology and risk assessment

S18-01
The exposome in practice

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The exposome is defined as a potential measure of the effects of life course exposures on health. It comprises the totality of exposures to which an individual is subjected from conception to death, including those resulting from environmental agents, socioeconomic conditions, lifestyle, diet, and endogenous processes. Characterization of the exposome could permit addressing possible associations with health outcomes and their significance, if any, alone or in combination with genetic factors. Exposomics work is in the context of the needs of 21st century hazard identification and risk assessment (paradigm of "pathway perturbation", e.g. US National Academy of Sciences volume, 2017). We have identified seven areas where Exposome research can be helpful, with reference to air pollution and water contamination and with emphasis on omics: causal assessment ("meet-in-the-middle"); mixtures; dose-response; cross-omics; calibration of health effects; longitudinal models; data integration. Exposome research shows that the investigation of omics and molecular pathways (e.g. metabolomics, methylome, proteomics) can identify early signs of damage from environmental agents and be used for prediction. In principle pathways are complex and "perturbation of a pathway" can be used to infer that there is a hazard and also to estimate risk.

S18-02
The potential of microfluidic systems in the identification of new biomarkers: highlight on a perfused proximal tubule model

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Biomarkers have immense potential to benefit healthcare and are destined to play a major role in the future of personalized medicine. However, commonly used methods for biomarkers identification, such as 2D culture, are poor predictors and hindered biomarkers discovery. By employing high-resolution, real-time imaging and non-invasive analysis of biochemical and metabolic activities of living cells in an organ context, Mimetas microfluidics (OrganoPlate® [1]) has a great potential to overcome development challenges and to advance biomarkers identification, such as in the assessment of nephrotoxicity [2].

Human RPTEC (SA7K clone, Sigma) and ciPTEC-OAT1 were grown against a collagen I ECM in a 3-channel OrganoPlate®, which allowed the culture and assessment of 40 independent kidney tubules [3]. Drug-induced toxicity was assessed by exposing kidney tubules to 4

known nephrotoxicants from 24h to 48h and evaluated by measuring miRNA-levels as putative biomarkers in the medium. Parallel to this, cell viability with a WST-8 assay and the presence of LDH in the supernatant were assessed [4].

Upon perfusion flow, kidney cell lines formed leak-tight confluent tubular structures in the OrganoPlate®. The exposure to different treatments revealed a significant decrease in cell viability and a dose-dependent release of miRNA in the media which may be good indicators of compound-induced cell death of proximal tubular cells.

These data show the potential of the OrganoPlate® for high-throughput screening and for analyzing metabolites and other secretory products (e.g. by PCR, ELISA, mass spectrometry), which may aid in the identification and development of novel biomarkers for toxicity, efficacy and diseased processes.

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S18-03
Lessons learnt from 'omics' technologies *in vivo* in the last decades

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The application of "omics" in toxicology started with transcriptomics in 1999, when Spencer Farr suggested that all toxicological relevant effects are accompanied by gene expression changes, and that similar toxicological mechanisms cause comparable expression changes. This raised great hopes that toxicological outcomes might even be predictable from shorter term studies. Experience over the last 30 years has brought the omics field back to reality, with mostly mechanistic applications in the context of phenotypic anchoring. Still, several gene expression databases for compound classes causing certain toxicities in rodents were developed and recently became public. With renewed efforts these now allow evaluation of signatures which may enable classification of carcinogenic potential at least for rodent hepatocarcinogens incl. assessment of human relevance. Furthermore benchmark dose modeling for transcriptomics data deliver mechanistically anchored short term *in vivo* study data for a first assessment of toxic levels for environmental chemicals associated with very few data. In addition, other omics data, including metabolomics and epigenomics, are being included into toxicity studies; the latter may especially be relevant for compounds affecting epigenomic regulators. This presentation will give an overview on lessons learned by 3 decades of 'omics' use, with case study examples highlighting specific applications.

S18-04
Use of biomarkers in the assessment of risk from environmental contamination by perfluorinated compounds: strengths and weaknesses

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Recent provisional guidelines from EFSA for PFOA, have used benchmark dose modelling of epidemiological data to derive biomarker guideline values. Endpoints judged to be caused by PFOA have included reduced birthweight, increased ALT and increased cholesterol. Cholesterol associations have been shown in numerous cross-sectional studies, however concern has been raised that this may be due all or in part, to confounding, with doubts also related to the lack of consistency with animal data with some results suggesting that high dose PFOA can reduce cholesterol in rodents.

Cross sectional studies of the association between PFOA and cholesterol are potentially vulnerable to confounding if the excretion or metabolism of cholesterol is correlated with excretion rates for PFOA, both pathways thus affecting measured serum concentrations of both pollutant and cholesterol. This may be plausibly mediated via renal or fecal excretion pathways. In the C8 study in West Virginia and Ohio, the largest population dataset of a community with PFOA pollution, the evidence of confounding and causality can be addressed directly. There were three different designs, in addition cross sectional analyses, with evidence of dose response relationships, which can illustrate the role of epidemiology based on biomarkers in helping to assess risk from PFAS.

In a longitudinal study of over 30,000 adults, higher PFOA exposure was associated with increased risk of hypercholesterolemia. In a nested longitudinal study of 560 adults, degree of fall in serum PFOA was association with degree of fall in cholesterol. In an ecological study of over 40,000 adults, higher degree of PFOA contamination was associated with raised cholesterol. These three designs are not vulnerable to confounding by, for example correlated fecal elimination of PFAS and cholesterol, yet they all showed significant associations pointing to there being a causal association between PFOA and cholesterol. There is likely some confounding in cross sectional data, and more work is needed to determine the causal proportion.

S19 | Endocrine disruption: identification of root causes

Supported by ECETOC

S19-01
ED identification in the EU and the use of weight of evidence

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Mode of action investigations have previously been used to predict or explain findings. Methodology, such as the WHO IPCS human relevance frame work, has been developed to enable systematic and transparent evaluation of evidence for modes of action.

This is first time that a mode of action rather than an end point has been used as the criterion for classification and this has brought challenges to the development of guidance on ED identification. In the legislation, a substance shall be considered as having ED properties if it meets all of the following criteria:

1.) it shows an adverse effect
2.) it has an endocrine mode of action
3.) the adverse effect is a consequence of the endocrine mode of action

ECETOC has developed a logical stepwise approach to evaluating the criteria which is based on using existing methodology for assessing data quality, weight of evidence and mode of action.

EU guidance has redefined criteria b) and c) as:

1.) it shows endocrine activity
2.) there is a biologically plausible link between the adverse effect and the endocrine activity

The redefined criteria raise issues:

- Are they actually equivalent to the legal criteria?

- How do they affect the logical flow of the evaluation?
- How do they shift the burden of proof?
- What are the implications for adequacy of data for registered products?

These questions will be explored.

S19-02
Dose-response relationship of single and combined exposure to ED chemicals *in vitro* & *in vivo*

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Well described yeast-based androgen or estrogen receptor transactivation assays (YAS, YES) were used to assess the combinatorial effects of binary mixtures of antiandrogenic (Vinclozolin and Flutamide) or estrogenic (Bisphenol A, Genistein, Trenbolone) compounds; all mediating effects via the androgen or estrogen receptor. Receptor mediated responses for combined exposure demonstrated an interaction (additive effects) at the low part of the dose response relationship. In absence of effects for the individual compounds, combination of these compounds did also not result in a measurable effect. At the higher end of the dose-response curve there is a reduced response (lower than additive effects), reflecting receptor saturation. *In vivo* investigations examined whether combined exposure to three antiandrogens (Flutamide, Prochloraz, Vinclozolin) result in interference with endocrine homeostasis when applied at very low dose levels, and whether the results of combined exposure are more pronounced than to the individual compounds. A pre-postnatal study design was applied with more parameters than regulatory testing protocols require (additional endpoints addressing hormone levels, morphology and histopathological examinations). Dose levels represented the lowest observed adverse effect level (LOAEL), the no observed adverse effect level (NOAEL), and the acceptable daily intake (ADI) for each individual substance. Anti-androgenic changes were observable at the effect level (LOAEL) but not at lower exposures. Nipple/areola counts appeared to be a sensitive marker of effect, in addition to male sex organ weights at puberty and gross findings. There is neither evidence for effects at low/very low doses, nor for (adverse) effects at the NOAEL dose. A non-monotonic dose–response relationship was not evident. Combined exposure at LOAEL produced enhanced responses for anogenital index, number of areolas/nipples, delayed preputial separation and reduced ventral prostate weight in comparison to the individual compounds. Overall conclusion: dose addition is a conservative estimate for combined exposure.

S19-03
Application of the EU criteria and guidance to identify endocrine disruptors: scientific perspectives

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The EU Regulations concerning plant protection products (pesticides) and biocidal products (biocides) incorporate hazard-based exclusion criteria: active substances deemed to possess certain hazards cannot, generally, be approved for use in the EU. Regulation 1107/2009 for pesticides states that an active substance shall only be approved if it is not considered to have endocrine disrupting (ED) properties that may cause adverse effect in humans or non-target organisms; whilst Regulation 283/2013 for biocides states that active substances shall not be approved if they have ED properties that may cause adverse effects in humans or that are identified in accordance with REACH as having ED properties. Under the regulations that dictate information requirements, if there is evidence that an active substance might have ED properties, additional information or specific studies shall be required to elucidate the mode/mechanism of action; and/or to provide sufficient evidence for relevant adverse effects.

The EU scientific criteria for the determination of endocrine disrupting properties of pesticides and biocides have been in use for several months now, as has their associated guidance document. In this time, the UK competent authority for biocides and pesticides has gained experience of their application to several active substances from both regulatory regimes. These encompass substances where we concluded that the criteria had not been met (no ED-related adverse effects in apical studies), where we concluded the criteria had been met (endocrine activity *in vitro* and *in vivo* with biologically-plausible link to adverse effects on endocrine organs and reproduction in intact experimental animals, of potential relevance to humans), and where we sought expert consultation to achieve consensus (mode of action of adverse effects, and their impact on function of the organ, not clear).

The starting point of the criteria and the additional information requirements is that there should be evidence of ED properties. Experience so far indicates that problems arise when the dataset isn't deemed to be comprehensive enough, even if higher-tier animal studies are available. For example, experts have recently agreed that pesticides with standard datasets were not EDs (no evidence of ED-mediated adverse effects or of endocrine activity in *in vitro* ToxCast screening tests), but because the datasets did not strictly follow the guidance document, two-generation or extended one-generation reproductive toxicity studies were requested; this seems to contravene animal welfare regulations and basic scientific principles. Another potential issue is how a substance will be dealt with that appears to exhibit only isolated findings potentially related to ED or conflicting results from a range of different endocrine activity tests. These situations raise the fundamental question of how such regulatory decisions should be made in a scientific, weight-of-evidence-based manner.

S19-04
The real causes of changes in trends of "Endocrine Related Diseases": an epidemiological perspective

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There is controversy over the causes of rising disease incidences of diseases potentially associated with xenobiotic endocrine active compounds. These diseases or health parameters include, testicular cancer, hypospadias, cryptorchidism, low birth weight, prematurity, autism, male infertility, and PCOS. We conducted a targeted literature reviews on the risk factors for testicular cancer, hypospadias and childhood obesity and combined these with routinely collected demographic data.

Results: Changes in birth order distribution and maternal age at first pregnancy explain a substantial proportion of the increases in these diseases: The change in the proportion of first born boys over time explains an increase of 26% in testicular cancer. The change in nulliparity and maternal age explain an increase of 34% in hypospadias prevalence. The change in family size by itself explains an increase of 24% in childhood obesity.

Conclusion: Changes in reproductive and demographic factors such as family size, parity and maternal age at first pregnancy have had a profound impact on disease trends of the last five decades. The impact of subfertility, today not being a key determinant of family size, on factors like low birth weight and prematurity needs to be further investigated.

S20 | Investigative Toxicology Leaders Forum (ITLF): Scientific advancements and case studies for the optimization of drug discovery

S20-01
DILI revisited – key results from the innovative medicines initiative MIP-DILI project

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Adverse drug reactions (ADRs) remain a challenge in modern healthcare, particularly given the increasing diversity of therapeutic drug modalities. Of the different ADRs, the liver is perhaps among the most susceptible to drug-related toxicity. Drug-induced liver injury (DILI) also remains a challenge for the pharmaceutical industry, contributes to the attrition of drugs in development, and is among the leading causes for post-marketing drug monitoring and market withdrawal.

A key driver in the challenge to predict human DILI is the multifactorial nature of the disease. This simply means that some of the single cell screening assays employed by Pharma, with refinement and optimization, can successfully facilitate first tier testing of compounds for hepatocellular toxicity and mitochondrial dysfunction, but cannot identify drugs that have idiosyncratic DILI.

As part of the global effort to improve on drug safety, the MIP-DILI Consortium undertook a 5-year public-private funded programme to identify current practices for DILI testing by Pharma to improve on the panel of *in vitro* tests currently employed and contribute to our understanding of the mechanisms of human DILI. Through a greater understanding of mechanisms that underlie many of the different forms of human DILI and by defining appropriate biomarkers *can these combined efforts improve upon existing and future test systems for the detection of drug liabilities and prediction of human DILI.* Central to these objectives, a roadmap proposing a three-tiered approach for use of current and future models for the prediction of human DILI was defined. The Roadmap encompasses mono- and multi-cellular assays and complex test systems integrating the phenotype and functional characteristics required of these models for use in drug development.

This paper provides an overview covering the key highlights and accomplishments by the MIP DILI consortium; greater understanding of DILI mechanisms, the adoption of the refined cell-based assays, protocols and progress towards the standardization of testing strategies by both academic industrial partners.

S20-02
Prevention and reversion of ALT increase by bile acid sequestration in dog treated with FGF401, a selective FGFR4 inhibitor

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The FGF19-FGFR4-βKlotho (KLB) pathway plays an important role in the regulation of bile acid (BA) homeostasis. Aberrant activation of this pathway has been described in the development and progression of a subset of liver cancers such as hepatocellular carcinoma (HCC), establishing FGFR4 as an attractive therapeutic target for such solid tumors. FGF401 is a highly selective FGFR4 kinase inhibitor being developed for HCC. In preclinical studies in mice and dogs, single or repeated doses of FGF401 led to induction of Cyp7a1 and BA biosynthesis, resulting in increased BA pool size, decreased serum cholesterol and diarrhea in dogs. FGF401 was also associated with increases in serum aminotransferases, primarily alanine aminotransferase (ALT), in mouse and dogs in the absence of any observable adverse histopathological findings in the liver, or in any other organs. We hypothesized that the increase in ALT could be secondary to increased BAs and conducted an investigative study in dogs with FGF401 and co-administration of the BA sequestrant cholestyramine to test this hypothesis. Here we show that co-administration of cholestyramine with FGF401 prevented and reversed FGF401-related increases in ALT in dogs in parallel to its ability to reduce BAs in the circulation by intestinal binding and increased fecal excretion. BA profile analysis revealed that effects of BA sequestration were most pronounced for secondary BAs of high hydrophobicity. In addition, FGF401-mediated increases in ALT correlated with increases in TLCA and TDCA, the major secondary BAs in dog plasma, suggesting a mechanistic link between ALT elevation and BA pool hydrophobicity. These data therefore confirm our hypothesis that the increase in ALT with FGF401 is likely secondary to BAs increase and can be prevented by cholestyramine.

S20-03
Elucidating the role of mitochondrial dysfunction in drug-induced intrahepatic cholestasis

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Drug-induced intrahepatic cholestasis (DIC) represents the most frequent clinical manifestation of drug-induced liver (DILI), with bile acids (BAs) being recognised as the causative agents of toxicity. Whilst it is recognised that BA-induced toxicity is multi-mechanistic, research in isolated mitochondria and HepG2 cells has revealed that BA toxicity and mitochondrial dysfunction occur simultaneously in DIC. However, much of this prior research has been conducted using single BAs and thus overlooked the effects that a combination of BAs would have on the mitochondria.

HepaRG cells are a more suitable cell choice for DIC studies as they differentiate into hepatocytes and biliary-like cells and have a dynamic biliary system characterised by functional biliary transporters. Therefore, the aim of this research was to investigate whether BA mixture-induced mitochondrial toxicity could be detected concurrently in HepaRG cells and isolated mitochondria.

The mitochondrial toxicity of the BA mixtures was examined in HepaRG cells using Seahorse respirometry, alterations in mitochondrial membrane potential (MMP) and an acute metabolic modification assay. These results were then compared with the mitochondrial dysfunction detected in isolated mitochondria by changes in MMP and structural modifications.

It was demonstrated that 1000 x BA mix resulted in significant MMP depolarisation and structural alterations in isolated mitochondria. By contrast, BA-induced mitochondrial toxicity was not detected in HepaRG cells, as there were no significant changes in oxygen consumption rate, MMP or ATP levels between glucose and galactose media. BA mixtures were deemed cytotoxic as 1000 x BA caused a significant decrease in protein and retained LDH following 2 weeks treatment.

Overall, the results suggested that BA-induced mitochondrial toxicity does not precede cytotoxicity when studied in a whole cell sys-

tem as opposed to when using isolated mitochondria. The toxicity of DIC is multi-mechanistic thus suggesting that a limitation of isolated mitochondria is their lack of cellular context and physiological relevance. This research has highlighted the importance of studying mitochondrial toxicity in different models simultaneously in order to gain mechanistic insight that is applicable to the *in vivo* pathophysiology.

S20-04
Liver mitochondrial toxicity in the context of hypoxia

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Drug-induced mitochondrial perturbation is associated with severe clinical toxicities, leading to drug withdrawals. Current *in vivo* preclinical toxicity testing is relatively insensitive to mitochondrial toxicity and is far removed from the metabolic context of compromised patient populations. An optimal testing mode would include a clinically relevant, system-wide physiological stressor, such as hypoxia. Here, we examined the effects of acute (2d) and prolonged (2wk) hypoxic exposure (10% O_2) upon the metabolic response to a mitochondrial CIII inhibitor (GSK932121A) or vehicle control (VC) in female Crl:CD(SD) rats (n=8/group). Assessment of mitochondrial respiratory capacity in the liver using high resolution respirometry demonstrated a 58% increase (normoxic vs. 2d hypoxic VC, $p<0.05$) in both maximal oxidative phosphorylation and electron transfer system capacities at 2d, but not 2wk, hypoxic exposure. This effect was absent with drug treatment. Lipidomics analysis using LC-MS demonstrated a clear shift in lipid profile at 2d, including a drop in triglycerides associated with *de novo* lipogenesis that was recovered at 2wks. This response was again absent in the drug treated animals. These results thus demonstrate an interaction between the metabolic response to 2d hypoxia and CIII inhibition in the liver, in turn implying that 2d hypoxic exposure may hold promise as an effective testing model for toxicity assessment of mitoactive compounds.

S21 | Comprehensive toxicological profiles in nanoformulations for blood brain barrier

S21-01
Cargo-influenced distribution of polymeric nanoparticles at biological barriers

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Purpose: The blood-brain barrier (BBB) is both a blessing and a curse as, on one hand, it protects the delicate homeostasis of the central nervous system (CNS) and protects the brain tissue from toxins, but at the same time it is an obstacle for neuropharmacology. During the last decade, we have investigated NPs made of different polymers and tested them regarding their ability to enter into CNS parenchyma.

Methods: To analyze their kinetic, the nano-systems were loaded with various fluorescent markers for imaging. We have mainly worked with polybutylcyanoacrylate (PBCA) NPs, poly (lactic-co-glycolic) acid (PLGA) NP and polyvinylpyrrolidone (PVP) NPs. The distribution of these polymeric, fluorescent NPs at the blood-retina barrier – which is a model of the BBB – was investigated by *in vivo* confocal neuroimaging (ICON). With this technique we image the retina of anaesthetized, living rats with microscopic resolution using a confocal laser scanning microscope. As the retina is a structure of the brain we can monitor *in vivo* and online the fluorescent NPs' distribution in the vessels and in the parenchyma of the CNS after intravenous injection.

Results: Loading polymeric nanoparticles with different dyes resulted in distribution of the fluorescence markers in different compartments and even a different kinetic of the particle-carriers themselves was detected. This means that any change in formulation can have a significant but hardly predictable influence the kinetic of NPs. As a consequence, no nano-carrier system can be declared in general as safe regarding keeping the loaded compound out of the CNS. Therefore, any new NP design needs an "individual *in vivo* profiling" regarding BBB passage.

S21-02
Extracellular matrix and nanoparticles interaction – breaching new barriers?

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Extracellular matrix (ECM) represents a complex network of variously modified proteins and the glycosaminoglycan, hyaluronan, highly organized in a form of a suprastructure which ultimately constitutes the cell microenvironment. The ECM compartments are classified as: the pericellular in the immediate vicinity of the cells and the "far away" intercellular compartment. Cells are embedded in this highly specialized network which regulates cell biological functions and defines tissue properties. Importantly, ECM is an indispensable part of all biological barriers and substantially modulates the interchange of the nanotechnology products through these barriers. The blood brain barrier (BBB) is a highly specialized type of tissue-endothelium interchange. The endothelial cells are endowed with efficient, but highly specific efflux mechanisms, such as the multidrug resistant proteins and P-glycoprotein-transport systems, which transport "forbidden" compounds back to the vessel lumen. The communication through the BBB is managed by the gliovascular unit (GVU) with established roles for astrocytes, pericytes and perivascular cells. The complex BBB/GVU structure maintains brain homeostasis, controlling molecule, ion and cell transportation into the brain tissue. In addition, the ECM compartment deposited between endothelial barrier and astrocytes/pericytes both structurally and dynamically regulates BBB function. The interactions of the ECM with nanoparticles (NPs) depend on ECM morphological characteristics and on the physical characteristics of the NPs and may be either deleterious or beneficial. Importantly, an altered expression of ECM molecules ultimately affects all biological processes including inflammation and will modulate NPs penetration and interaction with the tissues. Interactions between the NPs and BBB, with focus on the ECM components, in both health and disease milieu will be reviewed.

S21-03
New nanosized macro-molecular system and their interaction with biopolymers and living objects

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Methods of synthesis of amphiphilic derivatives of a number of water-soluble polymers, in particular, poly-N-vinylpyrrolidone, have been worked out. In experiments on cells and experimental animals, the harmlessness of the polymers obtained was shown.

The synthesized amphiphilic polymers are capable of spontaneous aggregation in aqueous solutions with the formation of nanoscale micelle-like aggregates and are suitable for modifying liposomal membranes. The influence of the structure of amphiphilic polymers on the tendency to aggregation has been established. Such systems can be used as carriers of poorly soluble and water-insoluble medicinal substances [3,4].

It was shown, what aggregates of amphiphilic polymers of N-vinylpyrrolidone proved to be suitable for use as carriers and modifiers of various proteins and peptides (blood factor IX, angiostatin, Bowman-Birk soybean proteinase inhibitor (BBI)). In these cases, immobilization with the use of polymeric aggregates increases the resistance of proteins to denaturing effects, and thereof their total biological activity.

Methods have been developed for the introduction into the synthesized amphiphilic polymers of various functional groups. For example, introduction of additional side aminoacid groups in the polymeric part of amphiphilic systems allows the use of aggregates as carriers of nucleic acids and their subsequent application for transfection in genetic engineering [5,6].

Using fluorescent labels and probes, it was shown that the immobilized substance introduced into larger size aggregates penetrates into the living cell due to endocytosis, localizing in the cytoplasm inside the endosome. On the other hand, when immobilized active agent is introduced in smaller-sized aggregates, it evenly spreads both in the cytoplasm of the cell and in its nucleus. When studying the transport of aggregates of amphiphilic polymers of N-vinylpyrrolidone in the body (rats), it was established that a fluorescent probe immobilized in aggregates of amphiphilic polymers, when injected into the tail of experimental animals, quickly reaches the vessels of the eye [7-9].

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S21-04
Bio-inspired nanoparticles in neuroscience

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Bio-inspired nanomaterials area has been continuously developing to overcome the toxicity of nanoparticles. Nature has inspired nanomedicine to use materials that mimic/resemble/reproduce the complexity of biomolecules. The central nervous system has a redoubtable blood brain barrier (BBB) which limits surgical, therapeutic and diagnostic interventions, so nanomaterials were one of the best therapeutical choices to overcome BBB. Nanoparticle can tackle BBB and neural tissue at the nano-scale level.

The two main directions using nanoparticles were exploited in neuro-imaging techniques and in targeted drug delivery. For the imaging techniques nanoparticles should escape the reticulo-endothelial system and further accumulate in the target tissue. So contrast agents, less than 50 nm in size, negative surface charge and high surface area can bind to the tissue biomolecules. Thus, the first bio-inspired contrast agent in neuro-science was developed in the early 90s where a polypeptide coated magnetite nanoparticle conjugated with a tumor specific antibody was developed. This first report showed that magnetic nanoparticles can be good MRI contrast agents and hence created a platform for developing new application of nanomaterials in both neuro-diagnostics and neuro-therapy.

Targeted delivery of drugs overcoming BBB took advantage of the bio-inspired nanomaterials. In this area bio-inspired surface modifications that intend to regulate the specific composition of the outer corona of nanocarriers can facilitate BBB penetrations. Therefore the receptor mediated endocytosis is one well known phenomenon that was exploited within. Viral pathogens that are nano sized are successfully crossing the BBB and further are localizing in the brain tissue. HIV particles (average size 120 nm) cross BBB by adsorptive transcytosis. Mimicking this known pathological process developed by viruses, nano-particles functionalized with HIV derived peptides could successfully deliver a drug through the BBB within brain parenchyma. The penetration ability of nanoparticles was searched in several neurological disorders, in brain tumors, in neurodegenerative diseases like AD, PD, cerebral palsy, and Huntington's disease. Another biological process that inspired nanoparticles application in neuroscience is the trans-endothelial migration of leukocytes. This phenomenon can take place due to increased permeability and hypertrophy of brain endothelia induced by cytokines like TNF, interleukins, interferons. Nanoporous silicon particles that were covered with leukocytes membranes were shown to transport and deliver a payload across BBB through receptor-ligand interactions.

The main groups of biomolecules that developed the nanomaterials and their applications in nanomedicine are presented to be used in neuro-diagnostics are neurotherapy.

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S22 | Advancing toxicological evaluations inresolving current policy controversies in GMO products

S22-01
Integrating multiple 'omics' analysis to study the effects of herbicide-tolerant crops

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Safety concerns arising from the consumption of herbicide-tolerant crops remains a controversial topic. In most countries, health risk assessment of genetically modified (GM) crops relies primarily on a compositional assessment which is used to establish "substantial equivalence" of a GM crop to its conventional counterpart. In addition, animal feeding trials can be done to evaluate potential toxicity and allergenicity. In this presentation, I explain how high-throughput molecular profiling technologies known as 'omics' can be used to improve the reliability of the risk assessment of GM crops. The example of the glyphosate tolerant GM maize NK603 will be used.

In order to understand potential metabolic effects due to the consumption of the NK603 GM corn, we evaluated compositional differences between the NK603 GM corn and its isogenic counterpart using metabolomics and proteomics. This revealed energy metabolism changes which can be linked to the insertion of the transgene [Mesnage *et al*., 2016]. The maize kernels analysed were previously used in a chronic study investigating the potential toxic effects arising from the consumption of the NK603 Roundup-tolerant GM maize in rats. We analysed the transcriptome and metabolome of liver and kidneys from this study [Mesnage *et al*., 2017]. There were no statistically significant differences that we could attribute to the consumption of the NK603 Roundup-tolerant GM crop (with our without Roundup application during cultivation). This suggested that the metabolic changes caused by the transgene insertion are not toxicologically relevant.

An area that remains relatively unknown are possible effects of the consumption of GM food crops on the gut microbial ecosystem. We recently investigated the relationship between faecal microbiota and plasma metabolome in rats fed NK603 and MON810 GM maize from the GMO90+ study [Mesnage *et al*., 2019]. There were no statistically significant differences in taxa abundance in the rat faecal microbiota, which showed that the consumption of the widely cultivated GM maize varieties NK603 and MON810 even up to 33% of the total diet had no effect on the status of the faecal microbiota.

A large number of animal toxicity tests have been performed with herbicide-tolerant crops. In my opinion, the main lesson learned from 20 years of animal feeding trials with GM crops is that these tests are difficult to perform and alternatives are needed. Animal feeding trials with whole food/feed such as GM crops are largely irreproducible because they have a low power to detect adverse effects. The use of 'omics' approaches can improve the predictive ability of these tests, as well as the accuracy of the comparative analysis of chemical composition. Future health risk evaluations would benefit from the use of high-throughput 'omics' technologies.

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S22-02
Adverse outcome pathways (AOPs) and challenges in chronic studies with GMOs

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Safety evaluation of food derived from genetically modified organisms (GMOs) remains a controversial topic despite years of research and the establishment of national and international regulatory frameworks. In particular, the need for conducting chronic feeding trials is a matter of debate since testing health risks of whole foods in such studies is an extremely complex undertaking, and relating any detected adverse effects conclusively to a specific characteristic of the food can be very difficult. Safety testing has therefore focused on comparative compositional analyses as well as intended and unintended consequences of the genetic modification. These include differential expression of proteins and other substances with potential toxicity and/or allergenicity, nutritional modification or the possibility of accumulation of pesticide residues or contaminants. Here, the concept of Adverse Outcome Pathways (AOPs) may be helpful in guiding appropriate testing strategies. An AOP defines a series of necessary key events that link a molecular initiating event to a final adverse outcome. It provides a scientific rationale for the processes that lead to the activation of the next key event, i.e. the key event relationship (KER), considering relevant exposure and internal doses. Particular emphasis is put on weight-of-evidence assessment and quantification of KERs. In the case of GMOs, the adverse outcome could for example be an allergic reaction as a consequence of induction of sensitization by a newly introduced or differentially expressed food protein. The concept of such an AOP has been introduced but no detailed work on its individual components has been carried out so far. However, the well-established AOP on covalent protein binding leading to skin sensitisation (https://aopwiki.org/aops/40) could serve as a starting point. Thus, the AOP framework has the potential to advance and facilitate future safety testing of GMOs as part of an integrated approach.

S22-03
Is the success of genetically modified food and feed at nature's cost: highlighting the potential risks of GMOs

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Global commercialization of GM food and feed has stimulated much debate over the potential of risks for the consumers' health. However, it is important to examine the variable potential risks of GM crops within the context of wider knowledge. Researchers and policy makers have an increasing interest in exploring unintended effects of transgenes associated with gene flow, flow of naked DNA, weediness and chemical toxicity. The current state-of-the-art knowledge reveals that GM crops might impart damaging impacts on the environment such as modification in crop pervasiveness or invasiveness, the emergence of herbicide and insecticide tolerance, transgene stacking and disturbed biodiversity. However, underpinning research also realizes that the influence of GM crops on a disturbance in biodiversity, development of resistance and evolution slightly resembles with the effects of non-GM cultivation. Improved experimental techniques for long duration studies are currently been conducted in leading labs working on GMO risk assessment targeting to discover associated risks. Improved strategies adopted by these studies aim to represent a proof-of-concept that could drive the current prototype of GMO evaluations. The need for up-to-date, valid and harmonized methods will bring to focus the attention of policy makers, regulatory authorities, governments and will help to authenticate the possible long term unexplored effects, risks and damages to environment, ecosystems, biodiversity, and health prior to the release of any GM crop, food or feed.

S22-04
Scientific challenges for GMO regulation in Europe – The EFSA GMO Panel Chair's perspective

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The Panel on Genetically Modified Organisms (GMO) at EFSA provides independent scientific advice on the risk assessment of food-related genetically modified plants and animals. This integrated risk assessment is based on the molecular characterization of the organisms containing the new genetic event(s), the assessment of their safety when organisms with this event or these events are used as food or feed and the assessment of their safety for the environment. In the EU, essentially all procedures for the evaluation of GMO applications are determined by the detailed requirements laid down in Regulation (EU) No 503/2013. Current challenges of the GMO panel include, therefore, the science-based implementation of this rigid legal framework. We expect that this will be facilitated in the upcoming future by advances in bioinformatic ("*in silico*") tools and the development of *in vitro* methods for the prediction of hazards and risks, particularly in the assessment of newly expressed proteins with regard to their potential toxicity, allergenicity or their ability to induce non-immunoglobulin-mediated immunopathologies. Long-term challenges may include the risk assessment of gene-edited organisms, the assessment of organisms generated by synthetic biology approaches and the risk assessment of gene drive applications. If you are interested in the activities of the GMO panel, please consider participation at the November 27th–28th meeting open to observers.
(https://www.efsa.europa.eu/en/topics/topic/gmo)

S23
Optimization of existing and construction of new testing strategies for skin sensitization potency

S23-01
Development of a guideline on defined approaches for skin sensitisation

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A number of non-animal methods addressing key mechanisms underlying the acquisition of skin sensitisation are now available as OECD Test Guidelines. None of these methods in isolation is considered to provide equivalent information to the animal tests. Thus the data generated by individual methods have to be used in conjunction with other relevant information. For the purpose of hazard identification and characterisation, assessments based on a weight-of-evidence approach, are not desirable since they imply expert judgment and possible divergent conclusions.

To facilitate consistent application and interpretation of non-animal data from different sources as well as acceptance of predictions under Mutual Acceptance of Data, the OECD is developing a Guideline on Defined Approaches for skin sensitisation. Within Defined Approaches data are combined using a fixed data interpretation procedure (DIP), thus reducing bias in subjective interpretations. The Defined Approaches currently under consideration use simple DIPs and provide information for hazard identification and potency subcategorization according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Defined approaches that are based on more complex DIPs will be evaluated in successive phases of the OECD project.

Although it is recognised that the defined approaches under evaluation do not represent a final solution for risk assessment purposes, they can nevertheless play a role in the safety assessment of skin sensitisers. An overview of the state of play of the development of a Guideline on defined approaches for skin sensitisation will be provided.

S23-02
Chemical mapping of skin sensitizers in a reconstructed human epidermis model using HRMAS NMR spectroscopy

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Allergic contact dermatitis is a reaction of the immune system resulting from skin sensitization to an exogenous hazardous chemical. The prevalence of this disease (15-20%) has increasedand, as there is no treatment other than symptomatic, the prevention relies on the evaluation of the sensitizing potency of chemicals prior to their introduction on the market. One of the alternatives to animal methods for risk assessment of chemicals is by measuring their reactivity towards epidermal proteins. So far, this was approached using isolated small reactive peptides in solution but these tests are far from reflecting the complex chemistry that takes place in a living epidermis. With the aim of replacing animal tests while maintaining the most similar reaction conditions to the human epidermis, we have developed a new method based on the use of quantitative High Resolution

Magic Angle Spinning (HRMAS) Nuclear Magnetic Resonance (NMR) to monitor *in situ* the reactions of carbon 13 substituted chemical sensitizers with nucleophilic amino acids in reconstructed human epidermis (RHE).

Four skin sensitizers were investigated, namely methyl methanesulfonate (MMS), cinnamaldehyde (CIN), methylisothiazolinone (MI) and *p*-phenylenediamine (PPD). We have been showing that the reactivity of chemical skin sensitizers can be quantified (nmol/mg of RHE) in RHE using a 1D Heteronuclear Single Quantum Correlation (HSQC) sequence and this was applied to quantify either the effect of the dose and/or of the reaction time. Compared to peptides in solution, reaction were found to be much faster in RHE together with a fast detoxication through the formation of GSH conjugates and metabolism via phase I/II enzymes.

For the first time, our proposed methodology that combines quantitative HRMAS NMR and RHE, allowed following and quantifying the reactivity of skin sensitizers with nucleophilic residues of epidermal proteins present in a complex 3D tissue. Thus, we were able to monitor, *in situ*,broader aspects of chemical reactivity like detoxication and metabolism that are not covered when using peptides in solution.

S23-03
A defined approach for skin sensitization potency integrating *in silico*, *in chemico* and *in vitro* cell data

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Despite the growing number of non-animal methods to predict skin sensitization, no single measurement is yet sufficient to predict sensitizer potency. Consequently, many testing strategies combining data from these methods with other relevant information have been proposed. Taking into account this rationale, and as part of our ongoing collaboration, ToxFinder has previously developed a defined approach (DA) for potency classification (weak, moderate, strong or extreme) based on linear discriminant analysis of *in silico*, *in chemico* and *in vitro* data. This DA showed a good predictive performance for LLNA potency categories, with an accuracy of 82.2%. Moreover, in two blind trials performed in collaboration with the Cosmetics Europe Skin Tolerance Task Force, from 50 sensitizers tested (including 10 challenging phase III chemicals), 96% were classified in either the correct or the adjacent potency category while only 4% were misclassified with more than one potency category.

In the present project we intend to optimize our previously developed model to become a single defined approach for skin sensitization potency allowing simultaneous hazard identification and potency categorization of chemicals. The method will be optimized to classify chemicals within five potency categories (non-sensitizer, weak, moderate, strong or extreme) using human data as training source. To derive the model, a dataset with the following discrimination variables will be created: 1) *in vitro* data: the EC_{30} values of chemicals assessed in THP-1 cells; 2) *in chemico* data: lysine and thiol depletion and 3) *in silico* data: molecular descriptors obtained from eDragon Software. Using the above data inputs (1-3) we will establish a discriminant mathematical model that will be further validated with a new set of compounds, in a blinded assay.

If successful, the proposed DA will be able to drive both hazard identification and potency categorization without the need for a two-tiered approach, presenting several advantages over other DA, namely less time consuming and expensive and also reducing the generation of false negatives and positives. An overview of the results obtained will be discussed.

S23-04
Practical application of existing and new testing strategies/ defined approaches for risk assessment of cosmetic compounds

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Cosmetics Europe has compiled a database of non-animal skin sensitization data and has used it to evaluate the defined approaches included in the OECD IATA case studies project. In the next step we have run case studies where the participants we asked to use these data as basis for their safety assessment. The sharing of case studies at a workshop demonstrated that the available Defined Approached (DA) can form an integral part of the New Generation Risk Assessment (NGRA) but alone are insufficient to draw a conclusion. Due to the variety of risk assessment needs and complexity in types of information that should be integrated in the skin sensitisation risk assessment, a guidance is needed to support NGRA for cosmetic ingredients.

To respond to this need, we have started building a framework which is based on the classical risk assessment workflow and relies upon the principles for next generation risk assessment of cosmetics developed by the International Cooperation on Cosmetics Regulation (ICCR). The key elements are that the risk assessment should address a clear question. It must be human relevant, exposure led, hypothesis driven and designed to prevent harm. A tiered iterative approach should be applied, starting with a thorough review of all of the available existing information. Available data from non-animal test methods can be utilised within any of the available DA, the choice of DA applied might be dependent upon the information available and the risk assessment question.

In conclusion, significant progress has been made in development and application of non-animal approaches in NGRA for skin sensitisation. The workflow presented here will help harmonize the risk assessment process while allowing sufficient flexibility for integrating different data and use for diverse chemical space.

S24 | Toxic epidemics: why should we still be worried in 2019?

S24-01
The opioid overdose crisis: the reasons for the worldwide threat

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Opioids represent the first cause of drug-induced fatalities in the US, with ~1000 Americans dying each week from opioid abuse or misuse. Partial reports confirm that opioids are responsible for a worldwide health issue including in some European countries. Several factors explain the increase in opioid abuse including the development of multiple molecules and formulations by the pharmaceutical companies, the facilitated opioid prescriptions to combat acute/chronic pain, the underestimation by physicians of the dependence risk in

the chronically opioid-treated patients and the spread of new opioid-based psychoactive substances (mainly fentanyl derivates) on the recreational scene. All these factors have contributed to the enhanced availability of opioids at home, while pathways resulting in dependence may facilitate cross-abuses. Opioid overdose is responsible for consciousness impairment, miosis and ventilation depression. Toxicity onset and duration are variable, depending on the opioid properties, formulation and route of administration. The dose is not the unique factor determining the overdose risk: gene polymorphisms, drug-drug interactions, additional interactions at other receptors contribute to explain the individual vulnerability. Naloxone, a competitive opioid receptor antagonist, is the first-line antidote to reverse opioid-related neuro-respiratory toxicity. Maintenance treatments are the cornerstone for the management of opiate dependence. Recently take-home intranasal naloxone programs were developed to allow laypersons administering the antidote to the opioid-overdosed person, preventing respiratory arrest onset and giving enough time to hospital transport. Abuse of opioid analgesics in combination with the heroin return and frightening spread of new opioid-based psychoactive substances represents a threat that requires international cooperation, law harmonization, and pharmacological approach using maintenance treatments and take-home naloxone.

S24-02
The new psychoactive substances (NPS)

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This talk will provide an overview of the NPS phenomenon, the emergence of these drugs, outbreaks of acute toxicity and fatalities associated with their use, the impact of legal responses and changes in their use and the acute harm associated with their use in recent years.

S24-03
The anticholinesterase pesticides

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Epidemics of poisoning with anticholinesterase and other pesticides through food contamination have been reported since the beginning of the Green Revolution in the 1950s. Contamination of food with parathion during transport by train in Kerala in 1958 affected 400 people and killed 40, causing widespread panic. Contamination of flour by parathion during storage at a European port in 1976 resulted in 79 cases and 17 deaths in three outbreaks after transport to Jamaica. More recently, in India during 2008, disposal of methyl parathion into drains resulted in contamination of water used to make chutney, causing two deaths amongst 65 cases, mostly children. Of note, consumers were not able to detect the pesticides in the food they ate. Patients presented to multiple hospitals with no apparent cause – in the last case, 33 patients presented within 15 min to one small hospital. With improved food hygiene and storage, such epidemics have not occurred in Europe for many years. However, the potential exists for intentional contamination of food stuff that would be rapidly distributed, locally or across borders, and result in many severely poisoned patients presenting to multiple hospitals with no obvious link. Health care systems need to be alert to the possibility of intentionally contaminated food causing sudden unexpected outbreaks of poisoned patients; food and agriculture authorities must have systems in place to rapidly trace and track such food. Clinicians need to be alert to the possible diagnosis of anticholinesterase poisoning and be confident in the resuscitation of these patients with atropine, oxygen, fluids and oximes.

S24-04
The toxic alcohol

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Methanol is one of the most widely used toxic alcohols throughout the world: approximately 70 million metric tons are consumed globally yearly. Each day, almost 200,000 tons of methanol is used as a chemical feedstock or as a fuel. Today the global methanol industry generates about $55 billion dollars per year. Mass or cluster acute methanol poisonings as a result of its use as a cheap substitute for ethanol occur frequently globally. In the twenty first century, the geography of methanol epidemics includes almost whole world, many developing and developed countries in Europe, Asia, and Africa. One of such modern age methanol "epidemics" was happened in the Czech Republic in 2012 with almost 140 cases and more than 50 deaths.

The long-term health effects of acute or subacute methanol exposure are not well studied. We performed a 6-year prospective cohort study of the subjects who survived acute methanol poisoning during the Czech Republic mass methanol poisoning outbreak. Of 84 patients who survived acute poisoning, 15 subjects died during the follow-up period including seven patients who died on cancer of different localizations. Therefore, the follow-up mortality was 18% during six years of observation.

We have found that acute methanol-induced optic neuropathy may lead to progressive chronic degeneration of ocular retina during the years following methanol poisoning in up to 24% of survivors. These patients demonstrated further visual loss in the following years. Brain lesions were detected in 52% of the survivors of methanol poisoning, mostly bilateral necrosis of the putamen; other vulnerable regions were the globus pallidus, brainstem, and subcortical white matter.

Evidence has accumulated over the last decades about the role of exposure to environmental toxic agents in chronic neurodegenerative diseases. Acute or chronic exposures to organic solvents are known to induce Parkinsonism in humans or influence the pathogenesis of Parkinson's disease due to the earlier loss of neuronal redundancy or damage to critical neuronal systems. From the experimental studies it is known that exposure to methanol causes depletion in dopamine levels and degeneration of the dopaminergic nigrostriatal pathway. However, further evidence is needed on the role that exposure to methanol plays in neurodegenerative processes. Therefore, prospective follow-up studies in the survivors of acute exposure to methanol may determine its role in the neurodegenerative processes developing during six or more years after acute poisoning, character and dynamics of these processes, and the effectiveness of therapeutic interventions.

S25 | Detection, assessment, management and communication of risk in mass human toxic exposures

S25-01
Warfare scenarios involving chemicals

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Recent events worldwide showed that the threat by chemical warfare agents has increased despite the destruction of most chemical weapons. It became obvious that former cold war scenarios in which state actors are involved need to be extended by poison attacks of rouge groups against unprotected civilians. Moreover, terrorist and murder attempts made the headline. Generally, nerve agents are the most toxic compounds and therefore represent the focus of this presentation.

In nerve agent poisoning early diagnosis is important for initiation of the immediate life-saving treatment by oximes and atropine as well as the identification of worried well to enable optimal utilization of limited resources. In detail, determination of acetylcholinesterase activity on-site allows confirmation of the clinical diagnosis of highly acute poisoning. Intoxication might result either from volatile cholinesterase inhibitors (e.g. sarin) but may also be due to a more slowly (hourly) progression of inhibition by persistent nerve agents such as VX especially after dermal exposure. In the latter cases, administration of an effective reactivator may even prevent development of signs and symptoms when the absorbed amount of poison is not too high. However, due to a longer persistence of V-substances in the body repetitive bolus doses or continuous administration of oximes may be necessary. The cholinesterase status monitor allows the estimation of the time frame for successful oxime application as well as the rationale of reactivator therapy (exclusion of aging (e.g. soman) or poisoning by nerve agents which are hard to reactivate (e.g. tabun).

Verification of exposure to nerve agents is not possible in the field but needs analysis of biomedical samples with sophisticated analytical methods. Innovative methods allow verification of nerve agent poisoning several weeks after the incorporation.

S25-02
Environmental contamination by organochlorine residues: Lindane manufacturing residues.

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The problems related to organochlorine residues have been a matter of concern for decades. Here we show the example of hazard and risk management strategies to deal with a heavily contaminated site in a very sensible area in the North of Spain due to the residues generated by a Lindane factory.

A chemical Company operating between 1975 and 1992 dumped about 6,800 Tm/year of solid residues and 300-500 Tm/year of liquid residues given an estimated amount of approximately 115,000 tons of waste products which were mainly dumped in two unlined landfills in a mixture composed of lindane itself, the other HCH isomers, benzene, chlorobenzenes, phenols and chlorophenols. These residues can be found in a solid and a liquid form, this last amounting to four thousand tons of a dense non aqueous phase liquids (DNAPLs) which constitute a particularly serious risk for the environment due to the proximity of the Gállego River.

Two approaches were implemented in parallel: Surveillance procedures to verify the impact on the environment, including atmosphere, aquifers, and surface waters and wildlife, and human general and occupational exposed population and research for remediation procedures aimed to try to dispose of solid and liquid waste. A third important point is to design communication strategies to inform the population in an open and clear way about the risk and the measures adopted to prevent it.

To survey the atmospheric contamination, devices to collect and measure particles, and HCH vapor were placed in several points. No significant contamination has been ever found in or near inhabited areas.

To survey aquifers contamination daily analysis inform on site from the exit point of the ravine and along the river's course. The aquifer samples, mainly in the dense phase, contain benzene and chlorobenzenes and HCH isomers in the range of ten to hundred grams per kg.

No human effects have been detected either in general or occupational potentially exposed population.

Decontamination strategies included the transfer of solid wastes to a new safe cell (250,000 m3 capacity), extraction of 20 tonnes of DNAPL by means of "pump and treat" techniques and a chemical oxidation technique to treat on-site the DNAPL still trapped in the soil fractures.

S25-03
Outbreaks by contaminated food and beverages

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Outbreaks of mass or cluster poisonings by chemical contamination of food or use of toxic alcohols as a cheap substitute for ethanol in alcoholic beverages present a serious challenge for public health throughout the world. Well-known historical examples are seed wheat treated with methyl mercury in Iraq, table oil adulterated with hexachlorobenzene in Turkey, or mercury-contaminated fish in the area of Minamata Bay, Japan. Examples of recent mass events are melamine poisonings from tainted infant formula in China or health problems of consumers of malathion-contaminated frozen foods in Japan.

Methanol poisoning outbreaks have been reported throughout the world in groups of people who ingested adulterated vodka, whiskey, sake, rum, and other alcoholic beverages. During 2000–2012, more than 50 methanol mass poisoning outbreaks with about 5000 poisoned subjects and more than 2000 fatalities had occurred worldwide. One of modern age methanol epidemics was happened in the Czech Republic in 2012 with almost 140 cases and more than 50 deaths.

During the outbreaks of mass or cluster poisoning by contaminated food and beverages the public health systems face serious challenges: great number of affected people in a short time, unknown source of poisoning, hardly predictable magnitude of event, delayed presentation and diagnosis, non-specific clinical signs and features, need for special diagnostic tools, limited availability of treatment resources, insufficient evidence of effectiveness and safety of therapeutic measures, often high mortality rate and prevalence of long-term health damage in the survivors.

S25-04
Risk communication in mass poisoning situations: "What do we do? Where do we go?"

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Crisis risk communication is difficult, but critical to appropriate incident management. Ideally, a well-informed public apprised of relevant facts by knowledgeable and reliable sources will dispassionately assess the personal applicability, need for and scope of response, and take appropriate action(s). Unfortunately, in mass poisoning situations, none of these factors are present in a timely fashion, leading to misinformation, fear, outrage, and distrust. This presentation begins with the definition of risk, then reviews the major tenets of communication in a disaster or stressful situation. Using real-world examples, this lecture synthesizes key components of risk assessment (hazard identification, exposure pathway, modifying factors, toxicity assessment) and translates them into effective public messaging. Examples of message maps provide an opportunity for participants to assess their own communication styles and practice developing actionable statements regarding environmental chemical exposures.

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S26 | Suitability of non-animal approaches in different industries: One size fits all?

S26-01
Non-animal testing approaches in the risk assessment of food and cosmetic ingredients in Europe

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Regulators and consumers mandate the use of animal-free approaches for the risk assessment of food and cosmetics ingredients. Animal testing is still predominant in the food ingredient industry, despite the recommendation to use alternative methods and efforts of organizations like ILSI Europe, EPAA, and others to foster development and acceptance of alternative methods. Since 2013, animal testing of cosmetic ingredients is prohibited in Europe, encouraging the development of non-animal methods (NAMs). On the downside, the development of new cosmetic ingredients is hampered, especially those requiring authorization. A nearly complete replacement of animal tests is achieved for skin and eye irritation. NAMs for skin sensitization are available as well but are not applicable to some compounds. For genotoxicity testing of cosmetics, micronucleus and comet assay in reconstructed skin were developed to replace *in vivo* follow-up tests. Also toxicokinetic evaluation is going animal-free: *in vitro* tests and physiologically based kinetic modelling are promising tools but not considered to completely replace *in vivo* tests. The biggest hurdle to overcome are repeated dose toxicity, reproductive/developmental toxicity and carcinogenicity studies for which no validated and accepted NAMs are currently available; tiered testing is used to limit animal numbers. Alternative approaches to risk assessment besides NAMs are gaining in importance: The Threshold of Toxicological Concern (TTC) approach is well established for food safety but is not accepted when specific data requirements must be met for regulatory approval. For cosmetics, the TTC approach is considered acceptable when dermal bioavailability is taken into account (internal TTC). Read-across and computational models are other possibilities to forego animal testing but both need proper validation, substantiation, and documentation to be accepted. The Adverse Outcome Pathway (AOP) concept is used to develop testing strategies for endpoints throughout different industries. Taken together, animal-free methods are already available for some – but not all – endpoints but acceptance by authorities is lagging. Alternative assessment methods like TTC, read-across, or computational methods are gaining importance in the food and cosmetic industries.

S26-02
Hurdles to the regulatory use of alternative methods for chemicals and 12 proposals to overcome them

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Alternative methods (and animal methods alike) have to fulfill certain requirements to be used in the chemical industry. Among those requirements are reproducibility, precision and practical applicability as well as predictivity and relevance.The REACH regulation defines toxicological standard information requirements in order to facilitate risk assessment and classification and labelling. Often, the information requirements refer to an animal method or the regulation, like CLP, was designed with animal studies and the data they are providing in mind. Obviously, method development is still needed to address the majority of toxicological endpoints, which are currently addressed by animal methods. While a wealth of new methods being developed (enabled by technological progress in biosciences), the regulatory use of these methods is lacking behind. This presentation is presenting hurdles to achieve regulatory acceptance and eventually regulatory use of a new method and introduces 12 proposals for improvements which are by illustrative examples of currently used or developed alternative methods:

1. Simplify validation process and speed-up regulatory acceptance.
2. Prioritize and guide replacement of *in vivo* testing for specific toxicological endpoints.
3. Review performance of existing methods and revise if needed.
4. Consider relevance of testing strategies.
5. Ensure availibility of equipment and biological materials.
6. Verify absence of licencing and ethical restrictions.
7. Adapt GHS to *in vitro* methods.
8. Develop and validate methods for mixtures.

9. Pre-define strategies and requested performance of methods.
10. Validate testing strategies – and accept them as full replacement.
11. Define requested precision – and assess and report limited precision of testing results (and method's predictivity).
12. Use 'value of information' to prioritize and validate methods.

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S26-03
Alternative approaches in the early phases of pre-clinical toxicology. What is really used in the pharmaceutical industry?

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The preclinical safety assessment preceding First-in-Man trials in the pharmaceutical industry is subject to a well-defined set of highly standardized *in vivo* and *in vitro* studies described by ICH (International Conference of Harmonisation) or OECD (Organisation of Economic Co-operation and Development) guidelines. Undoubtedly, this standardisation has contributed to a broad regulatory acceptance of the preclinical testing strategy across different regions. As a drawback this streamlined setting has the tendency to impede the adaption of new alternative approaches to safety testing required for drug approval.

The problem of late stage drug attrition and poor concordance between animal and human findings for some safety endpoints has led to the development of *in vitro* safety screens to frontload the safety assessment into early pre-clinical research. The increased output of medicinal chemistry has created an environment of *in vitro* investigative toxicity screening, which first started for genetic toxicity testing, followed by phospholipidosis screens and *in vitro* hepatoxicity assays. With a few exceptions (e.g. *in vitro* phototoxicity testing) these *in vitro* assays are not formally validated or described in guidelines. They require no regulatory acceptance since compounds which pass these tests are later investigated in the pivotal animal studies (i.e. these *in vitro* assays are not considered as replacements in the context of 3R). The assays are rather developed on a fit-for purpose basis, intended for "design-make-test" strategy, allowing a rapid application and adoption of *in vitro* assays within the companies. Yet, the results have been difficult to compare across companies due to differences in assay conditions and chemical space for which these *in vitro* assays are applied. However, these shortcomings are gradually being overcome by consortium approaches (IMI, IQ Pharma) that can help facilitate the evaluation of next generation *in vitro* tests such as micro-physiological systems and organs-on-a-chip. These latter technologies are often provided by commercial sources or co-developed by several companies, with the expected benefit of a greater standardization of testing by use of common protocols.

The paper provides an overview of alternative approaches across pharmaceutical companies and is largely based on recent activities of the Investigative Toxicology Leaders Forum (Beilmann et al. 2019, Altex).

S26-04
Establishing scientific credibility/validity of new approaches for different decision making contexts

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Validation is an indispensable step to facilitate/guarantee regulatory acceptance. Still, it is currently being identified by many as the main reason for the lack of sufficient advancement in the implementation of non-animal methods in a regulatory context. The principles of validation in a regulatory context were first established in the 90s and gained international recognition with the adoption of OECD Guidance Document No. 34 (GD 34) in 2005 (OECD, 2005), where validation is defined as the Process by which the reliability and relevance of a particular approach, method, process or assessment, is established for a defined Purpose. Even if these principles of validation remain relevant today and the process described in GD 34 was successful in pioneering the regulatory acceptance of alternative methods for less complex endpoints, an evolution of early practices is needed to embrace emerging technologies and the increased complexity of endpoints. Indeed, validation practice needs to keep pace with the considerable scientific advancements being made in the understanding of biological systems, the availability of increasingly sophisticated tools and techniques, the need for data integration to address complex endpoints, the increasingly evident lack of reliability and relevance of reference animal data to predict human effects, and the growing societal and regulatory demands for better protection of human health and the environment, to ensure that it continues to be fit for purpose. In 2004, a "Modular Approach to the ECVAM Principles on Test Validity" was proposed with the objective of making the validation process more flexible by breaking down the various steps of validation into seven independent modules, and defining for each module the information needed for assessing the scientific validity of a test method (Hartung et al. 2004). In order to maximize the flexibility and efficiency of study design, the modular approach to validation should be exploited to deliver fit-for-purpose validation strategies best suited to today's reality. Evolution of the modular approach includes more emphasis on initial method definition, better characterisation of biological relevance, explicit assessment of uncertainty, uncoupling the assessment of test systems and biomarkers to facilitate the deployment of new technologies/models across different fields of application (rather than assessing a method for a single use case scenario under a given regulatory framework), extending concepts of applicability domain, and increased use of performance standards. This talk provides a historical overview of the establishment and evolution of the principles of the scientific validation of alternative methods for toxicity testing as well as the challenges and opportunities for adapting the validation practices to keep pace with scientific progress whilst ensuring the protection of human health and the environment and best serve the needs of society.

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S27 | Neurotoxicity in the scientific and regulatory outlook

S27-01
Alternative neurotoxicity testing methods: performance characteristics and ability to predict chemical effects

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Neurotoxicity refers to any adverse effect of exposure to chemical, biological or physical agents on the structure or functional integrity of the developing or adult nervous system. Currently, the recognised test methods for the evaluation of the neurotoxicity potential of chemicals are the OECD Guideline 424 (Neurotoxicity studies in rodents) and 426 (Developmental Neurotoxicity Studies). Both these methods use complex *in vivo* tests which are often too laborious and expensive and might also not well reflect the human situation because of inter-species differences. Due to these reasons it is now recognised that the future of chemical safety assessment must move away from animal tests towards a combination of complementary approaches that address functional mechanistic endpoints tied to adverse outcomes of regulatory concern.

We performed a systematic literature search, collecting and appraising information on neurotoxicity *in vitro* methods for the period 1990–2017. As a benchmark for true positive studies, we collected neurotoxic compounds' modes of action (MoA). Articles were selected from title/abstract as well as full text screenings according to defined inclusion and exclusion criteria, which included accordance with the identified MoA. In addition, quality of studies was evaluated by using the ToxRTool, which is based on the Klimitsch criteria.

From a total of 6243 citations, 1351 papers were selected for full text screening and 228 were finally nominated for evaluation. These were based on 258 individual neurotoxic compounds and 23 compound classes, their MoA summarizing in 27 endpoint categories containing grouped endpoints, which reflect key events (KE) of neurotoxicity. The majority of studies were performed in rat cells followed by mouse, human, chicken and xenopus *in vitro* models. While rodent and chicken cells were mainly primary cultures, human studies were largely based on stem/progenitor cells and xenopus models were oocytes overexpressing certain neuronal proteins. Of those, we evaluated their applicability domains by the number of endpoint categories.

In summary, there is a good data base for rodent *in vitro* models assessing a variety of neurotoxicity endpoints. Considering species differences, the establishment of more human induced pluripotent stem cell-based endpoint measurements as test methods for regulatory use is desirable. A neurotoxicity *in vitro* test battery covering identified and relevant neurotoxicity MoA is recommended. Therefore, assays as test methods with relevant controls and standard operation procedures have to be set up for covering most important MoA. Chemicals representing compound classes with defined MoA need to challenge the *in vitro* testing battery thereby producing reliable reference data.

S27-02
Exploring chemically induced neurotoxicity mode of action

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OECD Guidelines 424 and 426 are the required test methods in the case of regulatory application of active substances with indication of potential neurotoxicity. Both these methods are based on laborious *in vivo* tests which are ethically questionable and scarcely informative on complex human health outcomes.

Experimental and mechanistic data, focusing on specific neurological pathways, tied to adverse outcomes of regulatory concern represent a valuable source to provide information on causal exposure-effect relationship and assist in the selection of complementary approaches to be used in an integrated testing strategy.

Several environmental chemicals and toxins, identified as toxic to the human nervous system, were analyzed for neurotoxicity Mode of Action (MoA) at molecular, cellular, organ and organism level, identifying the causal link between all the different levels (full MoA) or part of them (Partial MoA).

As a result, the selected compounds were sorted in four main MoA groups that share common key events associated to neurotransmission, ion channels/receptors, cellular endpoints and other. Data relative to neurotoxicity of chemicals with full or partial MoAs were then collected and analyzed to obtain information on species, the methods used to identify the effects, endpoints-specific controls, compounds effect and classification (true positive or negative, false positive or negative), in order to provide indication on the availability of *in vitro* methods/alternative organism able to address each selected MoA endpoint category and their predictivity.

As a general conclusion, we observed that all true positive and negative results were physiologically relevant based on the MoA analysis performed, indicating that each cell type/test system that resulted positive in the performed evaluation is suitable for neurotoxicity testing of selected endpoint categories. In particular, primary rodent cells addresses a large variety of endpoint categories. Encouraging results are obtained from human stem-/progenitor cells although more work is needed. Glia toxicity is underrepresented. Motor activity due to inhibition of cholinergic transmission is represented, as a complex behavioral read out, by C. Elegans, confirming the added value of a whole organism approach.

S27-03
Toward the regulatory application of DNT *in vitro* assays

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Development of human embryonic brain is a complex process going through a series of developmental stages that must occur in a given sequence an at the right time. The outcome is the human brain and each of these processes might be vulnerable to adverse effects from exposure to environmental chemicals or drugs. It is therefore time to recommend advancing the science and regulations related to DNT testing through a human relevant mechanistic DNT understanding and assessment. The goal can be achieved by developing a cost-efficient strategy based on a reliable in-vitro testing battery able to respond to the different regulatory problem formulations. Indeed, several steps have been taken, making an alternative DNT testing strategy a real possibility and ready for regulatory use. The list of activities also includes chemicals testing, which is a necessary step in order to fill the gaps identified through the analysis conducted so far. To this aim, data will be generated to provide the regulatory suit-

ability of the DNT testing battery and the OECD is developing a guidance for interpreting and integrating the data and inform regulatory decisions. It is therefore an expectation that this guidance will influence the regulatory approach on how DNT will be assessed for environmental chemicals.

S27-04
The use of zebrafish as an alternative model f or behavioural testing

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The zebrafish, *Danio rerio* is a well-recognized model species in developmental biology, with increasing use for toxicological and human disease research. Practical advantages such as the short generation cycle, 100–200 transparent embryos from one breeding pair, ease for automated high-throughput testing and genetic manipulation make them well suited for mechanistic research. The zebrafish genome shares 70–80% homology with the human genome and common pathways among vertebrates (e.g. neurotransmission) were demonstrated, pointing to the relevance of zebrafish as an alternative to mammalian models. Furthermore this metabolically competent, whole organism model displays numerous behavioural patterns, representing apical readouts for central nervous events. In this way, the zebrafish model is complementary to *in vitro* model systems and can be part of an integrated approach to testing and assessment (IATA), as is demonstrated for developmental neurotoxicity (Bal-Price et al., 2018; Selderslaghs et al., 2013). An overview of zebrafish behavioural assays commonly used for developmental neurotoxicity (DNT) and neurotoxicity (NT), as well as related technological developments for high-throughput screening will be presented. A few examples of compounds known to target the nervous system will be selected in order to demonstrate the strengths and the limitations of the zebrafish behavioural studies. The added value of these behavioural assays, next to the evaluation of early molecular and cellular events will be discussed in the context of adverse outcome pathways for DNT or NT compounds.

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S28 | Hepatotoxicity: mechanisms, new insight into liver function, and possibilities of *in vitro* prediction

S28-01
Insight into mechanisms of hepatotoxicity by two-photon microscopy and derivation of predictive *in vitro*/*in silico* systems

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In the present study, we introduce a toolbox for intravital two-photon-based imaging of the mouse liver. Key features of the imaging setup are a two-photon laser with a broad wavelength spectrum, long-distance objectives with high numerical aperture, and sensitive detectors like GaAsP. The presented methods can be applied to visualize hepatocytes, endothelial cells, Kupffer cells, stellate cells and infiltrating immune cells in the living organ. The flux of fluorescent bile salts and drugs from the sinusoid via Dissé space and hepatocytes into bile canaliculi can be quantified. For example alflatoxin B1 is taken up from asinusoidal blood into hepatocytes and enriched in the nucleus. Besides structural visualization also functional analyses are possible, such as quantification of the velocity of flow and diffusion coefficients in bile canaliculi and ducts. In contrast to prevailing thought, xenobiotics and bile acids do not flow in bile canaliculi. Rather, the canalicular network represents a 'standing water' in which flux of compounds is diffusion dominated; only in the downstream bile ducts flow sets in with a velocity of 1–1.5 μm/sec under basal conditions and up to 7 μm/sec after stimulation with secretin or taurocholic acid. Studying drug induced hepatotoxicity by acetaminophen revealed two simultaneously occurring death mechanisms, necroptosis in the pericentral region and a so far unknown death mechanism of hepatocytes that is initiated by dilatation and leakage of the bile canalicular hepatocyte membrane. The central role of bile acids in hepatotoxicity was documented by blocking APAP mediated cell death by FXR agonists that strongly reduced the concentrations of circulating bile acids.

S28-02
Computational modelling of the unfolded protein response upon chemical-induced cell injury

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Activation of cellular stress pathways occurs when cells are exposed to stressors such as many chemicals, and such activation has an important role in the development of organ failures such as drug-induced liver injury (DILI). One of these pathways is the unfolded protein response (UPR) pathway which is employed by cells to maintain proteostasis within the endoplasmic reticulum. Multiple sensors (IRE1α, PERK and ATF6) detect unfolded proteins and a complex downstream biochemical network of transcription factors and target proteins regulates protein folding and cell fate. Cells can adapt when the unfolded protein accumulation is limited yet upon severe cell injury by e.g. chemical exposure, sustained high levels of unfolded proteins could lead to cell demise. In order to obtain a quantitative understanding of the balance between adaptive and adverse UPR activation, we investigated the UPR pathway quantitatively in single cells using a combination of single cell microscopy-based quantification of UPR activity and dynamic computational modelling. Green Fluorescent Protein (GFP)-tagged XBP1, ATF4, BIP and CHOP reporter HepG2 cell lines were exposed to tunicamycin (Tun) at various concentrations and followed by live cell imaging for 24 hours. Dynamics of GFP-fusion UPR protein expression and cell numbers were obtained using in-house image-analysis pipelines. Based on the data for low concentrations where cells grew at unimpeded net rates, we constructed an ordinary differential equation (ODE) model to describe the underlying UPR signalling network. Incorporation of each of the three UPR signalling branches (IRE1α, PERK and ATF6) was required to obtain a good match to the experimental GFP-reporter data. Specifically, ATF6 activity was an important determinant in shaping the detailed dynamics of CHOP, which we confirmed experimentally by siRNA knockdown experiments and Western blot measurements of ATF6, i.e., at protein level. As a next step to under-

stand the transition from adaptation to adversity, we developed an ODE model at cell population level to describe the fate of cells including cell division and death and using the measured TF activity as an input to the model. TF activity could well explain the cell population dynamics at low and intermediate Tun concentrations but at high concentrations factors beyond the UPR TFs were required to explain the observed dynamics. Together, our models quantitatively link the dynamics of the UPR and of cellular adversity upon compound exposure. In the future, our models can contribute to the establishment of quantitative adverse outcome pathways for chemical safety assessment.

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S28-03
In vitro metabolome data and a comparison to the *in vivo* situation

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BASF and metanomics GmbH established the database MetaMap®Tox containing the plasma metabolome of more than 1000 compounds derived from 28-day studies in rats. Recently, a highly stable and reproducible liver *in vitro* model was established, in which the intracellular metabolome of HepG2 cells can be specifically altered through treatment with different hepatotoxicants. So far more than 90 different treatments have been analysed with this setup.

Within the BMBF- and ZonMW-funded project SysBioToP, different treatments known for their ability to cause drug induced liver injury (DILI) in clinal settings have been tested in different *in vitro* liver cell systems using imaging technologies, transcriptomics and metabolomics. We have analysed the intracellular metabolome of HepG2 cells treated with 9 DILI causing substances (amiodaron, azathioprine, ciprofloxacine, diclofenac, ketoconazole, nitrofurantoin, paracetamol, phenytoin and valproat) and vancomycin as a negative control. All of these, except for nitrofurantoin and ketoconazole, have also been tested in the *in vivo* 28-day rat study.

The *in vitro* metabolite changes were compared to the toxicity mechanisms of the respective substances. Common key events such as uncoupling of oxidative phosphorylation or impairment of beta-oxidation, e.g. for amiodarone, could be well related to metabolic changes *in vitro*. To compare the gained *in vitro* data with the *in vivo* data from the 28-day rat studies, the *in vivo* findings were compared to the known mechanisms as well.

The here described metabolomics platforms enable the direct comparison of *in vitro* and *in vivo* metabolome data. This provides valid information on the relevance of *in vitro* findings. The combination of mechanistic *in vitro* data with *in vivo* systemic toxicity data based on metabolomics provides a more wholistic understanding of DILI and can help to identify common key events in its development for different substances.

S28-04
Upregulation of glutathione in hepatocytes by the antibiotic Nitrofurantoin

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The antibiotic Nitrofurantoin (NFT) is applied for the treatment of bladder infections. In rare cases, adverse effects on liver function and integrity have been reported. NFT was therefore tested within the Systems Biology of liver Toxicity Predictions (SysBioToP) collaborative project. In the course of these investigations, we observed an upregulation of glutathione levels by subtoxic concentrations (< 100 µM) of NFT, both in the HepG2 human liver cell line and in primary human hepatocytes. Transcriptomics analysis revealed an induction of elements of cellular oxidative stress response pathways. The HepG2 cell model was therefore applied for the expression of fluorescent stress pathway reporters, based on bacterial artificial chromosome gene editing technology. Comparison of different cellular stress response pathways confirmed the activation of the Nrf2 pathway, ultimately leading to the expression of antioxidant enzymes. We could observe that the increase of glutathione, following NFT treatment, was the result of an elevated expression of glutamate-cysteine ligase (GCL) (catalytic and regulatory subunits). The induction of GCL, as well as the rise in glutathione in response to NFT, was prevented by siRNA-based knockdown of the transcription factor Nrf2.

In a next step, we tested whether glutathione induction by NFT would elevate the resistance of cells towards secondary stressors. In fact, cells pretreated with NFT demonstrated an increased tolerance against experimentally evoked mitochondrial impairment (rotenone), oxidative stress (paraquat), or proteasomal stress (bortezomib, MG-132). Based on these observations, low concentrations of NFT could serve as novel strategy to support the antioxidant capacity of the liver.

S29 | Species specific gastrointestinal (GI) toxicity in rabbits – what does it mean for prenatal developmental toxicity (PNDT) studies and their regulatory use?

S29-01
Gastrointestinal (GI) toxicity in rabbits – mechanisms and relevance for human

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Embryo-fetal or prenatal developmental (EFD/PNDT) studies are regulatory requested in two species (usually rat and rabbits) for pharmaceuticals, agrochemicals and (when produced in high volumes) for industrial chemicals. Whereas the rat is generally considered a straightforward and easy to handle test species, the rabbit poses several challenges for a Contract Research Organization (CRO). The main reason for this is the regular occasion of gastrointestinal toxicity in EFD/PNDT rabbit studies.

The rabbit has a system that: (1) allows a high food intake, (2) separates out the digestible and easily fermentable components of the diet, and (3) rapidly eliminates the slowly fermentable fibrous waste. Given that the system is developed for rapid elimination of

fibrous wastes, it is conflicting that the main driving force for the system is the presence of such indigestible fiber. Lack of this fiber is the most common cause of gastrointestinal disturbance in the rabbit.

These issues can occur due to toxicity caused by the test compound, but strikingly it occurs regularly in control animals. Moreover, the use of certain vehicles can also cause GI tract disturbances. Important is to use a low dose volume so the amount of vehicle being dosed is limited. However, this could result in the performance of an EFD/PNDT study in which the limit dose of 1000 mg/kg body weight cannot be reached. Propylene glycol has shown to be teratogenic in rabbits, but can be used without problems in rat EFD/PNDT studies.

These problems will lead to increased stress (and therefore increased sensitivity to toxicity), huge fluctuations in food consumption data, maternal deaths, and abortions. These issues will impact the toxicological evaluation of the study results, and might result in incorrect conclusions for human safety assessment.

S29-02
Rabbit PNDT studies: What are the regulatory consequences for plant protection products?

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The lowest observed and no observed adverse effect levels from prenatal developmental toxicity studies are used in conjunction with those from other mammalian toxicity assays to derive values for the Acceptable Daily Intake, the Acute Reference Dose (ARfD) and the Acceptable Operator Exposure Level.

A recent review of the basis for setting the ARfD for 130 plant protection products has shown that prenatal developmental toxicity studies have been used more than any other study type (~55%) to derive the ARfD. Of these, 58% were studies conducted in rabbits alone. In relatively few cases were the rabbit data judged to reflect a relevant acute effect; the rabbit data appear not to be well understood nor appropriately evaluated.

Rabbits in particular can be sensitive to bolus dose administration, not only to the test chemical itself but to the volume and nature of the carrier vehicle. This can trigger a variable response amongst individuals in the same treatment group and confound identification of the toxicity due to the test chemical. Some rabbits recover quickly whilst others may not. In the worst case, some individuals become inappetent, show progressive weight loss and in turn may resorb/abort their litters or necessitate removal from the study for welfare reasons. It is the response of these non-representative individuals in particular, which can result in misinterpretation of the data and the incorrect definition of effect levels and also, confusion between maternal and secondary, indirect, developmental effects.

Another difficulty in interpreting the nature of developmental effects in the rabbit is the high background incidence of spontaneous anomalies, compared with rat. The review revealed a lack of understanding that truly teratogenic agents produce a spectrum of change which is reproducible and not an assortment of unrelated findings which are not reproducible.

Interpretation of data from rabbit prenatal developmental toxicity studies is difficult for the non-expert. As a consequence, unnecessarily conservative judgements are being made resulting in the loss of important plant protection products with no real human health risk.

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S29-03
Alternative species and methods for embryo-fetal developmental toxicity studies for pharmaceuticals

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According to ICH S5 guideline, for most non-highly targeted pharmaceuticals, effects on embryo-fetal development should be evaluated in one rodent and one non-rodent species, with the rat and the rabbit as the default species. However, each species has its own limitations for certain pharmaceutical classes. The rabbit, due to the high gastrointestinal sensitivity, is unsuitable for some pharmaceuticals such as antibiotics. This toxicity is greater by oral administration and therefore an alternative route of administration (*e.g.* intravenous or subcutaneous) can be considered. On the other hand, as stated by the guideline, an alternative species may be appropriate and should be considered on a case by case basis based on the reproductive endpoints to be assessed. The *in vivo* mammalian models identified are the mouse, non-human primate, minipig, guinea pig, hamster, dog and ferret. The mouse is an alternative to the rat if considered more relevant and used as a species of choice for example surrogate antibodies. The non-human primate is essentially reserved for biotechnology-derived products as described in ICH S6 and not an alternative species for routine small molecule testing. The minipig could be considered for routine use in regulatory embryo-fetal development studies, and especially for small molecules that freely cross the epitheliochorial placenta. Indeed, the minipig has many metabolic similarities with humans and has advantageous reproductive physiology compared with the other large animal models such as the dog and non-human primate. The relative size and duration of gestation (112–115 days) in the minipig is, however, considered a drawback compared with routine smaller species. Therefore, alternative methodology has been developed by performing mid-term caesarean sections (60 days). In lieu of, or in addition to, the use of an *in vivo* mammalian testing, there is also a desire to employ alternative non-mammalian *in vivo*, *ex-vivo* and *in-vitro* approaches to test for embryo-fetal toxicity as described in the draft ICHS5(R3) guideline.

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S29-04
Rabbit gastrointestinal toxicity in prenatal developmental toxicity studies and its potential regulatory impact

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Introduction: Substances with antibiotic activity may disturb the microflora in the gastrointestinal (GI) tract and cause diarrhoea, enteritis and/or maternal death in rabbits [1]. ECHA launched a project

with the focus to compare maternal effects in rabbit and rat prenatal developmental toxicity (PNDT) studies (OECD TG 414) performed with REACH and Biocidal Products (BPR) substances.

Materials and methods: For 164 substances (74 REACH and 90 BPR substances) the results (as reported in the study summary) of PNDT studies in rats and rabbits with similar type of oral administration were analysed for maternal effects (mNOAEL and mLOAEL in mg/kg bw/d, type and severity of effects) and certain developmental effects.

Results: Changes in the amount or consistency of faeces (indicative of a GI effect) were reported in 27% rabbit and 2% rat PNDT studies. Excessive maternal mortality (>10%) in rabbits occurred in 23% of substances at or below the highest dose in the rat study without excessive mortality; the respective value for rats was 2%. mLOAELs or mNOAELs were ≥10-times lower in rabbits compared to rats in 9% of substances; the respective value for rats was 3%. Malformations and/or embryo-foetal mortality were observed for 18/164 substances in rat and/or rabbit PNDT studies. For 12/18 substances, developmental effects occurred in both species at a similar dose range in the absence of excessive maternal mortality. For 5/18 substances, developmental effects occurred in rats (in the absence of excessive maternal mortality); in 4 of these 5 substances, excessive rabbit maternal mortality was observed at or below the dose(s) which in rats showed developmental effects. The remaining substance of these 5 substances did not show developmental effects in rabbits even at higher doses than tested in rats. Only one of the 18 substances showed maternal and developmental effects exclusively in rabbits at maternally non or slightly toxic doses (50 and 150 mg/kg bw/d); this substance did not show maternal or developmental effects in rats (NOAELs 1000 mg/kg bw/d).

Discussion: The results are reviewed and discussed in the regulatory framework.

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S30 | Substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs); new challenges in their toxicological evaluation and risk management in REACH

S30-01
The chemical composition of UVCB substances – Challenges in characterisation

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Large number of constituents, large variability of composition and even partly unknown composition create challenges in identification and characterisation of the UVCB substances.

Generally, UVCBs require additional to composition information for their identification. This includes information on the identity of the starting materials and information on the process(es) used for obtaining the substance.

However, information on the composition is of primary importance for assessing a substance hazard.

UVCBs often create challenges also for analytical identification and quantification of the composition. In light of insufficient analytical information, other parameters may provide useful information on the composition. For example:

1.) Identity of the source materials, including compositional information.
2.) Process(es) used for obtaining the substance (e.g. reaction type and its mechanism).

Proper identification of a UVCB substance is essential for the correct joint submission of data by multiple REACH Regulation registrants of the same substance. To describe what is covered by the data jointly submitted, the registrants need to establish a Substance Identity Profile (SIP) and its technical reporting in the IUCLID dossier, a boundary composition. This boundary composition is particularly important as it is reporting all the compositions of the substance covered by the registration. The level of the compositional detail reported should enable the assessment of (potential) hazard of a substance.

S30-02
Assessment of the hazardous properties of UVCBs

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Structural similarity between substances is a pre-requisite for the adaptation of information requirements using predictions based on grouping of substances and read-across according to Annex XI, Section 1.5 of the REACH Regulation.

A substance registered under REACH may comprise a set of different chemical structures due to the presence of, for example, different constituents including impurities or stabilisers where present. By definition, the composition of multi-constituent substances and "substances of unknown or variable composition, complex reaction products or biological materials" (UVCBs) can include from several up to many chemical structures.

The Read-Across Assessment Framework (RAAF) was developed for mono-constituent substances. Its principles however may also be applied when assessing read-across approaches involving multi-constituent substances and UVCBs. However, to assess such read across approaches additional considerations are necessary to ensure that the chemical structures present within the substances are taken into account in the prediction (ECHA, 2017).

Due to the possibly complex nature in the composition of multi-constituent substances and UVCBs, a comprehensive characterisation of their identity and composition is essential for a sound read-across approach. Detailed information on the composition of the source substance and the test material actually used to perform the studies to be used as source data, is needed to establish their relevance to the target substance in terms of grouping and predictions. The allowed quantitative variations in the concentrations of the different constituents and the impact of combined exposure to several constituents are also relevant considerations.

As with read-across approaches for mono-constituent substances, supporting information is required to increase the robustness of the read-across hypothesis. Bridging studies, i.e. comparable studies on the source and target substance, may bring valuable supporting information in demonstrating that substances have similar properties for a particular endpoint.

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S30-03
How to establish read-across within a category of UVCBs – an industrial perspective

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Read across within groups or categories of substances is a well-developed way of providing toxicological data on substances that have similar properties or structures. It was a widely adopted approach in industry to help in assessing hazard and risk. In a more formal arena, categories and the read across was y used in the 1998 US EPA HPV challenge program and also the 1998 OECD HPV Chemicals programme in which industry was a key contributor. The application of read acorss for UVCBs, as opposed to single substances, provides a greater challenge to demonstrate a plausible and robust hypothesis for its application.

The methods used have undergone some development within industry and regulatory bodies and the approaches have been reflected in the original OECD guidance on categories and read across and its subsequent revision in 2014.

With the implementation of REACH, grouping of substances and read-across was given as a specific adaption to standard information requirements. It was widely used in the submissions for the first deadline in 2010 for higher tonnage substances. However, the was no clear EU guidance at that time. Subsequently the Read Acorss Assessment Framework (RAAF) has been developed.

The presentation will highlight the develop of approaches and give practical examples of complex UVCB categories that have bene successfully develop in the past 10 years, from petrochemicals to derivatives of plant derived products.

S30-04
Regulatory Risk Management of UVCB substances – challenges and effective implementation

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Hazard assessment (to support classification or risk assessment) together with information on uses and potential for releases and exposure is the starting point for authorities to initiate regulatory risk management actions. To further regulate a substance at a more global level (e.g. EU) confirmation of the hazard is usually the necessary first step (e.g. harmonised classification and labelling under the CLP regulation). Any decision to start a regulatory action implies some level of certainty on the hazard or risk identified.

Hazardous properties of substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs) are often (if not always) associated with the presence of single constituents. The hazard can come from either the presence of one individual constituent in the substance above its corresponding regulatory threshold and/or one or several (groups of) constituents. For those substances, we can expect a high variability in composition particularly in relation to those groups of constituents present at low concentrations (e.g. petroleum substances). Variability in composition is particularly difficult to assess when we deal with constituents of concern present around the threshold for classification. Some constituents of concern may be not present in some variants of the substance. Variation in composition can therefore lead to different hazard conclusion for the same substance, which requires specific attention when considering how to properly regulate the substance(s) and their uses in a proportionate manner.

Different regulatory tools may be used depending on what we want to achieve as regulators. We may need for instance to limit the presence of some constituent in mixtures or in articles or limit exposure to workers to some constituents. This will influence how to formulate the entries to be included in the different regulatory lists (e.g. Annex VI to CLP, Candidate list, Annex XIV, Annex XVII,). Therefore, when discussing the best regulatory management several options should be looked at such as (i) acting on the substance containing the constituents of concern above relevant regulatory threshold; (ii) acting on all substances containing the constituent(s) of concern or (iii) acting on the constituent(s) of concern as there may be a concern with its presence in substances, mixtures and articles.

When discussing those options, it is important to have sufficient knowledge on the level of composition information that it is possible to have on the substance(s) to be regulated. It is also important to consider both how industry is able to fulfil its obligations and how authorities are able to enforce the proposed regulatory measures.

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Short oral communications

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OP01-01
Human gut microbial glycerol dehydratase function: impact on chemical metabolism and toxicological relevance

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The heterocylic amine 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) is a mutagen and probable human carcinogen that reaches the human colon. Human gut microbial communities can metabolize MeIQx potentially altering its toxicity. However, understanding the toxicological relevance of the gut microbiome in chemical disposition and toxicity requires insight on the functional transformation capacities of the human gut microbiome, uptake potential of microbial metabolites, and the influence of microbial transformation on chemical toxicity. We found that MeIQx as well as a glucuronidated metabolite can be transformed to 9-hydroxyl-2,7-dimethyl-7,9,10,11-tetrahydropyrimido[2′,1′:2,3]imidazo[4,5-*f*]quinoxaline (MeIQx-M1) by the function of a microbial glycerol dehydratase enzyme. The mechanism of this transformation reaction appears to involve the microbial production of intermediate acrolein by a hitherto unrecognized endogenous means of acrolein production. To address whether microbial transformation influences intestinal transport of MeIQx, intestinal uptake of MeIQx and its microbial metabolite was quantified using rat intestinal segments. Results show that both compounds are similarly transported from the mucosal side to the serosal side of intestinal tissue. Physiologically based pharmacokinetic modeling, taking microbial biotransformation into account, suggests the requirement of high levels of intestinal acrolein to significantly alter the availability of MeIQx. Furthermore, *in vitro* evaluation of cytotoxicity and mutagenicity are consistent with the glycerol dehydratase-catalyzed transformation being a detoxification process. Moreover, metagenomics profiles from healthy individuals vs cancer patients suggest a protective role of the presence of this microbial gene. These findings suggest that gut microbial transformation of heterocyclic amines has the potential to reduce their toxicological impacts, but that further studies are needed to understand the concentration and biological fate of microbial acrolein produced in the human gut.

OP01-02
Mechanistic understanding of DILI using Metabolomics *in vitro*

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BASF and its daughter company metanomics established the database MetaMap®Tox containing the plasma metabolome of more than 1000 compounds derived from 28-day studies in rats. Recently, a highly stable and reproducible liver *in vitro* model was established, in which the intracellular metabolome of HepG2 cells can be specifically altered through treatment with different hepatotoxicants. So far more than 90 different treatments have been analysed with this setup.

Within the BMBF- and ZonMW-funded project SysBioToP, different treatments known for their ability to cause drug induced liver injury (DILI) in clinal settings, such as nitrofurantoin, amiodarone and diclofenac, have been tested in different *in vitro* liver cell systems using imaging technologies, transcriptomics and metabolomics. We have analysed the intracellular metabolome of HepG2 cells treated with 9 DILI causing substance and vancomycin as a negative control. The metabolome consisted of 236 unique metabolites, thereof 35 amino acids and derivatives, 11 carbohydrates and related compounds, 54 lipids, 14 energy metabolites, 6 nucleobases, 14 vitamins and cofactors as well as other miscellaneous or unknown metabolites. The metabolic changes in HepG2 cells induced by the test substances were compared for each test substance with the toxicity mechanisms described in literature. Amiodarone, as an example, is known to impair β-oxidation of fatty acids and to uncouple the oxidative phosphorylation. A huge set of fatty acids and lipids was significantly changed, going along with a significant increase of triacylglycerides. At the same time, a significant decrease of TCA cycle intermediates such as α-ketoglutarate, fumarate and malate, going along with an increase in pyruvate and alanine levels was found, indicating a misfunction in the TCA cycle as a secondary effect to the impairment of the oxidative phosphorylation. The here described metabolomics *in vitro* method using HepG2 cells is able to provide mechanistic understanding of DILI and can help to identify common key events in its development for different substances.

OP01-03

Importance of non-mitochondrial pathways in drug-induced hepatic steatosis: investigations with 12 steatotic drugs in HepaRG cells

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Steatosis is a liver lesion reported with numerous pharmaceuticals. Prior studies on a limited number of drugs suggested that impairment of mitochondrial fatty acid oxidation (mtFAO) could be a frequent mechanism leading to lipid accretion in liver [1-2]. However, increased *de novo* lipogenesis (DNL) and inhibition of very low-density lipoprotein (VLDL) secretion might also play a key role in drug-induced steatosis (DIS). The objective of our study, carried out in differentiated human hepatoma HepaRG cells, was to investigate these 3 mechanisms with 12 drugs able to induce steatosis in human: amiodarone (AMIO, positive control), allopurinol (ALLO), D-penicillamine (DPEN), 5-fluorouracil (5FU), indinavir (INDI), indomethacin (INDO), methimazole (METHI), methotrexate (METHO), nifedipine (NIF), rifampicin (RIF), sulindac (SUL) and troglitazone (TRO). Cells were exposed to drugs for 4 days with concentrations not exceeding 100xCmax. Neutral lipids were assessed by measuring Nile Red fluorescence using HCS microscopy. Among the 12 drugs, 10 induced a significant accumulation of lipids in HepaRG cells for non-cytotoxic concentrations: AMIO, ALLO, 5FU, INDI, INDO, METHO, NIF, RIF, SUL and TRO. Further investigations were thus performed with these drugs. mtFAO was evaluated by measuring the degradation of ^{14}C-palmitate into ^{14}C-ketone bodies. mtFAO inhibition was observed with AMIO (as previously reported [1,2]), INDO, RIF and TRO. Next, DNL was assessed by measuring the newly formed ^{14}C-lipids from ^{14}C-acetate, whereas VLDL secretion was evaluated by measuring apolipoprotein B (apoB) levels in cell culture supernatants. Higher DNL was observed with AMIO (as previously suspected [3]), INDO and SUL, while reduced secreted apoB levels were observed with ALLO, 5FU, INDI, INDO, RIF and TRO. Experiments in primary human hepatocytes (PHHs from 6 donors) showed that AMIO, ALLO, 5FU, INDI, INDO, RIF and TRO induced steatosis in at least one PHH batch. In conclusion, HepaRG cells appear to be a suitable model to investigate DIS. Besides mtFAO, DNL and VLDL secretion are 2 other important pathways that need to be investigated in order to better understand the pathogenesis of DIS.

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OP01-04

In vitro hepatic sulfation kinetics of selected bisphenols

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Bisphenols A (BPA) and S (BPS) are well-known endocrine disruptors. In the body, bisphenols are extensively metabolized, mainly to glucuronides and to a lesser extent to sulfates conjugates. Although sulfation represents up to 20% of BPA metabolic transformations, it is still poorly studied and there is no data on kinetic parameters for sulfation of BPA analogs. The purpose of the current study was to determine *in vitro* hepatic sulfation kinetics of selected bisphenols using human liver cytosol and in the next level to characterize their metabolism in human HepG2 cells. Additionally, estrogenic activity of two BPS sulfates formed in HepG2 cells (i.e. BPS sulfate and disulfate), was evaluated with transactivation assay using hERα-Hela9903 cell line.

BPA exhibited higher sulfation rates than BPS (v_{max}; 111.5 pmol/min/mg and 32.0 pmol/min/mg, respectively). Nevertheless, the obtained sulfation rates are still approximately 100 times lower than glucuronidation rates determined with human liver microsomes in previous study (Karrer et al., 2018). Both BPA and BPS exhibited comparable affinities toward sulfotransferases as determined with K_m values of 11.3 µM for BPA and 17.7 µM for BPS. In HepG2 cells, sulfation was predominant metabolic pathway, while glucuronides were barely detectable, which is in contrast with metabolites ratio determined in previous biomonitoring studies. Estrogenic activity was determined with OECD validated transactivation assay using hERα-Hela9903 cell line and BPS exhibited estrogenic activity with EC_{50} value of 4.81 µM, while BPS sulfate and disulfate were both without estrogenic activity.

The results revealed that sulfation represent important metabolic and also detoxification pathway as sulfates are without estrogenic activity. Secondly, we demonstrated that HepG2 cell line is not suitable model for *in vitro* studies of human metabolism.

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OP01-05

New insights into Montelukast metabolism – possible implications to the drug's adverse effects

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Montelukast (MTK), a cysteine leukotriene receptor 1 inhibitor, is currently prescribed for asthma treatment in adults and children 6 months and older. Recent evidence points towards additional therapeutic applications, namely as controller of neuroinflammation, which could be exploited in some neurodegenerative disorders (e.g. Alzheimer's disease), as chemopreventive and adjuvant in cancer therapy and as preventive agent in cardiovascular risk settings. Despite its benefits, MTK is associated with some adverse neuropsychiatric effects. MTK metabolism is poorly understood and the mechanisms underlying neuropsychiatric adverse effects remain unknown. To date, only five phase 1 and two phase 2 MTK metabolites have been identified and no significant interactions with bionucleophiles have been reported.

Taking into account the potential new applications of MTK, our initial goal was to evaluate the *in vitro* metabolism of MTK and its possible interaction with bionucleophiles. To address this question, we performed MTK incubations with human liver S9 and microsomal fractions, and with recombinant human cytochrome P450 systems (CYP 3A4, 2C8, and 2D6 expressed in baculosomes together with NADPH:P450 oxidoreductase), as well as with different peroxidases. The incubation products were analyzed primarily by high performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry.

In addition to the known MTK metabolites, we identified multiple new phase I metabolites that resulted from hydroxylation at different sites, S-oxidation, N-oxidation and oxidative dealkylation. In the presence of the adequate co-factors, we also identified new MTK-

derived conjugates, including several glucuronides. We also found that MTK appears to bind glutathione and cysteine, both under metabolic and non-metabolic conditions, suggesting that reaction with biologically relevant thiyl radicals is plausible *in vivo*, with no need for bioactivation. The involvement of a free radical-mediated pathway is potentially relevant to MTK–induced toxic events.

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OP01-06
Metabolism plays an important role in the *in vitro* hepatotoxicity of butylone, buphedrone, and 3,4-dimethylmetcathinone (3,4-DMMC)

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Synthetic cathinones, commonly known as 'bath salts', are one of the most abundant groups of new psychoactive substances monitored by the European Monitoring Centre for Drug and Drug Addiction [1]. Their growing use, along with the lack of human risk assessment, justify the need for further research.

This work aimed to determine whether butylone, buphedrone and 3,4-dimethylmethcathinone (3,4-DMMC) display toxicity to different *in vitro* hepatocyte models, and to evaluate their underlying mechanisms of toxicity. Our group has previously investigated the hepatic and neuronal toxicity of a few selected cathinones [2,3].

Primary rat hepatocytes (PRH), isolated through rat liver collagenase perfusion, human HepaRG cells and HepG2 cells were exposed for 24h to a wide concentration range of all drugs (1 µM–20 mM). Cell metabolic activity was determined using the MTT reduction assay. The same experimental design was used after PRH incubation with CYP450 inhibitors to assess metabolism influence on the observed toxicodynamics. Also, PRH were exposed to drugs at the concentrations that elicited 20%, 40%, 50% and 70% cytotoxic effect in the MTT assay to investigate for alterations in oxidative stress markers (reactive oxygen / nitrogen species and reduced and oxidized glutathione), mitochondrial homeostasis (membrane potential and ATP levels), cytoplasmic membrane integrity (through lactate dehydrogenase leakage assay) , induction of apoptosis (via evaluation of caspase-3, -8 and -9 activities and Hoechst 33342/PI staining) and autophagy (through analysis of formation of acidic vesicular organelles using acridine orange staining).

PRH were the most sensitive hepatocyte model (EC_{50} 0.158 mM for 3,4-DMMC; 1.21 mM for butylone; and 1.57 mM for buphedrone). All drugs induced oxidative stress, hampered mitochondrial homeostasis, disrupted cell membrane integrity and activated apoptosis and autophagy to similar extent for nearly all tested concentrations. Co-incubation of CYP inhibitors suggest that metabolism has a detoxifying role on 3,4-DMMC and butylone toxicity, while adding to buphedrone's toxicity through bioactivation.

Our results contribute to the growing body of information regarding synthetic cathinones toxicity, proving they elicit hepatic damage through distinct cellular mechanisms, and demonstrating that metabolism plays a key role in the substances' toxicity.

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OP01-07
Characterization of GCDC transport by human hepatic uptake transporters for *in vitro* testing purposes

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Background information: Bile acids and bile salts (BAs/BSs) contribute in several physiological processes including signaling pathways, absorption of fat, or elimination of cholesterol. Primary bile acids and their conjugates are formed in the hepatocytes, then excreted into the bile. Bile is depleted in the intestine where bacterial dehydroxylation and unconjugation occur, and the majority of the bile salt species gets reabsorbed into the blood and circulates back to the liver. Giving their detergent nature, high concentration of BAs/BSs intracellularly or in the circulation system can lead to cytotoxicity. Therefore, testing the effect of drug candidates with high hepatic clearance on the transport of BAs/BSs is an issue of critical importance. The most commonly used probe substrate in *in vitro* test systems is taurocholate (TC), although the concentration of taurine-conjugated bile salts in human is two/three-fold lower than that of glycine-conjugated species, whereas taurine conjugation is the main modification in rats.

Hypothesis: Using glycochenodeoxycholate (GCDC), one of the most relevant conjugated bile salt in human, as probe substrate in *in vitro* test systems might provide better prediction on the effect on enterohepatic circulation of bile salts.

Methods: HEK293 cells transduced with OATP1B1 and OATP1B3, as well as NTCP expressing CHO and HEK293 cells were used in uptake assay format. Proof of concept (POC) experiments were carried out with radiolabeled TC and unlabeled GCDC, sulfated GCDC and chenodeoxycholate-sulfate (3S-CDC) at two concentrations and two timepoints.

Results and conclusion: All bile salts were transported by OATP1B1 and 1B3 in a time- and concentration-dependent manner, while only TC and GCDC was picked up as substrates for NTCP. Since GCDC was efficiently transported by all three transporters, full transport characterization on OATP1B1, 1B3 and NTCP were conducted with tritiated TC and GCDC as probe substrates. Michaelis-Menten constants (Km) were around three-fold lower for GCDC than TC, showing higher affinity for that bile salt. Inhibitory effect of known substrates and inhibitors (atorvastatin, CCK8, diclofenac, pravastatin, telmisartan and troglitazone) on both probes was also tested. Based on the obtained data, and the higher *in vivo* relevance, the authors suggest replacing TC to GCDC for human *in vitro* test systems.

OP01-08

Hepatotoxic fungicides affect molecular targets associated with the AOPs for cholestasis and steatosis *in vitro*

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Several hepatotoxic fungicides of the triazole, pyrazole/anilide and strobilurine group like cyproconazole, epoxiconazole, propiconazole, fluxapyroxad or azoxystrobin show liver toxicity *in vivo*. While activation of nuclear receptors like CAR or PXR, subsequent enzyme induction and associated hepatocellular hypertrophy is the most prominent finding, some of these compounds have also been reported to cause hepatocellular vacuolization indicative of steatosis or cholestasis.

We analyzed the ability of several fungicides to activate nuclear receptors described as molecular initiators in the adverse outcome pathways (AOP) for steatosis (PXR) or cholestasis (FXR, PXR, CAR) in the human liver cell line HepG2 as well as expression of marker genes associated with either of the AOP (FASN, CD36, INSIG-1, CYP7A1, Slc10a1, etc.) in HepaRG cells. Gene expression analysis was performed using Human Molecular Toxicology Pathway Finder® low density RT-PCR arrays and targeted qRT-PCR. Active substances as well as two selected commercial products, respective combinations of active substances and some co-formulants were examined at subtoxic concentrations. In addition, AdipoRed staining was performed in HepaRG cells to address a functional endpoint.

Our results indicate that azoxystrobin activates FXR and related gene expression while on the other hand the azoles activate CAR and PXR. Several substances induced expression of steatosis and/or cholestasis related genes and showed lipid accumulation in AdipoRed staining. Products were more cytotoxic than the respective active substance combination. Effects at the transcript level at equimolar concentrations were more pronounced with products for some (e.g. CYP1A1/2) but not all of the molecular targets. Overall, our findings therefore suggest that key events of the respective AOPs can be measured as well in cell lines of human origin and the methodology may be useful to support the characterization of potential combination effects.

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OP02-01

Gene expression profiling of an *ex vivo* human placenta perfusion model following exposure to engineered nanomaterials

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With the rapid growth of nanotechnology, potential human exposure to engineered nanomaterials (ENMs) is expected to increase, thus raising concerns of possible adverse health effects in particular for sensitive populations such as pregnant women and the developing fetus. There is increasing evidence from animal studies that ENMs may affect pregnancy and fetal health by interfering with placental development and function. However, the underlying mechanisms are largely unknown and verification in human placental tissue is urgently needed to exclude species-specific differences. Therefore, our aim in the present study was to investigate the impact of two common commercial ENMs *i.e.* copper oxide (CuO; 10–20 nm) and polystyrene nanoparticles (PS; 70 nm) on human placental function and physiological signaling using the *ex vivo* human placenta perfusion model. Subsequently, global gene expression profiling was performed following a 6 h exposure to sub-cytotoxic doses of CuO (10 µg/ml) and PS (25 µg/ml). Interestingly, 1060 genes were differentially expressed upon PS exposure, while 271 were affected by CuO treatment. A total of 162 genes were commonly modulated after both ENMs exposures, compared to untreated placentas. Most differentially affected canonical pathways were related to hormone and chemokine/cytokine signaling. In addition, genes affecting the differentiation of stem cells, vascular functions and the regulation of the immune system were also disrupted, further supporting that ENMs may interfere with placental function. The observed dysregulation of cytokines, hormones and angiogenic factors may be associated with increased risk of pregnancy complications and disorders *such as* pre-eclampsia or intrauterine growth restriction. QRT-PCR validation of the obtained data is ongoing.

OP02-02

Grouping of representative nanomaterials is efficiently executed by combining high-throughput-generated biological data with physicochemical data

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Grouping of nanomaterials (NM) promises to serve effectively to reduce the extensive safety testing needs associated with regulatory risk assessment. Key challenges in this task are how to rapidly and cost-efficiently generate the needed data, and how to best combine structural material characteristics with biological effects data. Herein, we performed NM grouping from combining existing physicochemical data with high-throughput screening (HTS)-derived hazard assessment data generated in the human lung epithelial cell line BEAS-2B. Twenty-one NMs from the European Joint Research Centre's Representative Nanomaterials Repository (diverse nanoforms of substances ZnO, SiO_2 and TiO_2) and five reference chemicals were analyzed by HTS assays for cytotoxicity/cell viability (CellTiterGlo, Dapi-staining), oxidative stress (8-OHdG), apoptosis (Caspase-3), and DNA damage repair (γH2AX). Additionally, physicochemical data relevant for grouping of NMs under REACH (ECHA, 2017 Appendix R.6-1) were collated for 15 of the NMs, including from EU-funded projects (NanoReg2, caLIBRAte) and the OECD Testing Programme of Nanomaterials. The diverse data types were scaled, normalized and integrated using a newly developed scoring pipeline inspired by the US-EPA Toxicological Prioritization Index (ToxPi). Results demonstrated that the *in vitro*-derived hazard data permitted substance-based

grouping of the selected NMs, whereas integration of physicochemical data deepened the grouping of specific nanoforms within each substance group. Furthermore, a case study on 10 TiO_2 NMs showed that hazard-based grouping allowed for read across of physicochemical data between 6 NMs acting as source nanoforms and 4 NMs acting as target nanoforms. The ToxPi tool and scoring pipeline permitted transparent visualization of the final grouping, while giving equal weight to different types of data/results related to structure and biology. Overall, this study aligns fully with the ECHA recommendations for grouping of NM (Appendix R.6-1), i.e. i) to aim at identification of criteria for grouping nanoforms (and non-nanoforms) within one substance, and ii) to provide additional information beyond physicochemical data to support read across between nanoforms.

OP02-03
Pulmonary pro-inflammatory effects of alumina nanoparticles and hydrogen chloride gas mixtures on rats after single and repeated inhalations

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Environmental exposures to aerosol mixtures containing nanoparticles (NPs) and gases can occur especially after combustion processes. In defense and aerospace fields, NPs can be present in aerosols resulting from firing of solid composite propellants. These aerosols are complex mixtures among which alumina NPs (Al_2O_3) and hydrogen chloride gas (HCl) are found in high concentrations. Inhalation is the main route of exposure to these pollutants mixtures but their pulmonary toxicity is not known and understood yet. Therefore, the aim of this study is to investigate lung pro-inflammatory effects after inhalation exposure.

Wistar rats were exposed 4 hours (single exposure) or 4 hours a day during 4 days (repeated exposures) by inhalation in a nose-only exposure system to an aerosol containing Al_2O_3 NPs (primary sizes/crystalline polymorphs: 13 nm/gamma-delta) and/or HCl gas (5 ppm). Lung histopathology and bronchoalveolar lavages fluids (BALF) were analyzed 24h after the last exposure. In BALF, pro-inflammatory cytokines (IL1-β, IL-6, GRO/KC, TNF-α) were quantified using multiplex ELISA.

Lungs histological analysis revealed inflammatory tissues lesions following exposures to experimental aerosols containing NPs. Vascular congestions, alveolar edematous walls, presence of inflammatory infiltrates (lymphocyte and polynuclear cells) and epithelial cells pre-exfoliation were observed following exposures to NPs alone or mixed with HCl. Significant polymorphonuclear neutrophils increases in BALF were measured after single and repeated exposures, suggesting pro-inflammatory effects of Al_2O_3 NPs or mixtures aerosols. GRO/KC concentrations in BALF were significantly increased after both exposure scenarios to Al_2O_3 NPs associated or not with HCl. However, higher GRO/KC concentrations were quantified after repeated exposures. IL1-β and TNF-α concentrations were more significantly increased following repeated exposures.

Our study demonstrates the induction of early lung inflammation after Al_2O_3 NPs/HCl gas mixtures inhalation. Data also showed significantly increased pulmonary pro-inflammatory response after repeated exposures compared to single inhalation. Taken together, these results may help development of appropriate safety standards to protect health of exposed workers.

OP02-04
Lung toxicity of industrial particles

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The frame of the Nanoreg2 European project, which aimed at helping industrials partners to safely design nanomaterials, we studied *in vivo* toxicity of different nanomaterials produced by industry. Three industrial partners provided five nanomaterials. Two silicium nanomaterials were studied, one without and one with a carbon coating (named Si and Si-WC). Two carbon nanofibers with different degrees of purity and graphitization). And finally, one graphene. To study the lung toxicity of these products, we exposed rats to 0.1, 1 and 2 mg/kg of nanomaterials with one endo-tracheal instillation. Animals were then killed 24h, 3 and 28 days after exposure. Broncho-Alveolar Lavages (BAL) were performed in order to count and identify BAL cells but also to dose inflammatory cytokines secreted in BAL supernatant. Lungs were also fixed and analyzed by an histo-pathologist.

The coated Si (Si-WC) induced a greater lung inflammatory response which was also more persistent in time compared to the response observed with Si without coating. Si-WC treated group developed within the lung parenchyma, interstitial thickening which were slightly more intense than Si particle treated group. Bronchial alterations were presents only in the highest exposed groups, but slightly more frequently observed in Si-WC40 exposed groups, but a minimal dose related effects tend to be observed for both particles. A dose related effect was observed for the presence of particle rich macrophages density suggesting a high clearance activity, only for Si-WC40 treated groups.

The less pure carbon nanofiber induced a higher lung inflammatory response just after exposure which did not last in time. A very similar histologic lung profile was observed for both carbon nanofibers exposed groups with higher lesionnal score at early stages than late stages, suggesting an efficient clearance of the particles. No dose related effect has been observed. Bronchial alterations were observed only at the highest dose for both particles. However, a slightly more important number of bronchiolitis obliterans was observed after less pure carbon nanofiber exposure but tend to decrease in time. A dose related effect was observed for the presence of particle rich macrophages density suggesting a high clearance activity.

The graphene induced the most important inflammatory response, but it did not last in time. Alveolar and bronchi alterations were quite high after graphene powder exposure. The lesions were slightly less intense with time suggesting an ongoing recovery. No dose-related effect could be observed. Within the bronchi, hyperplasia associated with inflammation leading to bronchial obstruction (bronchiolitis obliterans) was observed for all doses and all times after graphene powder exposure. Particles rich macrophages were not as high as expected, suggesting a less efficient clearance process, thus consistent with more alveolar and bronchi lesions.

OP02-05

Silica nanoparticles induce the blood hypercoagulable state via miR-451/IL6R signaling pathway

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Background: Safety evaluation will be a prerequisite for nanomaterials in a wide range of sectors, including in chemical industries, medicine or food sciences. Yet, the toxic effects of SiNPs remain largely unknown. This study was aimed to explore the role of miR-451 on SiNPs-induced the blood hypercoagulable state *in vivo*.

Results: Histological and ultrastructural analysis manifested that SiNPs caused the vascular endothelial damage. Results from Doppler ultrasound showed that SiNPs could cause the reduction of blood flow velocity and impair the hemodynamic in SD rats. Using Tg(mpo:GFP) and Tg(fli-1:EGFP) transgenic zebrafish lines, SiNPs could induce neutrophil-mediated inflammation and impaired vascular endothelial cells. With the dosage increasing, SiNPs also markedly decreased the blood flow velocity, exhibiting a blood hypercoagulable state in zebrafish embryos. The expression level of MDA was elevated while the activity of SOD and GSH-Px were decreased in vessel tissues triggered by SiNPs, accompanied with the release of iNOS and decline of eNOS in blood serum. The coagulant factors TF, Fxa, vWF and PLT numbers were increased whereas the anticoagulant factors ATIII, TFPI and t-PA were decreased in blood serum. For in-deelp study, the microarray analysis showed that the down-regulated miR-451 could target the gene expression of *IL6R*, which further activated the JAK1/STAT3 signaling pathway triggered by SiNPs. Dual-luciferase reporter gene assay confirmed the target regulatory relationship between miR-451 and IL6R directly. Chemical mimics of miR-451 led to attenuate the expression of IL6R signaling pathway in vascular endothelial cells, while the inhibitor of miR-451 enhanced the activation of IL6R signaling pathway.

Conclusions: In summary, SiNPs could accelerate the blood hypercoagulable state via miR-451/IL6R signaling pathway.

OP02-06

Food-grade TiO_2 (E171) nanoparticles cross the human placental barrier: an *ex vivo* study on isolated and perfused placentae

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Purpose: Food-grade Titanium dioxide (TiO_2, E171 in EU) is used as white pigment and opacifying agent in foodstuffs, and contain up to 47% (number-based) of nanoparticles (NPs) [1]. Despite low intestinal absorption, dietary exposure is chronic (0.2–10 mg/kg/day) [2] and TiO_2-NPs accumulate in human systemic organs [3]. In rodents, perinatal exposure to TiO_2-NP models showed transplacental NP transfer with susbsequent health effects in offspring [4]. The current study aims at evaluating whether the nanosize fraction of E171 may cross the human placental barrier.

Methods: Term placentae and meconiums were collected with mother's consents. Basal titanium (Ti) levels were assessed by inductively coupled plasma-mass spectrometry (ICP-MS). Crystal TiO_2 forms among other particulate elements were assessed on placenta tissue sections by energy dispersive X-ray analysis (EDX) coupled with scanning electron microscopy (SEM). Placentae were perfused in a double open circuit for 30 min of equilibrium with Earle's medium (EM), followed by 1h of EM i) alone as control (n=2), ii) supplemented with E171 (15µg/ml, n=7) after particle dispersion by sonication (16min, 40% amplitude). Passive antipyrine transfer rate (≥20%) was used as viability biomarker. Passage of laser-reflective particles was evaluated by confocal microscopy on foetal exudate collected every 5min. Particle nature and size were analysed by SEM-EDX and Image J software.

Results: Basal Ti levels ranged 0.002-0.484 mg/kg in placentae (n=21), and 0.001-0.326 mg/kg in meconiums (n=3). Anatase and rutile TiO_2, and carbon particles were commonly found in term placentae. Laser-reflecting particles were detected in the foetal exudate 10min after E171 addition in the maternal side, reaching a plateau at 25–30min. SEM-EDX imaging showed all TiO_2 particles of diameters <200nm in the foetal side, 70–100% of them being NPs depending on the placenta. In conclusion, circulating TiO_2 accumulates in the human placenta then the meconium. A transplacental passage of food-grade TiO_2 (E171) particles was observed and mostly concerned NP fraction. Although the maternal-to-foetus TiO_2 transfer is low, both placenta accumulation and foetal exposure occur in human. These data emphasize the need of risk assessment in human of chronic exposure to TiO_2-NPs of dietary origin during pregnancy.

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OP02-07

Long-term effects of inhaled nanoparticles in rats – ceriumdioxide and bariumsulfate

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Ceriumdioxide (NM212) and Bariumsulfate (NM220) nanoparticles were tested according to the OECD test guideline no. 453; additions were made to the standard protocol to find evidence of inflammation and potential lung tumours with high sensitivity. Aerosol concentrations were concentrations 0.1, 0.3, 1 and 3 mg/m³ and 50 mg/m³ was tested, respectively.

Levels of cerium measured in the organs increased with higher exposure concentrations and over time. However, the accumulation only reached a very low level. Lung burdens of Barium were unexpectedly low during the first three months of exposure, due to fast clearance most probably by dissolution *in vivo*. Barium lung burdens increased thereafter.

Animals in all exposure groups showed chronic inflammation of the lungs, with stronger inflammation at higher exposure concentra-

tions. The level of particles present in the lungs was higher for barium-sulfate than for the highest level of cerium dioxide. The nature of the inflammatory tissue changes varied between the two nanoparticles.

Ceriumdioxide already triggered a chronic inflammation effect at the lowest dose, which was unexpected for insoluble nanoparticles without inherent toxicity. Despite chronic inflammation at all dose levels, no lung tumours were found which could be attributed to ceriumdioxide or bariumsulfate exposure. This may indicate that a particle-related, low-level chronic inflammation is not sufficient to cause tumour formation.

Low-level accumulation in extrapulmonary organs did not lead to any pathological changes. No evidence of further health impacts was found for ceriumdioxide and bariumsulfate beyond those already known for granular particles.

Acknowledgement: The project was carried out and funded jointly by the German Federal Environment Ministry, the Federal Environment Agency, the Federal Institute for Occupational Safety and Health and BASF SE as well as the EU project NanoREG and Cefic LRI. Histopathological examinations were performed by Fraunhofer Institute for Toxicology and Experimental Medicine and the biodistribution was analysed by the Federal Institute for Risk Assessment.

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OP02-08
Environmental risks associated with nanoscale zerovalent iron-based nanomaterials during remediation applications

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Nowadays, nanoscale zero-valent iron (nZVI) is one of the most used nanomaterials in the remediation industry. The high surface area and strong reductive power of nZVI lead to highly efficient degradation / removal of many organic and inorganic pollutants. Therefore, many successful applications were described worldwide during the last decade. Despite known positive aspects associated with nZVI (e.g. cost-effective application, degradation of persistent pollutants, etc.), potential adverse effects are still not well described; especially mechanisms of the toxic effects on the cellular level, changes in toxicity during the aging of iron nanoparticles or the effect on real microbial populations [1]. In the present work, the "environmental" fate (i.e. aging) of nZVI and derived materials was studied with emphasis to evaluate the toxicity towards microbial species, probably the most exposed organisms during nZVI applications. The results confirmed the hypothesis that over time, nZVI particles undergo oxidation which decreases the toxicity towards bacterial species. The X-ray diffraction analysis and used toxicological assays proved that the decrease in the toxicity greatly corresponds with the decreasing concentration of zerovalent iron and formed oxidation products of nZVI are thus less toxic. Moreover, the real influence of nZVI on the resident microbial communities from a contaminated site was explored. Using advanced technics of molecular biology and microbial biomass estimation via phospholipid fatty acid analysis, the long term effect (during 60 days) of a nZVI-based material on the resident microbes from the real site has been explored as well.

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PV01 | Poster Viewing 1

P01 – Biomakers of effects/exposure

P01-001

This abstract has been withdrawn.

P01-002
Effect of L-Glutamic acid and N-acetyl cysteine on carbon tetrachloride-induced oxidative stress in rats

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Carbon tetrachloride (CCl_4) is one of the most widely used toxicant. CCl_4-induced toxicity depending on dose and duration of exposure covers a variety of effects. The liver is especially sensitive to CCl_4 since it contains a large amount of the enzymes that change the form of the chemical. The kidney and brain and other tissues of the body are also sensitive to CCl_4. L-Glutamic acid (L-Glu) and N-acetyl cysteine (NAC) are antioxidants. Antioxidants play a critical role against CCl4 intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides. These amino acids are necessary for the synthesis of key molecules, such as glutathione, which involved in process of xenobiotics detoxification in reaction of conjugation with glutathione.

The present study was carried out to evaluate the antioxidant effects of L-Glu and NAC on CCl4- induced oxidative stress in rats. Experiments were conducted on albino Wistar rats (males) weighing 200–220g. The duration of experimental period was 24 hours. CCl4 (3ml/kg) administrated intraperitoneally to all experimental groups of rats. After that rats from the second and third experimental groups intraperitoneally received an aqueous solution of L-Glu and NAC. Rats of the control group were administered by the appropriate amount of saline. Activity of some antioxidant enzymes, intensity of peroxidation processes, some biochemical indexes in various tissues and blood of rats was studied.

Activity of antioxidant enzymes decreased and level of lipid peroxidation expressed by lipid hydroperoxides significantly decreased ($p \leq 0.05$) in group of rats when CCl4 only was administered. L-Glu and NAC treatment was found to increase antioxidant enzymes activity($P<0.05$) and decreased lipid hydroperoxides level. There was a difference between the CCl4 and CCl4 +L-Glu, CCl4 +NAC groups in others studied indexes.

The results obtained in this study show the protective action of L-Glu and NAC in carbon tetrachloride-induced oxidative stress in rats.

P01-003

Mother's residency (urban vs. rural) significantly influences newborns' sex hormone levels, IL-6 and micronucleus frequency

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The association of newborn health risks due to the mother's exposure to urban pollution has been investigated for decades but comparison of health risks with newborns whose mothers spent their pregnancy in agricultural areas is very limited. The purpose of this study was to compare for the first time IL-6, testosterone (T) and estrogen (E) levels, their ratio (E/T) and genome damage by micronucleus assay (MN) and nuclear bridge (NB) frequency between newborns born from mothers with urban or agricultural residency in order to assess the possible environmental endocrine effects and interaction between biomarkers pointing at different types of health risks. Fifty full-term newborns of both sexes, whose mothers were healthy and not occupationally exposed to any known carcinogen, were analyzed. All of the mothers filled in a questionnaire on life style, diet and residency. Multivariate analyses for dependent variables were done using generalized linear/nonlinear regression models using all effects models. Results showed significantly higher levels of E and E/T ratio in newborns of mothers from agricultural than from those born by mothers with urban residency. A lower level of E and E/T ratio was measured in newborns of mothers, who drank coffee every day, smoked and didn't eat fish. Testosterone was significantly higher in boys of mothers with agricultural residency than from mothers with urban residency. Residence had no impact on difference in MN frequency. IL-6 levels were higher in newborns of mothers with agricultural residency but also in those who lived close to the highway. NB levels were significantly associated with E and E/T ratio levels. A significant association between E levels and IL-6 and between E and T levels was found. Our results for the first time show a significant impact of mother's agricultural residency on sex hormones and IL-6 levels. Future research should focus on sex-specific effects of herbicide/insecticides on newborns' immunological and endocrine status. Increased incidence of cancer and chronic diseases in agricultural areas may have origin in transplacental exposure.

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P01-004

Assessment of mitochondrial function in peripheral blood mononuclear cells and platelets as potential surrogates for systemic mitochondrial perturbation

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Bioenergetic mitochondrial assessments are limited in *in-vivo* toxicity testing due to conflicting tissue requirements for mitochondrial isolation and pathology driving the need for bespoke investigative studies. Standard pre-clinical toxicity testing also relies on the use of animals which are generally young and metabolically healthy; as such they are relatively insensitive to compound-mediated mitochondrial perturbation, often remaining asymptomatic. This contrasts with patients whose metabolic capacity is often impaired by several factors; where a mild insult could result in a severe clinical effect. There are no easily accessible biomarkers to monitor compound effects on mitochondria *in-vivo* from a toxicology or pharmacology perspective. To compound this calcium loading and seahorse assays indicate that some *in-vivo* mitochondrial inhibition does not persist (or leaves an adapted phenotype) following mitochondrial isolation confusing interpretation [Broom *et al.* 2015]. To bridge this gap an exploratory study in healthy rats was conducted to assess the use of peripheral blood mononuclear cells (PBMCs) or platelets as a non-invasive method for detecting systemic mitochondrial perturbation by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in these isolated cells. Rats were dosed with a known electron transport chain (ETC) inhibitor or a mitochondrial uncoupler (both GSK compounds terminated for mitochondrial toxicity). The compounds provide discrete (clinically relevant) mechanisms of mitochondrial toxicity resulting in differing effects on OCR in mitochondria. ETC inhibition causes: drop in OCR and ATP production, adaptive shift to glycolysis, increased reactive oxygen species (ROS), drop in body temperature (BT) and (as no alternate route to ATP production exists) when glucose and glycogen are exhausted mortality. In contrast, uncoupling dissipates the mitochondrial membrane potential ($\Delta\Psi$) by shuttling protons from the inner membrane space to the matrix causing: Maximal ETC activity in a futile effort to recover $\Delta\Psi$, increase in OCR, drop in ATP production, shift to glycolysis and an increase in BT. As the ETC is still functional and uses all available substrates, an adequate dose results in prolonged hyperthermia and death before any substrate limitations. As circulatory cells are known to have altered energetics following activation by immune stimuli and thus potentially in response to tissue damage [Kramer *et al.* 2014] a comparator study was performed where cells were isolated from naïve rat blood and exposed to the two compounds to explore this effect and allow comparison to the *in-vivo* results. These studies serve as a proof of concept regarding our ability to detect mitochondrial changes in blood cell populations; that could provide a non-invasive route to assessing mitochondrial function in routine *in-vivo* toxicity studies for new chemical entities where mitochondrial function is of interest.

P01-005

This abstract has been withdrawn.

P01-006

Intra-erythrocyte chromium as an indicator of exposure to hexavalent chromium: *in-vivo* evaluation

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Thousands of employees are potentially exposed to hexavalent chromium (Cr^6) which is carcinogenic to humans.

There is currently no Cr^6 specific biological exposure marker. Although considered the most reliable biomarkers, the blood and urinary chromium concentrations are not specific for Cr^6 exposures. A previous *in vitro* study conducted on human blood samples has demonstrated that intra erythrocyte chromium (CrIE) is a specific indicator of the Cr^6 exposure. However, due to insufficient data, this assay cannot be proposed to the hygienists in routine.

This work aims to provide additional *in vivo* data in rat (useful for the improvement of PBPK models and the extrapolation across species for use in risk assessment) regarding the comparative kinetics of in-

corporation and elimination of Cr^6 in erythrocytes, plasma and urine.

Male Sprague-Dawley rats were iv injected with Cr^6 and/or Cr^3 solutions made from ammonium dichromate ($(NH_4)_2Cr_2O_7$) or chromium chloride ($CrCl_3$) dissolved in saline. Three doses of Cr^6 (corresponding to 0.13, 1.31 and 2.62 mg of Cr^6 per kg of rat), one of Cr^3 (corresponding to 1.31 mg Cr^3/kg rat) and one mixture Cr^6/Cr^3 (each at 1.31 mg/kg rat) were thus administered. Control groups were administered with saline solution only. Blood and urine were collected at different time points (until 48 h and day 90 for urine and blood, respectively).

Erythrocytes selectively incorporate Cr^6 at the expense of Cr^3 and Cr^3 has no effect on Cr^6 incorporation into erythrocytes. The Cr^6 incorporation into the erythrocytes is rapid (less than 10 min to reach the maximum) and the Cr remains trapped in the erythrocytes for a few days (quite stable for 2 days and 62% of the initial concentration in CrIE after 5 days). In addition, CrIE concentration is proportional to the amount of Cr injected. By way of comparison, the CrU concentration reaches a maximum 6 h after injection then returning to the threshold level in less than 24 h.

These results confirm the relevance of CrIE as a specific indicator of recent or older exposures to Cr^6. Since the life expectancy of human erythrocytes is longer than those of rat (120 days/60 days), a higher accumulation capacity and a slower elimination can be expected in human. Samples taken at the beginning and end of the workshift week could allow a good evaluation of the recent exposure to Cr^6.

P01-007
Cell-free, circulating microRNAs reflect air pollution-induced environmental health risks

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The WHO estimated that worldwide more than 80% of the people in cities are exposed to air pollution levels that exceed the air quality limits. A large source of air pollution originates from traffic emission which consists of a complex mixture of compounds that contributes to the pathogenesis of many diseases. In search of an early diagnostic biomarker for improved environmental health risk assessment, recent studies have shown that certain microRNAs (miRNAs) are responsive to exposure to traffic-related air pollution (TRAP).

Here, we present a genome-wide analysis of cell-free, circulating miRNAs (cimiRNAs) in a human healthy population exposed to different levels of TRAP. The cross-over study included blood sampling from 24 volunteers after 2 hours of resting or intermittently cycling at high and low TRAP exposure sites (4 scenarios per volunteer) in Barcelona, Spain. Real-time exposure of particulate matter (PM_{10}, $PM_{2.5}$, UFP and black carbon), nitrogen oxides (NO, NO_2) and carbon oxides (CO, CO_2) were measured during each intervention. Furthermore, next-generation sequencing analysis was used to quantify global cimiRNA levels across all subjects.

Associations between TRAP levels and cimiRNA levels were evaluated using multivariate normal models (False discovery rate <= 0.1). We identified 8 cimiRNAs to be associated with the mixture of TRAP and 27 cimiRNAs that were specifically associated with the individual pollutants NO, NO_2, CO, CO_2, BC and UFP. We did not find significant associations between cimiRNA levels and PM_{10} or $PM_{2.5}$.

Bioinformatics analysis revealed potential molecular mechanisms by which these cimiRNAs can target complex regulatory networks that are implicated in the development of major air pollution-induced diseases. These networks included among others the hub genes *TP53*, *VEGFA*, *IL6* and *PTEN* which have known roles in the pathogenesis of diseases such as lung cancer, asthma as well as multiple cardiovascular and neurodegenerative diseases. Further mechanistic studies are needed to confirm the regulatory roles of these cimiRNAs; however, this study presents a new avenue through which TRAP potentially induces human health effects. Furthermore, it provides novel evidence for the potential of global cimiRNA profiles to be used in biomarker based environmental health-risk assessment.

P01-008
Alcohol induced changes in the serum and placental metabolome during pregnancy

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Alcohol use during pregnancy is the leading preventable cause of developmental disability in children. Understanding the changes in the metabolome due to alcohol during pregnancy will enable to identify sensitive biomarkers of prenatal alcohol exposure and to find possible novel targets for treatment. Clinical value is obvious, since fetal alcohol spectrum disorders are under diagnosed conditions worldwide and no treatment options exist for prevention or alleviation of the symptoms.

We have analyzed first trimester serum samples from alcohol users (n=19), drug users (n=24), tobacco smokers (n=40) and controls (n=55), and placental samples from alcohol exposed (n=6) and control (n=6) pregnancies using untargeted liquid chromatography mass spectrometry based metabolomics. Samples were collected during routine clinical visits and used after an informed consent was gained from the mothers.

Increased levels of glutamate and decreased levels of glutamine and serotonin were associated with alcohol use during pregnancy in the first trimester serum samples. Furthermore, we found that in the placental tissue, alcohol use during pregnancy was associated with altered phospholipid levels. Especially the levels of phosphatidylethanolamines were increased in the placentas by alcohol.

These results give insight to the pathological processes caused by prenatal alcohol exposure, especially in the placenta. Furthermore, these results show that metabolomics can be used to pursue biomarkers of alcohol exposure during pregnancy. Especially placenta seems to be very interesting tissue for this purpose.

P01-009

Predictive toxicogenomics space modeling serves effectively to sensitive biomarker-based read across from capturing toxic mode-of-action of lowest-observable effect levels

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The predictive toxicogenomics space (PTGS) concept was previously developed for predicting cellular toxicity and organ pathology from analyzing transcriptomics data generated in cell culture experiments [Kohonen *et al*., Nat Commun, 2017]. Directed initially towards human drug-induced liver injury, the 14 gene component-based PTGS proved to effectively capture pathological states from analyzing human and rat hepatocyte data. Our extended work provides now a standardized PTGS-based biomarker analysis protocol that couples compound grouping and read-across with defining first the lowest-observable effect levels and toxic mode-of-action (MoA) to component or gene level. Scoring of microarray or RNA-seq data applying the U.S. EPA BMD Express 2.2 software identify initially points of departure (POD) biomarkers that are up to 100-fold more sensitive than previously identified biomarkers. The POD data in turn serves to connect the lowest observable toxic effect levels of agents under study to chemicals and drug molecules present in the Connectivity Map or the Library of Integrated Network-based Cellular Signatures perturbation classes. The novel PTGS-based protocol permits ab initio toxicity prediction to biomarker level of any agent coupled to potency, MoA and biological read-across data. Coupling of the biomarker data to key events in adverse outcome pathways is then a further dimension. Overall, quantitative PoD-focused biomarker discovery is bound to increase the applicability of *in vitro* and *in silico*-based data modeling for replacement of animal experiments in toxicity testing.

P01-010

Assessing Aflatoxin B1 exposure in humans by measuring Aflatoxin M1 in urine

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In humans, as well as in several other species; toxicology uses biomarkers as predictive tools to detect early exposure or damage caused by chronic exposure.

A biomarker is a measurable variation in the cellular or biochemical components, processes, structures or functions in a biological system or samples; these changes are induced by xenobiotics.

After the ingestion of AFB1 (AFB1) by human or animals, a metabolite called aflatoxin M1 (AM1) is produced and subsequently eliminated through urine and milk. In fact, AFM1 is a linear biomarker to estimate AFB1 exposure.

The main objective of the study was to assess AFM1 concentration in urine of a group of volunteers and relate it to previous AFB1 exposure.

A convenience sampling (non-probabilistic sampling), was used in Aguascalientes, Mexico. In the study both female and male individuals (≥18 years old) were included. The participants in the research signed informed consent.

The samples were analyzed in accordance to the directions specified on a commercial ELISA kit (detection range: 0–4.0 ng/mL; specificity and sensitivity: 100%).

The study was carried out over a 2-year period. During this time, AFM1 determination was performed in 660 urine samples.

Main results indicate that 46% male and 54% female were included in the study. The average age of volunteers was 20 year-old. Sixty percent of the samples had detectable levels of AFM1. The concentration of AFM1 in urine samples ranged from 0.15 to 0.40 ng/mL. The highest AFM1 concentrations were found on the >45 years-old volunteer group.

The results indicated an unnoticed AFB1 exposure that may cause subsequent damages, even cancer.

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P01-011

Regioselective synthesis of neoeriocitrin dihydrochalcone from naringin dihydrochalcone by *Bacillus megaterium* CYP102A1 and its effects on human cytochrome P450 activities

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Naringin dihydrochalcone (naringin DC) is well-known as an artificial sweetener with a strong antioxidant activity, that has potential applications in food and pharmaceutical fields. It is originally derived from the flavonoid naringin which occurs naturally in citrus fruits, especially in grapefruit. Naringin DC is a glycoside of phloretin which shows an inhibitory effect on active transport of glucose into cells by SGLT1 and SGLT2. It was suggested that naringin DC might be a potential therapeutic agent for the treatment of AD against multiple targets that include Aβ pathology, neuroinflammation and neurogenesis. A large set of natural compounds and their metabolites are known to effect on the catalytic activities of human cytochrome P450 enzyme, which are the major metabolizing enzymes. In this study, we have tried to find an enzymatic strategy for the efficient synthesis of potentially valuable metabolites from naringin DC. Effects of the naringin DC and its metabolites on P450 activities were studied. A set of *Bacillus megaterium* CYP102A1 variants was used to find efficient regioselective hydroxylases toward naringin DC. Human liver microsomes and recombinant human P450s were used to make metabolites of naringin DC. We found three highly active CYP102A1 variants to hydroxylate naringin DC among wild type (CYP102A1) and its 60 variants. Highly active variants produced one major metabolite and its chemical structure was determined by LC/MS and NMR. The major metabolite is neoeriocitrin dihydrochalcone (neoeriocitrin DC), which has a catechol structure of naringin DC. Inhibitory effects of the naringin DC and its metabolites on human P450 catalyzed reaction were observed. The synthesis of neoeriocitrin DC from naringin DC has been achieved by using biocatalytic strategy of CYP102A1 enzyme with highly efficient yields. At present, as neoeriocitrin DC is not commercially available, its biological functions have not been studied. This result suggests that neoeriocitrin DC can be used for further biological studies at the levels of cells and animals. Consumption of the naringin DC should be considered as a factor for the drug-drug interactions as the naringin DC show inhibitory effects P450 activities. Here, we reported an efficient synthesis of neoeriocitrin

DC from naringin DC by using CYP102A1 and inhibitory effects of naringin DC on P450 activities were shown.

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P01-012
Results from the Norwegian human biomonitoring study in the EuroMix project: Exposure to the pesticides boscalid and imazalil from the diet in Norway

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Background: The fungicides boscalid and imazalil were among the most frequently detected pesticides in the residues monitoring programs 2013-2017 in Norway.

The aim of the present study was to estimate the daily intake of these two pesticides and compare with measured concentrations in 24 h urine samples.

Methods: A human biomonitoring study was performed to study the exposure to chemicals present in food and personal care products (PCPs). In two 24 h periods two-three weeks apart, 144 participants (100 women and 44 men) kept detailed weighted food diaries and PCP diaries and collected all urine excreted. Individual-specific consumption data from both 24 h periods were used to estimate boscalid and imazalil exposure deterministically. A sensitive ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS-MS) method was developed to measure the boscalid metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (M510F01) and imazalil in the 24 h urine pools collected at Day 1.

Results: Overall, the estimated dietary exposure of boscalid and imazalil was comparable between males and females. In the lower bound exposure scenarios, the estimated dietary exposure of boscalid ranged from 0–0.9 µg/kg bw/day and the estimated exposure of imazalil ranged from 0–0.81 µg/kg bw/day.

In 99% of the samples M510F01was detected in concentrations from 0.04–15.03 ng/ml. There was a statistically significant difference between genders ($P<0.0001$) with a median concentration of 0.98 ng/ml for females, and 0.46 ng/ml for males. Imazalil was detected in 1% of the samples. One of the reasons for the low detection of imazalil in urine samples could be the choice of the biomarker. Comparisons with estimated exposure levels for both boscalid and imazalil will be presented.

Conclusion: Widespread human exposure to the fungicide boscalid as measured by one of its metabolites in urine samples was observed.

P01-013
Dietary exposure to phthalates in the European population from infants to the elderly

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Exposure assessment is one of the four pillars of chemical risk assessment carried out in EFSA. Exposure assessment methodologies can differ from one field to the other and this is of relevance when considering chemicals that are ubiquitous (such as phthalates) in different matrices and can contribute to an aggregate exposure. Several options are available to carry out exposure assessment, starting from crude conservative estimates following a tiered approach to refined exposure assessments based on individual food consumption data. EFSA selects the best approach on a case by case basis to guarantee the protection of EU citizens. Recently, EFSA received a mandate to update its 2005 risk assessments of five phthalates [1–5] which are authorised in the EU for use in plastic food contact materials: dibutylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), di-isononylphthalate (DINP) and diisodecylphthalate (DIDP). Dietary exposure (mean and 95th percentile) was estimated for different age groups from infants to the very elderly across 22 European countries by combining literature occurrence data with individual consumption data from the EFSA Comprehensive Food Consumption Database. Exposure estimates were assessed for the 5 phthalates individually and also as a group since some of them were placed into a Cumulative Assessment Group on the basis of co-exposure and due to sharing a common mode of action for toxicity. Data and methodology adopted to assess chronic dietary exposure to the named phthalates will be presented along with key results [6]. A comparison of results with reported exposure estimates obtained using other methodologies (such as biomonitoring and total diet studies) and the uncertainties related to the approach used will also be discussed

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P01-014

Hallmarks of ageing are interconnected in placental tissue and influenced by particulate air pollution exposure during pregnancy

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Background: Observations from experimental studies have put forth a "core axis of ageing" involving telomeres, mitochondria, tumour suppressor gene p53 (TP53), and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A). In this study, we hypothesized that these hallmarks of ageing in placental tissue are interconnected and influenced by early-life ambient air pollution exposure during pregnancy.

Methods: In 680 newborns of the ongoing ENVIRONAGE birth cohort, we measured protein levels of TP53 and PPARGC1A in cord plasma and telomere length and mitochondrial DNA (mtDNA) content in placental tissue. Daily ambient particulate matter with a diameter less than 2.5 µm ($PM_{2.5}$) was calculated for each participant's home address using a spatial-temporal interpolation model in combination with a dispersion model. The associations between prenatal $PM_{2.5}$ exposure and specific hallmarks of ageing were analysed with linear regression models, while accounting for covariates and potential confounders.

Results: $PM_{2.5}$ exposure averaged (SD) 13.5 µg/m^3 (2.5) over the entire pregnancy period. A 5-µg/m^3 increment in $PM_{2.5}$ exposure during the 3rd trimester was associated with 13.2% (95%CI, -19.3% to -6.7%) shorter placental telomere length, 11.2% (95% CI: -4.1 to -17.7) lower placental mtDNA content, and 7.4% (95% CI: 2.1 to 13.0%) higher TP53 protein levels. Telomere length and mtDNA content were linked [a 10% shorter telomere length was associated with a 4.8% (95%CI: 3.6 to 6.1%) lower mtDNA content], and we observed a negative trend between TP53 protein levels and telomere length (p = 0.08). PPARGC1A protein levels were not associated with mtDNA content.

Conclusions: Prenatal air pollution is associated between candidate hallmarks of ageing (telomeres, mitochondria, TP53) in placental tissue. This is the first observational study demonstrating some degree of interconnectedness between master regulators of the molecular circuit linking PM-induced telomere damage and compromised mitochondrial biogenesis.

P01-015

Selection process of relevant quantity data for the safety assessment of cosmetic products

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For a few years, papers on consumption of cosmetic products have been increasingly present in the scientific literature. Thus, the original problem of lack of data is gradually being replaced by choosing the most relevant data to be used for exposure assessment. The aim of this work was to develop a method to select quantity data of cosmetic products applied by consumers, to be used in the Margin of Safety calculation.

The method was based on a scoring of published studies. First, each study was analyzed according to 10 parameters defined as follows: 4 parameters assessing the study in its entirety (year, duration of exposure, statistical method, data homogeneity), 4 parameters assessing the data collection method (supervision, weighting of the product, instruction of use, personal product) and 2 parameters assessing the panel (size and geographical area of the population). Depending on its relevance level, each parameter was given a score of 10, 100 or 1000. Then scores obtained were weighted according to the importance of each parameter in order to choose the most realistic amount data. Different weighting factors were used, from 1 for the most important parameter to 9 for the less important. Finally, the overall score of the study was calculated by adding all the weighted scores.

Because no reference guideline is currently available for cosmetic products intended for babies, we used this method to determine the most relevant quantities to be used in safety assessment. Thanks to a previous work [Ficheux *et al.*, 2019] 8 studies were identified. As a result, this process led to the selection of the most relevant quantity data specific to European babies for 5 categories of products and specific to Asian babies for 3 categories of products. This method is going to be applied to other cosmetics such as sunscreen products.

This process allows the selection of the most relevant amount data based on recent consumption studies for specific populations. It can be useful as a new tool to choose more realistic data, especially when the daily amount proposed by the Scientific Committee on Consumer Safety [SCCS, 2018] is not representative of specific population as it is the case for babies.

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P01-016

Effects of sterigmatocystin on antioxidative enzymes and expression of Hsps in male Wistar rats

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Sterigmatocystin (STC), a precursor of aflatoxin B1, is mycotoxin which International Agency for Research on Cancer evaluated as Group 2B. STC was isolated in 1954 from *Aspergillus versicolor* and afterwards from other *Aspergillus* species producing it as aflatoxin B1 precursor or as final mycotoxin. Due to its structural similarity to carcinogen AFB1, STC carcinogenic potential was studied much more than its toxicity. The aim of this study was to evaluate the effect of STC on antioxidative enzymes and heat shock proteins (Hsp 70 and Hsp27) as parameters of oxidative stress. Male Wistar rats (N = 5 per group) were treated with single oral STC doses of 1/16, 1/8, and 1/4 of LD_{50} (10, 20 and 40 mg kg^{-1} b.w.). Control group was treated with corn oil which was used as STC vehicle. Catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in plasma, kidneys and liver were measured on a plate reader using commercial kits. Expressions of heat shock proteins Hsp70 i Hsp27 were measured in kidneys

and liver using Western blot methods. CAT activity did not change in organs of treated animals. GPx activity was significantly lower in liver of rats treated with 1/8 and 1/4 LD_{50}. Activity of SOD was significantly increased in kidneys of rats given 1/16 and 1/4 LD_{50}. Expression of Hsp70 was significantly increased in liver samples of rats treated with 1/16 LD_{50}; in kidneys the same dose augmented expression of Hsp70 but without significant difference to control group. Highest STC dose returned Hsp70 expression to control values. Expression of Hsp27 in liver and kidneys was not significantly changed at any STC dose. Taken together our findings suggest that acute oral exposure to STC causes oxidative stress both in liver and kidneys.

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P01-017
Combinatorial effects of pesticides on toxicologically relevant liver proteins in HepaRG cells

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Based on the steady increase of the world's population, the protection of plants and crops is essential. To yield sufficient food supplies, pesticides and biocides are widely used in agriculture. Everyday new substances get market approval and therefore the development of novel *in vitro* methods for the detection of potential cumulative effects are required, since the reduction of animal testing is worth striving for.

On the basis of mRNA expression analysis, a selection of important toxicologically relevant liver proteins was made and analyzed with mass spectrometry-(MS) based immunoassays to investigate potential mixture effects of pesticides. The workflow includes a tryptic proteolysis to yield proteotypic peptides of each analyte and an immune enrichment by use of Triple X Proteomics-(TXP) antibodies, which recognize short C-terminal epitopes. The analysis was performed in targeted parallel reaction monitoring (PRM) mode on an ultra-high performance liquid chromatography- mass spectrometry (UHPLC-MS) device. Quantification of the target analytes was done by use of stable isotopically labeled standard peptides. This project focused amongst others on toxicologically relevant proteins like cytochrome P450 enzymes (CYPs, phase I), UDP-glucuronosyltransferases (UGTs, phase II), as well as transporters (phase 0 & III).

As a well-established human hepatocyte system, HepaRG cells were used for the investigation of single and combinatorial effects of pesticides and biocides. 27 proteins were analyzed quantitatively in cells with 30 different single pesticides after 24 hours of treatment. For instance, induction effects were observed for CYP1A1, CYP1A2, CYP3A4, TNFRSF12A and S100P. Based on these results, substances were grouped according to their protein expression profile similarities in very weak, weak, moderate and very strong correlation (Pearson). Four mixtures were generated and HepaRG cells were treated for 24, 48 and 72 hours and analyzed afterwards. Combinatorial effects (additive effects) were observed for several analytes after mixture treatment.

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P01-018
GvHD: Non-clinical findings in the development of CAR-T cells projects

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Activation of the immune cell response targeting specific antigens and gene therapies are currently amongst the innovative and most promising frontiers for the care of hematological malignancies. In this context, ex-vivo gene modified autologous or allogeneic cells obtained and manufactured from human Peripheral Blood Mononuclear Cells (PBMC) demonstrate significant response in the treatment of different leukemia.

One of the major clinical complication (and limitation) of human cells administration in cancer patients is Graft versus Host Disease (GvHD) and different strategies have been developed to decrease this adverse reaction. The preclinical program should therefore take into careful consideration the different claims to properly evaluate the effects observed in preclinical species.

Based on the peculiarity of this therapeutic approach, customized preclinical safety programs have to be properly designed to at least identify the bio-distribution of the therapeutic cells, their persistency, and any adverse effects of cell administration. Particularly, the effect of lymphoid cells, presenting different modifications or obtained through different culture programs, to the host organism need to be investigated taking into account the different endpoints, while managing technical limitations of the animal models. The reaction of the exogenous cells versus the host environment and their relevance and predictivity for the clinical use has to be taken into account.

The experimental model and study plans used with different CAR-T cells projects and the effects observed with unmodified or mock cultured cells are presented, including bio-distribution and persistence evaluated through different bioassays (example flow cytometry, PCR technologies, immunohistochemistry, etc).

Clinical signs and histopathological findings from animals receiving unmodified cells and suggestive of immunological reactions following treatment with different cells preparations will be reported and compared to indicative changes observed in GvHD in models of animal disease.

P01-019
Diesel exhaust particle-altered inflammatory gene expression in alveolar macrophage cells relevant for lung toxicity

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Many epidemiological and animal studies have shown that particulate matter 2.5 (PM2.5) is associated with lung injury via induces the production of inflammatory cytokines, the generation of reactive oxygen species, and alteration in macrophage polarization. However, studies on the relationship between PM2.5 and the inflammatory response in alveolar macrophages are still unclear.

In this study, we used gene expression profiling and gene ontology (GO) analysis to investigate whether diesel exhaust particle (DEP), one of main PM2.5 occurred from motor vehicles in urban enhances the inflammatory response through increasing the expression of cytokines and chemokines in alveolar macrophage (AM) cells.

The gene expression profiles in murine AM (MH-S) cells following 3 hrs exposure to 100 μg/ml DEP were investigated using RNA-Seq

analyses. A combination of fold change ≥1.5 and p value <0.05 was used to define differentially expressed genes (DEGs). Overrepresentation of gene ontology terms representing biological processes and signaling pathway analysis was performed with bioinformatics tools (ExDEGA software, Ingenuity Pathway Analysis(IPA) and DAVID functional annotation tool).

The expression of 192 and 79 genes was up- and down-regulated >1.5-fold (p <0.05), respectively, after DEP exposure. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of these genes revealed significant enrichment in several key biological processes related to inflammatory response, TNF signaling pathway and cellular response to IFN-gamma. Genes related to these functions, such as CIITA, CCL2, CSF1, CXCL2, ANXA1, CX3CL1, TLR5, NLRP3 and AGER have the ability to elicit local and systemic inflammatory response. Also, through a comparative toxicogenomics database (CTD) analysis, we determined that 17 genes are linked to respiratory tract disease.

DEP exposure modulates expression of cytokines and chemokines in alveolar macophages important in the development of lung injury. This suggests that alveolar macrophage-mediated inflammation may contributes to PM2.5-induced lung damage and that the inflammatory genes expressed should be studied in detail, thereby laying the groundwork for development of novel therapeutic targets for PM2.5-induced lung injury.

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P01-020
Multiplex miRNA profiling for biomarker discovery and verification studies using the FirePlex® platform

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We have developed the FirePlex® Technology Platform to address the need for rapid and sensitive biomarker quantitation. Utilizing patented FirePlex hydrogel particles and a three-region encoding design, FirePlex assays allow for true, in-well multiplexing, providing flexible and customizable analyte quantification.

To facilitate miRNA biomarker discovery studies, we offer our standard FirePlex miRNA assays, for quantitation of 5–400 miRNA targets per sample and data acquisition on standard flow cytometers. For miRNA screening studies requiring faster workflows, we offer our high-throughput miRNA assays (miRNA-HT). The high-throughput assays allow for quantitation of 5-36 miRNA targets per sample and assay readout rapidly conducted on high-content imagers.

FirePlex miRNA assay combines particle-based multiplexing with single step RT-PCR signal amplification using universal primers. Thus, these assays leverage PCR sensitivity while eliminating the need for separate reverse-transcription reactions and mitigating amplification biases introduced by target-specific qPCR. Assy sensitivity is ~1000 miRNA copies per sample, with a linear dynamic range of ~5 logs. Assays can be performed without the need for RNA purification, making the FirePlex ideally suited for profiling in serum, plasma, exosomes, cell culture supernatants, urine, and directly from FFPE and tissues. The ability to multiplex targets in each well eliminates the need to split valuable samples into multiple reactions. Results are displayed and interpreted using the integrated, free-of-charge FirePlex Analysis Workbench.

Panels are available for biomarker discovery studies, as well as for specific research areas of interest. We also provide the option to design fully customizable miRNA panels for any sequence, from any species, at no additional cost.

Here we present the data from several studies investigating circulating miRNA profiles, as well as miRNA profiles obtained directly from FFPE tissues, using the FirePlex miRNA Assay Panels. Together, this novel combination of bioinformatics tools and multiplexed, high-sensitivity assays enables rapid discovery and verification of miRNA biomarker signatures from biofluid samples.

P01-021
Exposure of pregnant women to body moisturizer and anti-stretchmark care

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The European Regulation (EC) N°1223/2009 on cosmetic products defines pregnant women as a vulnerable consumer group (EU, 2009). Thus, a specific risk assessment with accurate exposure data is required. However, exposure values from the Scientific Committee on Consumer Safety guidelines (SCCS, 2018) do not consider pregnant women and little information is available in the literature. The aim of this study was to obtain consumption and real-life exposure values of two personal care products commonly used by pregnant women, body moisturizer and anti-stretchmark care.

The study was conducted on 43 French pregnant women enrolled thanks to a previous study on usage patterns of personal care products. The mean age of the subjects was 31 years old, 47.7% of them were at their 2nd trimester and 50% at their 3rd at the inclusion. The participants used their own product, either a body moisturizer or anti-stretchmark care or both, over a 3-week period according to their personal habits. To assess the exposure, products were weighed with a precise balance at the beginning and end of the study and the pregnant women were asked about their weight. Furthermore, the subjects recorded each application and the body areas where the products were applied in a follow-up form. Distribution data were generated with @Risk software.

Among the subjects, 24 were users of body moisturizer and 35 of anti-stretchmark care. 16 subjects used both products. The 90th percentile of daily frequency of use, amount and exposure were 1.46 use/day, 5.37 g/day and 84.63 mg/kg(bw)/day for body moisturizer and 1.83 use/day, 3.97 g/day and 60.91 mg/kg(bw)/day for anti-stretchmark care. The women mostly applied the body moisturizer on their legs (80% of the users), thighs (68%) and arms (52%) and the anti-stretchmark care on their belly (100%) and chest (57%).

This study provides actual exposure values and describes the consumption behavior of pregnant women for body moisturizer and anti-stretchmark care which could serve as a basis for the risk assessment.

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P01-022
Optimization of a 5-Fluorouracil-induced intestinal injury model in mice to construct a multi-scale predictive model of drug-induced intestinal toxicity

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Pharmaceutical industry faces an urgent need to improve early safety evaluation of new drug candidates. Computational systems toxicology could help identifying compounds with an acceptable safety profile in the clinic. However, its implementation within the drug discovery and development pipeline is still in its infancy. Our goal within the IMI project *Translational Quantitative Systems Toxicology* is to build a multi-scale predictive model of drug-induced gastrointestinal (GI) toxicity. To this end, we require new experimentation in clinically relevant animal models of GI toxicity – meeting both functional and phenotypic endpoints – that includes drug-response multi-omics profiles to identify the mechanisms underlying intestinal toxicity. Here, we describe a mouse model of 5-fluorouracil (5-FU)-induced GI injury that captures the pathophysiologic dynamics of the epithelium following exposure to chemotherapy. C57BL/6J male mice were treated with 5-FU at 20 or 50 mg/kg BID by IV bolus injection via a jugular vein catheter for 6, 24 or 96 h. The healing phase of the process was assessed in animals euthanized after a 48-h recovery period. Mice treated with 50 but not 20 mg/kg showed a progressive loss of body weight that reached 15% on day 4 and persisted after cessation of treatment. Also on day 4, 5-FU triggered a dose-dependent increase in diarrhea score, which was normalized after recovery. Histological evaluation demonstrated mucosal atrophy of the intestines in high-dose treated animals on day 4, with shortening of both villi height (32%) and crypt depth (7%), reduced crypt density (16%), and the presence of granulocytic infiltrates. Plasma citrulline levels were accordingly reduced. Despite a strong regenerative crypt hyperplasia, villus atrophy persisted in recovery animals. Regarding the impact of 5-FU on crypt cell death and proliferation, there was an increase in apoptosis at 6 h that peaked at 24 h, whereas inhibition of mitotic figures was first evident at 24 h but persisted until day 4. Ongoing omics analysis will shed light on the cell cycle signaling pathways affected by 5-FU. The biological processes identified will be integrated with our previously established computational model of epithelial cell dynamics to improve its predictivity for clinically relevant intestinal toxicity.

P01-023
A randomised, controlled study to evaluate the effects of switching from cigarette smoking to using a Tobacco Heating Product on Biomarkers of Exposure to cigarette smoke toxicants in healthy subjects

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Preclinical assessments and 5-day clinical studies have shown that toxicant emissions are lower and associated exposure is reduced when using the glo tobacco heating product (THP) compared to smoking conventional cigarettes. However, it is unclear if these reductions are sustained.

This study aimed to test the hypothesis that reductions in toxicant exposure observed in confined studies over 5 days are sustained over a longer period of 90 days in an ambulatory setting. Biomarkers of exposure (BoE) to cigarette smoke toxicants when smokers switch to using glo compared with smokers who continue to smoke were assessed.

This novel study, conducted in the UK (ISRCTN81075760), was approved by a local Research Ethics Committee and run in accordance with ICH-GCP. Subjects were of either gender and aged 23–55 years. Regular smokers were randomly allocated to either continue to smoke their own brand cigarettes (CTS) or switch to using glo for one year. A separate smoking cessation group consisted of regular smokers intending to quit who were provided with assistance to do so (NRT/varenicline/counselling). The final group were participants who have never smoked. For the 90-day exposure segment of this study, subjects attended a Screening Visit plus a total of 4 non-residential clinic visits. Urinary and breath BoE endpoints were assessed at days 1, 30, 60 and 90. Safety evaluations included adverse events, vital signs, clinical laboratory evaluations, physical examinations, electrocardiography, and spirometry.

The results show significant and substantial reductions in the levels of BoE in the smoking cessation group, as well as in the glo group compared to the CTS arm.

This study demonstrated that when smokers switched from smoking combustible cigarettes to using glo, their exposure to smoke toxicants decreased. This confirms that these reductions are sustained for at least 90 days in an ambulatory setting and suggests that glo has the potential to be a reduced exposure and/or reduced risk tobacco product. Further research is required to confirm whether these exposure reductions translate to reductions in smoking-related health risks. The continuation of this clinical study will examine changes in health effect indicators in subjects switching to glo for a period of one year.

P01-024
Changes in the mouse fecal microbiome upon cigarette smoke exposure and effect reversal upon switching to a potential RRP or cessation

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Cigarette smoking causes adverse health effects that may occur shortly after smoking initiation and lead to the development of inflammation and cardiorespiratory disease progression. The microbiome is susceptible to the influence of environmental factors, such as smoking, and recent studies have indicated microbiome alterations in smokers. To reduce the risk of smoking-related diseases, Philip Morris International is developing potentially Reduced-Risk Products (RRPs) to which adult smokers can switch instead of continuing to smoke cigarettes.

Here, groups of mice were exposed to either cigarette smoke (CS) from a reference cigarette; aerosol from two RRPs, the Carbon Heated Tobacco Product (CHTP) 1.2 and the Tobacco Heating System (THS) 2.2; or fresh air (Sham) over the course of six months. Two groups were exposed to CS over three months and switched to either CHTP 1.2 or Sham for the remaining three months. Fecal samples were collected from these groups of mice and subjected to next-generation sequencing-based microbiomics analysis in order to identify microbial taxa whose relative abundance is altered in response to aerosol exposure and changes in aerosol exposure. We identify taxa that are increased in abundance upon CS exposure, such as certain *Bacteroides* and *Akkermansia*.species, as well as species that are reduced in relative abundance upon CS exposure, such as certain *Lactobacillus* species. After two months of switching from CS to CHTP 1.2 or to

Sham exposure, one of the *Lactobacillus* species depleted by CS is increased significantly in both groups. These microbial changes could be important for understanding the effects of CS and of switching to RRPs on gut function and its relevance to disease via the microbiome.

P01-025
Prediction of interethnic differences in acetylcholinesterase inhibition upon chlorpyrifos exposure

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Chlorpyrifos (CPF) is an organophosphate (OP) insecticide. The exposure to CPF has been associated with acetylcholinesterase (AChE) inhibition in human red blood cell (RBC). RBC AChE inhibition has been used as indicator to define points of departure for risk assessment of CPF. The current study aimed at investigating interethnic differences in *in vivo* CPF exposure-related RBC AChE inhibition between the Chinese and Caucasian population. This was done by using physiologically based kinetic (PBK) models defined for both the Chinese or Caucasian population together with a reverse dosimetry approach to quantitatively convert concentration-response curves for RBC AChE inhibition to *in vivo* dose-response curves for these two populations. By doing so, the potential neurological risks for two targeted populations upon CPF exposure could be defined. The predicted *in vivo* dose-response curves for both populations revealed that CPF is 4- to 7-fold less toxic to Chinese than Caucasian as a result of interethnic differences in biotransformation. The average Chinese population appeared to be 4.6-fold slower in CPF bioactivation from CPF to Chlorpyrifos-oxon (CPO), 2.8-times more efficient in detoxcification from CPO to 3,5,6-trichloro-2-pyridinol (TCPy) and 2-times less efficient in detoxification from CPF to TCPy as compared to the average Caucasian popultaion, which could be partly explained by racial variation in the frequency of single-nucleotide polymorphisms (SNPs) for key enzymes involved. Collectively, these results highlight interethnic differences in CPF bioactivation and detoxification that may affect the ultimate risk and indicate that the newly developed PBK models for CPF coupled with reverse dosimetry are capable of predicting *in vivo* toxicokinetic of CPF and capturing possible interethnic differences in bioactivation and detoxification between the Chinese and Caucasian population.

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P01-026
Association between heavy metals in umbilical cord serum and DNA methylation of cord tissues in human

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Prenatal exposures to heavy metals are known to be associated with fetal development and adverse outcomes in later life, in which DNA methylations are currently considered as one of the possible mechanisms [1]. Whereas there might be a sex-specific association between exposure to heavy metals and DNA methylation [2], little is confirmed about the fact and the details.

The purpose of this study was to investigate the relationship between prenatal exposure to heavy metals and DNA methylation in offspring.

In a birth cohort study in Chiba (C-MACH), concentrations of heavy metals, mercury (Hg), manganese (Mn) and selenium (Se) in the Umbilical cord serum (UCS), and DNA methylation status in the Umbilical cord tissue (UCT) (a part of fetus) using a methylation array analysis, were examined and their association was analyzed by Spearman correlation adjusted by a false discovery rate in each sex of offspring.

Total 67 pregnant women who gave birth to 27 males and 40 females were participated in the end . Our previous study suggests that UCT is useful as an alternative surrogate for studying environmental effects on DNA methylation in human fetuses, compensating UC blood cells [3].

Only one locus was correlated to the concentrations of Hg in males and ten[sj5] loci were correlated to the concentrations of Se also in males, while no correlation was observed at any loci in females. There was no correlation between the concentrations of Mn and DNA methylation in either sex. The locus correlated to Hg concentration was on intron of gene body of HDHD1 gene on chromosome X and was a binding site for zinc finger protein CTCF (CCCTC-binding factor).

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P01-027

This abstract has been withdrawn.

P01-028
Chromosome damage in humans: from a group level indicator of genotoxic effects and cancer risk to an individual biomarker

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Cytogenetic biomarkers have for decades been used for assessing the genotoxic effects of human exposure to genotoxic carcinogens. A high frequency of chromosome aberrations in peripheral lymphocytes has been associated with an increased risk of cancer, and a similar relationship has also been found for lymphocyte micronuclei. Cytogenetic biomarkers have mostly been evaluated at the group level. This is reasonable, as cells with chromosomal aberrations or micronuclei are rare and their manual analysis is subjective and usually based on relatively low numbers of cells. A more extensive analysis has been time-consuming and expensive. However, the application of automated techniques is rapidly changing this scheme, as the number of cells scored can substantially be increased, while the time spent with the analyses and their expenses and the subjectivity are reduced. The most promising approach is offered by the reticulocyte micronucleus assay – the human equivalent of the rodent peripheral blood micronucleus test. As micronucleated reticulocytes are rapidly removed from blood circulation by the human spleen, micronuclei do not accumulate in normocytes in long-term exposure as they do in mice. The known time window from micronucleus induction in the bone marrow to the appearance of micronucleated reticulocytes in blood and their eventual disappearance from circulation makes it possible to apply the assay for following-up of genotoxic effects and for intervention studies. Due to the improved accuracy of the analy-

sis, reticulocyte micronuclei may become an individual biomarker of the effects of genotoxic carcinogens and cancer risk.

P01-029
Updating strategies for nonnegative matrix factorization to integrate cross omics layers

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Introduction: The ongoing development of high-throughput technologies has generated large and complex data sets of different omics layers, such as mRNA, methylation and protein expression. It is believed that the integration of these different layers should lead to a more complete understanding of cellular events. However, this integration step is not trivial, due to the different distributions and dimensions in each layer, and therefore an appropriate computational method has to be selected. Previous studies have shown the promising results of detecting clusters and features by applying Nonnegative Matrix Factorization (NMF). Here, we propose a multi-layer NMF with a prior knowledge integration workflow to detect both inter and intra relationships in all layers of omics information.

Method: The original NMF method by Lee and Seung decomposes one layer of information into a feature matrix W and a coefficient matrix H, by applying an update rule for both W and H. First, to take multiple omics layers into account, a new set of update rules has to be defined. Therefore, we introduce an update rule for H based on the omics layers W_i (i equals the number of omics layers). This will result in a clustering coefficient matrix H built from all omics layers and thus can be used to relate the different biological entities.

Second, the optimization problem for NMF is not necessarily convex and multiple local minima can be identified. Here, we hypothesize that initializing H with prior knowledge, a local minimum can be found associated with the features and clusters representing the different phenotypic endpoints or experimental conditions. This prior knowledge can contain information about exposure concentrations, compound information but also sample characteristics or disease development.

Results: The proposed multi-layer semi-unsupervised NMF workflow gives valuable information about sample clustering and features. The workflow has been evaluated with toy data, but also with gene expression and CpG methylation values from the NCI60 tumor cell line database. With the integration of those two platforms by our workflow, it becomes possible to obtain the relationships between CpG and gene data for different biological clusters. Future development of the workflow to handle time series data could allow for dynamic cross omics analysis.

P01-030
Using human biomonitoring for the risk assessment of polycyclic aromatic hydrocarbons in occupational exposures

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Background and Purpose: The Human Biomonitoring Initiative (HBM4EU) is a joint effort of 28 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020. HBM4EU is generating evidence of the current exposure of European citizens to chemicals and the possible health effects in order to assess the associated risks and support policy making towards human health protection. Polycyclic aromatic hydrocarbons (PAH) were considered one of the 1st priority substance groups to be addressed. In the scope of this project, the present work aimed to evaluate the added value of human biomonitoring (HBM) for the PAH risk assessment process, in the case of occupational exposure.

Methods: An extensive literature search was performed to identify scientific papers published between 2008 and 2018 that included air monitoring and HBM data in several occupational settings based in Europe. Among them, those papers presenting urinary 1-hydroxypyrene (1-OHP) quantification – the most common exposure biomarker of pyrene and a surrogate for exposure to PAHs mixtures – were selected. Based on the 1-OHP values the excess lifetime cancer risk (ELCR) for workers, concerning lung cancer, was estimated following the ECHA recent approach (https://echa.europa.eu/fi/applying-for-authorisation/evaluating-applications). ELCR values calculated using air and HBM data were compared.

Results: Based on the criteria described, only 7 out of 28 papers were considered for ELCR estimation. Overall, high ELCR values were estimated (several values higher than 10^{-4}). Moreover, for some studies (3 out of 7) the ELCR estimation using HBM data yielded values higher than those estimated from air monitoring data. This might indicate that, for those specific workplaces, transdermal absorption or even hand-mouth exposure can have an important role in the total exposure to PAH and that the HBM data allows a more accurate PAH exposure assessment. Nevertheless, these findings should be interpreted with caution, since ELCR estimates from air monitoring data are based on Benzo[a]pyrene (BaP) concentrations while HBM-based ELCR determination uses urinary 1-OHP concentration that reflects exposure not only to BaP but to all PAHs, irrespectively of sources or routes of exposure.

This work claims attention for two main aspects, namely: i) the exposure levels are still high in some occupational settings and ii) there is a need for developing new occupational studies, applying a set of exposure biomarkers or a more specific biomarker for BaP exposure, which would allow a better ELCR estimation for exposed workers.

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P01-031
Toxicity assessment caused by the insecticide methamidophos in bullfrog's tadpoles

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In Mexico, currently are sold various plagicides prohibited in other countries, thus it is important to determine its toxicity and how it affects to humans and animals. Methamidosphos is one of the above mentioned pesticides and belongs to a group of organophosphates that are characterized by causing neurological damage and alterations in various defense mechanisms. The purpose of this study was to assess the acute effects (median lethal concentration, LC50) as well as neurotoxic damage and the evaluation of oxidative stress markers in a non-lethal concentration.

It has been described that organophosphorus pesticides inhibit the enzyme's acetylcholinesterase activity, responsible of hydrolyzing acetylcholine, neurotransmitter of varied synapses, mainly in neuromotor plates and are precursors of increasing free radicals: O_2•, HO• and peróxidos:H_2O_2.

The excessive use of methamidophos on the agricultural fields close to acquifers, represents a level of risk to amphibian species, that is why the toxicity is evaluated in bullfrog's tadpoles, an animal with gastronomic importance, it is able to thrive in aquatic and terrestrial environments.

The results were, CL50 of methamidophos during the 48 h of exposure was 1.55 g/L. The methamidophos non-lethal concentration 0.155 g/L was used at 48 hours of exposure so as the acetylcholinesterase (AChE) inhibitory response, total protein levels and the antioxidant response that includes: the enzymes superoxide dismutase activity (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid hydroperoxidation (LPO) during 6, 12, 24 and 48 hours of exposure in bullfrog tadpoles.

It was established a drop in total proteins, in the entire period of exposure; acetilcholinesterase inhibition was demonstrated through the period of exposure. When assessing enzymatic activity, SOD increased significantly during the 48 hours compared to the control; on the other hand, the CAT had the highest peak at 12 h, being below the value control, it subsequently decreased; GPx showed no changes during the exposure, however, it was lower compared to the control; regarding to lipid hydroperoxidation, an increase was observed from 6 h until the end of the exposure time.

Conclusion: At sublethal concentration of 0.155 g/L methamidophos, oxidative stress and neurotoxic damage are generated in bullfrog tadpoles.

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P01-032
Regucalcin expression profiles in formalin-fixed paraffin-embedded (FFPE) samples: histological and molecular assessments for detection of sex steroid illicit administration

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The use of sex steroids in food producing animals is forbidden within the EU. Illicit growth promoters' cocktails are known to determine, together with increased meat production, several perturbations in different tissue biomarkers, which can be exploited to setup novel diagnostic tools [Benedetto *et al*., 2018; Pezzolato *et al*., 2016].

Recently, it has been shown that sex steroids induce a reduction of regucalcin (RGN) expression in calve testis [Cucuzza *et al*., 2017]. This effect can be detected by monitoring the expression of RGN at different levels (mRNA, proteins).

The aim of this work was to compare RGN mRNA levels with protein perturbations detected by immunohistochemistry (IHC) in testis.

FFPE testis samples of calves treated with nandrolone (n=10; 50 mg/head/week, four injections), 17β-estradiol (n=10; 5 mg/head/week, four injections), and a cocktail of the two hormones (n=10; 5+50mg/head/week, four injections) were analyzed by RT-qPCR with specific RGN assay and by IHC with rabbit anti-RGN polyclonal antibody (Sigma).

Gene expression data were analyzed using GenEx software for relative quantification (ΔΔCt). The IHC was evaluated by pixel analysis (NIS-Elements 4.5) for the quantification of the percentage of positive staining area.

Relative quantification (RQ) results demonstrated that androgens induce a 2.85 fold reduction (RQ mean 0.35, range 0.21-0.58, $p<0.05$), estrogens induce a 4.16 fold reduction (RQ mean 0.24, range 0.17–0.34, $p<0.01$) and the association of the two hormones induces a 11.1 fold reduction (RQ mean = 0.09, range 0.05–0.14, $p<0.01$) of RGN mRNA levels compared to untreated animals (RQ mean =1, range 0.74–1.34). The IHC showed a significant reduction ($p<0.0001$) in RGN expression in all treatments (nandrolone, 17β-estradiol and their association) compared to the control group.

The good correlation between RT-qPCR and IHC applied to FFPE testis samples confirms RGN as a useful biomarker to detect illegal administration of sex steroid hormones in veal calves.

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P01-033
Comparison of the tyrosinaemic potential from exposure to HPPD inhibitors of herbicidal & medicinal use

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The class of chemicals known to be inhibitors of 4-hydroxyphenylpyruvate (HPPD) can cause ocular toxicity in rats. The mechanism has been clearly defined and key is a sustained elevation of plasma tyrosine (tyrosinaemia) above a threshold due to blockage of tyrosine catabolism. This communication addresses the human relevance of this mechanism of action (MOA) for dietary exposure to herbicidal HPPD inhibitors.

One HPPD inhibitor (nitisinone) is recommended as the medication of choice for the treatment of Hereditary Tyrosinaemia Type I (HT-1), a life-threatening inborn error of tyrosine catabolism, and is administered from birth. It is a highly potent HPPD inhibitor, designed under continuous administration to block the tyrosine catabolic pathway completely, and induces a moderate tyrosinaemia. Today, the dose level and frequency of administration have also been clearly defined by medical agencies; however clinical trial information on induced tyrosinaemia is available from the early development period.

HPPD inhibitors have also been developed as herbicides in a variety of crops, such as corn, cereals or rice and for one of these compounds (mesotrione) human data on plasma tyrosine concentrations following oral dosing are available. Herbicidal HPPD inhibitors have a lower potency of inhibition of HPPD than nitisinone (based on toxicodynamic and toxicokinetic differences), and produce less marked tyrosinaemia at equivalent dose levels.

A comparison of the tyrosinaemic potential in humans of the two HPPD inhibitors via medicinal use and from dietary exposure to herbicide residues has been made. The medical use of nitisinone induces a moderate tyrosinaemia through continuous daily exposure which, in 5% of patients, can cause reversible ocular toxicity that can be managed by dietary adjustment of tyrosine/phenylalanine. Although some of the low potency agrochemical HPPD inhibitors are also capable of causing ocular toxicity in rats, dietary exposure to residues is negligible and is thus highly unlikely to cause any tyrosinemia and hence ocular toxicity in humans.

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P01-034
Exploring the effect of anticancer drugs doxorubicin and mitoxantrone on cardiac mitochondrial plasticity using a proteomic approach

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The current anticancer therapies have increased the number of cancer survivors, although the inherent cardiac side effects of some drugs are also increasing among survivors [1,2]. The cardiotoxicity of doxorubicin (DOX) and mitoxantrone (MTX) may be linked to the cardiac aging process, although the molecular modulation is not understood so far [3]. So, our goal was to study the effects of DOX and MTX in the molecular mechanisms harbored in the heart of adult male CD-1 mice (3 months) and compare them with old CD-1 mice (18 months). All animals were injected with 6 intraperitoneal administrations twice a week for three weeks: control mice received saline solution and DOX- and MTX-treated mice received a total cumulative dose of 9 mg/kg and 6 mg/kg, respectively. During the entire experimental period, animal welfare was assessed daily, and mice were euthanized one week after the last injection. The experiments were performed with the approval of the Portuguese National Authority for Animal Health (General Directory of Veterinary Medicine) (reference number 0421/000/000/2016). After excising, aliquots of whole cardiac tissue homogenate and enriched mitochondrial fractions were prepared and analyzed by immunoblot and enzymatic assays. Enriched mitochondrial fractions were characterized by mass spectrometry-based proteomics (GeLC-MS/MS). Data highlighted a decrease on mitochondrial density for both DOX- and MTX-treated and aged animals, as assessed by citrate synthase activity. Additionally, DOX treatment led to an increase in the ETFDH-to-ATP synthase ratio. GeLC-MS/MS analysis of enriched mitochondrial fractions resulted in the identification of 693 different proteins, assigned to the biological processes "small molecule metabolic process", "oxidation-reduction process" and "carboxylic acid metabolic process", according to String v10.5 [4]. From the PLS statistical analysis, no proteome signature could be associated to each group, although the drugs induced down-regulation of branched-chain amino acid metabolism and fatty acid beta-oxidation with no clear connection with the cardiac aging process. Taken together, our data points to a modulation of mitochondrial plasticity induced by the anticancer drugs DOX and MTX. Indeed, the decrease on mitochondrial density may be associated to mitochondrial adaptations, such as metabolic shift to fatty acid beta-oxidation. Thus, more than alterations noticed in isolated cardiac mitochondria, these drugs seem to modulate mitochondria biogenesis.

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P01-035
Perfluorinated compounds in women of reproductive age exposed to contaminated drinking water in the Veneto Region, Italy

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Per- and polyfluoroalkyl substances (PFASs) have been widely produced and used for many years as water repellants and protective coatings in industrial and domestic products and due to their use and persistence to degradation they are widespread around the globe. Humans are generally exposed to low levels of these chemicals principally through diet, but consumption of drinking water can be an important source of exposure in communities living in areas where PFASs have contaminated water supplies. A well-known example is represented by the water contamination that occurred in Ohio and West Virginia and was investigated starting from 2006 by the C8 Project. Such project included the biomonitoring of PFAS in 69,030 subjects from six contaminated water districts.

A major episode of PFAS water contamination occurred in Veneto, a region in the North-Est of Italy. The contamination, identified in 2013, was originated from a chemical plant that has been producing PFASs in the area for decades. Contamination had affected also drinking water where the presence of several PFASs had been detected. On this evidence, a human biomonitoring study was carried out.

The study included a group of women of reproductive age, a population group which raised major concerns for the local sanitary authorities and the population because of the possible PFAS effects on maternal health and on foetal growth and development. PFAS concentrations were measured in a group of 121 exposed women (E) of reproductive age (20–40 years old), and in a group of 80 women (NE) from the same region living in areas not exposed to contaminated drinking water. Serum samples were analyzed for PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFBS, PFHxS, and PFOS. About 250 µL of serum were spiked with labelled internal standards. Extraction was performed with acetonitrile, reduced and transferred to an autosampler vial to undergo instrumental analysis. Instrumental analysis was carried out by HPLC interfaced with a triple quadrupole mass spectrometer operated in the electrospray negative mode.

Mann-Whitney and Spearman tests were used to assess differences between groups in the concentrations of serum contaminants and correlations with determinants of exposure. The characteristics of study participants (age, body mass index (BMI), residence area) together with information on lifestyle in relation to water use and consumption were considered in the evaluation of results.

Serum concentrations of most of the analyzed contaminants were significantly higher in the E group, years of residence in the municipalities and BMI appear to be the most important determinants of exposure.

PFOA levels assessed in the contaminated areas resulted to be in the concentration range found to be associated with pregnancy induced hypertension in the C8 study, and much higher than the range of levels associated with adverse effects on birth weight and development in published studies.

P01-036
Development of a dietary-PTU model of gradual thyroid disruption (hypothyroidism) in the mouse

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Recommendations for the evaluation of thyroid disruption are very scarce and limited. It can include summary evaluation of histological structure of the gland and/or thyroid hormone plasma concentrations . We aim at determining which one of histological modification or hormonal concentrations (TH and/or TSH) is the more sensitive biomarker of thyroid disruption.

Our approach was to develop a model of gradual thyroid disruption (hypo) in mouse to analyze the relationship between circulating TH /TSH concentrations and quantitative parameters characterizing the thyroid architecture. The goal of the current study as a first step was to identify treatment conditions associated with major modification of both hormone levels and thyroid structure and low dose inducing more moderate modification to determine the range of PTU doses that could be used to produce a full scale dose-response relationship.

Materials and methods: Swiss adult male mice were allocated to 3 groups (n = 4 each), a control fed with a standard iodine concentration diet (0.5 ppm) and two groups PTU-treated animals fed with iodine deficient diet (0.03-0.05 ppm), supplemented by PTU (10 and 1000 ppm). Animals were observed daily, blood was collected on days 0, 14 and at the end of a 28-day treatment. The thyroid were sampled at the end of the treatment. Thyroid histological structure and morphometric measurements (thyroid follicular density, colloid area, epithelial surface using NIH's ImageJ software) were analyzed on hematoxylin-eosin and PAS stained sections respectively. The mean Activation Index (AI), expressed by the epithelial volume/colloid volume ratio was calculated for each group.

Results: Major functional, macroscopic (enlargement) and histological changes were observed in PTU treated groups without clear clinical signs of hypothyroidism or changes in bodyweights. In both treated groups, thyroid parenchyma was modified with diffuse and/or focal follicular hyperplasia associated with epithelial hypertrophy of moderate (10 ppm) to severe intensity (1000 ppm). At the low dose, at day14, TT4 was decreased by 23% and fell below the assay limit of quantification (5 ng/ml) by the end of the treatment. In the high PTU dose, TT4 was already much lower than in the low dose at day 14 and below assay detection limits for most of the animals. Mean AI of the 10 ppm group was three-fold higher than in control. In the highest PTU dose group, the thyroid histological organization was so modified that is was not possible to determine an AI. From this results we identified a range of PTU doses from 1 to 100 ppm in iodine-deficient mice as a way to obtain different degrees of hypothyroidism to model the relationship between the two main types of parameters used to characterize thyroid disruption.

P01-037
Activation of keratinocytes in response to multi-exposure of a cosmetic sensitizer in a reconstructed epidermis

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Keratinocytes (KCs) are the main component of the epidermis, an epithelium in continuous self-renewal. The four distinct layers are characterized by the differentiation status of keratinocytes: the undifferentiated basal layer, the stratum spinosum, the stratum granulosum differentiated additional and the stratum corneum with dead corneocytes. During their maturation process, KCs move from the basal to the upper layer and orchestrate immune responses if microbes and molecules enter the stratum corneum due to mechanical or pathological skin barrier defects.

In certain diseases such as allergic contact dermatitis (ACD), KCs play a key role since they are the first cells to encounter the contact sensitizer (CS) in the skin. KCs contain enzymes that have metabolic activity to transform prohaptenes into biologically active haptenes, facilitating protein binding to form the antigenic complex. In addition, by expressing chemotactic factors and inflammatory cytokines when exposed to CS, KCs could initiate the immune response.

In this study, we investigate how repeated exposure to CS influences the process of epidermal differentiation. To answer this question, a 3D skin model composed of KCs (NIKS cell line) grown on a matrix of collagen and primary human fibroblasts was used. All along the differentiation time, the skin model was exposed to low concentrations (0.1 mM & 0.25 mM) of cinnamaldehyde (CinA), a well-known electrophilic compound. At the end of the differentiation, the 3D skin model was analyzed by immunohistochemistry, western blot and RT-qPCR. A biochip was also carried out to highlight new canonical pathways in order to propose new genes of interest.

Our results show that repeated exposure to CinA induces a slight increase in skin thickness and a lower percentage of apoptotic cells. An induction of filaggrin expression is measured in response to a chronic exposure to CinA. In addition, the transcription factor Nrf2 is activated and antioxidant genes such as *ho-1* and *nqo-1* are also

induced. Preliminary results from the microarray show a high degree of segregation between groups and the analysis is currently under study.

This work shows that a low concentration of CS can modify the epidermis and seems relevant for cosmetic products often used with low doses of sensitizing molecules.

P01-038
Roles of Nrf2 protein in environmental chemicals' toxicity: Toxicogenomics data mining

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Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a protein encoded by NFE2L2 gene. It has a role in antioxidant proteins expression regulation, especially those that protect against the oxidative damage induced by injury and inflammation. Nrf2 might have an important role in oxidative stress and toxicity defense, while its activation is being used as biomarker of chemical damage. The aim of our study was to explore the influence of environmental chemicals on NFE2L2 gene using the toxicogenomics approach. Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org) was the main data mining tool for our analysis. Set Analyzer CTD tool listed 783 chemicals that interact with NFE2L2. Top environmental chemical influences for NFE2L2 gene were: sulforafan, sodium arsenite, tetrachlorodibenzodioxin, tobacco smoke, 2-tert-butylhydroquinone, resvertarol, paraquat, quercetin and cadmium chloride. SetAnalyser CTD tool listed 2,321 diseases connected with NFE2L2 gene. For the top 10 curated diseases (fatty liver, hepatomegaly, acute kidney injury, hyperglycemia, liver neoplasms, hepatocellular carcinoma, skin neoplasms, pulmonary fibrosis, gastrointestinal diseases and non-alcoholic fatty liver disease) NFE2L2 gene played a role in the ethyology and might be used as a biomarker. This can be connected with exposure to some chemicals. For example, paraquat, herbicide which causes severe pulmonary fibrosis, decreases the activity of NFE2L2 protein and expression of NFE2L2 mRNA. Tobacco smoke decreases the expression of NFE2L2 protein as well. However, it is important to consider the role of NFE2L2 gene as a therapeutic target in the treatment of some diseases. This gene has been listed in the CTD as a possible therapeutic target for cardiovascular diseases (heart failure and vascular system injuries). Sulforaphane and resvertarol, which increase activity of NFE2L2 protein and expression of NFE2L2 mRNA, might be used as a prevention of cardiovascular diseases. These substances also inhibit the reaction of other chemicals that decrease the expression of NFE2L2 protein. These results provide a basis for further *in vitro* and *in vivo* investigation of the molecular mechanisms behind Nrf2 role in environmental chemical's toxicity (project 46009III).

P01-039
BMD analysis of *in vitro* and *in vivo* whole transcriptome TempO-Seq dose response data

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Gene expression (Gex) dose response measurements with Benchmark Dose (BMD) statistical analysis provide a sensitive and data-rich basis for chemical risk assessments and determination of human health guidance values. Unfortunately, this approach has been hampered by high costs and complexity of RNA-seq technologies and the low reproducibility of such assays. A targeted expression profiling assay with efficient NGS-based readout, TempO-Seq®, facilitates BMD analysis by drastically reducing cost per sample, simplifying high-throughput workflows, and providing the reproducibility required for quality dose response data. The assay proceeds directly from cell lysates without RNA extraction or reverse transcription; consistency of results is high without input normalization; and data analysis is fully automated (requiring no bioinformatics expertise). Furthermore, the assay allows for proportional attenuation of highly expressed targets, and its high tolerance to RNA damage and degradation allows Gex measurement of fixed archival tissue samples. We used TempO-Seq to compare dose response results from rat cell lines treated with fenofibrate *in vitro* vs. FFPE tissue from rats treated *in vivo*. Data is fine-grained enough to allow step-by-step analysis of cellular responses, with the expression activity associated with the PPARa agonist mode of action of fenofibrate increasing with dose and time. Both *in vivo* and *in vitro* BMD analysis shows dose and time-dependent activation of the expected processes (lipoprotein lipase activity, fatty-acid beta oxidation, triglyceride biosynthesis). Cellular amide metabolic processes, proposed as a mechanism of action for fibrate drugs, are detected as the second most sensitive pathway to fenofibrate treatment. We extended this dataset with analyses of cells and rats treated with β-estradiol, N-(2-Fluorenyl) acetamide, phenobarbital, 5,6-benzoflavone, and amiodarone, showing that cell line treatment provides a reasonable model for *in vivo* effects. BMD analysis of TempO-Seq dose response data permits the ranking of pathways that describe the mode of action of drugs, the comparison of *in vivo* to *in vitro* validates the utility of *in vitro* assays, and this approach will ultimately permit cross species analysis (e.g. animal *in vivo* to human *in vitro*).

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P01-040
Development, testing, parameterisation and calibration of a human PBPK model for the plasticiser, Hexamoll® DINCH using *in silico*, in-vitro and human bio-monitoring data

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A physiologically based pharmacokinetic (PBPK) model for Hexamoll® DINCH (diisononyl-cyclohexane-1, 2-dicarboxylate) was developed to interpret the biokinetics in humans after single oral doses. The model was parameterised with *in vitro* and *in silico* derived parameters and uncertainty and sensitivity analysis was used during the model development process to assess structure, biological plausibil-

ity and behaviour prior to simulation and analysis of human biological monitoring (HBM) data. The model provided good simulations of the urinary excretion (Curine) of two metabolites; cyclohexane-1, 2-dicarboxylic acid mono hydroxyisononyl ester (OH-MINCH) and cyclohexane-1, 2-dicarboxylic acid mono carboxyisononyl ester (cx-MINCH) from the biotransformation of mono-isononyl-cyclohexane-1, 2-dicarboxylate (MINCH), the monoester metabolite of DINCH. However, good simulations could be obtained, with and without, a lymphatic compartment. Selection of an appropriate model structure was informed by sensitivity analysis which could identify and quantify the contribution to variability in Curine by parameters, such as, the fraction of oral dose (FracDose) that directly entered the lymphatic compartment via the lacteals in the gut and therefore by-passed the liver and the fraction of MINCH bio-transformed to cx-MINCH and OH-MINCH (FracMetabcx, FracMetabOH). By constraining FracDose, FracMetabcx and FracMetabOH within biologically plausible limits the presence of a lymphatic compartment was deemed an important model structure. Furthermore, the use of sensitivity analysis is important in the evaluation of uncertainty around *in silico* derived parameters. By quantifying their impact on model output sufficient confidence in the use of a model should be afforded. This type of approach could expand the use of PBPK models since parameterisation with *in silico* techniques allows for rapid model development. This in turn could assist in reducing the use of animals in toxicological evaluations by enhancing the utility of "read across" techniques.

P01-041
Identification of urinary metabolites of diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A plus) using microsomes and electrochemistry – application in exposure assessment study following dermal application

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Uvinul A Plus (DHHB, Diethylamino Hydroxybenzoyl Hexyl Benzoate) is used in personal care products as an effective UV filter. It is considered to be safe and penetrate poorly through human skin. Human metabolic pathways of DHHB are not described yet, thus no validated biomarker is available for the assessment of internal dose following oral, dermal or combined exposure. In this work we applied electrochemical reactor and human liver microsomes (HLM) to simulate and study first phase metabolism and select potential candidate which may serve later as urinary biomarker of exposure. Finally, study on urinary elimination of metabolites following controlled dermal exposure was performed on 6 volunteers. Application amount of commercial personal care product containing 3% of Uvinul A Plus was 1 mg/cm^2. The approximate applied dose of DHHB was 30 µg/cm^2.

Several oxidative metabolites corresponding to hydroxylated products and mono- and di-N-dealkylated products were generated by both electrochemical reactor and HLM. Additionally, product of hydrolysis 2-(4-(diethylamino)-2-hydroxybenzoyl)benzoic acid (DHBA) was detected in HLM incubations but as expected, it was not observed in electrochemistry (EC) experiments. The range of tentatively identified metabolites generated by HLM and EC allowed to develop targeted LC-MS/MS method for their determination in human urine. DHBA was quantified in the samples using authentic standard.

Among human DHHB metabolites 2-(4-(diethylamino)-2-hydroxybenzoyl)benzoic acid (DHBA), was eliminated in urine in the highest amount and was not present in the pre-exposure samples. Thus DHBA may be considered as a potential urinary biomarker of exposure to DHHB. The study provides first experimental data on DHHB human skin penetration and suggest human biotransformation pathways. It also presents methodology employing electrochemistry for better characterization of possible biotransformation products.

P01-042

This abstract has been withdrawn.

P01-043
Prenatal exposure to parabens and triclosan and assessment of possible health impacts

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Background: Parabens (PBs) and triclosan (TCS) are antimicrobial agents widely used in personal care products such as deodorants, shampoos and shower gels, mouth pastes and washes, cosmetics, etc., making exposure to them inevitable. Problems in reproductive and respiratory system, thyroid gland's dysfunction and cancer are the most frequently reported health problems.

Purpose: The aim of this study was to assess the prenatal exposure to PBs and TCS and the potent health impacts to both mothers and infants.

Methods: 100 pregnant women aged 35.2 ± 5.8 years old participated in the research. Urine samples were collected during 1st or 2nd trimester of pregnancy. Liquid – liquid extraction with ethyl acetate and analysis using a liquid chromatography – mass spectrometry system was performed. Questionnaires regarding maternal and infants' somatometric characteristics and lifestyle habits were also completed.

Results: Statistical analysis of questionnaires data showed that 30.2% of the participating women suffered from thyroid gland's problems, followed by gynaecological problems (29.2%), allergies (27.4%) and respiratory and other problems (6.6%). Concerning the current pregnancy, 18.8% of the women reported health problems and 17.6% suffered early pregnancy. Somatometric characteristics of the infants did not show significant differences between the two sexes. Analysis of urine samples showed that 64.0%, 8.0%, 13.0% and 81.0% of them were positive for MePB, EtPB, BuPB and TCS, respectively. Mean levels of positive (>LOD) samples were 378.5 ng/ml for MePB, 23.2 ng/ml for EtPB, 34.1 ng/ml for BuPB and 50.6 ng/ml for TCS. Health problems during pregnancy were not significantly correlated with measured analytes. Infant's somatometrics were also not correlated with urine levels of MePB, EtPB, BuPB and TCS.

Conclusion: TCS presented the highest positivity rate, while MePB the highest mean concentration level. Concerning the other analytes, positivity rates followed this order MePB > BuPB > EtPB. It is remarkable that MePB mean concentration level was one order of magnitude higher in comparison with the mean levels of the rest analytes.

P01-044
Assessment of drinking water chlorination by-products in view of multiroute exposure

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Most chemical disinfection methods are accompanied by the formation of a huge number of disinfection byproducts (DBPs) in treated drinking water (DW) through reaction of the chemical disinfectant with naturally occurring inorganic and organic matter in the source water. In some cases number of them exceed 500. A number of DBPs cancerogenic or cause target-organ toxicity (including repro- and developmental toxicity). As a result population are chronically low-level exposed to a very large number of DBPs. At the dose levels tested in laboratory experiments, a number of these DBPs were either carcinogenic or caused target-organ toxicity, including reproductive/developmental toxicity. These dose levels are high compared with the low levels found in water. In our previous research the algorithm for evaluation integrated toxicity of complex mixtures of DBPs in DW [http://rspch.by/Docs/instr-015-1118] was developed and tested. It recommended for use on the stage of substantiation of the choice of DW disinfection method which will pose the lowest risks to public health.

For routine surveillance of DW classic approach are used – confirmation of compliance with national standards on DBPs. In Belarus 6 DBPs of chlorination have hygienic standards and routinely monitored. At the same time last scientific data and estimates have allowed to strengthen the regulation of volatile chlorination by-products (THM) in developed countries: for chloroform 0.06 mg/l with the total content of the priority 4 THM 0.1 mg/l. In Belarus the guideline value for chloroform is 0.2 mg/l (3.3 times less stringent). It based only on per oral intake of DW, while THM are volatile and express hazard also through inhalation and percutaneous exposure while bathing, showering and housework. For revision of guideline values for THM in DW the research is needed.

The purpose of this ongoing research is to conduct complex health risk assessment of DBPs mixtures in DW considering multiple routes of exposure.

In research we use 3 methods: 1) assessment of complex exposure with priority THMs based on results of laboratory assessment of THMs levels in DW and accounting of the specific contribution of 3 routes of exposure with all household DW use; 2) assessment of internal doses of THM due to the complex intake of THM from DW at home on the basis biomonitoring data of THM levels in blood and urine (biomarkers of the exposure) of the population living in areas exposed to the harmful factor (Minsk); 3) the study of the genetic polymorphism effect of enzymes involved in the metabolism of THM (CYP450, GSTT), determination of sensitive groups of the population to the exposure to THM (by genetic sensitivity biomarkers).

Provisional results show the added value of biomonitoring data for complex risk assessment, they characterize of internal exposure with THM. The results of research will be used for revision of guideline values for THM in DW.

P01-045
Risk assessment of traditional alcoholic beverages

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Purpose: Traditional Greek spirit beverages, tsipouro and tsikoudia, are very popular, consumed both bottled and in buck quantities, while many locals especially in agricultural areas are distilling their own fermented grape pomaces for private consumption. We analyzed distillates for the identification of chemical compounds produced during the primary metabolism of the fermentation process and a risk assessment approach was implemented in order to evaluate the magnitude of human risk.

Materials and methods: Totally, 56 drinks from the Greek market were collected, stored at -20ºC and analyzed using either clinical chemistry analyzer or by gas chromatography coupled with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry detector (GC-MS).

Results and discussion: The concentration of cancinogenic compounds (ethanol, acetaldehyde), higher alcohols (isobutanol, isoamyl alcohol), esters (ethyl acetate) and methanol were measured in order to estimate the potential cancer risk and the dietary intake of the other compounds. European Food Safety Authority (EFSA) margin of exposure (MOE) was used for cancer risk characterization, while the no-observed-adverse-effect-level (NOAEL), the oral reference dose (RfD) and data from the Integrated Risk Information System (IRIS) were used in order to make estimates of the health risk assessment of the other compounds, in terms of the Health Risk Index (HRI).

Conclusion: The margin of exposure approach (MOE) for carcinogenic compounds, such as ethanol and acetaldehyde, was found to be less than 500 (mean value) well below to 10,000 as suggested by EFSA for public concern. Contradictory, the risk assessment of non carcinogenic compounds, such as alcohols, aldehydes and esters, identified a specific compound, the isobutanol, with health risk index (HRI) greater than 1, making those spirits possible of inducing health side effects (nausea, dizziness, headache and stupor) in case of huge consumption.

P01-046
Biodistribution of the new psychoactive stimulant 3,4-dimethylmethcathinone (3,4-DMMC) in Wistar rats assessed by gas chromatography-mass spectrometry (GC-MS)

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3,4-Dimethylmethcathinone (3,4-DMMC) is a new psychoactive stimulant belonging to the first group of synthetic cathinones detected via the EU Early Warning System in 2010. As the pharmacokinetics of this drug is still unknown, the aim of this study was to validate a GC-MS methodology for the quantification of 3,4-DMMC in biological matrices and further apply it to the *in vivo* study of the drug biodistribution profile in Wistar rats.

Adult female Wistar rats weighing 250–300 g were administered 20 or 40 mg/Kg 3,4-DMMC i.p. After 1h or 24h, rats were anaesthetized and euthanized for collection of blood, brain, liver, heart, kidney, muscle, adipose tissue, lung, spleen and gut. Blood samples were cen-

trifuged at 1,600xg for 15 min at 4°C, and plasma was separated and precipitated with 5% HClO4. Organs were homogenized (1:4 w/v) in ice cold 100 mM phosphate buffer (pH 7.4) and centrifuged at 3,000xg for 10 min at 4°C. All supernatants and plasma were subjected to a solid phase extraction, and the obtained residue was derivatized with trifluoroacetic anhydride prior to GC-MS analysis. The method was fully validated in plasma using methylone as internal standard. The validation of the method consisted on the evaluation of the limit of detection and limit of quantification (4 ng/mL and 13.5 ng/mL, respectively), linearity (with correlation coefficients above 0.9937 and within the concentration range 78–2500 ng/mL), selectivity, inter-day and intra-day precision (CV% always lower than 15%), accuracy (always between 80–120%) and recovery (78–98%). The inter-day and intra-day precision, accuracy and recovery were evaluated at 3 distinct concentrations (78, 625 and 2500 ng/mL). All these parameters met with the international acceptance criteria for bioanalytical methods, indicating good linearity, recovery, precision and accuracy of the method, with no interferences. The analysis of biological samples showed that after 1 h the drug distributed to all the analysed organs in a dose-dependent manner, achieving higher concentrations in spleen, lung, kidney and brain; but was not detected after 24 h. 3,4-DMMC has a rapid and extensive distribution as noted with amphetamines. To our knowledge, this is the first *in vivo* biodistribution study of 3,4-DMMC.

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P01-047
Effect of polystyrene nanoplastics on the polychaete *Hediste diversicolor*: a multibiomarker approach

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Plastics became emergent pollutants all over the globe. Their increased production and persistence in the environment raise concerns about their impact on marine life. Polystyrene (PS) is one of the most produced plastic polymers, used in a large number of single-use/low reuse products and is among the most frequently reported in the aquatic environment. Once in the marine environment, these plastics, like many others, will slowly break down into increasingly smaller particles, becoming more available for biota and threatening organisms both in the water column and sediments, as they tend to gradually sink to the ocean floor.

Polychaetes usually are the most abundant group in marine ecosystems and support much of the diversity at higher trophic levels. As benthic organisms, they are not only exposed to waterborne contaminants but also to contaminants present in the sediments. Thus, this study aims to assess the effects of PS on biochemical endpoints associated with oxidative status and energy metabolism, behaviour and regenerative capacity.

Hediste diversicolor specimens were collected in a reference site in Ria de Aveiro lagoon (Portugal), and after acclimatization they were exposed, for 28 days, to five different concentrations of 100 nm PS particles (0.0; 0.005; 0.05; 0.5; 5.0; 50.0 mg/L).

The results showed that burrowing activity of the organisms exposed to 0.005; 0.05 and 0.5 mg/L was significantly affected, with organisms taking more time to bury. The regeneration capacity, typical of these organisms, was not significantly different among tested concentrations, but a slight decrease was observed in exposed organisms.

PS demonstrated the ability to affect biochemical endpoints of the tested polychaetes. Overall, the antioxidant enzymes glutathione peroxidase (0.005 and 0.05 mg/L) and catalase (0.05 to 50 mg/L PS and the enzymes of phase II of biotransformation glutathione-S-transferases (0.05 to 50 mg/L PS were sensitive to PS exposure, displaying decreased activities. In contrast, an increase of electron transport was observed in organisms exposed at 0.05 to 50 mg/L of PS. Protein oxidation was reported in organisms exposed at 0.05 to 50 mg/L of PS.

Overall, the results highlight that PS induces alterations in the studied polychaetes, which may present potential impacts at the population level.

P01-048
Biomarkers of exposure to estrogen-derived reactive metabolites: mass spectrometry-based methodologies to identify protein covalent adducts

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Estrone (E1) undergoes a CYP450-catalysed hydroxylation at position C-16, yielding 16-α-hydroxyestrone (16-α-OHE1) that is a reactive metabolite with the ability of covalently modifying the lysine residues of proteins, involving the formation of a Schiff base. This intermediate can be subsequently stabilized by two distinct mechanisms: via reductive stabilization, yielding the α-hydroxyamine adduct or via Heyns rearrangement, yielding a stable ketoamine adduct.

Upregulated levels of 16-α-OHE1 were identified in autoimmune [1] and in pulmonary hypertension patients [2] and the formation of 16-α-OHE1-derived protein covalent adducts is thought to have a role in the onset of some of these pathologies [3]. Therefore, the development of analytical methodologies capable of unequivocally identifying and quantifying these adducts is a relevant pursuit.

We report herein the development of highresolution mass spectrometry-based methodologies for the identification of 16-α-OHE1 covalent adducts formed with the blood proteins hemoglobin and human serum albumin. The methodologies developed will be crucial towards the evaluation 16-α-OHE1-derived protein adducts as biomarkers of exposure to estrogen-derived reactive metabolites and as diagnosis tools of diseases more prevalent in women.

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P01-049

DNA methylation patterns associated with seric metals concentration. Accessing effects of pollutants on human epigenetic modifications.

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Anthropogenic activities increase the exposure to metals and the major sources are drinking water and contaminated food. Despite knowledge about toxic potential of these compounds as well as its implication in non-transmissible chronic diseases, it is not yet established how it contributes to the aetiology and progression of these diseases. Large-scale genomic studies allow thousands of regions to be evaluated simultaneously and can provide a global approach for clinical studies. This study aims to analyze the effect of metals seric concentration on DNA methylation for further inferences regarding health problems related to environmental exposures. This is especially important because recently some environmental disasters occurred in Brazil, increasing exposure to toxic metals. DNA were extracted from women (n = 42) and used to 450k beadchip methylation analysis, results are represented in beta values format which varies from 0 to 1. Serum was used to metals determination using ICP-MS, 15 metals were evaluated (Mg, Cu, Zn, Mo, Li, Rb, Sr, Se, Mn, Ni, Co, Cd, As, Al, Hg). Bioinformatics analysis were based on the Champ package pipeline. Singular Value Decomposition Analysis (SVD) was used to find the correlation of principal components and biological factors, bumphunter algorithm to find the metals-related Differentially Methylated Regions (DMRs), and linear regression models to find Differentially Methylated Positions (DMPs). Results: SVD analysis revealed that Hg, Al, Mo, As, Cd, Mn, Ni and Co contribute significantly on global DNA methylation. The number of significant DMRs for each metal were: Mo:21, Mn:17, Ni:18, Co:82, Cd:25, Al:56, Hg:32, and DMPs were Mo: 234, Mn:377, Ni: 271, Co:2, Cd, 1095, Al:245, Hg:567 ($p<0.05$). Conclusion: Seric metals concentration was related to DNA methylation beta values, and this may lead to pathway disruption due promoting changes in gene expression. This is an important topic in the field of chronic and non-communicable diseases which are the most important causes of death worldwide. This kind of study is important to support decisions regarding safety and legislations about metals usage and environment.

P01-050

Biomonitoring of phthalate metabolites in urine from pregnant women in Crete, Greece

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Introduction: Exposure of pregnant women to phthalates was evaluated from the biomonitoring of six phthalate metabolites (MEHP, MEHHP, MEOHP, MiBP, MnBP, MBzP) in maternal urine.

Methods: A total of 100 urine samples were collected. Metabolites were deconjugated with enzymatic hydrolysis and extracted with 2 ml ethyl acetate for 20 min for three times. A solid phase extraction (SPE) procedure was applied to the organic extracts as a further cleanup process. The final extract was evaporated to dryness and reconstituted in methanol prior to instrumental analysis with LC-MS.

Results & Discussion: Positive samples were from 27% (MEHP) to 54% (MiBP) at median concentrations 17.9, 4.9, 41.5, 28.1, 46.7 and 6.1 ng/ml for MEHHP, MEOHP, MiBP, MnBP, MBzP and MEHP, respectively. MEHHP and MEOHP which are the oxidative metabolites of DEHP were significantly correlated with each other ($r_s = 0.92$, $p < 0.001$) and also MnBP with MEOHP ($r_s = 0.41$, $p = 0.02$) and MiBP ($r_s = 0.95$, $p < 0.001$) and MBzP with MiBP ($r_s = 0.70$, $p < 0.001$) and MnBP ($r_s = 0.63$, $p = 0.002$), indicating their common sources of exposure. MEHP was significantly associated with frequent use of plastics for food storage ($p = 0.026$). No significant associations came up for head circumference, birth weight and length, allergies, respiratory problems or other abnormalities.

Conclusion: Phthalate metabolites were detected in urine from pregnant women indicating their acute exposure to the pollutants during pregnancy. Use of plastics for food storage was proved to be a source of exposure to the compounds. It was found that acute maternal exposure had no effects for the infant development and health.

P01-051

Selective citation in scientific literature on the human health effects of bisphenol A

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Introduction: Bisphenol A is highly debated and studied in relation to a variety of health outcomes. This large variation in the literature makes BPA a topic that is prone to selective use of literature, in order to underpin one's own findings and opinion. Over time selective use of literature, by means of citations can lead to skewed knowledge development and a biased scientific consensus. In this study we assess which factors drive citation and whether this results in the overrepresentation of harmful health effects of BPA.

Methods: A citation network analysis was performed to test various determinants of citation. A systematic search identified all relevant publications on human health effect of BPA. Data were extracted on potential determinants of selective citation, such as study outcome, study design, sample size, journal impact factor, authority of the author, self-citation and funding source. We applied random effect logistic regression to assess whether these determinants influence the likelihood of citation.

Results: 169 Publications on BPA were identified, with 12,432 potential citation pathways of which 808 citations occurred. Positive studies have a 1.5 times greater chance of being cited compared to negative studies. Additionally, authority of the author and self-citation are consistently found to be positively associated with the likelihood of being cited. Overall, the network seems to be highly influenced by two highly cited publications, whereas 60 out of 169 publications received no citations.

Conclusion: In the literature on BPA, citation is mostly driven by positive study outcome and author-related factors, such as high authority within the network. Interpreting the impact of these factors and the big influence of a few highly cited publications, it can be questioned to which extent the knowledge development in human literature on BPA is actually evidence-based.

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P01-052

This abstract has been withdrawn.

P02 – Clinical toxicology

P02-001
Target safety assessments: evaluation of the toxicological risk of targeting FRS (Phenylalanyl-tRNA Synthetase) in the treatment of Malaria

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Phenylalanyl-tRNA synthetase (FRS) is a highly conserved enzyme that catalyzes the ligation of phenylalanine to its cognate transfer tRNA during protein synthesis. Due to its vital role as part of the translational machinery, FRS has been identified as a potential target to treat the malaria parasite. However, since target-related toxicity accounts for > 50% of all drug project failures, it is vital to understand the potential unintended consequences of target modulation in non-plasmodium (mammalian) species to assist in the determination of the required plasmodium/human safety ratio. We conducted a comprehensive *in silico* target safety review to understand the role of FRS in normal physiology as a basis for evaluation of the potential toxicity of FRS inhibitors. Based on published literature, it is clear that eukaryotic cells harbour two different types of FRS: the heterotetrameric cytosolic alpha (FRSA) and beta forms, and the monomeric mitochon-

drial forms. Pathogenic variants in FRS2 (encoding the human mitochondrial FRS) have been associated with phenotypes ranging from spastic paraplegia to fatal infantile Alpers encephalopathy. FRSA knockout mice are homozygous lethal. Heterozygote phenotypes include abnormal bone morphology, decreased bone mineral density, decreased circulating chloride and sodium levels, impaired glucose tolerance and increased total body fat amount. Based on these observations, we predict that potential target organs of toxicity caused by inhibition of FRS could include bone, immune system, kidney, liver, muscle, and the nervous system. Specifically, there may be a risk of abnormal bone development, perturbed glucose metabolism, immunosuppression, nephrotoxicity, reduced liver function, myopathy and an increased risk of epilepsy. Based on this toxicological profile, inhibition of host FRS could be a serious limitation; therefore, the specificity and selectivity of compounds will be a key for their success. However, a single genomic copy of mitochondrial FRS is targeted to the parasite mitochondria and is exclusive to malaria parasites within the apicomplexan phyla, hence drug targeting of FRS presents a unique opportunity to potentially target malarial FRS specifically. Nonetheless, it would be sensible to conduct an early rodent investigative study looking at in life effects and potential target organs to help identify whether the risks our *in silico* analysis has identified actually occur *in vivo* with inhibitors of FRS.

P02-002
Blood level measurement of urea and creatinine in methamphetamine abuser patients

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Purpose: The methamphetamine ($C_{10}H_{15}N$), known as the "*Crystal*", is a psychoactive substance and a stimulant for the nerves. Due to its psychomotor stimulating features such as increased alertness, euphoria and mood elevation, the drug can be greatly abused. The chronic methamphetamine abuse may cause damage in some organs including brain, heart, and liver of human. The study was aimed to measure the blood levels of urea and creatinine as kidney function indices in methamphetamine abuser and to predict urea dependent drug toxicity.

Methods: The specimens included 52 serums of people who had more than one year of history of drug addiction and consumed between 1-2 grams of crystal per day. They were tested for serum urea and creatinine levels. Experiments were performed in fasting mode using a quantitative diagnostic kit. Normal values for this method for urea and creatinine were 20-43 and 0.8-1.4 mg/dL, respectively.

Results: The mean age of patients was 29.61 ± 5.39 years. The mean values obtained from serum urea were found to be 17.98 ± 3.49 mg/dL. The highest and lowest values of urea were 23 and 10 mg/dL, respectively. The mean serum creatinine level was 0.90 ± 0.13 mg/dL and the highest and lowest of that were obtained as 1.10 and 0.60 mg/dL, respectively.

The study concludes; the creatinine levels in methamphetamine abusers are not significantly differed in comparison with normal population, whilst the blood urea level of these patients can be decreased due to disorders in protein synthesis which accompanied by methamphetamine toxicity.

P02-003
Determination of the most susceptibility of bacteria to antimicrobial agents in endophthalmitis

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Purpose: Bacterial endophthalmitis is a rare and serious complication that may occur as a result of eye surgery. In this disease, the role of timely diagnosis and treatment as well as the use of appropriate antibiotics to prevent blindness is very important. The aim of this study was to laboratory survey for determination of the most antimicrobial susceptibility of pathogens producing bacterial endophthalmitis.

Methods: The samples were culture positive isolates of vitreous (n=101) from patients who had been referred, due to bacterial endophthalmitis, to Farabi Ophthalmology Hospital of Tehran University of Medical Sciences in 2013. Determination of the maximum susceptibility to antibiotics in the common bacterial pathogens agent of the disease was performed on the basis of laboratory standards and antibiotic disc diffusion method. The antibiotics disc were included of Cefazolin(CZ), Ceftazidime(CAZ), Chloramphenicol(C), Amikacin(AN), Ciprofloxacin(CP), Trimethoprim(SXT), Gentamycin(GM), Vancomycin(VA), Oxacillin(OX), Imipenem(IMP).

Results: Our results showed that, in this disease, the most common gram positive and negative bacteria are Staphylococcus epidermidis (35.58%) and Pseudomonas aeruginosa (12.5%), respectively. Among gram-positive species, Staphylococcus epidermidis was found to be most susceptible (100%) to CZ antibiotic. Whilst for gram-negative bacteria, Pseudomonas aeruginosa was shown to be most susceptible (100%) to CP, GM, IMP antimicrobial agents and also (91.67%) to AN antibiotic. In the light of this study physicians would be able to have a predictable susceptibility pattern for treatment of bacterial endophthalmitis.

P02-004
Predicting the need for hospitalization of intoxicated patients: a pilot study

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Background: Intoxicated patients are frequently admitted to the Emergency Department (ED) whereas hospital admission is not always necessary. No predictive models exist that could improve ED triage of these patients. In a pilot study, we aimed at identifying potential predictors for developing such a model.

Methods: We conducted a prospective cohort study of ED presentations involving intoxications in a Dutch University Hospital during a 1.5-year period (January 2015–July 2016). The primary outcome was "necessary hospitalization". This outcome was determined in retro-

spect by selecting patients according to their Poisoning Severity Score (moderate & severe categories) and/or the need for treatment on the ward. Potential predictors were covariates available in the first hours following ED presentation, including vital signs and findings based on clinical examination, ECG and laboratory analysis. After multiple imputation of the missing values, selection and prediction optimization were achieved using an Elastic Net regularization. The predictive performance was evaluated by using a cross-validation approach.

Results: 417 ED presentations were included for analysis. In 190 cases (45.6%), hospitalization appeared necessary in retrospect. The strongest risk factors for a necessary hospitalization (factors with OR > 1) were: ingestion of at least one modified-release preparation, hypotension, and pH <7.37. Normal glucose and pH values were strong protective factors (with OR <1). The expected severity, based on the reported exposures (mg product/kg bodyweight) and our Poisons Center's comprehensive toxicological database, was also predictive. AUCs for predicting a necessary hospitalization were on average 0.70, depending on the included predictors. The calibration plots showed a good fit of the data.

Conclusion: This pilot study identified predictors of necessary hospitalization of intoxicated patients. Our findings should be confirmed in a study including a larger number of patients.

P02-005
The carbonylation pattern in type 2 diabetes using capillary electrophoresis

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Purpose: Type 2 diabetes like many other chronic diseases, is strongly related to oxidative stress. One of the biochemical consequences of oxidative stress is the carbonylation of the proteins. The aim was to find a pattern in the electrophoregrams of carbonyl proteins from patients with diabetes.

Materials and Methods: Samples from patients with type 2 diabetes were evaluated using derivatization with 2,4-dinitrophenylhydrazine and analysed using a capillary electrophoresis (CE) method. Electrophoretic separation was performed using an aqueous electrolyte system: 20 mM borate buffer with pH 9.0 and 15% w/w dextran 70. Injection was performed in the hydrodynamic mode at 0.5 psi for 10s, and the applied voltage was – 25 kV. The detection was performed at 370 nm, 365 and 214 nm.

Results: CE method offers qualitative information about the carbonylated species through electropherograms and the peak characteristics. Based on the retention time (RT), the peaks resulted from samples from patients with type 2 diabetes were grouped within 10 groups. We determined the average number of peaks for every patient serum sample, found in each RT group. This is an index showing the fragmentation degree which leads to the formation of more carbonylated species that have very close molecular mass. More than one third of the samples exhibited peaks in half of the groups. A suitable measurement as an indicator of a protein carbonylation pattern was the percentage of patients whose serum samples issued peaks in a certain RT group. 75% of the samples had carbonyls with RT inside group with lower RT values, meaning that in type 2 diabetes mellitus, carbonylation most frequently occurs on smaller fractions. We also observed a specific fragmentation dynamic.

Conclusion: A preliminary pattern of protein carbonylation associated to type 2 diabetes mellitus was issued. Further studies are needed to elucidate the chronology of protein chain lysis and carbonylation.

P02-006
Spectrum of acute drug toxicity during the most popular house and techno party in the world

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Background: Since 1991, the Street Parade, world's most popular house and techno parade in Zurich, is still a mecca for ravers. One Saturday in every August, about one million visitors celebrate this initially peaceful event which stands for love, freedom and tolerance. However, extensive drug abuse has also been commonly seen. The prevalence of acute drug toxicity (ADT) due to novel psychoactive substances (NPS) during the Street Parade is unknown. Therefore, the aim was to investigate the drug spectrum of acute intoxicated patients from the Street Parade presenting in the Emergency Department (ED).

Methods: We investigated consecutively urine samples of acute intoxicated patients who participated at the Street Parade and presented in a Swiss tertiary care ED in 2017 and 2018. The endpoints were the analysis of the drug spectrum and assessment of the prevalence of ADT by NPS. Samples were analyzed by a screening method using liquid chromatography coupled to high-resolution mass spectrometry. Substances were identified by their theoretical exact mass and by comparing acquired tandem mass spectrometry (MS/MS) to library spectra.

Results: In total, we analyzed 47 urine samples. Ten patients presented with symptoms of ADT but only a wide spectrum of different medications was detected. In 20 patients (42.5%), alcohol without any other drug was identified. Finally, 17 intoxicated patients (36.2%) consumed drugs plus alcohol. The three leading drugs were cocaine (21.3%), 3,4-methylenedioxymethamphetamine (MDMA) (19.1%) and tetrahydrocannabinol (THC) (17.0%) followed by methamphetamine (8.5%), methylphenidate (6.4%) and 2.1% for each lysergic acid diethylamide and amphetamine. Furthermore, one patient (2.1%) showed an abuse of NPS (methylon) in combination with alcohol, cocaine and MDMA. An overdose of methamphetamine occurred in five patients in 2018 whereas no overdose of methamphetamine was detected in 2017.

Conclusion: Cocaine, MDMA and THC in combination with alcohol are the most prevalent drugs in Street Parade patients whereas NPSs are still rare. Methamphetamine intoxications seem to increase. Thus, future preventive strategies need to sensitize the rave scene about the drug spectrum and possible health consequences.

P02-007
Prevalence of clinical intoxications: a study of drug intoxications profile in an emergency department of a Portuguese hospital

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Human intoxication processes have been one of the most serious public health problems due to the lack of control and prevention of intoxication associated to easy access of the population to a high number of substances with a high degree of toxicity. Acute intoxications represent one of many causes of admission to hospital emergency services. The profile of clinical intoxications in Portugal is not well established and therefore its assessment is of utmost importance to help healthcare professionals to respond more efficiently and adequately to intoxications episodes.

This work describes the retrospective and descriptive analysis of the adult patients who were classified as eventually intoxicated (overdose or poisoning) by the Manchester Triage System at the time of entry into the emergency department of the Hospital da Senhora da Oliveira in Guimarães city in the period of January 1, 2017 to May 31, 2018.

Over the studied period, of the 837 possible intoxications cases observed, 221 patients were seen with a drug-related intoxication and 492 with alcohol-related intoxication.

Of the drug-related intoxications studied, 78.7% involved female individuals, whereas 21.3% were male. The average age was approximately 44 years old. Most of these intoxications were voluntary (96.8%), and 54.8% of those without suicidal ideation. In 99.5% of the episodes, the administration route was oral. The majority of patients had mono-intoxication (84.6%) and drug and alcohol intoxication accounted for 10.6%. The pharmacological group more frequently mentioned were the anxiolytics, hypnotics and sedatives (54.8%), followed by (42.1%) and antiepileptics and anticonvulsants (15.4%). The average number of drugs involved in intoxications was 2. Intoxicated individuals received mostly gastrointestinal decontamination treatment, such as gastric lavage (67.0%) and activated charcoal (58.5%). The antidotes were given in 20.21% of the intoxications, where flumazenil represented 87% followed by acetylcysteine (13%). Most exposure patients (67.5%) were admitted.

This work contributed to the documentation and identification of the occurrence of clinical intoxications in Portugal and highlight the need of the improvement in the prevention and education in this field.

P02-008
Nutritional modulation of environmental toxicity and implications in inflammatory diseases

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Exposure to environmental pollutants is associated with the development of many diseases through multiple mechanisms including the induction of chronic inflammation. Many organic pollutants are persistent and express high stability and ubiquity in the environment. For example, coplanar polychlorinated biphenyls (PCBs), which act as an agonist of the aryl hydrocarbon receptor, exert toxic effects on the endothelium and associated vasculature. Atherosclerosis, a chronic inflammatory disease initiated by vascular endothelial cell dysfunction, remains the leading cause of death worldwide. Furthermore, PCB-induced toxicity has been linked to increased expression of pro-inflammatory caveolin-1, the major structural protein in caveolae membrane domains. Caveolae are particularly abundant in endothelial cells, where they play a major role in the regulation of vesicular trafficking and signal transduction. PCBs are also known to affect the cellular redox status, which may initiate antioxidant responses through nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling. Our data show that PCB toxicity is modulated by cross-talk between caveolae and Nrf2 signaling. Cav-1 silencing (siRNA treatment) increased levels of Nrf2-ARE transcriptional binding, resulting in higher mRNA levels of the antioxidant genes glutathione s-transferase and NADPH dehydrogenase quinone-1 in both vehicle and PCB-treated systems. Nutrition may function as a modulator of vulnerability to environmental insults. Increasing evidence suggests that diets high in plant-derived bioactive food components (e.g., polyphenols) and omega-3 lipids are associated with a reduced risk of chronic inflammatory diseases such as atherosclerosis. Current data suggest that endothelial cell dysfunction and inflammatory events induced by exposure to persistent environmental pollutants such as coplanar PCBs can be downregulated by polyphenols, such as flavonoids, as well as by omega-3 PUFAs. Our data suggest that PCB-induced inflammation is a trigger of cardiovascular disease risks and that dietary polyphenols and omega-3 lipids exhibit anti-inflammatory protection via caveolae and cytosolic Nrf2 signaling.

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P02-009
Toxicity/adverse effect predictions based on computational toxicology techniques and large-scale databases

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Understanding the features of chemical structures related to the adverse effects of drugs is useful for identifying potential toxicities/adverse effects of new drugs and chemical products. This can be based on the limited information available from post-marketing surveillance, assessment of the potential toxicities of metabolites and illegal drugs with unclear characteristics, screening of lead compounds at the drug discovery stage, and identification of leads for the discovery of new pharmacological mechanisms. This present study developed techniques used in computational toxicology such as quantitative structure-activity (toxicity) relationship (QSAR/QSTR) analysis to investigate the content of large-scale spontaneous report databases of adverse effects such as FDA Adverse Event Reporting System (FAERS; JAPIC-AERS) and Japanese Adverse Drug Event Report database (JADER). Furthermore, volcano plotting, a new visualization method for clarifying the relationships between drugs and adverse effects via comprehensive analyses, will be introduced. These analyses may produce a great amount of data that can be applied to drug repositioning.

P02-010
Transcriptomic approach to improve the understanding of 5- fluorouracil (5-FU) induced intestinal toxicity *in vitro* and *in vivo*

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5-fluorouracil (5-FU) is a classical cytotoxic agent widely used in cancer therapy that has been associated with adverse drug reactions (ADRs) in several organs, including the Gastrointestinal (GI) tract. 5-FU has shown to induce acute toxicity in small and large intestines, supported by patients' reports of diarrhoea, nausea and abdominal pain that often lead to interruption of cancer treatments, impairing patients' quality of life and survival to the disease. Nevertheless, the understanding of the molecular mechanisms underlying 5-FU toxic-

ity and how these relate to the ADRs experienced by patients is limited. In this study, we aim to expand our knowledge by establishing 5-FU induced transcriptomic responses and cytotoxicity in different models. *In vitro* human intestinal organoids, derived either from colon or small intestine (SI), were exposed to 0, 10, 100, 1000 μM of 5-FU. The *in vivo* study consisted in exposing mice to 0, 20 and 50 mg/kg of 5-FU. Both *in vitro* and *in vivo* exposures are based on PBPK model calculations considering the doses recommended to cancer patients. Following the *in vitro* exposure, cell viability and apoptosis were assessed as functional endpoints. Moreover, gene expression profiles of non-exposed *versus* exposed samples were also assessed for both models. Transcriptomics was measured by performing RNA sequencing, after which the most affected biological pathways and respective differentially expressed genes (DEGs) were evaluated. Cell cycle, DNA damage/repair, p53 signalling, mitochondrial ATP synthesis, metabolism and apoptosis were amongst the most altered pathways unveiled by the *in vitro* assays, demonstrating time and dose effects, particularly in colon organoids. In addition, comparison of the functional and transcriptomic outcomes is evaluated between both *in vitro* and *in vivo* experiments. In further studies, the molecular responses will be used to build a multi-scale predictive model of drug-induced intestinal toxicity. Taken together, this study provides insight into possible toxicity mechanisms as well as the *in vitro* to *in vivo* translation of results generated in organoids. Moreover, it potentially leads to a step towards the improvement of the quantitative systems toxicology (QST) in predicting 5-FU effects in intestines.

P02-011
Metabolomics evaluation of urine from PCa patients by GC-MS and NMR spectroscopy

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Prostate cancer (PCa) is one of the most common types of cancer in men. In this work, 41 PCa and 42 non-cancer (control) urine samples were analyzed by GC-MS (direct injection after derivatization) and ^{1}H NMR spectroscopy in order to obtain a comprehensive PCa metabolic signature. Multivariate statistical analysis was used to evaluate the ability of the GC-MS and ^{1}H NMR urinary metabolic profiles to distinguish PCa from controls. The created discriminant models were further validated using an external validation set (n=18 PCa and n=18 controls). The GC-MS model presented a sensitivity of 94%, a specificity of 84% and an accuracy of 92%, whereas the ^{1}H NMR model presented a sensitivity of 78%, a specificity of 94% and an accuracy of 86%. In GC-MS approach we disclosed 15 metabolites significantly altered in PCa (including 3 unidentified compounds) and in ^{1}H NMR approach we revealed 12 metabolites significantly altered in PCa (including 3 unidentified compounds). Among them, 12 metabolites were found over-expressed in PCa cases, namely sarcosine, propylene glycol, oxalic acid, threose and threitol (identified through GC-MS), and leucine, valine, 2-hydroxyvalerate, 2-hydroxyisobutyrate, pyruvate, acetone and hydroxyacetone (identified through ^{1}H NMR), while 9 metabolites were down-expressed, comprising gluconic acid, arabitol, fucitol, ribitol, mannitol, glucose and *myo*-inositol (identified through GC-MS) and 2-furoylglycine and trigonelline (identified through ^{1}H NMR). To the best of our knowledge, this is the first study reporting significant alterations in the levels of propylene glycol, oxalic acid, threose, threitol, hydroxyacetone, fucitol, mannitol, 2-furoylglycine and trigonelline in PCa biological samples. Based on these results, we were able to associate PCa metabolic signature to the dysregulation in 14 biochemical pathways, being the majority of these pathways associated with amino acids and energetic metabolism. So, our results prove the potential of GC-MS and ^{1}H NMR metabolic signatures for discrimination of PCa patients from control subjects and towards a better understanding of the metabolic dysregulations associated with PCa progression and development.

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P02-012
The role of exosomes from human MSC 3D cultures in wound healing

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Exposure to toxic agents frequently leads to cutaneous toxicity, which often induces skin irritation and impairs the healing process. The current available therapeutic options often fail to promote full tissue regeneration, and therefore novel strategies to develop effective therapies for improving the healing process are needed. In this context, mesenchymal stem cells (MSCs) gained relevance due to their role in tissue regeneration via paracrine mechanisms. Recently, the secretion of exosomes has been suggested as a dominant mechanism by which MSCs exert their healing function. Our study aimed at evaluating the role of exosomes, derived from umbilical cord matrix-derived MSCs primed by 3D culturing, on cutaneous wound healing using *in vivo* methodologies paired with integrative proteomic analysis. As such, the whole secretome was obtained by collecting and concentrating the culture media conditioned by MSCs in 2D (CM2D) and 3D (CM3D). Exosomes were isolated from CM2D (Exo2D) and CM3D (Exo3D) by size exclusion chromatography. Size distribution of the isolated exosomes (135 ± 54 nm and 265 ± 37 nm for Exo2D and Exo3D, respectively) pointed out the influence of the culture system in its morphology, however without compromising the presence of CD9 and CD81 exosomal surface markers. Moreover, proteomic analysis of the isolated exosomes revealed that 3D conditions lead to higher protein diversity than the 2D environment. Indeed, Exo3D show 18 specific proteins, some of which are involved in cell chemotaxis, division and proliferation. Accordingly, the evaluation of the effect of Exo3D/2D and CM3D/2D in skin regeneration using an *in vivo* rat wound-splinting model suggested a significantly higher therapeutic potential of exosomes over the MSC secretome. Macro-

scopic observations show that Exo-treated wounds exhibited accelerated wound closure when compared to control wounds. Accordingly, histological examination revealed that Exo3D-treated wounds show an improvement in the healing profile, by promoting wound margin closure and complete tissue regeneration with hair re-growth. Overall, the results suggest that 3D MSCs-derived exosomes promoted wound healing, granting their potential new role as active players in cell-free-based therapies for different pathological or toxicological contexts. Moreover, *omics* approaches may help on the identification of new markers involved in the healing process and ultimately improve therapeutic outcomes.

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P02-013
Manganese in the diets of infants and young children: A review of manganese in the diets of infants and children by the UK Committee on Toxicity.

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Manganese is an essential micronutrient in the human diet, but high chronic exposures have been associated with a range of neurological signs and symptoms which combine, in severe cases, to cause a Parkinson-type syndrome called manganism. It is unclear whether children are more sensitive than adults but there is a large body of literature linking high manganese exposure, primarily measured using biomarkers such as concentrations in hair, tooth or blood, with neurodevelopmental effects in children such as IQ decrements and attention deficit hyperactivity disorder. Humans may be exposed to manganese found naturally in the environment and from industrial processes. In the UK, manganese exposure in workers, and the general public, from industrial activity is minimal, but there are uncertainties over the impact of dietary manganese on the neurological development of infants and young children.

The UK Committee on Toxicity have reviewed manganese exposure of UK infants and young children using data from the analysis of food samples and two dietary surveys: the national diet and nutrition survey, and the diet and nutrition survey of infants and young children. They have compared these exposures with current health-based guidance values, primarily that set by the WHO in their Guidelines for Drinking Water Quality. The Committee found that estimated exposures from the diet exceed current health-based guidance values for manganese in nearly all age groups. There is considerable uncertainty on the degree to which manganese in the diet is absorbed in the gastro-intestinal tract, and there are some inconsistencies in the data on adverse effects, such as contradictory sex-related differences and the nature of the dose-response relationship. There are also uncertainties in the exposure assessment. Therefore, the health risk from manganese in the diets of infants and young children is unknown. The aim of this poster is to highlight the limitations in the database on manganese and identify further research to help close these data and knowledge gaps.

P02-014
Poisons and poisonings by snakes of medical importance in Angola

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Snakebite is considered a neglected tropical disease responsible for high morbidity and mortality in Asia and sub-Saharan Africa. In Angola the scenario is unknown.

The objectives of this study were: to evaluate the performance of health professionals towards to snakebite poisonings in four Angolan regions; ii) to biochemically analyse the venoms of the snakes collected in these regions; iii) to evaluate the immunogenicity of the venoms and produce the experimental antivenom serum.

A three-step methodological design was carried out. First-step – a prospective cross-sectional descriptive observational field study including 151 health professionals; Second-step – Biochemical characterization of the venoms of captured snakes, including: i) protein content of the venoms and characterization of their electrophoretic profiles; ii) determination of the glycosylation profile of the venom proteins; iii) evaluation of the proteolytic activity; iv) detection of the phospholipase activity; and v) evaluation of the hyaluronidase activity in a sample of venoms of eigth snakes. In the third step – the evaluation of the immunogenic potential of Angolan snake venoms in a murine animal model and detection of the antigenic components against the murine sera.

The results of the present investigation allowed to conclude that: 1 – there is a low level of knowledge of health professionals in the diagnosis, evaluation and therapy of Angolan snakebites; 2 – the clinical manifestations of Angolan snakebites can be local, systemic, ophthalmological and neurological; 3 – the venoms of snakes involved in ophidian accidents, have a remarkable intraspecies biochemical variability, related to the sex of the animals and their regions of origin. 4 – Venoms of snakes of medical importance in Angola are immunogenic. Viperid venoms (*B. arietans*, *B. gabonica*) were more immunogenic than the elapidic venom (*N. nigricollis*) and the murine sera produced recognized a considerable number of venom components.

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P02-015
A fatal case related to heroin injection

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Objectives: To report an accidental death caused by injection of heroin. A 23-old man, who was very strong, was found dead after heroin injection for half an hour in a car. The death scene investigation showed evidence of acute intoxication with previous doses of heroin consumption after an abstinence period time.

Methods: The forensic autopsy revealed no wound in his body, and his lip, fingernails and toenails were all cyanosis. Pathological examination: respiratory failure pneumonia, and heart lesions. A systematic toxicology analysis was performed by UPLC-QTOF and gas chromatography-mass spectrometry (GC-MS), Morphine, codeine, O6-monoacetylmorphine were founded in heart blood and urine, then they were identified and quantitated by ultra-high-performance liquid chromatography-mass Spectrometry (UPLC-MS/MS), Alcohol was determined by gas chromatography-flame ionization detector (HS-GC/FID) with headspace injection.

Results: The concentration of the drugs were as follows: Morphine 0.331μg/mL, codeine 88.0ng/mL, and O6-monoacetylmorphine undetected in the heart blood. Morphine 0.387μg/mL, codeine 0.106μg/mL, and O6-monoacetylmorphine 1.48μg/mL in the urine. In heroin-related deaths blood morphine concentrations vary substantially, from nanograms to milligrams per liter [1], these data cannot be used in isolation to diagnose an overdose death. O6-monoacetylmorphine in the urine can be used as a biomarker for heroin consumption. Pathological examination of cardiac lesion was consistent with organ damage caused by heroin abuse.

Conclusion(s): O6-monoacetylmorphine, as a biomarker for heroin consumption, has a shorter survival time in the blood, usually only morphine is detected [2], so blood and urine analysis were performed simultaneously that should be helpful to identify heroin abuse or fatality, Cardiopathy is the organ damage associated with long-term heroin abused [3]. So it could be determined that the victim died from a heroin overdose based on the concentration of substances in blood and urine for this case.

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P02-016
Olanzapine induced hepatotoxicity is investigated by individual susceptibility and metabolomics

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Hepatotoxicity is one of the deleterious effects of antipsychotic drugs. Hepatic function is monitored by serum aminotransferase levels. However, serum aminotransferases may not be liver-specific and sensitive. Alpha-glutathione S-transferase (α-GST) may be liver specific due to having greater cytosolic concentration, shorter half-life and smaller molecular weight than aminotransferases. GST enzymes catalyze the biotransformation and detoxification reactions of many drugs. Single nucleotide polymorphisms on GSTs can change the enzyme activities and therefore in drug response. Antipsychotic drugs and psychotic disorders can change metabolomics and are related to individual susceptibility. We aimed to investigate whether α-GST can be a better indicator of hepatotoxicity rather than others and whether the polymorphisms on GST enzymes have an effect on hepatotoxicity among individuals. Blood samples were taken from 30 patients, who have psychotic disorders, treated with olanzapine at 3 different time periods: T1, before medication; T2, 10 days after medication and T3, 3 months after medication. *GSTT1, M1* and *P1* genotyping was performed by PCR-RFLP. Serum α-GST enzyme activities were measured by ELISA. We observed statistically significant increase in α-GST enzyme activity (p=0,047) and alanine aminotransferase (ALT) levels (p=0,006) in T2 compared to those in T1. However, the percentage increase in ALT between T1 and T2 was greater than that in α-GST. We did not find any significant association between α-GST enzyme activities and GSTs variations. Schizophrenia-specific metabolomics pattern was observed and furthermore, tryptophan levels were high as we expected.

P02-017

This abstract has been withdrawn.

P02-018

This abstract has been withdrawn.

P02-019
Screening and regulatory approaches to risk assess *in vitro* chemical mediated changes in thyroid function

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The hypothalamic-pituitary-thyroid axis (HPT axis) is conserved across vertebrate evolution. Perturbation of thyroid hormone homeostasis (THH) can lead to adverse effects in thyroid function affecting growth, metabolism and cognitive function. In utero, appropriate thyroid hormone concentrations are absolutely required for normal nervous system development. Chemical disruption of THH can occur via a number of mechanisms, including increased hepatic thyroid hormone clearance, inhibition of iodide transport into the thyroid (sodium/iodide symporter), inhibition of iodide oxidation (thyroid peroxidase) and inhibition of thyroid hormone deiodination (deiodinases). To understand the effect of chemicals on these functions the following assays are utilised. 1. *in vitro* primary hepatic thyroid hormone metabolism (multiple species), 2. thyroid peroxidase inhibition (multiple species), 3. deiodinase inhibition (rat and human), 4. sodium/iodide symporter inhibition (rat). Rat sodium iodide symporter inhibition and rat and human deiodinase 1,2 and 3 inhibition assays are currently being validated. Here we report the validation of *in vitro* rat, dog, pig and human TPO inhibition and *in vitro* primary hepatocyte metabolism of thyroxine (T4). In concurrence with the literature, TPO inhibition by 6-propyl-2-thiouracil (PTU) shows broad sensitivity across the species tested. in rat (IC_{50} 2.2 μM) Dog (IC_{50} 17.7 μM) pig (IC_{50} 7.6 μM) and human (IC_{50} 50.9 μM). T4 metabolism by pri-

mary cultures of human and rat hepatocytes show a consistent dose response induction (approximately 2 fold over vehicle control) in response to reference item administration. This suite of assays will be used to generate data in support of the current requirements for endocrine disruption hazard assessment. Alternatively, we are currently adapting our platform of assays (standalone Regulatory platform (GLP)) to a high throughput format to allow these *in vitro* assays to be used to generate data to study chemical endocrine disruption data early in your discovery program.

P02-020
Using of liquid mass spectrometry for detecting testosterone in blood plasma

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The purpose of our work is the development of the method for testosterone determination in blood plasma of rats that allows to identify the threat of reproductive toxicity of lambda-cyhalothrin. It is well-known that the determination of testosterone is very important for the estimation of various diseases. At the same time, the application of liquid mass spectrometry in the practice of laboratory diagnosis can largely solve controversial interpretations, regarding the significance of diagnostic of the results of the definition of testosterone using various enzyme immune sets. Also, liquid mass spectrometry provides a number of indisputable advantages in relation to the enzyme immune method, which allows more accurately differentiate male hypogonadism state.

The proposed method of determination of testosterone is based on liquid extraction of biological objects, purification of extracts on Strata NH2 cartridges (55µm, 70A), Phenomenex and chromatographic separation on a reverse-phase column with using a liquid chromatograph Shimadzu LC-30A in a gradient moving phase, detection and quantitative analysis with using mass detector LCMS-8050. Based on experimental data, the metrological characteristics were obtained and are the following: the detection limit (LOQ) at 0.01 ng/ml, coincidence (Sr) -0.15%, intra-laboratory reproducibility (SR) -0.25%, extended vagueness (U) (at P = 0.95) -0.5%. It was shown that the using of liquid mass spectrometry makes it possible to determine the testosterone and establish common reference intervals for laboratory diagnosis.

The influence of lambda-cyhalothrin doses of 0.3 mg/kg, 3 mg/kg and 10 mg/kg on the level of testosterone in the blood plasma of rats was established with a high degree of reliability (P = 0.01) and discussed.

Thus, the developed method provided for the study of antiandrogenic activity of the lambda-cyhalothrin test sample. This method allows to determine the level of testosterone in plasma and to establish a violation of spermatogenesis.

P02-021
Development of a new chromatographic screening method for the determination triazole metabolites in raturine using High-resolution Hybrid LC-Orbitrap

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Pesticide exposure is typically done based on residue data from food monitoring (raw commodities) and food consumption databases. Biomonitoring is an alternative and can bring added value for chemical risk assessment. Upon uptake, most pesticides are rapidly metabolized and excreted. Therefore, urine analysis typically comes down to measurement of pesticide metabolites as biomarkers of exposure. Major bottlenecks in biomonitoring of of pesticides:

- most suited metabolites (biomarkers) are often not known
- the dynamic of individual metabolites origination is not known
- analytical standards are not available
- most of metabolites are fairly more polar compare to parent compound
- analytical methods are not available

The main strategy of this work is development of screening method for triazole urinary metabolites using LC-HRMS. We used triazole pesticides as the most common fungicides. The method was applied to analysis of rat's urine samples. Various pesticide-biomarkers were identified. Metabolites were detected through non-targeted analysis followed by both suspect screening analysis of samples before and after exposure. The most selective and sensitive metabolites will be used in developing quantification method. Development of dedicated targeted methods is our next main step.

P02-022
Activation of xenobiotic-sensing nuclear receptor PXR increases blood pressure and stimulates plasma renin activity

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Metabolic syndrome involves several related conditions exposing to diabetes and cardiovascular disease. The main features are obesity, impaired fasting glucose, elevated blood pressure and dyslipidemia.

Pregnane X receptor (PXR) is a nuclear receptor that was originally identified to regulate drug metabolizing enzymes and drug transporters, i.e. mechanisms involved in the detoxification of xenobiotics. Subsequently, this xenobiotic receptor has been recognized to possess much broader regulatory functions, PXR has been shown to promote several components of the metabolic syndrome including dyslipidemia and impaired glucose tolerance. In the current study we addressed the question if PXR activation affects blood pressure.

We conducted a clinical trial on healthy volunteers to study the effects of rifampicin (well establish ligand for human PXR) on blood pressure and performed ambulatory 24-hour blood pressure monitoring. The design of the study was randomised, single-blind with blinded study personnel, placebo-controlled and cross-over. Rifampicin 600 mg a day or placebo was dosed on each arm for a week and the 24-hour blood pressure was monitored at the end of each arm. Rifampicin induced both the systolic and the diastolic blood pressure and also the heart rate. Furthermore, after rifampicin treatment the plasma renin level was increased.

Since PXR expression is mainly limited to liver and intestine, we hypothesized that 4β-hydroxycholesterol (4βHOC), an LXR ligand, known to be significantly increased in human circulation after treatment with PXR agonists, could mediate the increase in plasma renin level. To test this hypothesis, we established a stable expression of LXRα in Calu-6 cells, which constitutively express renin. Treatment of these cells with 4βHOC increased renin expression. However, relatively high 4βHOC concentration was required for induction. In summary, these results establish blood pressure elevation via renin activity as a novel function regulated by PXR.

P02-023

This abstract has been withdrawn.

P02-024
Severity use of drugs of abuse and cell aging

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Introduction: Telomeres are repeated 5′-TTAGGG-3′ sequences at the end of chromosomes, which maintain genomic stability. It is known that drugs of abuse can provoke shortening of telomeric ends. The aim of our study is to evaluate if the shortening depends on the severity of usage and the type of drug.

Methods: Blood samples were collected from sixteen opiates and/or cannabis abusers. Metaphase spread leukocytes were isolated from peripheral blood telomeres length were measured by 3D Quantitative Fluorescence *in situ* Hybridization procedures with a (C3TA2)3 PNA probe. The severity of cannabis abuse was calculated as cigars x week and the severity of opiates abuse as gr x week, medium heavy (< 5 cigars per week) and hard heavy abuse (> 5 cigars per week) were used to describe the extent of abuse.

Results: Cannabis use anged from 7 to 26 years and weekly consumed cigarettes varied between 1 to 200 cigs. Opiates use ranged from 2 to 26 years and the consumption of heroin ranged from 1 to 39 gr/week. Negative correlation was observed ($r = -0.564$, $p = 0.045$) of 1st quartile of short telomere length and the severity of opiates abuse. Additionally, tendency of association ($p < 0.100$) was found between opiates abuse and 3rd quartile of telomere length. In an alternative way of analysis, using categorical opiate abuse (< 5 vs > 5 gram/week), resulted to significant difference at measured median Short Telomeres Lenght with $p = 0.035$.

Conclusion: A possible effect of opiate heavy abuse on telomere length was observed, while abuse effect of cannabis comparison was questionable.

P02-025

This abstract has been withdrawn.

P02-026
Detection of colchicine from biological samples of two death by QuEChERs-UPLC-MS/MS

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Background: In recent years, the number of cases involving murder and suicide using clinical drugs is climbing. Colchicine which is used mainly for the treatment and prevention of gout is widely used in China.

Cases Brief: A woman appeared vomiting and diarrhea after drinking a cup of tea which was poisoned by colchicine, and died of multiple organ dysfunction syndrome (MODS) 7 days later. A man committed suicide by taking colchicine tablets orally due to huge property losses caused by fraud and died after emergency treatment.

Method: Using 5% methanol-acetonitrile as extractant, 30 mg NaCl and 20 mg anhydrous $MgSO_4$ as salting-out agent, and purified by 20 mg C18 powder. UPLC-MS/MS uses acetonitrile as organic phase, 5 mmol ammonium formate -0.1% formic acid-water as aqueous phase, MRM mode, m/ z 400.5 → m/ z 358.2 as a quantitative analysis of ion pairs.

Result: Fatal levels of Colchicine was detected from both deceased. In the female deceased, the blood concentration after dialysis treatment is 8.05 ng/mL, bile is 44.8 ng/mL, liver is 39.8 ng/mL, and kidney is 45.2 ng/mL. In the male deceased, the blood concentration is 16.6 ng/mL.

Conclusion: The QuEChERS-LC-MS/MS method is simple to operate, high sensitivity . It can be used for accurate and rapid detection of colchicine in biological samples.

P02-027
Xanthones as potential P-glycoprotein modulators at the intestinal barrier: *in vitro* and *ex vivo* studies

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P-glycoprotein (P-gp) is a membrane efflux pump belonging to the ATP-binding cassette (ABC) transporter superfamily, with a polarized expression in barrier and excretory tissues [1]. Due to its wide range of substrates and to its large efflux capacity, P-gp has an outstanding impact in the pharmaco/toxicokinetics of xenobiotics [2]. This mechanism is particularly important at the intestinal level, significantly reducing the intestinal absorption of xenobiotics, limiting their access to the target organs, thus resulting in a decrease in their toxicity [3]. Consequently, P-gp induction and/or activation has been proposed as a therapeutic strategy in intoxication scenarios [4]. In fact, several xanthones derivatives have been shown to protect cells against toxic xenobiotics by increasing P-gp expression and activity [5,6].

The aim of the present study was to investigate the potential effect of 6 newly synthetized xanthones (X1, X2, X5, X6, X12, X16) on P-gp expression/activity. *In vitro* studies were performed in SW480 cells and some xanthones were able to significantly increase P-gp expression [X1, X2, X6 and X12 significantly increased P-gp expression to 113%, 119%, 139% and 122%, respectively, when compared to control cells (100%)] and activity [X1, X5, X6 and X12 significantly increased P-gp activity to 130%, 133%, 113% and 119%, respectively, when compared to control cells (100%)], 24 hours after exposure, as observed by flow cytometry and spectrophotometry, respectively. Additionally, in a short incubation period of 90 minutes almost all the xanthones significantly and immediately increased P-gp activity [X1, X2, X5, X6 and X12 significantly increased P-g activity to 121%, 120%, 128%, 126% and 135%, respectively, when compared to control cells (100%)], thereby behaving as P-gp activators given the short period of exposure. Furthermore, the protection afforded by these xanthones against the cytotoxicity induced by mitoxantrone (MTX, 10 µM), a toxic P-gp substrate, was evaluated by the MTT reduction assay. However, the xanthones failed to protect against MTX-induced cytotoxicity. Nevertheless, the most promising compound, X12, was tested for its ability to increase P-gp activity *ex vivo*, using rat everted intestinal sacs and rhodamine123 (RHO123) as a fluorescent substrate (300 µM). A significant increase in RHO123 efflux was observed in the presence

of X12, an effect selectively blocked by zosuquidar (10 µM), a third-generation P-gp inhibitor. Therefore, the obtained results demonstrated P-gp involvement in the increased RHO123 efflux, confirming the *in vitro* results concerning the X12 P-gp activation potential.

Taken together, the obtained *in vitro* and *ex vivo* results suggested the P-gp activation potential of some of the tested xanthones and highlighted a potential source of new P-gp inducers and activators, disclosing new perspectives in the therapeutics of P-gp substrates-induced intoxications.

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P02-028
Poisons centre enquiries relating to synthetic cannabinoids receptor agonists (SCRAs) in the UK, 2009-2018.

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The misuse of new psychoactive substances (NPS), in particular SCRA, has recently been an important public health issue because of their potential to cause serious clinical effects. These previously uncontrolled drugs of misuse have sometimes been perceived as safe alternatives to cannabis. SCRAs include a diverse group of compounds with various chemical structures that bind to CB1 and CB2 receptors with different affinities resulting in different potencies and toxicological profiles. There is limited information available on the incidence and changes in time of SCRA-related toxicity, therefore the present study evaluated this using poisons centre enquiry data collected in the UK over the last decade.

Clinical enquiries to the UK National Poison Information Service (NPIS) were reviewed retrospectively to ascertain the incidence of reported NPS–related toxicity from January 2009 to December 2018. NPS were defined as drugs of misuse that were not legally controlled in the UK prior to 2009. SCRA related enquires included the use of those branded products likely to contain a SCRA.

Over the 10 years of the study, 4158 episodes involving NPS toxicity were reported to the NPIS, of which 2510(60%) involved a SCRA or SCRA-containing products. Of those exposed, 67% were 30 years of age or younger, 75% were male, 23% female and in 2% sex was not recorded. The median age of the users with known age 24 years (range: 10–78). The incidence of SCRA-related enquiries increased between 2009 and 2015, but has subsequently decreased significantly (P=0.0041) and this decline has continued since.

In conclusion, up to 2015 SCRA-related toxicity was becoming increasingly common,, especially in younger people and males, but has since declined in frequency. While the introduction a generic drug control law based on psychoactivity (The UK Psychoactive Substances Act, May 2016) may have made a contribution, other factors are also likely to be important, including the use of pre-existing legislation to restrict sales via headshops and websites.

P02-029
P-glycoprotein modulation by xanthonic derivatives: a strategy to fight Alzheimer's disease

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P-glycoprotein (P-gp) is the best characterized member of the ATP-binding cassette (ABC) transporters superfamily. Regardless its contribution to the multidrug resistance (MDR) in neoplastic cells, this ATP-dependent efflux pump was also found to be constitutively expressed in the apical surface of normal human epithelial tissues. Noteworthy, its great efflux capacity of a wide diversity of substrates, and its cellular polarized expression in several excretory and barrier tissues (e.g. Blood-Brain Barrier (BBB)) make this protein vital in the defense of susceptible organs, by limiting the absorption and distribution of either toxic xenobiotics (e.g. colchicine) or endogenous substrates (e.g. amyloid beta (Aβ) peptide) [1]. For this reason, P-gp can be faced as a potential disease-modifying pathway when positively modulated (activated and/or induced), in several pathologies/diseases, including in Alzheimer's disease (AD), where one of the main pathological factors to be considered is the accumulation of Aβ peptide, a P-gp substrate [2].

Xanthonic derivatives have been previously reported to act as P-gp modulators [3]. Accordingly, the key goal of this work was to evaluate the induction or/and activation potential of six newly synthetized xanthonic derivatives in the *in vitro* model of the human BBB, the hCMEC/D3 cell line [4]. Furthermore, the neuroprotective effect of the most promising xanthone against the Aβ-induced cytotoxicity was also assessed.

The newly synthetized xanthonic derivatives demonstrated to interact with P-gp, leading to an increase in P-gp expression and transport activity 24 hours after the incubation, and to an immediate increase in P-gp activity after a short incubation period of 90 minutes, indicating not only P-gp induction, as well as a direct pump activation. Additionally, one xanthone significantly protected hCMEC/D3 cells against the cytotoxic effect induced by the Aβ peptide, being this neuroprotective effect selectively blocked by zosuquidar, a third-generation P-gp inhibitor, thus confirming P-gp involvement in the observed neuroprotection.

Therefore, P-gp induction/activation, by increasing the efflux of Aβ peptide, can be faced as a potential prevention/treatment therapeutic approach in AD.

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P02-030

This abstract has been withdrawn.

P03 – Ecotoxicology

P03-001
Increased consumption of seaweed in human nutrition: a new source of exposure for toxic element?

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The use of seaweed for human consumption dates back to ancient times in many regions of Asia and Africa. Western countries begun to introduce algae in their diet since the 1980s and the quantities of products imported each year are continuously increasing in the EU. Seaweed are rich in proteins, carbohydrates, vitamins and trace elements with undoubted beneficial effects for human health. However, due to the environment of collection and poor sanitizing treatments to which seaweed are subjected before consumption, they can be a potential source of intoxication and/or food poisoning. The ability of seaweed to accumulate iodine and heavy metals, arsenic in particular, which may constitute a danger to consumer health, is also well known. The increase in consumption was followed by an increase in attention by the European Food Safety Agency (EFSA), which recommends the collection of information on both production /import data and potential risks to human health. Seaweed for human consumption belong to three large groups: brown algae (Phaeophyceae), red algae (Rhodophyceae) and green algae (Chlorophyceae). The algae are mostly collected in nature, in coastal areas close to the coast, where can be found the greater concentration of polluting factors of different nature. In this study, 100 samples of algae were examined, including 51 Phaeophyceae, 33 Rhodophyceae, 12 Chlorophyceae and 4 mixed preparations. Evaluations of iodine and heavy metals were conducted using ICP-MS technique, with interesting results. The concentration of lead and cadmium did not differ from that of other food products. With regard to the total and inorganic content of arsenic and total iodine, values were higher than those indicated in the EU Commission Recommendation (EU) 2018/464 of 19 March 2018 on the monitoring of metals and iodine in seaweed, halophytes and seaweed products. Recommendation 2018/464 specified that the ingestion of seaweed products, in particular dried products, might lead to a dangerously high intake if these products contain more than 20 mg of iodine per kg of dry matter and/or 2 mg per Kg of total arsenic. This research was funded by the Italian Ministry of Health (PRC 2016017).

P03-002
Plant Protection Products: an ecotoxicological assessment of active substances and associated metabolites

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Quantitative Structure Activity-Relationships (QSARs), commonly known as (Q)SARs, are computational models used to predict physicochemical, biological and environmental fate properties of compounds simply based on their chemical structure. The importance of these software's is already accepted by the scientific community since they follow the 3Rs principle – Refinement, Reduction and Replacement, related with the use of animals for testing purposes. Moreover, these predictions are becoming more accurate with the continuous development of more relevant, reliable and adequate (Q) SAR models.

In this study, the software OECD (Q)SAR Toolbox was used to perform an ecotoxicological assessment of 21 active substances (registered by Ascenza Agro S.A.) and 48 of their metabolites. The main goal of this study was to investigate the ecotoxicological trend of metabolites compared with their active substances. Results show that 77% of the metabolites are equivalent or less toxic than their parent compounds, and 45% of these metabolites share their parental toxicophore. Overall, it was not possible to establish a connection between an active moiety and the presence of an ECOSAR alert.

P03-003
Embryotoxicity of selected organic UV filters on zebrafish (*Danio rerio*)

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Organic UV filters are able to absorb ultraviolet radiation and are extensively used in many cosmetics products. Over the past decade,

their application has increased steadily as a consequence of growing concerns of negative effect of UV radiation.

Although UV filters are present in the aquatic environment at comparatively low concentrations, these levels are biologically relevant and pose a significant growing risk for aquatic organisms. The aim of this study was to assess the acute embryotoxicity of selected two organic UV filters (2-ethylhexyl 4-methoxycinnamate EHMC, phenylbenzimidazole sulfonic acid PBSA).

As a model organism we used zebrafish (*Danio rerio*), which belong to one of the model fish organisms commonly used in toxicity tests to evaluate negative effects of various chemicals, which are occurring in the aquatic ecosystem. Toxic effects were studied using evaluation of lethal endpoints, development disorder, and other sublethal endpoints such as hatching rate, formation of somites, and development of eyes, spontaneous movement, heartbeat, blood circulation, pigmentation, or edema at 24, 48, 72, and 96 hours post fertilization. The embryonal toxicity test was performed through the modified method of Fish Embryo Acute Toxicity (FET) Test (OECD guideline 236). Newly fertilized zebrafish eggs were exposed to various concentrations of a single substances and their mixtures as well. In our experiment, we focused especially on testing low environmentally relevant concentrations of organic UV filters, which are usually found in surface water. Moreover higher concentrations were tested in order to reveal if the effects on exposed organism might be dose dependent. Our results showed that higher concentrations cause mortality and changes in development.

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P03-004
POPs in muscles of farmed deer

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Human activity results in the release of many toxic substances into the environment. One of the particularly toxic groups is persistent organic pollutants (POPs), such as chlorinated and brominated dioxins, polychlorinated biphenyls and polybrominated diphenyl ethers. Scientific research indicates the relationship between the POP environment contamination and toxicity to both animals and humans. Specific toxic effects to humans can include reproductive disorders, disruption of the immune system, damage to the nervous systems, and developmental and carcinogenic effects. Wild deer accumulate a high concentration of dioxins and PCBs in their muscles, which was shown in our previous study. Sum of PCDD/PCDF/DL-PCBs was in the range from 0.96 to 10.80 pg WHO-TEQg^{-1} fat, and NDL-PCBs from 1.57 to 20.47 ng g^{-1} fat. The aim of the present study was to assess the concentration of selected POPs in the muscles of farmed deer and compared them with muscles of free-living deer. The content of 52 toxic congeners of PCDD/Fs, PBDD/Fs, DL-PCBs and NDL-PCBs, and PBDEs in samples of farmed deer muscles (*Capreolus capreolus* L and Cervus *elaphus* L, *Dama dama* L) was determined. The gold standard in POPs analysis, HRGC/HRMS method with isotope dilution technique (IDMS) was used. The levels of PCDD/Fs and PCBs, PBDD/Fs as well as PBDEs, were much lower than tissue levels of free-living deer. Sum of PCDD/PCDF/DL-PCBs was in the range from 0.19 to 3.39 pg WHO-TEQ g^{-1} fat, PBDD/PBDF levels were from 0.04 to 0.22 WHO-TEQ g^{-1} fat, and NDL-PCBs from 0.06 to 24.26 ng g^{-1} fat. All these results were definitely lower than the maximum admissible levels for the livestock meat (1259/2011/EU). The PBDE congener concentrations were in the range of 0.27 to 0.94 ng g^{-1} fat. In the case of PBDEs and brominated dioxins and furans, there is no limit set by the European Union. The results indicate that farmed deer accumulate much fewer pollutants in their tissues than wild animals, which often live in a polluted environment. Chlorinated pollutants are bioaccumulated in higher concentrations than brominated ones. Because venison is a fairly popular source of food for humans, the meat of wild animals may pose some health risk to its frequent consumers. The meat from farmed deer is safer.

P03-005
Study of the impact of gold nanoparticles on representative of aquatic ecosystem

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Nanoparticles (NPs) have diverse applications in electronics, medical devices and cosmetics. With their increasing production and growing usage, a rise of concentrations of NPs in the environment is expected. Therefore, investigating the potential aquatic toxicity of nanomaterials has become an important issue.

To better understand the potential ecotoxicological impact of gold nanoparticles (AuNPs) released into freshwater environment, the *Daphnia magna* 48-h immobilization test was used. The experiment was carried out on the basis of OECD guideline 202 (ČSN EN ISO 6341). The toxicities of three suspensions of AuNPs (PVP 11.5 d.nm, PVP 15.2 d.nm, citrate 1.1 d.nm) and ionic form of gold were assessed. The particle suspensions used in the toxicity tests were characterized by Transmission Electron Microscopy and by Dynamic Light Scattering. Concentrations were chosen on the basis of the range finding test. The swimming behavior of *D. magna* and visible uptake of AuNPs were investigated and compared as well.

D. magna showed the highest sensitivity to $AuCl_3$ (48hEC50 = 0.591 $mg.l^{-1}$) and citrate form of gold (48hEC50 = 78.919 $mg.l^{-1}$). All the gold species in this study caused abnormal swimming by the *D. magna*. The gold nanoparticles were ingested by the *D. magna* and accumulated in the gut.

The ecotoxicity of AuNPs varies considerably according to the particle sizes. The smaller sized NPs were more toxic compare to larger ones. These organisms are good bioindicators for assessing the acute toxicity of environmental contaminants.

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References are available within the author.

P03-006
Reproductive and developmental toxicity of tebuconazole to Caenorhabditis elegans

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Tebuconazole (TEB) is a triazole internal absorption fungicide, widely used in agriculture, and there are a large number of residues in

crops, water and soil. In this study, the alternative model Caenorhabditis elegans (C. elegans) was used to estimate the reproductive and developmental toxicity of TEB at five concentrations [0 control (M9 solution), 0.01μg/L, 0.1μg/L, 1μg/L and 10μg/L] according to the LC50 test results (1.87mg/L). In order to determine the reproductive toxicity of TEB, L4 C. elegans larvae was exposed to TEB for 24, 48 and 72h. After 24h exposure, a significant decrease in brood size was detected in nematodes exposed to 10ug/L TEB($P<0.01$) and showed dose-dependent reduction. After 72h exposure, the brood size in each exposed group reduced dose-dependently compared with the control group ($P<0.01$).However, there was no difference in generation time between control and the exposed. Moreover, after 24h exposure, the fluorescence intensity of distal-tip cells (DTCs) in each exposure group significantly decreased compared with the control ($P<0.01$) and showed a dose-dependent reduction. After 72h exposure, the fluorescence intensity of DTCs decreased significantly compared with the 24h and 48h exposure group, which showed a significant dose-dependently reduction. Meanwhile, the oocyte numbers were time-and dose-dependently reduced when exposed to TEB ($P<0.01$). In the developmental toxicity assessment, parent generation (P0) was exposed to TEB for 24h using L4 C.elegans larvae, but F1 generation was not exposed to TEB. The body length of parent and F1 generation was dose-dependently reduced ($P<0.01$). And the length of F1 generation was shorten than that of parent. Meanwhile, the body width of P0 showed dose-dependently decreased with the increasing of TEB exposure, while the body width of F1 showed dose-dependently increased. The body width of F1 generation is wider than that of its parent generation at the same dose level. The results show that TEB can produce reproductive toxicity with reduced brood size, oocyte number and fluorescence intensity of DTCs and intergenerational developmental-toxicity with shortened body length and biphasic body width. Brood size, oocyte number, DTCs, body length and body width can be sensitive indicators of reproductive and developmental toxicity.

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P03-007
Comparison of the ecotoxicological effects of PPCPs on *Artemia franciscana* and *Aliivibrio fischeri* in automated and manual systems

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Pharmaceuticals and personal care products (PPCPs) represent a group of chemicals, that includes human and veterinary drugs, disinfectants and fragrances used in household substances and personal care (e.g. body cleaning product, sunscreens). The U.S. Environmental Protection Agency (EPA), has categorized PPCPs as emerging organic pollutants; few data are available to define the impact on the environment and human health, such as endocrine-disrupting effects, teratogenicity and carcinogenicity. Previous studies have classified PPCPs as pseudo-persistent, because these chemicals are continuously introduced in the ecosystem. Coastal areas and aquatic environments are the most threatened. The interest on the adverse effects of these substances on the environment has increased in the last years, as well as the development of innovative early warning systems able to detect their presence. The purpose of this study was to compare the ecotoxicological effects of several PPCPs on two different organisms: *Artemia franciscana*, a brine shrimp, and *Aliivibrio fischeri* (NRRL-B-11177), a luminescent bacterium. Aiming at this, ten ingredients normally used in sunscreens were tested, including conservation agents, solvents, surfactants and chelators. *A. franciscana*-based ARTOXKIT M (Microbiotests, Belgium) was used as a preliminary assay to measure the mortality effect at 24 h (APAT-IRSA 8060). The fully automated analyzer Easychem® Tox Lab and the manual system Microtox® M500 were used to evaluate the inhibitory effects on the bacterial light emission at 5, 15 and 30 minutes (EC50) (ISO 11348-3:2009). A Wilcoxon signed-rank test was performed to evaluate EC50 variations associated with PPCPs tested in different systems. Despite shortest time of contact, *A. fischeri* resulted to be more sensitive to PCBBs than *A. franciscana*. For all the tested chemicals, the EC50 values of *A. fischeri*, were observed to be lower than *A. franciscana* mortality concentration ranges. Importantly, it was observed that automated and manual methods for performing the *A. fischeri* test gave comparable results at 15 and 30 minutes ($p > 0.05$).

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P03-008
A novel sensor for behavioural toxicity testing with freshwater and marine bivalves: preliminary results

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Various types of sensors have been developed to detect valve gape of molluscs as behavioural endpoint of toxicity assays. Electromagnetic inductance instruments, video monitoring techniques, Hall sensors, optical fibre, laser detector and other mechanical systems have been investigated as detection technologies of valve movements. This study aimed to develop and test a novel distance measuring system, based on an infrared proximity sensor and an open source hardware platform. It is able to measure gapes remotely, with high resolution (> 0.1 mm) and without any disturbing attachments on different sizes of shells (height > 2 mm). The valve-gaping behaviour of freshwater and marine adult bivalves (*Anodonta cygnea*, *Unio mancus*, *Sinanodonta woodiana*, *Pisidium casertanum*, *Chamelea gallina*, *Venus verrucosa*, *Callista chione*, *Ruditapes philippinarum*) have been registered every 30 seconds by using the novel sensor. Apart from *P. casertanum*, all molluscs have been exposed for 72 hours at 0,49 mg/L of hexavalent chromium [Cr(VI)], as reference toxicant in ecotoxicological studies. Five ranges of valve gapes (VG) have been established for the

frequency calculation of results: VG ≤ 20%, 21–40%, 41–60%, 61–80% and ≥ 81%. Both in control and exposed mollusc groups, the average amount of time percentage spent in each VG range has been evaluated per species. A descriptive analysis using the average values has been conducted. Overall, Cr(VI) produced a general reduction of VGs in all tested species. For *A. cygnea*, *V. verrucosa* and *R. philippinarum*, there were little changes between exposed molluscs and controls. On the other hand, the behaviours were particularly different for *S. woodiana* and *C. gallina*. For both species, the exposed groups spent more time in the VG ≤ 20%, while the controls respectively in VG = 61–80% and VG ≥ 81%. Concerning *P. casertanum*, two individuals were observed directly on the field for 12 days. They showed dissimilar behaviours, one individual registered a flapping graph with prevalence in VG ≤ 20%. The pattern of the other one was almost always fixed at VG ≥ 81% without any valve movements, probably due to the presence of seven juveniles ready to be released. Generally, each species showed behavioural rhythm of valve movements to be investigated. In addition to VG that is a sensitive parameter to Cr(VI), more registrations and analyses are necessary to elaborate other behavioural parameters of tested species useful for increasingly sensitive toxicity testing.

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P03-009
Perchlorate toxicity in organisms from different trophic levels

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Perchlorate (ClO_4^-) is an emerging inorganic pollutant widely distributed in the environment, derived from natural and anthropogenic sources. It is considered a potent endocrine disruptor that affect the iodine fixation by the thyroid gland, impacting metabolism, reproduction and development in the biota. However, there are few reports of its ecotoxicological impact on wildlife. The objective of this work was to evaluate the adverse effects of perchlorate exposure on different models, HEK, N2a and 3T3 cell lines, *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Eisenia fetida*. Perchlorate exhibited similar toxicity against tested cell lines, with LC_{50} values of 19, 15 and 19 mM for HEK, N2a and 3T3, respectively. In *V. fischeri* the toxicity, measured as reduction of bioluminescence, was considerably lower (EC_{50} = 715 mM). The growth of the freshwater algae *P. subcapitata* was impaired by perchlorate with an LC_{50} value of 72 mM, and the toxic response on *D. magna* was greater (LC_{50} = 5 mM). Finally, in the earthworm *E. fetida*, perchlorate induced avoidance behavior, weight loss, decrease egg production and hatchling, as well as morphological and histopathological effects, such as malformations, dwarfism and necrosis, displaying an LC_{50} of 56 mM in soil. In conclusion, exposure to perchlorate has a significant impact on the survival, development and reproduction of organisms from different trophic levels.

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P03-010
A combined *in vitro*/risk assessment approach to identifying aquatic environmental risks of cosmetic products: A case study of UV filters

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Personal care products, such as sunscreen UV filters, can profound impact aquatic ecosystems, raising concerns about their sustainability. Therefore, enhancing and promoting eco-friendly products in cosmetic industry requires robust methods for assessing the environmental impacts. *In vitro* screening assays have been proposed as key components of a future testing paradigm for mechanism-base regulatory ecotoxicology; and improvements in predicting ecotoxicity risks of products can be achieved by combining cell-based models (*in vitro*) with risk assessment tools. Grupo Boticário has developed an environmental risk assessment tool, named IARA™, which integrates data of bioaccumulation, biodegradation, acute toxicity and PEC/PNEC ratio, providing risk quotients for ranking cosmetic ingredients according to their levels of environmental risk (high-moderate-low). However, the quality of toxicity data available poses a challenge to the reliability of risk assessment. Aimed to improve the accuracy in predicting environmental risks of cosmetics ingredients, we have developed a combined *in vitro*/risk assessment approach based on *in vitro* fish cytotoxicity testing (MTT assay, ZFL cell line – Danio rerio normal liver) and IARA™ matrix. Cytotoxicity data of eight UV filters (ethylhexyl methoxycinnamate, homosalate, diethylamino hydroxybenzoyl hexyl benzoate, titanium dioxide, phenylbenzimidazole sulfonic acid, ethylhexyl salicylate, zinc oxide, polysilicone-15) were used to verify the efficacy of our approach in ranking the aquatic toxicity risks of sunscreen UV filters. High to low cytotoxicity was observed to ZFL cells exposed (96-well plates, monolayer cells, 24 hours) to all tested UV filters (0.01–100 g/L). Applying these *in vitro* cytotoxicity data into IARA™ matrix improved the prediction of environmental risks of UV filters compared to risks estimated by database data-driven IARA™, demonstrating the relevance of controlling

the quality of toxicity data. In summary, the combined *in vitro*/risk assessment approach herein proposed can drive the safer aquatic environmental use of sunscreens, framing them into the sustainable context.

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P03-011
Effect of the electrochemical advanced oxidation process on the ecotoxicity of a solution composed of norfloxacin in presence of sodium sulphate

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The presence of antibiotic compounds in surface waters is an emerging environmental issue since many of these substances are not biodegradable, toxic and capable of accumulating in aquatic organisms. In this sense, numerous researches have promoted the advanced electrochemical oxidation as a promising alternative technique to treat wastewaters containing toxic and refractory organic pollutants.

Ecotoxicological bioassays based on Lactuca Sativa seeds and bioluminescent bacterium have been carried out to analyse the ecotoxicity of a contaminated solution composed of norfloxacin (NOR), an antibiotic widely used, in sodium sulphate solution, as supporting electrolyte before and after the treatment by an electrochemical advanced oxidation process. The effect of some process variables (pH, anode material, reactor configuration, applied current, supporting electrolyte concentration...) on the toxicity evolution is evaluated.

The toxicity limit of the effluents contaminated with norfloxacin in the presence of sodium sulphate has been determined using statistical tools. The EC_{50} value obtained with Lactuca sativa seeds for norfloxacin is 336 $mg{\cdot}L^{-1}$ and this value decreases if sodium sulphate is added to the solution. However no synergy is observed.

All the samples treated by electrochemical oxidation are more toxic than the starting solutions. This is mainly due to the formation of persulphates due to the oxidation of the sulphates present in the solution. Since the boron-doped diamond anode (BDD) is able to produce more persulphates than the ceramic anodes, samples treated using the BDD anode present higher toxicity values.

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P03-012
Evaluation of the toxic effects of livestock drinking water by *in vitro* studies

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Introduction: Water is a necessary component of human and animal life, but often the role of water in livestock feeding is underestimated. In European context the drinking water quality standards for human consumption are regulated by the Directive 98/83 EC, while in the zootechnical sector no specific rules concerning the qualitative and health characteristics of water are defined.

Many chemical contaminants have been identified in surface drinking water deriving from industrial, agricultural practices, pharmaceuticals, detergents, personal care products, and disinfection treatments.

The aim of present study is to evaluate toxicological effects of livestock drinking water using *in vitro* studies.

Materials and Methods: For sampling activity of drinking water, six sites with different characteristics, have been selected throughout the Piedmont region (north-western Italy). The samples were analysed to assess:

- evaluation of presence of substances with estrogenic activity by ER CALUX
- identification of viral agents (Hepatitis A, E and Norovirus)
- identification of potential bacterial agent
- determination of presence of microcystin and cylindrospermopsin
- concentrations of trace metals

Results: Results show no positivity for microcystin, cylindrospermopsin, viral and bacterial agents as expected in drinking water.

The metals didn't exceed regulatory levels; the different levels of magnesium, iron, copper and zinc detected in some samples reflected the characteristic of water related to the different zones of collection.

No estrogenic activity was detected by ER CALUX bioassay demonstrating the absence of substances able to induce endocrine perturbation.

Discussion: Results show the healthiness of the livestock drinking water tested and demonstrate the low risk for food producing animals and indirectly the poor risks of contamination for final consumers.

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P03-013
Assessing the impact of the lead waste on environmental objects

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Production wastes consider as the toxic factor of the habitat forming the risk of chemical pollution of water, the soil and air and, as a result, having potential toxicity for live organisms including humans.

The purpose of the work is to determine the degree of toxicity of lead sludge for biological objects of the environment.

Methods. Learned lead sludge contain cadmium, lead and arsenic. Studies of Ecotoxicity of lead sludge on *Tetrahymena pyriformis W.* were carried out in acute, subacute and chronic experiments. The toxicity of lead sludge in the *Eisenia foetida* test model was studied in an acute experiment. Phytotoxicity of lead sludge was studied on seeds of higher plants.

Results. The toxicity on *Tetrahymena pyriformis* is caused by the impact on processes of cellular division and growth among generations leading to decrease in population decline. Observed decrease in vital activity of populations of *Tetrahymena pyriformis* during the whole period of observation, death of organisms, decrease in stability of the cell membranes of ciliates to the adverse effects of the environment. No mutagenic activity.

After a seven-day exposure of lead sludge in the test model of *Eisenia foetida*, the mean lethal concentration LC_{50} =15,36 (7,39–31,93) g/kg was established. Changes in the behavioral reactions of animals in the form of reduced motor activity, reducing the rate being burying in the ground.

Test results on phytotoxicity. Noted the effect of inhibition of the development of the roots of seedlings of cucumbers equal to 40,31%, radish – 32,28%, oats – 56,02%, which exceeds the threshold of phytotoxicity, equal to 20%, for each crop of seed, and indicates the presence of phytotoxic action of waste. The most sensitive were oat seeds – the average effective dilution (ER_{50}) was 1,8.

Thus, it is possible to characterize the lead sludge waste as having a significant adverse effect on the biotic elements of the environment.

P03-014
Ecological risk assessment of pesticides in groundwater in Saiss plain of Morocco

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In this study a pesticide monitoring survey took place in 21 wells of Saiss plain in Morocco in September 2017 and January 2018 to explore 4 different scenarios of risk. For this purpose, passive samplers were deployed between 14 to 20 days in 21 traditional wells from the study area and 28 pesticide compounds were analyzed including fungicides, insecticides, herbicides and their metabolites.

The Scenarios 1 and 2 were used to depict the risk of failing to meet the good groundwater chemical status as defined by WHO (The measured environmental concentrations (i.e. the mean MECs) of each pesticide per sampling site over the survey period were used to assess the first scenario and the max MECs were used to assess the second scenario). The Scenarios 3 and 4 were used to assess for the first time the ecological risk in groundwater bodies, defined as the likelihood of hazard to the groundwater communities stably residing in the 21 wells that may be affected by pesticide contamination (the mean MECs (Scenario 3) and the max MECs (Scenario 4)). The ecological risk was assessed through a new procedure called GERAp (Groundwater Ecological Risk Assessment due to pesticides). The main results of this study highlighted that: 1) the Scenario 1 provided information of little use for risk managers; 2) chlorothalonil, dicofol, chlorpyrifos methyl, bifenthrine were the compounds most frequently detected using the highest concentrations measured in the monitoring period; 3) a high ecological risk were found in 6 wells of Saiss plain due to 13 insecticides (scenario 3); 4) some pesticides that were banned in Morocco should be kept monitored in the next surveys because they showed a persistent occurrence in some wells such as DDE, DDT and endosulfan; 5) DDE, Diazinon and Permethrine are expected to damage groundwater communities at concentrations that are lower than the present legal limits (scenario 4).

P03-015
Comparing approaches to acute fish toxicity testing across sectors and regions to identify opportunities to advance the 3Rs

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Submitted on behalf of the NC3Rs ecotoxicology working group.

The acute fish toxicity test has been a mainstay of ecotoxicology for over 30 years. The aim of the test is to establish the concentration of test material which causes the death of 50% of the exposed fish (LC_{50}). As such, there is the potential for significant suffering over the 96 hours of the test. Assessment of the potential for acute fish toxicity is a core requirement under many regulatory frameworks across the world. Whilst in many sectors and regions there is still a requirement to generate *in vivo* test data, alternative appraoches to assess this endpoint are increasingly being accepted. The UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) ecotoxicology working group includes experts from government agencies, academia and a range of industries. The working group has compared approaches accepted across sectors and regions to identify differences and where there is scope to further apply the 3Rs.

Following this comparison, the working group have identified a number of aspects of acute fish toxicity testing requirements where there may be potential for refinement, reduction or replacement of *in vivo* studies, and where approaches taken in one region or sector could be more widely adopted. These include when it may be necessary to generate data in multiple species or for products as well as individual active ingredients or whether data can be extrapolated to reduce the number of studies conducted, exposure considerations (for example whether chronic toxicity data may be more relevant) and use of alternative approaches such as quantitative structure-activity relationship models. These factors will be discussed and opportunities for sharing experience in assessing acute toxicity across sectors, regions and species (including fish, birds and mammals) highlighted.

P03-016
Pharma pollution as a selective pressure

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Pollution may act as a selective pressure. A great importance to define how pollution could drive selective pressure should be given to the kind of pollution: acute or chronic pollution. When the toxicological "death dilemma" is applied to selection it is possible to conjecture: i. if all individuals in a population share the same threshold above which death occurs stochastically, acute pollution can not produce significant selection into a population; ii. if the tolerance threshold is distributed among the individuals in a population and its exceedance leads to certain death, acute pollution can cause genetic drift of alleles. Moreover, chronic pollution may reduce the number of genetic variants in a population and select only those could give a high fitness in individuals.

One other aspect to take into consideration could be the generation time of the species considered, due to high generation turnover could fix faster the genetic variants than species have low turnover.

Between 1900 and 2000, the increase in world population was three times greater than during the entire previous history of humanity, an increase from 1.5 to 6.1 billion in just 100 years. In order to satisfy the therapeutic needs of the human population, the increasing production of pharmaceuticals and the subsequent presence of pharmaceuticals in the environment are unavoidable. Patient use of medicines is the principal pathway by which pharmaceuticals (prescription and over the counter) find their way into the aquatic environment. Typically, a fraction of the medicines taken by patients is excreted and enters waterways. To a lesser extent, pharmaceuticals can enter the environment through improper disposal of medicines and from manufacturing discharges.

The selective pressure of chronic exposure to pollutants into environment can positively select, for example, more efficient detoxification pathways.

In the last years particular concerns have been expressed on compounds that mimic hormones and can disrupt reproduction and development in animals. It can be hypothesized that the pressure of chronic exposure to exogenous hormones into environment could lead, for example, to the reduction of endogenous production of spe-

cific hormones or reduction of specific membrane/nuclear receptors. But what happen if the exogenous stimulus, i.e. pollution from medicinal hormones, decrease abruptly? Taking into account the short time period and the physiological importance of the endocrine system compared to the detoxification pathways, paradoxically it can be hypothesized that the sudden loss of this selective pressure can be considered itself a selective pressure opposed to the previous one. Such selective pressure will be directly proportional to the selective pressure placed by pollution and could cause effects comparable to those of acute pollution.

In conclusion, the measures aimed at remedying situations of chronic pollution should be carefully evaluated and modulated over time.

P04 – Environmental toxicology

P04-001

This abstract has been withdrawn.

P04-002
PM2.5 exposure impairs sperm quality through testicular damage dependent on NALP3 inflammasome in mouse

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Exposure to ambient fine particular matter (PM2.5) has been clearly associated with male reproductive disorders. However, very limited toxicological studies were carried out to investigate the potential mechanism underlying the PM2.5-induced sperm quality decline. In the present study, we established a real time whole-body PM2.5 exposure mouse model to investigate the effects of PM2.5 on sperm quality and its potential mechanisms. Sixty male C57BL/6 mice were randomly subjected to three groups: filtered air group, unfiltered air group and concentrated air group. Half of the mice from each group were sacrificed for study when the exposure duration accumulated to 8 weeks and the rest of the mice were sacrificed when exposed for 16 weeks. Our results suggested that PM2.5 exposure could induce significant increase in circulating white blood cells and inflammation in lungs. PM2.5 exposure induced apparently DNA damages and histopathologic changes in testis. There was significantly decreased sperm density of mice, which was paralleled with the down-regulated testosterone levels in testes tissue of mice after exposure to PM2.5 for 16 weeks. The numbers of motile sperms were decreased and sperms with abnormal morphology were increased after PM2.5 exposure in a time-depended and dose-depended manner. PM2.5 exposure significantly increased the expression of the major components of the NACHT, LRR and PYD domains-containing protein3 (NALP3) inflammasome, accompanied by the increased expression of miR-183/96/182 targeting FOXO1 in testes. The present data demonstrated that sperm quality decline induced by PM2.5 could be partly explained by the inflammatory reaction in testis which was as a consequence of systemic inflammation. The molecular mechanism was depended on the activation of NALP3 inflammasome accompanied by miR-183/96/182 targeting FOXO1 in testes.

P04-003
A case report of unknowing ingestion of *Brugmansia Suaveolens* Leaves presenting with delirium in Sri Lanka

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Background: Self Poisoning carries a high mortality and morbidity in Sri Lanka, and has a case fatality ratio of 9% but is under-evaluated. Poisoning cases of *Datura* or *Brugmansia,* which come under the *Solanacea* family, in other countries were almost always due to ingestion of seeds.It contains alkaloids like scopolamine, atropine and hyoscyamine which cause an anticholinergic toxindrome by blocking the muscarinic acetylcholine receptors of the nervous system. There have been a very few reported cases of accidental ingestion of *Brugmansia* seeds among children, but hardly any reported cases of *Brugmansia* leaf poisoning among adults in Sri Lanka.

Case presentation: A 60 year old female presented with acute delirium, and agitation. She had ingested a kanji drink made from leaves from her garden prior to the onset of symptoms, until which she was previously well. She had urinary retention, mydriasis, and sinus tachycardia. She was managed supportively with activated charcoal, hydration, and 1.5mg IV Midazolam to calm the patient. The delirium completely resolved within 15 hours. Her CT brain was normal and urine for toxicology was negative for illicit drug substances. After regaining consciousness she admitted that she made the kanji drink containing an unknown plants leaves from her garden, which we identified as *Brugmansia suaveolens, w*ith the help of a native medicine physician and specialist in botany. she did not require the antidote physostigmine and recovered fully.

Conclusion: Although seeds are the most toxic plant part in most cases of *Brugmansia* poisoning, leaves also have a significant degree of toxicity. It is important that medical professionals promptly recognize the features of anticholinergic syndrome and have a high index to suspect *Brugmansia* poisoning and start prompt treatment to improve outcome. Further research can be recommended on the degree of awareness of toxicity of toxic plants in the Sri Lankan community, and measures must be taken to improve awareness among the general public of toxic effects of plants and recognizing such plants by their appearance in order to prevent toxicities and fatalities.

P04-004
Establishment of an animal model of allergic inflammation caused by atmospheric dust

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Purpose: Atmospheric dust is strongly suspected of contributing to upper respiratory allergy symptoms and has been called the Japanese national disease. However, to the best of our knowledge, there has been no replicate study on the atmospheric dust-specific immune responses. The establishment of an animal model is the current bottleneck in transitioning from epidemiological studies to basic studies. Therefore, our study aimed to investigate the atmospheric dust-specific inflammatory responses in the footpad of adjuvant-sensitised mice by measuring cytokine/chemokine production.

Materials and methods: Atmospheric dust was collected on the filters of a central ventilation system in a building in the centre of the Beijing city (CRM No. 28: Japan National Institute for Environmental Studies). Male BALB/c mice were used in all experiments. 500 μg of atmospheric dust was gently mixed with 50 μl of PBS and 2% of Tween 80. This mixture was emulsified using an equal volume of complete Freund's adjuvant (CFA) or incomplete FA (IFA). 100 μl of this emulsion

was subcutaneously injected at the base of the tail with CFA and IFA, on days 0 and 14, respectively. Control mice were injected with only CFA or IFA. On day 28, 100 µg of atmospheric dust in 10 µl of PBS and 2% Tween 80 was subcutaneously injected into the footpad, and footpad swelling was measured using a dial gauge calliper at 24, 48 and 72 h after injection. Cytokine/chemokine mRNA production was then measured by real-time polymerase chain reaction, and the classification and ratio of lymphocytes was determined for the isolated footpad and nearby popliteal lymph node by flow cytometric data.

Results: At 24 h after subcutaneous injections, injection of atmospheric dust alone in mice footpads with atmospheric dust sensitisation caused them to be enlarged to approximately 1.3 times the size of those without atmospheric sensitisation. We also confirmed increases in cytokine mRNA expression of at least seven types in the footpads and increases in CD3+, CD4+ or CD8+ T lymphocytes and CD3− and CD19+ B lymphocytes. These results strongly suggested that atmospheric dust provokes an allergic immune response.

P04-005
Biological response modulation of human reconstituted airway epithelium repeatedly exposed to $PM_{0.3\text{-}2.5}$

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Over the past decades, air pollution has risen dramatically worldwide. Many epidemiological studies have shown associations between respiratory and cardiovascular diseases and airborne pollutants, including particulate matter (PM) playing a major role in the human health disorders.

In order to understand the PM's effects on the biological activity, the present study evaluates the impact of two types of fine particles ($PM_{0.3\text{-}2.5}$) with similar aerodynamic diameter but different chemical compositions (polycyclic aromatic hydrocarbons, heavy metals …): PM_{traf} from traffic zone and PM_{ind} from industrial zone.

To mimic the real human exposure to $PM_{0.3\text{-}2.5}$ an innovative 3D *in vitro* model was used: a human airway epithelium reconstituted, nasal origin, co-cultured with human airway fibroblast (MucilAir™-HF, Epithelix®).

The epithelia were exposed to PM at 45 or 90µg.cm^2 twice a week for two consecutive weeks. 48h after each exposure, the culture medium on basal side was collected and inflammatory response was assessed by ELISA assay. At the end of each week, the membrane integrity was evaluated using the TEER (transepithelial electrical resistance) measurement and part of the epithelia was sacrificed for histological analysis and gene expression assessment. RNA extraction was conducted in order to analyse the PM's influence on inflammatory and oxidative stress gene expression by RTqPCR.

Our results showed no loss of integrity whatever the PM tested, while a significant increase of the inflammatory response appears. IL-8, IL-6 and GM-CSF secretions were higher after PM_{traf} exposure compared to PM_{ind}, probably due to its chemical composition. In addition, the cytokine secretions were the highest during the first week of exposure, certainly due to the adaptation capacity of the 3D-model. Besides, some modulation of gene expression appears after PM exposure: increase of inflammatory biomarkers (IL-8, IL-6 and GM-CSF) and decrease of oxidative stress biomarkers (NOS and SOD).

The major outcome of this study is the demonstration that, 1/the origin of particles, influencing their chemical composition, acts in the development of respiratory disorders, and 2/the reconstituted human epithelium is adapted to evaluate the impact of repeated exposures to environmental pollutants.

P04-006
Toxicity of nonylphenol and nonylphenol ethoxylate in *Caenorhabditis elegans*

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Nonylphenol (NP) and its ethoxylated isomers (NPEOs, e.g. NP-9) are compounds used as raw materials for many industrial processes. These chemicals and their metabolites are commonly found in environmental matrices. The objective of this work was to evaluate physiological and neurotoxic effects of NP and NP-9 in *Caenorhabditis elegans*. Lethality and locomotion were assessed in larval stage L4, whereas growth was carried using the L1 stage of the wild strain N2, exposing worms to different concentrations of NP and NP-9. GFP transgenic strains *mtl-2*, *gst-1*, *gpx-4*, *gpx-6*, *sod-4*, *hsp-70* and *hsp-4* were employed to evaluate the activation of signaling pathways related to cellular stress, whereas RT-qPCR was utilized to measure mRNA expression for different genes associated with neurotoxicity (*unc-30*, *unc-25*, *unc-49*, *dop-3*, *dat-1*, *mgl-1*, *eat-4*, *glt-3*, *glt*-6) and oxidative stress (*mtl*-1 and *mtl*-2). The nematode lethality was concentration-dependent, with 24h-LC_{50} values of 122 and 3215 µM for NP and NP-9, is the value of the LC_{50} for NP-9 respectively. At non-lethal concentrations, locomotion and body length were significantly reduced by both xenobiotics, although NP was always more potent. GFP activity suggests NP and NP-9 activate ROS-mediated pathways, and in the case of glutathione peroxidase, the concentration-response curve for NP was bimodal, a typical endocrine disruption response. Nonylphenol significantly inhibited the transcription of several genes related to neurotoxicity, such as GABA, glutamate and dopamine; whereas NP-9 down-regulated GABA, glutamate and stress oxidative-related genes, although dopamine and glutamate displayed a non-monotonic concentration-response curve. In summary, NP and NP-9 induced neurotoxic responses in *C. elegans* through mechanisms that involve ROS and disturbances of the GABA, glutamate and dopamine pathways, effects observed at environmentally-relevant concentrations. Colciencias-UniCartagena (Convocation No. 727 of 2015 Res. 513, July 2015).

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P04-007
The association of exposure to fine airborne particulate matter with cardiovascular diseases in Beirut Lebanon

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Ambient air pollution represents a worldwide environmental risk factor for cardiovascular diseases (CVD). In Lebanon, it was estimated that annual median concentrations of $PM_{2.5}$ exceed the recommended levels set by the WHO air quality guidelines. One study reported a potential for association between exposure to ambient air pollution and CVD in Lebanon. However, and due to the lack of air monitoring stations, the investigators used individual questionnaires about outdoor air pollution as proxy instead of quantitative air pollutant measurements. No studies in Lebanon applied a modeled spatial distribution of air pollutants to investigate this association. This study hence aimed to determine the relationship between the level of exposure to airborne particulate matter and coronary artery disease (CAD) in the city of Beirut using a modeled spatial distribution of $PM_{2.5}$.

This study builds on a cohort of subjects living in the city of Beirut who were recruited between March 2014 and December 2017 under the Vascular Medicine Program of the American University of Beirut Medical Center. Data were collected for demographics, smoking habits, comorbidities, and exact place of residence. In addition, the coronaries of all participants were visualized by cardiac catheterization. In parallel, a spatial distribution for a representative meteorological situation in Beirut was obtained by simulating the transport of $PM_{2.5,}$ based on an accurate emissions inventory of the diesel generators, using the physically-based mesoscale and micro-scale dispersion model system GRAMM-GRAL. The modeled distribution of $PM_{2.5}$ was used to determine the level of $PM_{2.5}$ exposure at the participants' place of residence.

Preliminary results from 340 subjects revealed that obstructive CAD (defined as 50% or more obstruction in at least one of the coronaries) was significantly associated with the levels of $PM_{2.5}$ among smokers (OR 1.052, 95% CI (1.016–1.089)) per 1 $\mu g.m^{-3}$ rise in $PM_{2.5}$ concentration. These data suggest that ambient air pollution represents an additive risk to smoking on CVD. Further analysis is ongoing to include comorbidities and associations with cardiac index. This knowledge is a starting point for assessing the impact of $PM_{2.5}$ on CVD and potentially driving public health interventions to reduce air pollution.

P04-008
Environmental pollution: a 3D skin model to assess protective properties of cosmetic ingredients

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Ambient air pollution has become a major risk factor of health damage, as world population exposure increases. Consequences of pollutants on skin physiology are extensively reviewed, however some mechanisms of penetration and action remain unclear.

Our previous publication [Quantin *et al*., 2018] showed an impact of pollutants on inflammation and induction of cytochromes P450 through the AhR pathway in keratinocytes. This study aims at identifying exposure scenarios that mimic real conditions, resulting in an increase of particles absorption. We determined cell viability and biomarkers of skin exposed to pollution in a 3D skin model to highlight the potential protective capacities of skincare ingredients.

A 3D reconstructed human epidermis (RhE) VitroDerm (VD), internally validated and used in routine for skin irritation assessment, was exposed to a standardized mixture of pollutants, Urban Dust SRM 1649b for 48 hours at reactive doses. To validate the use of VD in the specific context of pollution, several endpoints identified in an internal review of the literature were investigated: cell viability by MTT assay and skin barrier function through histological staining of filaggrin. Then, cells were exposed to different conditions to determine a potential enhancing effect in Urban Dust absorption. Finally, protective effect of cosmetic ingredients was assessed.

The VD model showed promising response to pollution as we observed a decrease of cell viability after exposure to pollutants and a change in the identified biomarkers. Our model allows to assess the enhancing capacity of environmental factors, therefore increasing pollutants skin passage. These experimental conditions previously determined were used to observe the effect of pollution on skin barrier function through filaggrin expression as well as the efficacy of protective ingredients with the VD model.

In conclusion, pollution impacts skin physiology at different levels, altering skin barrier function through the expression of filaggrin. Moreover, pollutants passage can be enhanced by some environmental conditions. Further studies will allow us to determine whether exposure to other external factors, such as ultraviolet (UV) or cigarette smoke, could impact the skin barrier function.

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P04-009
Effect of ferulic acid on airway damage and the change of TGF-β1/Smads signaling pathway induced by atmospheric PM2.5 in asthmatic rats

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Purpose: To explore the protective effect of Ferulic Acid (FA) on the respiratory tract injury and the change of TGF-β1/Smads signaling pathway induced by $PM_{2.5}$ in asthmatic rats.

Method: SD rats were randomly divided into the following seven groups (n = 8 for each group): control group, model (OVA, ovalbumin) group, $PM_{2.5}$ (OVA+6.0 mg/kg$PM_{2.5}$) group, FA (19.4) group (OVA+6.0 mg/kg $PM_{2.5}$+19.4mg/kg FA), FA (38.8) group (OVA+6.0 mg/kg $PM_{2.5}$+38.8mg/kg FA), FA (77.6) group (OVA+6.0 mg/kg $PM_{2.5}$+77.6mg/kg FA) and positive control group (OVA+6.0 mg/kg $PM_{2.5}$+dexametha-

sone). OVA-sensitized rats were used to build the asthmatic rat models. Rats were exposed to OVA for sensitization and challenge, and rats in the control group were sensitized and challenged only using saline. From the first day of the experiment, different treatments were given to rats with gavage capacity of 5ml/kg for 28 days continuously. At 29th day, rats were hocused with 5mg/kg chloral hydrate, exposed to $PM_{2.5}$ by tracheal instillation for three times, then ultrasonic atomizing inhalation for 30 min for 5 consecutive days, The femoral artery blood and the lung tissues of the rats were collected respectively. The activities of superoxide dismutase(SOD), methane dicarboxylic aldehyde(MDA) in serum were detected by colorimetric method, The contents of TGF-β1, Smad3, Smad2 and Smad7 in lung tissue were detected by ELISA. The pathology of lung tissues was measured by HE staining.

Results: Compared with $PM_{2.5}$ group, in the FA (19.4) group and FA(38.8) group, the contents of MDA in serum were significantly decreased (*p<0.05*) and the contents of TGF-β1, Smad2 and Smad3 in lung tissue were obviously decreased (*p<0.01*) and SOD activity in serum and Smad7 content in lung tissue were increased (*p<0.01* or *p<0.05*).The contents of MDA were significantly decreased at the FA (38.8) and FA (77.6) group. The positive group and the FA intervention group could improve the bronchial injury by reduction of inflammatory cell infiltration and mucus secretion on pathological sections.

Conclusion: A different concentrations of FA could effectively inhibit the effects of $PM_{2.5}$ on respiratory tract injuries in asthmatic rats. These protective effects may be achieved by inhibiting oxidative injuries and regulating TGF-β1/smads signaling pathway.

P04-010
Modelling of uptake, depuration and bioconcentration of arsenic, zinc and copper mixtures in juvenile milkfish (*Chanos chanos*)

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This study investigates the uptake, depuration and bioconcentration of arsenic (As), zinc (Zn) and copper (Cu) in juvenile milkfish, *Chanos chanos*. A 14-day exposure experiment was conducted to assess the time-integrated uptake and depuration of As, Zn and Cu by juvenile milkfish during exposure to each of these three chemicals and in various combinations, As-Zn, As-Cu and Zn-Cu. These three chemicals were chosen for the experiments, because they are found in culture ponds of juvenile milkfish in the blackfoot disease (BFD) area, southwest Taiwan. The uptake rate constant (k_1) and depuration rate constant (k_2) as well as the bioconcentration factor (BCF) of juvenile milkfish were analyzed based on a simple toxicokinetic model. The k_1 values for As, Zn and Cu in milkfish exposed to single chemicals were 846.49 ml g^{-1} d^{-1}, 682.32 ml g^{-1} d^{-1} and 13.08 ml g^{-1} d^{-1}, respectively, while k_2 values were 4.27 d^{-1}, 2.02 d^{-1} and 1.24 d^{-1}, respectively. The k_1 and k_2 values of the chemicals accumulated in milkfish were As > Zn > Cu when the fish were exposed to single chemicals. It indicates that uptake and depuration of As by milkfish occur more rapidly than the other two chemicals. The values of BCF of As, Zn and Cu were 198.42, 337.92 and 10.52, respectively. The results demonstrate that milkfish exhibited a greater ability for Zn accumulation than As and Cu. The decrease of k_1 (from 846.49 ml g^{-1} d^{-1} to 361.51 ml g^{-1} d^{-1}), k_2 (from 4.27 d^{-1} to 3.23 d^{-1}) and BCF (from 198.42 to 112.08) of As accumulation in milkfish was observed when Zn was added into the As stock. The values of k_1 and BCF were decreased (from 846.49 ml g^{-1} d^{-1} to 842.69 ml g^{-1} d^{-1} for k_1 and from 198.42 to 161.94 for BCF, respectively), while the value of k_2 was increased (from 4.27 d^{-1} to 5.20 d^{-1}) when the As stock was in combination with Cu additive. The k_1, k_2 and BCF values of Zn in milkfish were enhanced (from 682.32 ml g^{-1} d^{-1} to 841.93 ml g^{-1} d^{-1} for k_1, from 2.02 d^{-1} to 2.26 d^{-1} for k_2 and from 337.92 to 373.34 for BCF, respectively) when the fish were exposed to As-Zn mixture, while the BCF value was reduced (from 337.92 to 301.84) with higher values of k_1 (from 682.32 ml g^{-1} d^{-1} to 771.39 ml g^{-1} d^{-1}) and k_2 (from 2.02 d^{-1} to 2.56 d^{-1}) when exposed to Zn-Cu mixture. When milkfish were exposed to As-Cu mixture and Zn-Cu mixture, the values of k_1 of Cu in milkfish were reduced (from 13.08 ml g^{-1} d^{-1} to 8.94 ml g^{-1} d^{-1} for As additive and from 13.08 ml g^{-1} d^{-1} to 7.05 ml g^{-1} d^{-1} for Zn additive, respectively) and the values of BCF also decreased (from 10.52 to 8.01 for As additive and from 10.52 to 5.49 for Zn additive, respectively), while the value of k_2 for As additive was relatively lower (from 1.24 d^{-1} to 1.12 d^{-1}) and that for Zn additive was relatively higher (from 1.24 d^{-1} to 1.28 d^{-1}). The BCFs for the binary mixtures are lower than those for the single chemicals, which suggests that there is inhibition of one chemical accumulation by the other ones.

P04-011

This abstract has been withdrawn.

P04-012
Mercury speciation of preserved historical sludge to estimate risks from sludge entrapped under the reclaimed area of Minamata Bay, Japan

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A large amount of methylmercury (MeHg) was directly discharged into Minamata Bay and created the Minamata disease. Then, sludge with high levels of mercury (exceeding 25μg/g on dry basis) was entrapped under reclaimed land of the bay. The objective of this study was to obtain data on potential MeHg pollution risks possibly caused by sludge leakage from reclaimed land in Minamata Bay, Japan by analyzing preserved sludge as well as current sediment samples and to answer concerns from residents. In this study, we performed a survey of Hg concentration and speciation in preserved sludge samples collected before the start of the dredging project (i.e., 35–50 years ago) and compared those results to sediment collected recently (July 29 to June 1st 2015) in Minamata Bay. The total mercury (THg) on wet basis was 0.18 μg/g in the control (n=1), 6.1 μg/g (range: 0.83–12.2) for current sediments (n=5), and 241 μg/g (range: 22.4–3620) for the preserved sludge (n=4). MeHg was 0.71 ng/g, 3.7 ng/g (range: 1.71–8.5), and 108 ng/g (range: 7.8–503) for the control, current bay sediments, and preserved sludge, and MeHg percentage was 0.41%, 0.12% (range: 0.051–0.21), and 0.03% (range: 0.014–0.049), respectively. For all samples, the %MeHg decreased exponentially with increases in the THg concentration. An X-ray absorption fine structure analysis suggested that the main chemical form of the preserved sludge is β-mercury sulfide (β-HgS) and our data showed that the extractability of THg to the seawater was much lower than that of MeHg. Results indicate that, although MeHg was directly discharged from the company into Minamata Bay during the Minamata epidemic and that the preserved sludge had extremely high THg concentrations, we can estimate that, in the unlikely event of sludge leakage from reclaimed land, the risk of MeHg from the reclaimed land into Minamata Bay is low and far below the levels which caused the Minamata epidemic.

P04-013
Toxicity of diuron metabolites in human cells

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Diuron is a phenylurea herbicide which is commonly used across the globe. It is known to be toxic to aquatic organisms and animals such as rats. Diuron is metabolized to equally or more toxic compounds, N-(3,4-dichlorophenyl)urea (DCPU), N-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and 3,4-dichloroaniline (DCA). In the literature, metabolism of diuron has been linked with the development of urothelial cancer in rats. According to our earlier study, diuron is toxic and possibly genotoxic to human cells. In this current study, we wanted to pursue the effects of these diuron metabolites on cell viability, cell proliferation and ROS production. Studies were carried out using human cancer cell lines: BeWo (placental choriocarcinoma), MCF-7 (breast adenocarcinoma) and CaCo-2 (colon adenocarcinoma). According to our results, all the metabolites reduced the viability in all studied cell lines with BeWo cells being the most sensitive. Relative cell counts (indicating cell proliferation) were statistically significantly reduced by DCPMU in CaCo-2 cells and DCA in MCF-7 cells. DCPU slightly reduced the relative cell counts in all the cell lines. ROS production was statistically significantly increased in the case of DCA in all the cells and DCPMU in BeWo and MCF-7 cells. DCPU statistically significantly increased the production of ROS in BeWo cells. Experiments for potential effects of DCPU on ROS production in MCF-7 cells are yet to be carried out. In conclusion, our data indicates that diuron metabolites are cytotoxic probably through induction of ROS production. Sensitivity of BeWo cells (representing human placenta) to diuron metabolites implicates for possible fetal toxicity.

P04-014
Distribution of cyfluthrin in brain regions, induction of dopamine depletion and up-regulation of oxidative stress and inflammation markers

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Taking into account that several classes of pesticide exposure causing lesions in dopaminergic neurons, the widely usage of cyfluthrin could be a serious public health concern. The present study aimed (i) to explore cyfluthrin oral absorption and its distribution in CNS and elimination in male Wistar rats after single oral dose of 20 mg/kg bw in order to determine in plasma and brain regions (hypothalamus, striatum, hippocampus and frontal cortex) toxicokinetics parameters. Cyfluthrin concentrations in plasma and brain were quantified by LC/MS; (ii) to determine dopamine (DA) and its metabolites levels after cyfluthrin treatment (20 mg/kg bw, orally 6 days); and (iii) to investigate also in these brain regions the expression of genes linked to apoptosis (Bax, Bcl2, casp-3), oxidative stress (Gpx1, Nrf2, Sod2, Cox2), proinflammation (Cox2, Il-1β, Il-6, NF-κB, TNFa) and DA metabolism (TH, DT, rD1, rD2, MAOA, MAOB) by Real-Time PCR. All experimental procedures involving animals were conducted in accordance with the ethics requirements and authorized by the Institutional Animal Care and Use Committee of the Complutense University of Madrid**.** Our results demonstrated: (i) Cyfluthrin crosses the blood-brain barrier. Plasma and nervous tissue kinetics showed an extensive oral absorption of cyfluthrin and a slow elimination (T1/2β range 17-23 h). The ratio AUC(0-24) tissue/AUC(0-24) plasma for cyfluthrin was 3.17 for hypothalamus. (ii) Cyfluthrin modulates the level of neurotransmitters, cyfluthrin produced a loss of DA and their metabolites contents in striatum (-81%), hypothalamus (-69%), prefrontal cortex (-55%) and hypoccampus (-53%) with respect to control. (iii) Of the genes examined, in hypothalamus tissue, changes in mRNA levels (fold change >1.5) were observed on cyfluthrin-up-regulated Gpx1, Cox2, Il-1β, NF-κB, TNFa, rD1 and MAOB genes. These results imply cyfluthrin as a dopamine neurotoxin and a possible environmental risk factor for neurodegenerative diseases.

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P04-015
Effect of prenatal treatment with Valproic acid on offspring investigated by phase contrast X-ray CT

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Purpose: Prenatal exposure of antiepileptic drug Valproic acid (VPA) increases the risk of having offspring with autism spectrum disorder (ASD). Neuroimaging studies play an important role for insights into changes in the brain structure of ASD. However, due to the small absorption differences between soft tissues of brain, conventional imaging techniques cannot depict internal structural of brain without contrast agent. Thus, imaging system with high spatial resolution is required. Now, phase-contrast X-ray CT with less than 36μm spatial resolution depicted soft tissue structural changes in various animal model diseases. Here, brains of VPA-exposed offspring were imaged by phase-contrast X-ray CT.

Methods: Autism rat was created by exposure of rat fetuses to valproic acid (600 mg/kg) on the 12.5th day of gestation (VPA). Normal Control rat was created using normal saline at the same condition. 13 weeks old 6 VPA and 3 normal rat's brains were used in this study. Rat's brains were extracted under anesthesia and fixed with 10% formalin for imaging. A two-crystal X-ray interferometer-based phase-contrast X-ray imaging system was used for the observations with an X-ray CCD camera with a 2560 x 2100 pixel sensor of pixel size: 6.5 x 6.5 μm^2 and 17.8-keV synchrotron radiation. After imaging, Hematoxylin-eosin (H&E) staining was performed to examine abnormal histopathological structures.

Results and Conclusion: High spatial resolution phase-contrast X-ray CT without contrast agent clearly depicted the anatomical structures of brain, cortex, thalamus, corpus callosum, hippocampus, and amygdale depending on different densities. Mild to moderate expansion of ventricle was found in VPA group. In statistical analysis, increased density of hippocampus, thalamus and amygdale were observed in VPA group compared to control group. The significantly increased density was shown in hippocampus especially in dentate gyrus. This increased density might be associated with various neurodegenerative processes leading to ASD. H&E stain also revealed decreased neuronal size and increased cell packing density in dentate gyrus that is consistent with increased density.

This study demonstrated that phase contrast X-ray CT enabled to detect the fine inner structural changes in brains of VPA-exposed offspring.

P04-016

The toxic effects and underlying mechanims of $PM_{2.5}$-induced cardiomyocytes apoptosis and cardiac dysfunction

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Background: Although the strongly causal associations were between fine particulate matter ($PM_{2.5}$) and cardiovascular disease, the toxic effect and potential mechanism of $PM_{2.5}$ on heart was poorly understood. This study was aimed to investigate the toxic effects and involving mechanisms of $PM_{2.5}$-induced cardiomyocytes apoptosis and cardiac dysfunction.

Methods and Results: *In vitro*, $PM_{2.5}$ markedly augmented cardiotoxicity including oxidative damage and apoptosis in cardiomyocytes AC16 as well as epigenetic alteration. The cell viability was decreased while the levels of LDH release, ROS generation and MDA were increased in a dose-dependent manner induced by $PM_{2.5}$. Followed by the activities of SOD and GSH-Px were declined. Mitochondria damage and apoptosis induced by $PM_{2.5}$ was observed with the protein levels of Caspase-3, Caspase-9 and Bax were up-regulated while the anti-apoptotic protein, Bcl-2 was down-regulated. DNA methylation profiling revealed a significant gene-*ADRB2* was involved in the cardiac relative GO and KEGG pathways. Mechanistic study showed the role of *ADRB2* hypermethylation in inhibitng the β2AR and the downstream pathways, such as PI3K/Akt and p53 pathway in $PM_{2.5}$-treated AC16. The transgenic cell lines showed overexpression of *ADRB2* weakened the $PM_{2.5}$-induced cardiomyocytes apoptosis in opposite way, but was augmented by PI3K inhibitor. The aboved cardiac toxicity induced by $PM_{2.5}$ was also consistent with *in vivo* study using animal model via echocardiography, TUNEL staining, ultrastructural and histopathological evaluation.

Conclusions: Our results demonstrated that *ADRB2* hypermethylation and mitochondria-mediated apoptosis pathway played a critical role in $PM_{2.5}$-induced cardiac dysfunction.

P04-017

Cigarette smoke extract produces superoxide in aqueous media by reacting with bicarbonate

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Cigarette smoke (CS) contains free radicals, reactive oxygen species (ROS) and other pro-oxidants. In addition, CS is capable of stimulating cells or tissues to generate ROS by activating cellular ROS sources. Hence, oxidative stress plays a large role in toxicity of smoking. Here, we found CS to generate superoxide in cell-free, aqueous solution and characterized chemical reaction producing superoxide. CS was generated from the mainstream smoke of 3R4F reference cigarettes and vapor-phase cigarette smoke extract (CSE) was prepared by passing CS through an impinger containing phosphate-buffered saline (PBS). CSE was added to biocompatible aqueous solution such as water, Dulbecco's modified Eagle media (DMEM), Hank's balanced salt solution (HBSS), PBS or blood plasma, and superoxide was measured using water-soluble tetrazolium salt-1 (WST-1). CSE produced superoxide only in DMEM and HBSS. In the experiments using aqueous solution containing each component of HBSS, bicarbonate (HCO_3^-) was proved to be responsible for superoxide production. Detection of superoxide by WST-1 was abolished by superoxide dismutase or a superoxide scavenger TEMPOL, but not by catalase or a hydroxyl radical scavenger mannitol. Chemical species detected by WST-1 was confirmed again to be superoxide using electron paramagnetic resonance spectroscopy. Considering the superoxide-producing chemical reaction between peroxy acid and bicarbonate, peroxy acids in CSE were assumed to be a culprit for superoxide generation. Indeed, by pretreating CSE with peroxidase to remove peroxy acids, superoxide generation in bicarbonate solution was reduced significantly. In addition to CSE, tar-phase of CS, so-called cigarette smoke condensate, also produced superoxide in the presence of bicarbonate. Taken together, CS can generate superoxide in aqueous media containing bicarbonate, and the substrates of peroxidase such as peroxy acids, at least in part, participate in such chemical reaction. These results suggest that bicarbonate may be a critical determinant of oxidative toxicity of CS in biological environments and the experimental conditions such as bicarbonate concentration in aqueous media should be carefully considered in *in vitro* toxicity study of CS.

P04-018

Chemical characterization of industrial- and road traffic-influenced fine particles ($PM_{0.3\text{-}2.5}$) and impact on xenobiotic metabolizing enzymes gene expression in human reconstituted airway epithelium

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Atmospheric fine particulate matter ($PM_{2.5}$) was recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC). Despite the relationship already established by epidemiological studies between PM exposure and the onset of cardiorespiratory diseases, the physiopathological mechanisms responsible for these diseases remain poorly understood.

For a better understanding of the PM's health impact, the present study aims to evaluate the impact of two samples of fine particles ($PM_{0.3\text{-}2.5}$) differing by their emission sources (traffic vs industry) and thus by their chemical composition (heavy metals, paraffins, polycyclic aromatic hydrocarbons …).

The chemical characterization showed that PM collected under industrial influence (Ind-PM) were 8-folds more concentrated in PAHs and 1.5-folds in metals than PM under traffic influence (Traf-PM), while Traf-PM were 2-fold enriched in paraffins. However, metals composition was clearly influenced by industrial emissions in Ind-PM (Al, Ca, Co, Fe, Mg, Mn, Ni, V et Zn…) and by vehicles and traffic emissions Traf-PM (Ba, Cu, Mo, Sn…).

Then, for biological assessment and to mimic the real human exposure to fine particles, an innovative 3D *in vitro* model was used: a human airway epithelium reconstituted, nasal origin, co-cultured with human airway fibroblast (MucilAir™-HF, Epithelix®). Epithelia were exposed to PM at 45 or 90µg/cm^2 twice a week for two consecutive weeks. At the end of each week, part of the epithelia was sacrificed for the evaluation of xenobiotic metabolizing enzymes gene expression by RTqPCR. A significant dose-dependent induction of *CYP1A1, CYP1B1* and *HMOX* genes expression was observed after exposure to Ind-PM and to a lesser extent to Traf-PM. Such an induction could be responsible for the formation of PAHs-reactive metabo-

lites that could lead to an increase in DNA damages by adduct formation. Thus, a genotoxicity study will be led to assess the potential impact of these two samples on genome.

P04-019
Cord blood acrylamide levels and birth size, and interactions with genetic variants in acrylamide-metabolizing genes

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Introduction: To date, 3 epidemiological studies have consistently shown an inverse association between prenatal acrylamide exposure and birth size. According to the Developmental Origins of Health and Disease hypothesis, suboptimal prenatal development likely predisposes to inferior health throughout life.

We investigated the association between acrylamide and glycidamide to hemoglobin adducts in cord blood and birth size, and the interaction between acrylamide and polymorphisms in acrylamide-metabolising genes. Through this, we aimed to probe the causality of the inverse relationship between acrylamide and fetal growth.

Methods: In 443 newborns of the ENVIR*ON*AGE (ENVIRonmental influence *ON* AGEing in early life) birth cohort, we investigated the association between prenatal acrylamide exposure (acrylamide and glycidamide to hemoglobin adduct levels (AA-Hb and GA-Hb, respectively) in cord blood) and birth weight, length and head circumference.

Furthermore, we studied interaction with single nucleotide polymorphism (SNPs) in *CYP2E1* (rs2480258, rs915906 and rs11101888), *EPHX1* (rs1051740) and *GSTP1* (rs1695 and rs1138272).

We used multiple linear regression for the statistical analyses.

Results: The effect estimate for a 10 pmol/g hemoglobin increase in AA-Hb was -40 grams (95% CI: -71,-9; p: 0.01), for birth weight, -0.17 centimeters (95% CI: -0.31, -0.03; p: 0.02) for length, and -0.13 centimeters (95% CI: -0.24, -0.01; p: 0.03) for head circumference. For GA-Hb, the effect estimates were -53 (95% CI: -90, -0.16; p: 0.005), -0.24 (95% CI: -0.41, -0.08; p: 0.004) and -0.11 (95% CI: -0.25, 0.03; p: 0.11), respectively. The associations were similar or stronger in newborns of non-smoking mothers.

There was no statistically significant interaction between acrylamide exposure and the studied genetic variations. However, there were stronger inverse associations with birth weight and head circumference among newborns with homozygous wildtypes alleles for the *CYP2E1* SNPS and with variant alleles for a *GSTP1* SNP (rs1138272), especially in children of mothers who did not smoke during pregnancy.

Conclusions: We observed an inverse association between prenatal dietary acrylamide exposure and prenatal growth. In addition, the interaction pattern (although not statistically significant) with SNPs in *CYP2E1* is an indication for the causality of this association. Larger other studies are needed to corroborate this finding.

Given the consistent results of the good quality epidemiological studies that were performed to study the link between acrylamide and birth size, and the data on possible interaction with SNPs in *CYP2E1*, suggesting causality, preventative measures leading to reduced exposure of pregnant women to acrylamide are urgently called for.

P04-020
Health risks associated with cigarette sidestream smoke inhalation

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Cigarette sidestream smoke particulate matter (CSSP) is a common source of indoor air pollutants for nonsmokers. We measure the contents of metals and polycyclic aromatic hydrocarbons (PAHs) in CSSP emitted from Long Life cigarettes, a leading brand in Taiwan. 29 metals and 17 PAHs are found in CSSP. CSSP-bound metals may increase the chance of developing cancer by 9.27 ~ 20.93 x 10-6 and the hazard quotient for non-cancer toxicity by 0.496 ~ 0.286 when a Long Life cigarette is smoked in a 60-m^3 poorly ventilated room. In contrast to Western cigarettes, cadmium is the primary toxic metal present in Long Life CSSP and accounts for more than 90% and 80% of metal-associated cancer and non-cancer risk, respectively. PAHs that are carcinogenic and probably carcinogenic to human comprise about one fifth of the total PAH mass. Carcinogenic potency is equivalent to 144 ng benzo[a]pyrene (BaP) per cigarette. When smoking occurs in a 60-m^3 room, CSSP-bound PAHs increase cancer risk by a 1.44 x 10-6 chance per cigarette. In addition, the concentration of PAHs in the room is equivalent to 2.4 x 10-6 mg/m^3 BaP, which is above the reference concentration for developmental toxicity recommended by US Environmental Protection Agency. High concentrations of CSSP are cytotoxic. Elevation of AhR expression in lung cells can attenuate CSSP-induced ROS generation and cytotoxicity. However, metals and PAHs are not the causes for cytotoxicity and have no effect on AhR activity.

P04-021
Convolution neural networks for training the unbalanced toxicity assessment data and analyzing chemical functional group

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Deep learning models with the capability of the automated feature extraction have been developed and outperformed the traditional statistical models in the toxicity prediction recently. However, there is little consideration of data imbalance between 'active' and 'inactive' chemicals in the dataset, and efficient extraction of the activated substructure of the chemicals. In this study, the methods of training the models in the data-imbalanced conditions and interpreting the chemical substructures highly related to the toxicity from the model are proposed with convolution neural networks.

First, a convolution neural network is designed to predict the binary outcome of chemicals; 'active' or 'inactive' to toxicity in the given ligands. Second, a hybrid method of oversampling of active chemicals and down-sampling of inactive chemicals is utilized for training the model in the imbalanced data conditions in order to prevent overfitting. Third, the activated substructures are extracted by tracing back of convolution layers' output and filters. For efficient extraction, filters of each convolution layer in the model are designed with the recent SMILES representation methods.

The experimental results with TOX21 datasets showed that (i) the proposed training method improves the prediction accuracy of chemicals' activity to toxicity by 10% of AUC, (ii) the extracted substructures from analyzing convolution layer's filters is validated with

chemical functional group from the literature reviews. These results have potentials to in-Silico toxicity analysis for the prediction of unknown chemical's toxicity effects and for finding the substructures for toxic ligand binding. Testing the unknown chemicals for screening the toxicity assessment and validation of a new substructure founded as toxicity biomarker will be conducted as future works.

P04-022
Explanation of estrogenic activity in waste water treatment plant effluents

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There are many concerns about the effects of endocrine disruptors (EDs) on the environment and human health. Especially aquatic ecosystems are highly affected by waste water treatment plant (WWTP) effluents as a secondary source of pollution. Hormonal activity of a sample can be revealed by using two approaches: analytical methods combined with prediction modelling and ecotoxicological assays. Nowadays, with the development of highly sensitive analytical methods, a large spectrum of EDs is being quantified in all matrices. Detected concentrations can then be used for the calculation of the total hormonal activity of the sample on the basis of the compounds respective potentials. Correspondingly, with the second approach, certain hormonal activity of the whole sample can be determined by ecotoxicological assays. Discrepancies between results obtained from both approaches are plenteously described in the literature.

By comparing our results for more than 20 samples of WWTP effluents we tried to explain these differences. Estrogenic activity was observed in more than one third of the samples (0.66±0.06 – 4.27±0.63 ng/L of 17β-estradiol equivalent). Most frequently detected analytes were bisphenol A, irgasan and estrone. Due to additive effect of EDs, we focused on determining the limits of detection of selected EDs by a newly developed liquid-chromatography method. Afterwards, we determined the estrogenic activity (EC_{50}) of every single substance employing the yeast *Saccharomyces cerevisiae* with human estrogenic receptor. Based on the potency of the compounds we then expressed the estrogenic activity of a mixture as 17β-estradiol equivalent (EEQ) using the quantified limits of detection. No sample exceeded this calculated value (2.48 ng/mL EEQ). Our results showed that the selected analytes explained the majority of the total estrogenic activity in the examined effluents.

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P04-023
Analysis of the biological effects of Persistant Organic Pollutants (POPs) on human leukocyte cell lines and peripheral blood mononuclear cells

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Despite the ban on the manufacturing and application of polychlorinated biphenyls (PCBs), their environmental persistence as well as their ability to bioaccumulate in tissues of living organisms (Persistent Organic Polutants, POPs) remain a great concern. Within the context of the European EnviroGenomarkers program (www.envirogenomarkers.net) the effect of POPs, on genome-wide expression as well as CpG methylation of leukocyte DNA of healthy volunteers was studied. The epigenetic exposure profile showed extensive and highly statistically significant overlaps with published profiles associated with the risk of future B-cell chronic lymphocytic leukemia (CLL) as well as with clinical CLL, suggesting an etiological link between exposure and CLL. A thorough toxicogenomic study of human leukocyte exposure to POPs *in vitro* is therefore necessary to validate the results of the population study and elucidate the role of POPs in haematological cancers. Therefore, cytotoxicity (by the MTT assay) and genotoxicity (double or single strand breaks using the COMET assay) of three selected POPs (HCB, PCB118 and PCB153) in human leukocyte cell lines (Jurkat and U937) as well as in Peripheral Blood Mononuclear Cells (PBMCs) were examined. Increased cytotoxicity was observed, regardless the cell line and incubation period used, only at concentrations greater than 50 μM, whereas, no notable genotoxicity was apparent at all incubation periods and concentrations. Only PCB153 induced a marginal but disputable, in terms of biological relevance, increase in DNA damage. However, using the LuMA assay, a dose-dependent global DNA hypomethylation was observed in PBMCs treated with both PCB118 and PCB153 at concentrations as low as 5 μM. The epigenetic *in vitro* CpG modulation observed might provide a causal association between POPs exposure and haematological cancers.

Samples from the *in vitro* experiments were sent for whole genome methylome (Illumina Infinium Methylation EPIC 850K microarray) and transcriptome analysis (polyA, RNA-seq using the Illumina Sequencer_HiSeq4000). The preliminary results from the biostatistic as well as bioinformatic analyses will be presented.

P04-024
Assessment of Roundup® cardiotoxicity on guinea-pig isolated Langendorff perfused heart and human induced pluripotent stem cells derived cardiomyocytes

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Introduction: Although pesticides are known to come with benefits for agriculture, there is a lot of controversy surrounding the use of these substances because of suspected hazardous health effects. Roundup®, one of the most popularized pesticide, is composed of Glyphosate, its active ingredient, and adjuvants. The purpose of this work was to examine the effects of Roundup® exposition on heart function using isolated guinea-pig (GP) perfused heart and human Induced Pluripotent Stem cells derived cardiomyocytes (hiPSC-CM) models.

Methods: Haemodynamic and ECG parameters were recorded on isolated GP heart using the Langendorff method (3 doses of Roundup®, acute exposition) while contractility (impedance) and electrophysiology (MEA) of up to 9 doses were recorded in Cor.4U hiPSC-CM using the xCELLigence RTCA cardio ECR platform (acute and chronic exposition, up to 24h).

Results: On isolated GP heart experiments, Roundup® increased ECG parameters and decreased heart rate only at 100 μM. An atrioventricular block occurred in 1 of 5 preparations. A concentration-dependent decrease of contractile function was observed at 10 and 100 μM.

Acute and chronic exposition to Roundup® also induced modification of hiPSC-CM electrophysiology and contractile function. At the top concentration of 1 mM, hiPSC-CM stopped beating and Cell Index was rapidly and drastically affected, corresponding to cardiomyocytes death.

Conclusion: Both models were able to detect cardiotoxicity of Roundup® mainly characterized by a massive decrease of the heart's contractile function and troubles in electrical conduction. The effects observed in man after Roundup® intoxication, as reported in the literature (moderate ~360 µM to severe intoxication ~5 mM), were consistent with those observed in these *in vitro-ex vivo* models. These predictive and sensitive assays can be useful for cardiotoxicity assessment of pesticides and to a larger extent to compounds other than medicines developed by pharmaceutical industries.

P04-025
Toxicity and degradability of widely used personal care products

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Endocrine disrupting compounds (EDCs) belong among widely discussed and studied pollutants in last decade. Well known synthetic and massively used estrogens 17α-ethynylestradiol and bisphenol A are commonly detected in the environment and the methods to eliminate these compounds are studied extensively. Nevertheless, not only studied compounds contribute to the hormonal activity in the environment. We have focused on personal care products (PCPs) daily used as antimicrobial compounds in dental hygiene. Hormonal activity of octenidine – OCT, hexadecylpyridinium – HDP, menthol – MEN, eucalyptol – EUC, limonene – LIM, thymol – THM, sanguinarine – SAN, hexetidine – HEX, chlorhexidine – CHX was examined by the yeast *Saccharomyces cerevisiae* with human estrogenic receptor (ER)/androgenic receptor (AR) tests and the human cell line tests CXCL12-T47D and AIZ-AR for confirmation of estrogenic and androgenic activity, respectively. None of the tested compounds were identified as agonists of steroid receptor, but five of the tested compounds (OCT, HDP, CHX, THM and MEN) were able to inhibit estrogenic and/or androgenic pathway. The Schild analysis was used to identify direct binding on the receptor. Only THM and MEN were determined as antagonists of steroid receptors. The mechanism of antagonistic property of OCT, HDP and CHX requires further studies. Since the consumption of these antimicrobial agents is immense, a degradation study was also performed. *In vivo* and *in vitro* experiments were carried out with a representative of white-rot fungi, very potent degraders of persistent organic pollutants. *Irpex lacteus* and extracellular enzyme manganese-dependent peroxidase were used for degradation tests. *In vivo* experiments showed that *I. lacteus* was able to degrade more than 70% of CHX and OCT in 14 days. *In vitro* experiments showed 52%, 30% and 27% degradation of CHX, OCT and HDP, respectively.

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P04-026
The novel mathematical model for the quantitative analysis of antagonist-receptor interactions

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The quantitative analysis of drug-receptor interactions developed by Schild and Cheng-Prusoff is widely used for the assessment of antagonist effects. These methods are derived from the Gaddum equation. However, the Gaddum equation is derived for the simple law of mass action and does not consider that the compounds can have different slopes of their curves. This means that the slope parameters (Hill coefficients) of dose-response curves are always treated to be equal 1. Simplification in the Gaddum equation often leads to an inaccurate estimation of the equilibrium dissociation constants of the competitive antagonists, which is the key characteristic of the receptor ligands. In our previous study, we described the development and validation of a new mathematical model for mixture toxicity. Using this model, we derived a novel form of the Gaddum equation which contains the original hill coefficient of the agonist. Standardized *in vitro* yeast reporter gene assay (BMAEREluc/ERα) has been used for the validation of the proposed model and several known estrogen antagonists have been measured by Schild and Cheng-Prusoff method. Our mathematical model significantly reduces the differences in values calculated by the Cheng-Prusoff and Schild methods and yields more accurate estimations of antagonist affinity. This novel form of the Gaddum equation could improve hazard identification and dose-response assessment of chemical compounds.

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P04-027
Cellular response and extracellular vesicles characterization of human macrophages exposed to $PM_{2.5}$

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Exposure to atmospheric fine Particulate Matter (PM) is one of the major environmental causes involved in the development of inflammatory lung diseases, such as chronic obstructive pulmonary disease (COPD) or asthma. When PM is penetrating in the pulmonary system, alveolar macrophages represent the first line of defense, in particular by triggering a pro-inflammatory response, and also by their ability to recruit infiltrating macrophages from the bone marrow. The aim of this study was to evaluate the toxicological and inflammatory responses of infiltrating macrophages after exposure to PM. Extracellular vesicles (EVs) production has been evaluated following their exposure to $PM_{2.5}$. Finally, the ability of these EVs to convey information from PM exposed macrophages to pulmonary epithelial cells was evaluated.

Undifferentiated infiltrating macrophages respond to fine particles exposure in a conventional manner, as their exposure to $PM_{2.5}$ induced the expression of EMXs such as CYP1A1 and CYP1B1, the enzymes involved in oxidative stress SOD2, NQO1 and HMOX as well as pro-inflammatory cytokines in a dose-dependent manner. Exposure to PM also induced a greater release of EVs in a dose-dependent manner. In addition, the produced EVs were able to induce a pro-inflammatory phenotype on pulmonary epithelial cells, with the induction of the release of IL6 and TNFa proinflammatory cytokines. These results suggest that infiltrating macrophages participate in the pro-inflammatory response induced by $PM_{2.5}$ exposure and that EVs could be involved in this mechanism.

P04-028
The influence of the *Cucurbitaceae* and their selected plant secondary metabolites on structurally related phenoxy acid herbicides removal and phytotoxicity mitigation

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The aim was to evaluate the effects of *Cucurbitaceae* (zucchini and cucumber) and their selected plant secondary metabolites (PSMs: ferulic and syringic acids) on 1) removal rate of phenoxy acid herbicides (2,4-D and MCPA) with chemical structures resembling those of PSMs, and 2) phytotoxicity mitigation. The former was measured using chromatographic and molecular methods enabling to determine the removal rate of studied herbicides and presence of bacterial *tfdA* genes responsible for its degradation; and the latter using Phytotoxkit test. The research aimed also to determine the 3) influence of 2,4-D and MCPA, as well as artificial PSMs application, on cucurbits condition measured as changes in their morphometric and physiological parameters.

The obtained results demonstrated that although the removal of 2,4-D reached 100% irrespectively of PSMs spiking or not, the phytotoxicity remained very high. In contrast, in variants with MCPA+PSMs the herbicide removal was 99%, whereas in sample without PSMs was almost two-fold lower. Also phytotoxicity was found to be lower in samples spiked with MCPA+PSMs.

The results show also that simultaneous application of studied herbicides and PSMs contributes to increased detection of herbicide degradative genes. Samples spiked with herbicides+PSMs demonstrated a mean two-fold increase in detection of *tfdA* genes in comparison to those amended only with herbicides. Such an increase in *tfdA* genes detection demonstrated that PSMs can enhance the biodegradation of structurally similar phenoxy acid herbicides. 16S rRNA gene sequence analysis revealed high homology to soil bacteria *Rhodoferax saidenbachensis*, *Burkholderia* spp.and *Cupriavidus* spp. commonly known to be involved in biodegradation processes of phenoxy acid herbicides.

As far as the studied herbicides are used to selectively control the growth of dicotyledonous weeds, its application can also affect the condition of cultivated plants. Obtained results demonstrated that application of these herbicides influences on cucurbits condition measured as lower plant biomass, leaves surface area and length, and chlorophyll content. Simultaneous application of both phenoxy acid herbicides+PSMs, alleviated this inhibitory effects, however this positive influence was observed only in the case of zucchini variants.

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P04-029
Toxic effects on *Caenorhabditis elegans* from sedimented dust in an urban area of northern Colombia

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Sedimented dust is a heterogeneous mixture whose potential sources are soil erosion, atmospheric deposition, and anthropogenic activities. It is the dominant fraction of air pollutants in urban areas carrying toxic substances such as heavy metals, pesticides, and polycyclic aromatic hydrocarbons, among other chemicals. The objective of this work was to evaluate the toxic effects of sedimented dust extracts from an urban area crossed by a railway line at northern Colombia on *Caenorhabditis elegans*. Urban dust samples were collected in 21 points at the municipality of Aracataca (Magdalena) Colombia, a location influenced by coal transport on trains, intensive agriculture, and some urban traffic. A reference sample was taken 3 Km northeast of the urban area. Aqueous extracts (K medium) were obtained from dust particles (< 75 µm), and synchronized nematodes (L1 and L4) were exposed to the extracts evaluating lethality, growth, locomotion, reproduction, as well as gene expression with transgenic GFP strains: *mtl-2* and *sod-4*. ICP-MS analysis was performed at points with low, medium and high lethality. Lethality varied between 0.88% and 60.2%, and surprisingly, nematode size increased in 85.7% of the samples, whereas locomotion was inhibited between 0.77% and 46.1%. Some extracts promoted egg hatching. The *mtl-2* and *sod-4* expression increased moderately in most samples, suggesting metal exposure. Trace elements concentrations (ppm) in analyzed samples increased in the order Ba > Zn > Sr > Pb > B > Rb > Ce. Growth and locomotion showed a negative association with Zr and Rb concentrations, respectively. In short, urban dust extracts impacted physiological parameters in *C. elegans*, such as survival, growth, and locomotion, modulating gene expression related to metal exposure and oxidative stress. Colciencias-Unicartagena, 785-2017.

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P04-030
Toxicity of organic matter originated from *Microcystis aeruginosa*

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Nowadays, the eutrophication of surface water results in a massive increase in algal and cyanobacterial growth associated with the occurrence of water blooms. Due to the decomposition of the biomass, the concentrations of algal organic matter (AOM) including cyanobacterial toxins also increase in water reservoirs which are frequently used as a source of drinking water. The main problem arises during the drinking water treatment processes because the majority of dissolved organic compounds serve as precursors for the formation of potentially toxic disinfection by-products (DBPs) in drinking water. The mentioned compounds (e.g. cyanotoxins, DBPs) are currently considered to be a threat to drinking water quality due to their adverse effect on human health.

A method employing liquid chromatography with tandem mass detection (LC-MS/MS) has been developed and optimized for the determination of selected types of cyanobacterial toxins such as microcystins (MCs), anatoxin, cylindrospermopsin, nodularin. The fully optimized method has been used for the detection of cyanotoxins in a sample containing dissolved organic carbon (DOC) of cyanobacterium *Microcystis aeruginosa*. Cyanotoxins detected in the sample were: anatoxin (0.02 µg/mg of DOC), MC-RR (0.78 µg/mg of DOC), MC-YR (0.22 µg/mg of DOC), MC-LR (0.74 µg/mg of DOC), MC-LY (0.02 µg/mg of DOC) MC-LW (0.69 µg/mg of DOC) and MC-LF (0.02 µg/mg of DOC).

Moreover, toxic properties of the studied AOM-containing sample have also been established using Thamnotoxkit F with the crustacean *Thamnocephalus platyurus* for which LC_{50} was determined at 20.2 mg/l of DOC. Furthermore, IC_{50} for root growth inhibition of plant *Lepidium sativum* was established (180.3 mg/L of DOC). The growth of other tested organisms (*Saccharomyces cerevisiae, Bacillus subtilis, Escherichia coli*) was not affected by the exposure to organic matter originated from *M. aeruginosa* and the growth of alga *Desmodesmus subspicatus* was even stimulated.

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P04-031
Aromatase activity in the presence of penconazole and essential metals

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Triazoles are agrochemicals or pharmaceuticals used for protection of crop or skin against fungi or mildew. The mechanism of their action lies in the inhibition of sterols biosynthesis [1]. It is known that essential metals significantly influence the behaviour of azoles in the manner of (i) complexes creation with them [2], (ii) changes of redox behaviour [3] and (iii) degradation pathways [4]. It is not clear how the cocktail effect of other present biological active chemicals modify the inhibitory effect of azoles (penconazole) to aromatase. We provide the experiments in the gas phase to clarify the reaction mechanisms modifications as an effect of essential metals presence.

Acknowledgments: The research was supported by by GA CR (project No. 18-01710S).

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P04-032
Migration of pesticide of the derivative class of phenoxyacetic acids in soil-water system

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Introduction: Study of the migration of phenoxyacetic acid class pesticide in soil water system.

Materials and methods: Derivative of the phenoxyacetic acid class (IUPAC name (4-chloro-2-methylphenoxy)acetic acid) is a broad-spectrum herbicide. According to hygienic classification of pesticides by degree of danger, it is a highly hazardous compound due to its carcinogenic effect (hazard class 2C in Russian Federation), according to the classification of the IARC – hazard class 2B. Substance in form of aqueous solution was introduced into the upper 20 cm layer of soil filtration columns. (Figure 1) in triplicate in concentrations: maximum recommended application rate in agriculture is 0.52 mg/kg; 10 times lower of the maximum rate - 0.052 mg/kg; 10 times higher of the maximum rate of 5.2 mg/kg. The experiments carried out in the most extreme conditions, using a model of soil standard. The determination of the substance in water carried out by LC/MS equipment consisted 1290 Infinity LC system with triple quadrupoles mass spectrometer Triple Quad 6460 (Agilent Technologies, USA) with negative ESI MRM mode, LLOQ - 0,0025 mg/L (Figure 2). Samples were taken daily 5 times a week for a month (until the content of substance in water decreases at the level of its maximum permissible concentration (MCP) into water bodies (0.003 mg/L).

Research results: It was established, that the maximum migration of a substance from soil to water was observed on the 9th day. (Figure 3). The content of the substance in water was: at 0.052 mg/kg of soil - 0.075 mg/L of water; at 0.52 mg/kg - 0.49 mg/L; at 5.2 mg/kg - >100 MPC mg/L, respectively. A strong correlation was found between the content of substance in the soil and in the water. The correlation coefficient is r = 0,997. Based on the regression equation there was established a threshold value of 0.0026 mg/kg of soil.

Conclusions: 1. Established a strong correlation relationship between the concentration of the substance in the soil and in the water filtrate. 2. The threshold value by the migration-water hazard indicator at the level of 0.0026 mg/kg of soil.

P04-033

Newborn telomere length and the prenatal exposome: findings from the ENVIRONAGE birth cohort

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Background: The exposome encompasses all exposures over an entire life as from conception onward. Telomere length (TL) is marker of biological ageing and TL at birth may predict disease susceptibility later in life. Telomeres can be considered as cellular memories of exposures to oxidative stress and inflammation, and therefore TL may be a proxy for assessing the exposome. We evaluated the potential of TL at birth as a proxy for the prenatal exposome.

Methods: In the ENVIRONAGE birth cohort, Flanders, Belgium, we measured cord blood TL in 1200 mother-newborn pairs using a qPCR method. We collected data on maternal external and internal factors of the exposome. We used linear regression models to associate maternal residential air pollution and traffic exposure, temperature exposure, maternal internal indicators of inflammation and diet, education, BMI, and lifestyle factors during pregnancy with cord blood TL. Models were adjusted for parental age, newborn sex, gestational age and month of delivery.

Results: We found that high particulate matter ($PM_{2.5}$) and black carbon (BC) air pollution and temperature exposures during pregnancy were negatively associated with cord blood TL. Higher traffic density near the residential address was negatively associated with cord blood TL. Furthermore we found that increased maternal plasma insulin and homocysteine levels were associated with shorter TL, but no associations were observed for folate, and IL-6. Low maternal education and a high BMI independently were associated with shorter TL. Maternal lifestyle factors during pregnancy including, diet, physical activity and alcohol consumption did not strongly predict newborn TL.

Conclusion: Our results show that TL, already at birth, may capture some of the individual exposure factors of the prenatal exposome. However, to explore additive effects of multi-exposures from the exposome, an integrative assessment and an exposomics approach modeling strategy will be applied in the future to fully evaluate these multi-exposures in association with newborn TL.

P04-034

Activation of NRF2 and AHR signaling pathways and autophagy by ambient fine and quasi-ultrafine particles in human bronchial epithelial BEAS-2B cells

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Particulate matter (PM), a major class of air pollutants, represents a heterogeneous complex of inorganic (*e.g.* metals, ions), organic (*e.g.* polycyclic aromatic hydrocarbons: PAH), and biological (*e.g.* pollen, fungi) components. PM have been widely studied, but the relationship between its chemical components and its toxicity is still unclear. In this work, we also sought to compare the toxicological effects of the organic and inorganic fractions of ambient $PM_{2.5\text{-}0.3}$, on the one hand, and the organic fractions of ambient $PM_{2.5\text{-}0.3}$ and $PM_{0.3}$ in human bronchial epithelial lung BEAS-2B cells, on the other hand.

Physico-chemical characterizations of $PM_{2.5\text{-}0.3}$ and $PM_{0.3}$ were carried out using GC-MS for PAH, O and N-PAH, and n-alkane quantification, ICP-AES for major and trace elements quantification, ionic chromatography for ion quantification, X-ray diffraction for crystalline phase study, and SEM-EDX for morphology and elemental composition at particle scale. After 6 and 24h of exposure at low doses (3 and 12µg/cm^2), comparisons were made between the toxicological effects of native $PM_{2.5\text{-}0.3}$ to its organic extractable matter ($OEM_{2.5\text{-}0.3}$), and non-extractable matter ($NEM_{2.5\text{-}0.3}$), and between those of $OEM_{0.3}$ and $OEM_{2.5\text{-}0.3}$. Redox status and xenobiotic metabolizing enzymes were also studied by evaluating expression of several genes implicated in both these signaling pathways (*AHR, ARNT, CYP1A1, CYP1B1, GSTA-4, and EPHX1; NRF2, NQO1, KEAP1, SOD and HMOX; RT-qPCR*) and oxidative damage (carbonyl proteins, 8-isoprostane, and 8-OHdG; Elisa). Autophagy was evaluated through ATG5, LC3b, Beclin1, and PARKIN (Western-blot).

Concentrations of organic compounds (PAH, N and O-PAH, and n-alkane) were higher in $PM_{0.3}$ than in $PM_{2.5\text{-}0.3}$, thereby supporting the strong influence of combustion processes on $PM_{0.3}$ emission. All the fractions were able to induce NRF2 and AHR signaling pathways, and autophagy. However, by comparing the different fractions derived from $PM_{2.5\text{-}0.3}$, native particle lead to the highest activation of these underlying mechanisms. Finally, $OEM_{0.3}$ was found to be the most toxic, probably due to its richness in organic compounds. Taken together, these results supported the toxicity of both inorganic and organic fractions of $PM_{2.5\text{-}0.3}$, and showed the crucial role played by $PM_{0.3}$ in PM toxicity.

P04-035

Total arsenic and arsenic speciation of cereals and seaweeds in South Korea

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Arsenic (As) has different toxic effects on human depending on its chemical species with unique characteristics. Inorganic As (iAs), including arsenite (As (III)) and arsenate (As (V)) is more toxic than organic As and is known to cause cardiovascular disease and cancer. As an important dietary source of iAs exposure, there needs to monitor As species in various cereals. In addition, seaweed, which is rich in dietary fibers and minerals, is a popular type of seafood in South Korea, and it is known that the concentration of As in seaweed is relatively high. The annual consumption of seaweed in South Korean is close to 5 kg/person, and more than 50 kinds of seaweed are used as foods. In this study, total As (tAs) and six iAs species, including As (III), As (V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC)) were monitored in barley (n=16), oats (n=13), glutinous rice (n=20), corn (n=18), black rice (n=12), wheat (n=5), white rice (n=20) and brown rice (n=17) for cereal samples and in kelp (n=33), laver (n=25), sea mustard (n=25), agar (n=20), seaweed fulvescens (n=17) and gulfweed (n=12) for seaweed samples. For the present study, tAs was analyzed using inductively coupled plasma-mass spectrometry, ICP-MS, and 6 species of As were analyzed by HPLC coupled with ICP-MS. It was found that low level of As in corn, barley, oat and wheat samples, and relatively high level of As in rice were found, and there is a positive correlation between tAs and iAs concentration in cereals. In seaweed, there was

no observative correlation between tAs and iAs concentration, and high level of tAs was detected in seaweeds, whereas level of iAs was quite low except for gulfweed. This study will be used to assess potential risk of As species from cereal and seaweed consumption.

P04-036
A 28-day repeated oral dose toxicity study of 5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphinan-5-yl)methyl methyl methylphosphonate in mice

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5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphinan-5-yl)methyl methyl methylphosphonate (PMMMP) is one of the novel phosphorus based-flame retardants. Because PMMMP is known to be an indoor contaminant, possible exposure of human to PMMMP has been concerned. However, there have been few reports in terms of safety assessment for PMMMP. In the present study, we investigated a repeated oral dose toxicity of PMMMP using mice. Six-week-old male CD-1 mice were treated with PMMMP (containing 20% bis[(5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphorinan-5-yl)methyl] methyl methylphosphonate as a contaminant) by gavage at doses of 100, 300 or 1000 mg/kg/day for 28 days. The typical toxicological parameters were analyzed at necropsy. There were no treatment-related clinical sign and significant changes of body weight and food consumption. The serum phosphorus level was significantly increased in 300 and 1000 mg/kg/day PMMMP-treated groups, but there were no changes in serum calcium level in all PMMMP-treated groups. The absolute and relative adrenal weights were significantly increased in all PMMMP-treated groups except the relative weight at the low dose. Histopathological examination revealed no treatment-related changes in any organs. Therefore, we concluded that the increases in serum phosphorus level were due to the treatment with phosphorus agent, but not toxicological effects. Also, weight changes in adrenals might have no toxicological significance. Thus, there were no significant toxicological changes in any parameters in the present study. Hence, no adverse effect level of PMMMP under the present experimental condition in male mice was estimated to be greater than 1000 mg/kg/day.

P04-037
Pesticide residues in peppermint, chamomile and bladder herbal teas sold in Estonia

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Using medicinal plants for treating illness is one of the oldest method to cope with diseases. According to WHO currently about 80% of Worlds' population is primarily relying on herbal remedies when falling ill. It is well known that there are many contaminants and residues in herbal remedies that may cause harm to consumer – such as pesticides. To avoid harm from these substances it is essential to control the quality of herbs prior consumption.

The aim of this study was to determine pesticide residues in commercially sold peppermint, chamomile and bladder tea herbs. All samples were bought from local pharmacies and supermarkets to include as many producers possible at given time. Samples were prepared with extraction with hexane followed by silica column cleaning. The chamomile samples in repeated analysis were prepared by standard method: Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method EN 15662. The analysis were carried out with Agilent Technologies 7890B gas chromatography, Agilent Technologies 5977A mass-selective detector, Agilent MassHunter Qualitative Analysis B.07.00 and Agilent MassHunter Quantitative Analysis B.07.00 programs. The pesticides were selected based on EU Pesticide Database and Statistics Estonia Database.

Results: Residues of 5 pesticides were detected in 8 samples of peppermint, 4 samples consisted pesticide amounts exceeding allowed limits. In chamomile residues of 6 pesticides were detected in 7 samples, 2 samples consisted residues in quantifiable amounts, none exceeded EU limits for pesticide residues. Bladder tea herbs contained more than 14 different pesticide residues, from which 4 were detected in quantifiable amounts.

Conclusion: The origin of pesticide residues in organically produced herbal teas is unclear, but the amounts are in trace levels and therefore pose no substantial risk on their own for consumers health. There is a risk for synergistic effect with such wide array of different pesticides depending on consumer habits. Some herbal teas sold in supermarkets exceeded EU limits for pesticides and should be avoided.

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P04-038
The *Fusarium* mycotoxins effect on glutathione system in broiler chicken

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In temperate climates *Fusarium* mycotoxins, including deoxynivalenol (DON), fumonisin B1 (FB1) and T-2 toxin are the most relevant contaminants of cereal grains and they very frequently co-occur. Combined exposure might cause additive, synergistic or antagonistic toxic effects, but little is known about the actual multi-mycotoxin risk, yet. It is well known that *Fusarium* mycotoxins provoke oxidative stress, which is neutralized by the glutathione system.

The aim of this study was to investigate the intracellular biochemical and gene expression changes in case of multi-mycotoxin exposure, with attention to certain elements of the glutathione system, which is thought to be a major defence at the onset of low-level oxidative stress. *In vivo* study was performed in broiler chicken in a short-term (72 hours) feeding trial with low (T-2 toxin: 0.25mg/kg; DON: 5 mg/kg; FB1: 20 mg/kg) and high (T-2 toxin: 0.5mg/kg; DON: 10 mg/kg; FB1: 40 mg/kg) doses of multi-mycotoxin exposure. Liver samples were taken, in which some parameters of the glutatione system, reduced glutathione (GSH) concentration, glutathione peroxidase (GPx) activity and changes in the gene expression of glutathione peroxidase4 (*GPx4)*, glutathione synthetase (*GSS*), and glutathione reductase (*GSR*) were measured.

The results revealed that multi-mycotoxin exposure caused significant differences in the amount and activity of the glutathione

redox system as a function of the exposure time. *GPx4* and *GSR* expression was decreased in the low dose group and *GSS* expression was elevated in high dose group on day 1 as compared to the control. The results showed how the *Fusarium* multi-mycotoxin exposure activated the antioxidant defence system as a consequence of low level oxidative stress by its individual effects.

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P04-039
Food levels of 9 bisphenol analogues in Catalonia (Spain): comparing canned vs non-canned foodstuffs

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Bisphenols (BPs) comprise a wide group of chemicals used in the manufacture of polycarbonate plastics and epoxy resins. Traces of BPs may be contained in a broad range of products, such as food containers, dental fillings, medical devices, toys, thermal paper, and surface coatings of can, among others. Although bisphenol A (BPA) has been the most largely used analogue, it is being gradually replaced by some alternatives, labelled as "BPA-free", that could be less toxic. However, since the chemical structure of these analogues is very similar to that of BPA, their endocrine disrupting potential could be similar or even higher. Diet accounts for more than 99% of the exposure to BPs, being food containers a potential source of high importance. Unfortunately, data relative to food concentrations of not only BPA but especially the rest of BP analogues are still very scarce. Therefore, an accurate chemical analysis is needed to evaluate the potential exposure to these group of chemicals. This study was aimed at determining the levels of 9 bisphenol analogues (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE, BPAP, and BPP) in 40 food samples obtained from a duplicate diet study. Samples were divided into 2 groups: 1) canned food; and 2) fresh food, packed in glass containers or other BP-free materials. Samples were homogenized, and BPs were determined combining QuEChERS extraction with a dispersive liquid-liquid microextraction (DLLME), followed by a gas chromatography coupled to mass spectrometry. BPA was present in all the canned foods, with levels ranging between 3.45 and 88 µg/kg. Canned chicken also showed traces of BPB (3.86 µg/kg). On the other hand, BPA was also found in 9 out of 26 non-canned foods, with mushrooms presenting the highest concentration of BPA (9.56 µg/kg). Oil samples, both in can and glass, contained measurable levels of BPB (1.25 and 0.85 µg/kg, respectively). Additionally, fresh chicken also had BPB (4.19 µg/kg). Finally, BPE could be also quantified in nuts and mushrooms in glass (mean levels: 12.35 and 2.40 µg/kg, respectively). Our results are very valuable to assess the dietary intake of these chemicals and to understand the role and the distribution of each analogue in the industry.

P04-040
Levels of *Alternaria* toxins in feed for farm animals – a preliminary study

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Purpose: Among the mycotoxins, Alternaria toxins seem to be one of the most important group. This is about 70 compounds of secondary metabolites of *Alternaria alternata* which toxins as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), and altertoxins (ATX) are described to induce harmful effects in animals, including fetotoxic and teratogenic effects. However, data on the sensitivity of farm animals are very limited and do not allow the estimation of tolerance levels for individual toxins and mixtures thereof. However the presence of those compound was reported in wheat, sorghum, and barley, and in oilseeds such as sunflower and rapeseed, tomato, apples, citrus fruits, olives and several other fruits and vegetables, the data about animal feed are scarce [1]. The aim of this study was to survey the presence of six *Alternaria* toxins in a different kind of feed and feed material (227 samples of swine feed, poultry feed, silage and grains) with LC-MS/MS method.

Methods: Analytes (AOH, AME, TeA, ATX, altenuene ALT, tentoxin TEN) were extracted from feed samples (1g) with 4 ml of solvent extraction (ACN:H_2O:HCOOH; 79:20:1 v:v:v) by 30 min of horizontal shaking. After extraction, 100 µl of supernatant was transferred to a plastic tube. After evaporation (N_2, 40°C), the dry residue was dissolved in 100 µl of 50% MeOH and centrifuged (14 000 rpm, 30 min). Finally, the sample was transferred to orange vials and determined with LC-MS/MS technique (chromatograph Nexera X2 coupled with tandem mass spectrometer LCMS 8050, Shimadzu). The chromatographic separation was obtained using Kinetex C18 column (100×2.1 mm, 2.6, Phenomenex) and as a mobile phase 100 mg/l $NH_4(CO_2)_2$ and MeOH (pH ~7.8). The method was validated: limits of quantification (LOQ) ranged between 5.0 and 50 µg/kg; the repeatability and reproducibility (expressed as CV, %) were between 4.0–35% and recoveries were ranged from 65% to 150%.

Results: The results show a relatively high occurrence of Alternaria toxins in analysed feed samples. TeA, AOH and TEN were determined in 54-56% of all samples; ALT, ATX and AME in 9, 15 and 21% of all samples, respectively. Over 73% of the poultry and swine feed samples were contaminated with TeA in the range of 10–980 µg/kg. The feed materials (grains) contained TEN in the relatively low concentration (mean 8.5 µg/kg) but in 77% of surveyed samples. The AOH was determined in 62% of silages samples (mean 17.5 µg/kg).
The rate of the occurrence and range of concentration shows a significant level of feed contamination with Alternaria toxins. Due to the lack of knowledge about the toxicity of those compounds on different animal species and its residues in food of animal origin the comprehensive study in this area have to be conducted.

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P04-041
Assessment of heart rate variability under exposure to GSM 900-MHz signal from mobile phone in healthy young people

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Purpose: Recently a National Toxicology Program has issued their technical reports (NTP TR 595 [1] and NTP TR 596 [2]). They found that high exposure to radio frequency radiation (RFR) used by cell phones was associated with clear evidence of tumors in the hearts of male rats. In this context and given the large number of mobile phone users worldwide, the present study was focused on the effect of RFR of

mobile phone on the heart rate variability (HRV). The aim of the present study was to analyze, in healthy subjects at rest, the influence of exposure to GSM 900MHz on HRV parameters.

Participants and Methods: Twenty-six young healthy volunteers participated in the experiment. The volunteers were selected following a routine clinical examination. Inclusion criteria included regular sleep habits, no medication, no chronic disease or disability, no recent acute illness, no smoking, and no neurological or psychiatric illness. Participants having a history of cardiovascular disease were excluded. Volunteers participated into two ECG recording sessions. For each session, the participants were exposed for 26 min to sham or real GSM RF exposure. Exposure to RF EMF was performed by a commercial dual-band GSM mobile phone (Nokia 6650). The HRV was evaluated by both time domain and frequency domain analysis. Standard deviation of all R–R intervals (SDNN) and root-mean-square of successive differences (RMSSD) were measured in the time domain analysis of HRV. For frequency domain parameters, spectral analysis was performed by using fast-Fourier transform method. We analyzed the very low-frequency component (VLF), the low-frequency component (LF), and the high-frequency component (HF). The LF and HF powers were also converted into normalized units (LFnu, HFnu). Sympathovagal balance was expressed as the LF/HF ratio.

Results: Analysis of time domain HRV parameters showed that SDNN was significantly higher during the exposure session when compared to the control session.

Analysis of frequency domain HRV parameters demonstrated that absolute values of LF power and total power were significantly increased during exposure ($p = 0.0463$ and $p = 0.0427$ respectively). However, VLF, HF, LF n.u, HF n.u and LF/HF ration were not affected.

In conclusion, it seems that most HRV parameters were not affected by GSM signal in our study. The weak effect observed on frequency domain HRV LF or total power is likely to represent a random occurrence rather than a real effect.

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P04-042
Aluminum increases colorectal cancer cell metastasis through Smad2/3 signaling pathway

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Aluminum (Al) is an abundant element found in environment and in foodstuffs. Human body, such as the digestive system is continuously exposed to Al. However, the effects of Al to the intestinal epithelium have rarely investigated. Particularly, the influence of Al in the metastasis of colorectal cancer cells has not been reported. Therefore, we investigated whether Al influences on the metastasis of human colorectal cell line, HT-29. Cells were treated with Al for acute (72 h, 1–4 mM) and chronic (30 weeks, 100–200 μM) exposure schemes. Results showed that cells treated with Al either acute or chronic exposures promoted migration and invasion of cells. The acute exposure of cells to Al decreased cell adhesion, whereas the chronic exposure increased cell adhesion. To further study the underlying mechanisms, expression of the genes and proteins associated with cancer metastasis were measured in cells. Acute exposure of cells to Al decreased both mRNA and protein levels of E-cadherin, while vimentin and snail were increased. Chronic exposure of cells to Al, however, did not alter expression of vimentin. Furthermore, nuclear translocation of Smad2/3 and gene expression of MMP-7 and 9 increased in cells treated with Al. These results indicate that activation of Smad2/3 is a key signaling pathway in cancer cell metastasis due to Al. It seems that the exposure of Al to the digestive track is a potential risk factor in the initiation of metastasis.

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P04-043
Developmental and toxicological joint effects of selected fungicide mixtures in zebrafish embryo

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An increase in the use of pesticides to control pests that attack agricultural and wine crops (eg, mildew and powdery mildew) has been observed over the years due to climate changes. These compounds, once applied, are subsequently detected at residual levels in food and freshwater. In this sense, natural-based products have been studied as possible ecofriendly alternatives. However, there is the need to assess the impact that these compounds to aquatic ecosystems. As such, the objective of this study was to evaluate the toxicological effects of a mixture of commonly used synthetic fungicides or Mix 1 at environment relevant concentrations (azoxystrobin (10 μg/mL), tebuconazole (5 μg/mL) and mancozeb (0.5 μg/mL)), a mixture of natural compounds or Mix 2 (Equisetum extract (6.25 μg/mL), Mimosa tenuiflora extract (8 μg/mL) and thymol (0.2 μg/mL)), with fungicidal properties, as well as to the mixture of both classes of fungicides or Mix 3 (all compound at previous concentrations descript) in the development of the zebrafish embryo.

Embryos in the blastula stage (~2h post-fertilization) were exposed for a period of 96h to the Mixes described before. During exposure, the mortality, spontaneous movements, heartbeat, hatching rate, malformations effects were evaluated. At the end of exposure, the activity of the enzymes superoxide dismutase, catalase, glutathione reductase, glutathione S-transferase, lactate dehydrogenase, acetylcholinesterase and carboxylesterase as well as the levels of the reduced and oxidized forms of glutathione, lipid peroxidation and reactive oxygen species were evaluated.

All the animals showed a normal development although exposure to the Mix 2 induced an increase in glutathione peroxidase activity. The exposure to the Mix 1 showed no differences relative to the control group. Similarly, no significant differences between the Mix 1 and Mix 2 were observed.

The results of this study show that exposure to the synthetic compounds at environmental relevant concentrations was not effective in inducing embryo-physiological alterations and oxidative-, neurotransmission- and energy-related changes on zebrafish embryo. On the other hand, and as expected, the results show that natural compounds potentially induce antioxidant activity. Overall, the results obtained deserve further studies.

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P04-044
Used of urtica dioica and capsicum frutescens for pathogens' management

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In Morocco, vicia faba is considered the most important legume for both food and feed. However, the ravages caused by pathogens remain hardly controlled. The objective of the present study is to evaluate the efficacy of Urtica dioica and Capsicum frutescens' extracts in protecting broad beans against Bruchus rufimanus infestation. To this end, the research was conducted at the National Institute for Agricultural Research in Meknes (INRA) during the 2015-2016 crop year. The experimentation was conducted in pots with four replicates to evaluate the efficacy of aqueous extracts of urtica dioica and capsicum frutescens on five varieties of Vicia faba beans. The cultures were spread with the aqueous extract of these two plants from the beginning of flowering to maturity. The results of the study showed that the aqueous extract of c. frustescens at 100g/l dose, decreased the rate of infestation of beans by bruchids by 9.49% while for the other treatments, no decrease was recorded. In addition, an increase in yield was notified for the aqueous extract of u. dioica at the dose of 50g/l with a percentage of 1.86%.

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P04-045
Copper exposure reduces the lifespan in *Caenorhabditis elegans*

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Environmental pollution from heavy metals has proven to be a major global environmental problem. As one of the most widely heavy metals, copper exposure does cause harm to human health. This study conducted *Caenorhabditis elegans* (*C. elegans*) model to explore the effect of copper on lifespan. The synchronized L1 stage *C. elegans* worms were exposed to different concentrations of copper (0, 0.01, 0.1, 1 and 10 mg/L) for 48 h, respectively. To evaluate copper effect on lifespan in *C. elegans*, we performed lifespan assay, body length assay and brood size assays in worms after copper treated. AM141 worms were utilized for PolyQ aggregation assay to evaluate aging, and the number of poly(Q) aggregates was counted with the epifluorescence microscope. Aging process response genes including daf-2, age-1 and daf-16 were measured by quantitative RT-PCR (qRT-PCR). Fitness-related traits including developmental rate, brood size and lifespan were important in life history of *C. elegans*. Brood size was used to measure capacity of reproduction, our results showed that brood size under different copper concentrations (0, 0.01, 0.1, 1 and 10 mg/L) were 234.11 ± 16.00, 198.77 ± 7.51, 171.00 ± 35.34, 146.11 ± 24.36 and 128.63 ± 32.20 ($P < 0.05$), respectively. What's more, body length of *C. elegans* were 346.53 ± 29.21, 322.86 ± 38.20, 291.49 ± 32.35, 287.08 ± 36.50, 279.16 ± 35.14 μm ($P<0.05$), respectively. The result of lifespan assay showed that the longest longevity of each group were 23 days, 21 days, 20 days, 19 days, and 18 days ($P<0.05$), respectively. Besides, the average longevity was 21.00 ± 1.76, 18.70 ± 1.42, 18.30 ± 1.95, 17.40 ± 1.71 and 16.80 ± 1.48 days ($P<0.05$), respectively. Then, the number of poly(Q)40::YFP aggregates revealed that copper promoted PolyQ-YFP accumulation in the muscle cells of AM141 worms. The result of qRT-PCR showed copper could promote the expression of *daf-2* and *age-1*, and inhibit the expression of *daf-16*. In conclusion, our findings suggest that copper could inhibit growth and development of worms, and suppress survival and lifespan of *C. elegans*.

P04-046
Mercury-induced cellular damage is associated with enhanced mitochondrial DNA damage, oxidative stress and mitochondrial dysfunction

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Background: Alterations in mitochondrial function has been associated with several pathological conditions induced by exposure to environmental xenobiotics. Here we investigate mercury induced cellular toxicity and its associated changes in mitochondrial structure and function.

Methodology: Human dermal fibroblast cells were treated with mercuric chloride ($HgCl_2$), changes in mitochondrial structure and function were assessed. DNA damage assessment was done by long-

amplicon PCR and alterations in mitochondrial structure/mass by Mitotracker red/Nonyl-acridine orange dye staining. Functional changes were analyzed by detection of cytosolic/mitochondrial ROS, mitochondrial membrane potential, activities of respiratory complexes, aconitase activity, ATP and mitochondrial GSH levels. Induction of mitochondrial biogenesis was measured by changes in expression of PGC1-α and Nrf1.

Results & Conclusion: $HgCl_2$-treated human dermal fibroblast cells showed higher lesion frequency in mitochondrial DNA versus nuclear DNA indicating higher sensitivity of mitochondrial genome. Initial increase in mitochondrial ROS was accompanied by a gradual increase in cytosolic ROS levels and this was paralleled by loss of mitochondrial membrane potential along with decline in mitochondrial respiratory complex activity, depletion in MMP, aconitase activity and respiratory enzyme complexes along with lowering of ATP levels. Decrease in mitochondrial function was associated with enhanced mitochondrial fission. Elevated expression of Nrf1 and PGC-1α indicated the involvement of mitochondrial biogenesis during mercury intoxication that may contribute to maintenance of mitochondrial mass. We infer that mitochondrial biogenesis act in a harmonious way to maintain cellular integrity and prevent cell death after mercury exposure in fibroblast cells.

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P04-047

This abstract has been withdrawn.

P04-048
Lithium, selenium, cobalt and other elements in scalp hair from a group of young Spanish adults

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Human scalp hair has been suggested as an appropriate tissue to determine body burden of various metals although this raises controversy in the scientific community due to different factors influencing their content in this matrix. Lithium (Li), selenium (Se) and cobalt (Co) have been linked to mood and brain function, recent reviews have reported an inverse association for these metals in human scalp hair with suicide rates. As a pilot study, we have determined the levels of these metals, including arsenic (As), mercury (Hg) and lead (Pb), in scalp hair from 37 young adults (20 to 24 years-old; 28 female and 9 male) from different towns in the Community of Madrid (Spain). After appropriate pre-treatment of samples following previous methodologies, metals were monitored by ICP-MS. The limits of detection (ng/g) were: Li (1.98), Se (5.14), Co (0.75), As (2.0), Hg (1.0), Pb (1.0). The evaluated concentrations (median and percentiles provided in ng/g) were as follows: Li 5.44 (4.45, 7.47), Se 309.04 (255.47, 331.79), Co 7.53 (2.78-17.04). Concentrations for As (0.014), Hg (1.72) and Pb (0.64) were presented as arithmetic means in µg/g. Although our results are not reliable due to the differences in the number of participants by sex, levels of Se and Hg were significantly higher in males, which is in accordance with similar studies and could be related to the higher and significant intake of fish and shellfish previously reported in male participants in this group of population by our team. In general, the presence of these metals in the Spanish group's hair were within those highlighted in different studies performed in healthy Caucasian young adult populations. However, levels of Li, Co and Se were much lower than reference ranges reported for a Japanese population, which although from a different ethnic background, might suggest that the intake of these essential elements could be compromised in the Spanish group. Our results, although preliminary, might indicate that the intake of Li, Co and Se should be carefully monitored in Spanish young adults, as these have been recognised as essential metals for optimal brain function.

P04-049
Effect of the bread-making process on mycotoxin levels

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Wheat is a cereal very susceptible to contamination by mycotoxigenic fungi. In this research, it is analyzed the stability of Aflatoxins B1, B2, G1, G1, zearalenone and enniatins A, A1, B and B1 during baking process of sliced bread. Therefore, sliced bread is made with previously contaminated flour with strains of fungi producers of these mycotoxins. Levels of these mycotoxins produced on sliced bread are analyzed with liquid chromatography coupled with a triple quadrupole mass spectrometry (LC-QqQ-MS), previously the extraction of nine mycotoxins was optimized with methanol. The highest reduction in mycotoxin levels was found in the first fermentation (first proof), while the lowest reduction was observed in the baking stage. The effect of baking in sliced bread was determined by four different temperatures and times: 180°C (100 minutes), 200°C (60 minutes), 220°C (40 minutes) and 220°C (15 minutes). The results showed that maximum reduction occurs during the fourth condition and further reduced mycotoxin level was obtained with zearalenone, while aflatoxins B2 and G1 present greater reduction than the B1 and G2 and the lower decrease in the mycotoxin level in sliced bread was observed in enniatins. The mycotoxin reduction during sliced bread making is minimum, so it is important that the presence of fungi and mycotoxins are controlled in raw material.

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P04-050
Association between blood lead, high sensitivity C-reactive protein and metabolic syndrome

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Purpose: Environmental exposure to toxic heavy metal such as lead and systemic inflammation have been suggested as risk factors

for cardiovascular disease. However, little is known about the association between environmental lead exposure, elevated inflammation marker and metabolic syndrome. The aim of this study was to investigate the association between blood lead, high sensitivity C-reactive protein (hsCRP) and metabolic syndrome.

Methods: Data used in this study was from the Korea National Health and Nutrition Examination Survey (KNHANES) in 2016 and 2017. There were 13,037 subjects who attended all the tests for metabolic syndrome. Among them, 5,258 subjects who were tested for blood lead hsCRP were included in the analysis.

Metabolic syndrome was diagnosed when the subject had three or more of the following measurements: abdominal obesity (waist circumference of greater than 90 cm in men, and greater than 85 cm in women), high triglyceride level (150 mg/dL or greater), low HDL-cholesterol level (less than 40 mg/dL in men or less than 50 mg/dL in women, high blood pressure (systolic blood pressure of 130 mmHg or greater, or diastolic blood pressure of 85 mmHg or greater), high fasting blood glucose (100 mg/dL or greater). Blood lead concentrations were divided into quartiles based on the distribution. hsCRP levels were divided into three categories: low (less than 1 mg/dL), moderate (1 ~ 3 mg/dL), and high (greater than 3 mg/dL).

Logistic regression analyses were performed to calculate odds ratios (OR) of blood lead level and hsCRP level for having metabolic syndrome.

Results: There were 1,290 subjects (24.5%) who were met the criteria of metabolic syndrome. The median of blood lead was 1.603 μg/dL (interquartile range 1.199 ~ 2.145 μg/dL). Mean values of hsCRP and blood lead were statistically significantly higher in those who had metabolic syndrome ($p < 0.001$, respectively).

Blood lead level was statistically significantly associated with metabolic syndrome. Compared to the lowest quartile of blood level (< 1.199 μg/dL), OR of the highest quartile of blood level (> 2.145 μg/dL) was 1.548 (95% confidence interval [CI] 1.168 ~ 2.051), after adjusting for age and sex.

hsCRP level was also statistically significantly associated with metabolic syndrome. Compared to the lowest level of hsCRP (< 1 mg/dL), OR of the highest level of hsCRP (> 3 mg/dL) was 2.988 (95% CI 2.275 ~ 3.926), after adjusting for age and sex.

These association was not significantly changed after adjusting for blood lead level and hsCRP simultaneously.

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P04-051
Proposal of next-generation system in big data era based on chemical data science – Integrated toxicity research support system adapted to the new era

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Introduction: Changes in toxicity research approach and computer environments.

At present, toxicity research needs to be developed in consideration of the mechanism of AOP, and application of powerful data analysis methods such as artificial intelligence is required. On the other hand, computer technology is rapidly advancing in both hardware and software. As a result, next-generation system is required to apply totally different ideas and technologies from the past.

Purpose: Current system features and limitations and problems. Most of the systems currently deployed are designed / developed to achieve the best performance according to individual goal. Therefore, as the external / internal environment changes, it becomes difficult to adapt the originally designed function to the new environment. Changes in toxicity research methods and approaches: Toxicity studies themselves also need to consider toxicity mechanisms such as AOP. In such a case, a system that does not consider mechanisms can't be applied to the latest toxicity studies.

Changes in the computer related environment: Computer-related environmental changes are extremely rapid. In a short period of time, the conventional technology becomes old, and it is necessary to introduce and adapt a new technology.

Conclusion: In the latest toxicity evaluation research, an approach considering the toxicity development mechanism such as AOP is important. As a result, in addition to the conventional toxicity prediction software, coordination with a system having a toxicity mechanism analysis function is required.

As described above, the development of toxicological data analysis methods and the advancement of computer-related technologies (including big data and AI) require technologies and approaches that are different from conventional system construction. In this poster, we propose the next-generation system for toxicity research and evaluation by computer.

P04-052
First detection of *Acanthamoeba* spp. and *Balamuthia mandrillaris* in different water ecosystems in Leicestershire (UK)

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Acanthamoeba spp., *Naegleria fowleri and Balamuthia mandrillaris* can produce severe brain infections in immunocompetent and immunocompromised individuals. These free-living amoebae (FLA) have a worldwide distribution. Despite the rarity of brain infections by these organisms in the United Kingdom (UK), generally linked to travelling exposures, the incidence of *Acanthamoeba* keratitis (AK) is significantly higher in the UK than in other European countries or the United States. However, to date, isolation of these pathogens in the UK is limited to *Acanthamoeba* spp., mainly in drinking water supplies. Three sets of 30 water samples were collected, according to the US Environmental Protection Agency (EPA) method 1623, from different open water systems in Leicestershire (UK) per season between March and November 2018 using a portable water pump connected to a foam filter module. Water samples were collected in the same locations each season from: 15 ponds (in public parks)/water reservoirs; 7 from the River Soar; 2 from a canalised section of the River Soar, Grand Union canal; 1 from the River Biam and a marina near the River Soar; 4 from lakes highly frequented for fishing or leisure (John Merricks', Kings Lear's; Bennion Pools Fishing and Abbey park). Water samples were concentrated using the IDEXX® Filta Max system according to manufacturer's instructions and EPA method 1623; DNA was extracted using a FastDNA® Kit. Real-time PCR was used to detect these FLA according to previous methodologies. To our knowledge, these FLA were detected for the first time in 12/90 (13.3%) of the monitored samples in Leicestershire. *N. fowleri* was not detected in any sample; whereas *Acanthamoeba* spp. was detected in 11 water samples (12.2%) in all three seasons and environments monitored except the marina, which may suggest a wide environmental distribution of this pathogen in England. *B. mandrillaris* was found in John

Merricks' lake (1.1%) in Spring 2018, which is the first report of the presence of this pathogen in the UK. Our results highlight a potential risk for human health that should be carefully considered due to the high number of users of these water environments, particularly of the River Soar. Awareness of the presence of these pathogens and specific control measures should be provided to users of these open water systems.

P04-053
Exposure to TBBPA impedes vascular growth and disturbs metabolic pathways during early development in zebrafish

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Tetrabromobisphenol A (TBBPA), a widely-used brominated flame retardant, has been applied in a good number of industrial and commercial products. Of TBBPA's adverse health effects, impact on development is the primary concern. Epidemiological and animal studies have revealed an association between exposure to TBBPA and developmental problems. However, the effects and the underlying mechanisms of developmental defects resulting from TBBPA exposure are largely unknown. The vascular system which supplies oxygen and nutrients, maintains homeostasis and protects from toxic agents, is crucial for tissue development. Disruption of vascular development has been directly correlated with miscarriages, birth defects, maternal placental complications, and neurodevelopmental problems. In this study, we investigated the impacts of TBBPA on early vascular development using a zebrafish model. Zebrafish embryos were continuously exposed to waterborne TBBPA ranging from 0.5 to 300 μg/L starting from 2 hours post fertilization (hpf). Fluorescent images of vasculatures in kdrl:eGFP zebrafish were acquired using confocal microscope. Quantitative RT-PCR was applied to assess the mRNA levels. TBBPA-exposed zebrafish larvae did not exhibit significant difference in mortality, hatching rate, malformation and body length at 72 hpf. TBBPA exposure at 100 and 300 μg/L resulted in a delayed growth of common cardinal vein (CCV). The expression of genes related to angiogenesis and differentiation of endothelial cell, including Notch2, Hey2, Cdh5, Fli1a, Tal1, Rag1 and Npas4l, was suppressed in TBBPA-treated larvae. In addition, TBBPA exposure led to a reduction in the expression of Hmgcra and PPARγ, critical genes in lipolysis pathway. Strikingly, the enzymes responsible for glucose metabolism, including Hk1, Gk, Pk and Pepckc, dramatically decreased in zebrafish larvae exposed to TBBPA. The results reveal that developing vasculature in zebrafish is a sensitive target for TBBPA exposure. The findings indicate that TBBPA inhibits vascular development, and disturbs lipid and glucose metabolic pathway, which provide new insight into the mode of action of TBBPA upon developmental exposure.

P04-054
Effect of DEHP and DBP on steroidogenesis of adrenal gland in male Wistar rats

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Adrenal gland is a less focused endocrine organ for the endocrine disrupting effect of endocrine disrupting chemicals (EDCs) leading to the neglected study of steroidogenesis as the target of EDCs. The effects of two extensively used phthalate esters viz. di-ethyl hexyl phthalate (DEHP) and di-butyl phthalate (DBP) on adrenal gland were observed in Wistar rats in the present study to check the susceptibility of adrenal gland and steroidogenesis in it against the exposure of these extensively used plasticizers which are well known EDCs. Wistar rats were divided into seven groups (n = 6) and received the treatment for fourteen days. Group I was control and received only corn oil which is used as vehicle. Group II, III and IV received daily dose of DEHP of 250 mg/kg-BW, 750 mg/kg-BW and 1500 mg/kg-BW respectively while group V, VI and VII received daily dose of DBP of 100 mg/kg-BW, 500 mg/kg-BW and 1000 mg/kg-BW respectively. The comparative microscopic study of histological slides of endocrine glands i.e. pituitary, pineal, thyroid, parathyroid, adrenal gland and testes revealed the susceptibility of adrenal gland towards the DEHP and DBP. Steroidogenesis was analyzed by molecular docking of DEHP and DBP with the enzyme proteins of involved in steroidogenesis using Maestro Schrodinger 9.4 software showing the potential of DEHP and DBP to inhibit these proteins comparable to the known inhibitors of these enzymes. The mRNA expression study of the enzymes of involved in the steroidogenesis i.e. StAR, 3β-HSD, CYP21A1, CYP1B1 and CYP11B2 on exposure to DEHP and DBP by real time PCR has also assessed the sensitivity of the steroidogenesis towards DEHP and DBP. The mRNA expressions of StAR and CYP1B1 were up-regulated in dose dependant manner on exposure to DEHP and DBP. The expression of CYP21A1 was slightly up-regulated on DBP exposure but in case of DEHP it was comparatively more up-regulated. It was vice-versa in case of 3β-HSD that mRNA expression was slightly up-regulated on DEHP exposure and comparatively more up-regulated on exposure to DBP. CYP11B2 was down regulated on exposure to both DEHP and DBP. The present study gives a unique approach to elucidate the novel mechanism of endocrine disruption by EDCs through the analysis of the sensitivity of adrenal steroidogenesis on exposure to DEHP and DBP.

P04-055
Heavy metal evaluation in rescue dogs

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Purpose: Increasing environmental pollution caused by heavy metals, which are released by industrial and mine activities, is an important worldwide problem (Allan, 1997). Concentrations of lead, cadmium, arsenic, and mercury are strongly influenced by this type of discharge and are most frequent in wastewater. In Brazil, the Brumadinho dam disruption, on January 25, 2019, resulted in one of the largest mine tailings disasters. The dam rupture released about 12 million cubic meters of tailings. Initially, three dogs were used to retrieve victims. These animals remained for two months at the accident site, having direct contact with the mud contaminated by toxic discharge. In this context, the objective was to analyze metals in their blood circulation.

Methods: The mine, named "Córrego do Feijão", is located in the Brazilian county of Brumadinho, in the State of Minas Gerais. Blood samples from three adult male dogs were obtained for toxicological exams. The blood was collected and stored in tubes free of trace elements, with heparin. Measurements of aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), lead Pb) and mercury (Hg) were performed by atomic absorption.

Results: The animals showed the following mean values (mg/kg): Al -2,506; As – 0,011; Cd – 0,036; Cu – 1,449; Pb – 1,009; Hg – 0,010. High blood concentrations of Al, Cu and Pb were detected. Aluminium is non-essential and toxic element. Biologically reactive aluminium is present throughout the body and while it can rarely be acutely toxic, less is understood about chronic aluminium intoxication. Aluminium is a silent, if not potentially highly disruptive, visitor to biological milieus, which means that it piggy-backs upon essential bio-

molecules hijacking both their form and function (Exley, 2016). Pb is among the more common toxic metals present in our environment. The primary site of action of Pb is the central nervous system, and exposure to this metal is associated with several neurobehavioral alterations (Bradbury and Deane, 1993). The dogs did not show any acute symptoms, such as abdominal pain, constipation, and anemia. So, the chelation therapy was not recommended. Cu is an essential element, but toxicity is caused by excess in the body. Since the dogs showed no clinical symptoms or alterations in the complementary tests, it was suggested to remove the animals from the area, rest for a month and then reassess.

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P04-056

Evaluation of the effect of perfluorooctanesulfonate (PFOS) on DNA damage and highly reactive oxygen species generation in human peripheral blood mononuclear cells (*in vitro* study)

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Introduction: Perfluorinated compounds (PFCs) are commonly, widely produced substances used in industry since 1950. Due to their chemical properties and extensive using in consumer products such as textiles, food or coating for cookware, they are persist in the environment and have been detected in wildlife and humans. One of the most widely used PFCs is perfluorooctanesulfonate (PFOS), which was included in the group of organic pollutants (POPs) by Stockholm Convention. Up to now, the mechanism of PFOS action on human peripheral blood cells (PBMCs) has been poorly investigated. Taking the above into consideration we have assessed the potential of PFOS to generate highly reactive oxygen species (ROS, mainly hydroxyl radical) and to induce DNA damage in PBMCs.

Material and methods: PBMCs were separated from buffy coats by a density gradient method using Histopaque. The final concentrations of the compounds were in the range from 0,02 to 100 μM. In order to detect DNA single strand-breaks (SSBs) comet assay was employed. Highly reactive oxygen species were analyzed by flow cytometry using 3'-(p-hydroxyphenyl)-fluorescein (HPF).

Results and conclusion: Flow cytometry analysis (3'-(p-hydroxyphenyl)-fluorescein staining) showed that PFOS increased the intracellular highly ROS (20-100 μM) and the increase was concentration-dependent. However, observed changes were not statistically significant. Interestingly, comet assay analysis showed that PFOS (0,5-100 μM) induced DNA damage and no statistically significant alterations in this parameter were found only in PBMCs treated with the lowest tested concentration (0,02 μM).

Collectively, obtained results suggest that DNA damage and highly ROS generation not occurs in PBMCs of general population. It may be also concluded that PFOS induced DNA damage in tested cells in the concentrations which may enter the human body as a result of occupational exposure.

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P04-057

Environmental factors related with chronic kidney disease of unknown origin (CKDu) of Centro America: relationship of 28 element profile concentrations in drinking water with the prevalence in communities of Nicaragua.

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Mesoamerican nephropathy (MeN), also known as chronic kidney disease of unknown etiology (CKDu) is a public health problem in rural communities causing thousands of deaths mostly (but not exclusively) young male of the Centro America Pacific coast with extensive volcanic activity.

The relationship of ionic profile in drinking water with the prevalence of CKDu was evaluated in an ecologic epidemiological study studying the concentration of 28 elements by ICP-MS in rural communities in Nicaragua affected by high or medium prevalence of CKDu. Moreover a preliminary assessment of the biomonitoring of elements in hair has been also performed.

The mean values of some elements were higher that WHO and EU guideline. Some samples showed high K, Ca, Ni, As and Se. In the communities with high CKDu prevalence, significantly higher concentrations were found with Mg, Ca, Fe, Ba, Sr. On the contrary, Cu, Cr, As, Se, Cd were higher in areas of medium prevalence. The cases of high Mg and Ba over the guidelines were found only in areas of high prevalence. The special case or As: although median is below the guideline it concentration are considered higher than usual in most drinking waters, however is higher in the group of intermediate prevalence

By other side, the binary ratios of some elements as Ni/Na, showed predictive value. Although we cannot still stablish a cause-effect relationship, there are significant statistic correlations among elements. Two polarized groups are observed: one include Mg, Ca, Sr, Co and other AS, Ni, CD with positive correlations intragroup end negative intergroup.

Environmental factors (profiles of elements in drinking water) influence the prevalence of CKDu, which deserves to be evaluated in other affected regions.

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P05 – Genotoxicology and carcinogenesis

P05-001

Genotoxicological studies of novel food sources: alkaline comet assay

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The Russian system of complex biomedical research of genetically modified organisms (GMO) of plant origin includes general toxicological studies as well as specific types of toxicity studies, such as genotoxicological, reprotoxicological, immunotoxicological, allergological. The feature of this approach is the use of various experi-

mental models to identify possible unintended effects of genetic modification.

According to a modern concept of mutagenesis the mechanism of chromosomal aberrations associates with the molecular disorders induction which lead to DNA helix break. Therefore the use of a two-level approach, that includes the assessment of DNA structure integrity by the Alkaline Comet Assay (OECD 489) and Mammalian Bone Marrow Chromosomal Aberration Test (OECD 475), is of high diagnostic value when studying the genotoxic GMO effect.

Long-term experience of DNA comet research in framework of GMO safety assessment allowed to form a database (historical control) of DNA damage levels in the liver, kidney, bone marrow and rectum of healthy adult C57Bl/6 male mice (age 70–90 days). In total more than 100 animals (of control groups), 400 organs and 40 000 cells were examined and analyzed. Based on the data, the ranges of physiological fluctuations of DNA fragmentation levels for bone marrow cells were determined as 7.49 ± 0.38% (from 4.61 to 9.82), for liver as 7.22 ± 0.31% (from 5.62 to 9.45), for kidney as 7.65 ± 0.39% (from 5.15 to 9.54) and for rectum cells as 7.92 ± 0.40% (from 4.63 to 10.00) .

The possibility of historical control use provides more correct interpretation of data, that are obtained in genotoxicological studies of new GMO within the State registration procedure; such approach is consistent with current trends in toxicological studies.

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P05-002
Safety assessment of genetically modified soybean: potential genotoxicity

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The potential genotoxicity evaluation of genetically modified organisms includes the Bone Marrow Chromosomal Aberration Test (OECD 475) and the Alkaline Comet Assay (OECD 489) within *in vivo* experiment on C57Bl/6 mice. The two-level approach based on performing of DNA integrity assessment along with changes in chromosome structure detectable by microscopic examination of the metaphase stage of cell division, allows to register both DNA helix break and chromosomal aberrations in genomes.

The aim of this study was to investigate the possible genotoxic risk of the genetically modified (GM) fat-free soybean flakes containing GM line MON87701×MON89788. The 40 days experiment was performed on male mice of C57Bl/6 line with the initial bodyweight of 16–18 g. The animals were divided equally and randomly into two groups receiving soybean flakes from traditional (control group) and GM (exposure group) soybean flakes in the diet throughout the entire study.

The DNA structure integrity of the exposure group rats did not have significant differences from the control animals and averaged 7.65 ± 0.19% and 7.73 ± 0.20% in the bone marrow, 5.87 ± 0.55% and 5.47 ± 0.17% in the kidneys, 5.68 ± 0.21% and 5.62 ± 0.30% in the liver, 5.61 ± 0.20% and 5.91 ± 0.25% in the rectum, respectively. The percentage of cells with structural chromosomal aberrations in the bone marrow of the exposure group did not have significant differences from control animals and averaged 2.0 ± 0.4% and 2.1 ± 0.4% of damaged metaphases, respectively.

Thus, genotoxicological research did not reveal any genotoxic effect of soybean flakes containing GM line MON87701×MON89788GM compared to the flakes produced from traditional soybean. The levels of DNA structure integrity and of chromosomal aberrations were similar in the control and exposure groups.

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P05-003
Influence of genotoxic asarone isomers on DNA strand break repair mechanisms

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α- und β-asarone are phenylpropanoids occurring in essential oils and rhizome of the plant species *Acorus Calamus*, which are used as food supplements, for tea or as flavoring compounds for sweets or alcoholic beverages. Both isomers are classified to be carcinogenic in rodents and are known for their genotoxic properties [1-3]. The mechanism of action underlying these genotoxic effects is not elucidated so far, which prevents an adequate risk assessment. Considering the toxic potential of the asarone isomers cytochrome P 450 monooxygenase-mediated oxidation seems to be a crucial step because the generated epoxide induces DNA adduct formation. This metabolic activation is postulated to be responsible for genotoxic and mutagenic effects of asarone isomers *in vitro* [4].

Data on persistence of DNA damage and the cellular response to these genotoxic effects are limited. Absent mutagenic effects in mammalian cells as well as a decrease of DNA adduct levels after 6 h suggest that DNA damage is recognized and signaling cascades are activated, which especially initiate DNA repair. Preliminary tests focusing on DNA repair showed that after 24 h DNA strand breaks were completely repaired. In a next step different key elements of DNA repair mechanisms were investigated, with a deeper focus on double strand break repair. An increase of phosphorylated histone H2AX, which is considered as marker for double strand breaks, was determined after short-time incubation up to 2 h. Furthermore, there are first indications that single strand break repair mechanisms are involved as well, demonstrated by a rapid repair of oxidative DNA damage in the Comet assay and *via* immunofluorescence of 8-Oxo-dG. To sum up, it can be said that activation of DNA repair mechanisms seems to play a crucial role in the cellular response to genotoxic asarone isomers or their respective metabolites. The potential contribution of base excision repair is currently under investigation.

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P05-004
Comparative study of *Salmonella typhimurium* tester strains TA1537, TA97 and TA97a in mutagenicity evaluation of tobacco products

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The Ames test is widely employed to assess mutagenicity using bacteria. The Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 471 states that at least five tester strains should be used for the Ames test, and *Salmonella typhimurium* TA1537, TA97 and TA97a can be used interchangeably. The Ames test has been

conducted to examine the mutagenicity of tobacco products and to compare relative mutagenicity of cigarette smoke. It is well known that cigarette smoke shows clear mutagenic activity in some tester strains including TA1537, however, few studies have been reported using strains TA97 and TA97a. Thus, here, we compared strains TA97, TA97a and TA1537 in terms of their sensitivity to detect a mutagenic response and the discriminatory power to distinguish between different types of cigarette.

We selected four types of test cigarette (i.e., 3R4F, 1R6F, 100% single grade burley, and 100% single grade flue-cured) for use in this study. The cigarette smoke condensate (CSC) derived according to International Organization for Standardization smoking conditions was subjected to the Ames test. The assay was performed according to OECD TG471 in the presence and absence of metabolic activation by S9.

Regarding sensitivity, we compared the minimum dose where a significant increase in the revertant colonies was observed in a dose-dependent manner for each strain. In the presence of S9, all three strains showed clear, positive responses for all the CSCs. Especially a significant increase in revertant colonies was observed for TA97a at a lower dose than for the other two strains. In the absence of S9, TA97 showed a positive response to the CSCs, whereas the other two strains did not due to growth inhibition. Regarding the discriminatory power, in the presence of S9, the three strains provided consistent results in terms of the mutagenicity rank-order (i.e., burley > 3R4F ≈ 1R6F > flue-cured) and the ability to discriminate statistically dose responses found in the different CSCs.

We suggest that *S. typhimurium* strains TA97, TA97a and TA1537 have different sensitivities in detection of a positive mutagenic response, whereas the three strains are comparable in their ability to discriminate between different types of cigarette smoke. Further investigation is needed to understand the mechanism underlying the difference in sensitivity between the three strains in mutagenicity assessment of cigarettes.

P05-005
In situ detection of DNA double strand breaks by immunofluorescent γ-H2AX staining in mice exposed to multiwalled carbon nanotubes

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Phosphorylation of histone H2AX (γ-H2AX) at serine 139 is an acknowledged biomarker of DNA double strand breaks in cultured cells and tissue biopsies. However, γ-H2AX *in situ* staining has rarely been used to detect genotoxic effects of nanomaterials.

The application of immunofluorescent (IF) γ-H2AX staining on tissue samples has many advantages. The same paraffin embedded tissues can be used for both histopathology and γ-H2AX analysis, which allows implementing the γ-H2AX assay also on previously conducted *in vivo* studies. The detection of γ-H2AX *in situ* enables the localization of the genotoxic effect in tissue-specific structures and even cell types. Analysis by microscopy can easily discriminate between cells with different levels of DNA damage and apoptotic cells.

The purpose of this study was to further evaluate the use of IF γ-H2AX staining for the genotoxicity assessment of nanomaterials *in vivo*. Groups of C57BL-6 female mice were exposed to three doses (10, 40 and 80 µg/mouse) of multiwalled carbon nanotubes (MWCNTs; Mitsui-7) by single pharyngeal aspiration. Lung samples for genotoxicity (Comet assay) and histopathological evaluation were collected 24 h and 28 d post-exposure and the results were compared to a negative control group. The IF γ-H2AX staining was performed on formalin-fixed paraffin-embedded lung samples after deparaffination and antigen retrieval by boiling. An autostainer was used for primary (rabbit monoclonal anti-gamma H2AX phospho-Ser139) and secondary (goat anti-rabbit IgG) antibody incubations and for tyramide amplification of the fluorescent signal (Alexa Fluor™ 488 Tyramide SuperBoost™ Kit; ThermoFisher Scientific) according to manufacturer's instructions. Samples were counterstained with 4',6-diamidino-2-phenylindole and digitized with 20x fluorescent scanning. Expression of γ-H2AX foci was analyzed using marker counter module of a digital microscope application. For each sample, all nuclei in four randomly selected annotations (200 µm x 200 µm) were classified as negative, weak positive (≤ 3 foci), positive (> 3 foci), or apoptotic (pan-stained nucleus).

The results showed a dose-dependent induction of γ-H2AX positivity 24 h post-exposure. 28 days later, the effect of the MWCNT exposure was lower, although the percentage of γ-H2AX positive nuclei remained elevated in the lungs of the exposed mice. These results were in line with comet assay data (% of DNA in tail) from the same animals. Hence, it has been shown that IF γ-H2AX staining can be used to complement the comet assay for monitoring DNA damage induced by nanomaterials *in vivo*.

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P05-006
Value of cooperation of industry & regulatory agencies example: cancerogenicity

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Introduction: Carcinogenicity studies are the most time-consuming, costly and resource intensive non-clinical investigations required for pharmaceuticals and chemicals. Therefore, the test strategies must be thoroughly analyzed to select the best options. The International Conferences on Harmonization (ICH) published first recommendations in 1997 [1], and later research data analyses [2] contributed to the optimization of the evaluation processes for bioassays.

This paper discusses the acceptability of the proposed alternative transgenic animal models as substitutes for long-term studies and about the extended ICH reconsiderations for the need to conduct such assays. The purpose of this paper is to stimulate the Industry and Regulatory Agencies to continue identifying common targets and cooperating to find optimal solutions.

Methods:

1. Selection of animal models:

European Public Assessment Reports (EPARs) were reviewed for drugs which received market authorization between 2010 and 2018. The focus of the review was the determination of the animal models selected for carcinogenicity testing.

2. Need for carcinogenicity studies?

An update of the ongoing discussions within the expert groups of the ICH S1A will be summarized [3]. The discussions over the past years attempt to justify a reduction of studies and number animals in carcinogenicity investigations [4].

Results:

1. Selection of Models

The review showed that 557/614 of the initially authorized medicinal products continued to hold a marketing authorization. No

carcinogenicity studies were needed for generic medicinal products (n=156), biosimilar medicinal products (n=28), medicinal products with informed consent applications (n=31), for fixed combination preparations (n=52), for hybrid applications (n=33) or for biotechnology-derived medicinal products (n=75). For 104 products, carcinogenicity bioassays with the active substance were conducted. For these 104 products, 54.8% were tested using a traditional / conventional strategy (long-term carcinogenicity bioassays in rats and mice), and in 26% the ICH-recommended transgenic alternative approach (one long-term assay in rats plus a short-term transgenic mouse study) was used. The remaining drugs were submitted to different designs.

2. No Need for Cancerogenicity Studies

Some reasons why carcinogenicity studies are not needed, are listed in the previous paragraph. Additional justifications could be: the lack of tumor-inducing mechanisms, limited exposure due to short-term indications (e.g. anesthetics, diagnostics), unequivocal genotoxic compounds (assumption of trans-species carcinogenic effect), low life-expectancy of 2–3 years for the target patients or drugs only used topically with no systemic exposure [1].

In 2011 [5], an industry group [Sistare *et al.*] reported the outcome of a data review comparing the results from chronic toxicity studies with long-term carcinogenicity investigations. This review showed that chronic repeat dose studies could predict a negative outcome of long-term bioassays, if there are no signs of pre-neoplasia, genotoxicity and/or hormonal perturbation. These results led to an ICH-sponsored program to test this hypothesis: sponsors were asked to submit a prediction of the carcinogenic potential of their product in a Carcinogenicity Assessment Document (CAD); regulators were to decide about virtual waivers for carcinogenicity studies (starting from 2015) and finally the value of the prediction was to be checked against the real carcinogenicity studies conducted.

By the end of 2018, 48 CADs were received by drug regulatory authorities (DRAs): 24 of them were assessed by Industry as Category A/B (A=likely in rats, irrelevant in humans; B=highly unlikely in both rats and humans) and only 12 CADs associated with Category 3A/B by DRAs.

Conclusions:

1. The favorites of alternative models are transgenic mouse models with activated oncogenes (TG.rasH2 & TG.AC) or inactivated tumor suppressor genes ($p53^{+/-}$). The incidences of tg models increased from 5.5% before 2010 (6) to 26% before 2018.

2. ICH S1A revision: The data collection is finalized. The review process continues. By now, there exists in 61% of the cases concordance between regulators and industry. For 7/28 no need for long term assays would be requested.

The final revision of ICH S1A is expected in 2020.3. Thorough cooperation between Regulatory Agencies and Industry experts can result in finding optimal solutions for issues.

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P05-007
Possible threshold setting for aniline toxicity with transgenic Big Blue® Fischer344 rats.

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Aniline, an environmental contaminant and residue/impurity in products, has been identified as a human health concern due to the occurrence of splenic tumors in rats. There are carcinogenic mechanisms proposed; genotoxicity via direct interaction with DNA (non-threshold), or an indirect, threshold-based mechanism via the accumulation of iron and the generation of oxidative stress after methemoglobin (MeHb) formation (see proposed AOP). We attempted to clarify whether aniline is mutagenic or not in transgenic rats. Toxicological mechanisms were also investigated concurrently.

Male Fischer 344 Big Blue® rats were given daily oral doses of 0, 25, 50 or 100 mg/kg/day aniline for 28 days. Mutant frequency was measured in liver, spleen and bone marrow at Day 31. Aniline concentration in plasma, MeHb in blood, erythrocyte (RBC), hemoglobin concentration (Hb), peripheral immature erythrocyte (reticulocyte: RETI) were also measured on day 3 or 4 of dosing and on completion of dosing (day 28 or 29). A portion of spleen was subjected also to histopathology.

Exposure to aniline was confirmed by dose-dependent plasma concentrations but there was no increase of mutant frequency in any tissues. Around day 4, MeHb was increased dose-dependently, while decreased Hb and increased RETI were limited to the top dose. At termination, decreased RBC and Hb and increased RETI were also observed from the lower doses as increase of MeHb became prominent. Iron deposition was confirmed in spleen by Prussian blue staining.

Overall, aniline is not considered to be a direct mutagen. After MeHb formation and subsequent erythrotoxicity, iron deposition in spleen and compensatory erythropoiesis in bone marrow (increase of RETI) was observed, which is in line with the proposed mechanism. A threshold-based risk assessment can be applied for aniline.

P05-008
Exposure to dioxin modulated distinct responses of two subtypes of diffuse large B-Cell lymphoma cells determined by computational prediction and gene expression profile

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The incidence of non-Hodgkin lymphoma (HNL) has increased dramatically worldwide especially in developed countries. Environmental exposure to dioxin, have been implicated correlated with non-Hodgkin's lymphoma (NHL) in epidemiological studies. Dioxin is a persistent organic compound containing polychlorinated biphenyl structure, and enable to activate aryl hydrocarbon receptor (AHR) and modulate inflammation in immune responses. Diffuse large B-cell lymphoma (DLBCL) is the most common type of NHL, and classified into two major biologically distinct molecular subtypes: germi-

nal center B-cell (GCB) and activated B-cell (ABC). Patients with ABC DLBCL have been observed presenting substantially worse outcomes of treatment and poor prognosis. Thus, this study used the approaches of computational prediction and gene expression profiling to characterize the modulation of exposure to dioxin in distinct responses of two subtypes of DLBCL for investigating the potential genetically based indicators toward treatment of DLBCL.

This study firstly performed a computational method for the analysis of differentially expressed genes (DEGs) respectively in DLBCL tissues and in human cell lines exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; the most toxic dioxin) according to the microarray datasets from ArrayExpress. The results of the constructed gene-network showed that the biological pathways associated with DLBCL development included regulation of cell cycle phase transition, type I interferon production, Wnt signaling pathway, programmed cell death, and inflammasome-related genes. In the comparison of SU-DHL2 cells (ABC-like) with SU-DHL4 cells (GCB-like), TCDD treatment activated AhR with no obvious effect on NLRP3 and Caspase-1, which was leading to cell cycle arrest at the S to G2/M phase transition and reduce apoptosis in dose-dependent. Additionally, the distinct pathway in two subtypes of DLBCL cells presented ATF4-CREBBP-CYP1A1-TP53-CDKN1A-CTNNB1-RAC1-TLR4-NFkB-AKT1-GSK3. The current study indicated that exposure to dioxin could reduce the sensitivity to inflammasome and cause cell proliferation underlying reduction of apoptosis potentially for lymphogenesis and drug resistance of treatment. These novel target genes for lymphomagenesis identified in this study can provide a reference for disease prevention and cancer treatment.

P05-009
Mutagenicity assessment of a battery of compounds using a miniaturized-Ames test

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The bacterial reverse mutation test (Ames test) is a robust test, with an internationally agreed protocol (OECD 471), that is included in several standard genotoxicity testing strategies for pharmaceuticals (ICH) or food additives/contaminants (EFSA).

Several miniaturized versions of the Ames test have been used scaling down the original approach, reducing not only material costs but also time.

The aim of this work was to assess the predictivity of a 6-well plate miniaturized-Ames test comparing the results with the standard Ames test and increasing historical data. For that purpose, OECD Test Guideline 471 was followed using 5 *Salmonella typhimurium* strains (TA97a, TA98, TA100, TA102, and TA1535) with and without metabolic activation.

For that purpose, genotoxic compounds with different mechanisms of action (4-nitroquinoline 1-oxide [4NQO], cisplatin, colchicine, etoposide, methyl methanesulfonate [MMS] and potassium bromate) and non genotoxic compounds (TritonX-100, fluometuron, D-mannitol, ethylenediaminetetraacetic acid and Tris(2-ethylhexyl) phosphate) were evaluated at different concentrations in the miniaturized 6-well plates version of the Ames test. Furthermore, commonly used positive controls for each strain were included in each assay at one concentration: 4-nitro-o-phenylenediamine (NPD), mitomycin C, sodium azide (NAAZ), 2-aminofluorene and 2-aminoanthracene.

As expected, MMS, cisplatin, 4NQO and etoposide were found to be positive at least in one or more strains with and/or without metabolic activation. Results from positive controls were within historical data. Thus, 100% (11/11) of concordance with the standard Ames test was found but with the advantage of using 10-fold less quantity of compound.

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P05-010
Mode of action and human relevance for amisulbrom-induced rodent liver tumors

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Amisulbrom is a sulfonamide fungicide active ingredient and has been reviewed by Japan, EU, and US. In rodent carcinogenicity studies of amisulbrom, the incidences of hepatocellular adenomas increased in male/female rats and male mice. Amisulbrom has no genotoxic potential. In order to investigate the mode of action (MoA) and human relevance of amisulbrom-induced liver tumors in rodent, we conducted two experiments; 1) evaluating constitutive androstane receptor (CAR)-mediated mode of action using CAR KO rats, and 2) evaluating the effects on human hepatocyte using chimeric mice with humanized liver. In the experiment 1, Wild type (WT) and CAR KO rats received amisulbrom at 20,000 ppm or phenobarbital sodium salt (NaPB) at 500 ppm via the diet for 7 days. There were increased body weight relative liver weights, liver Cyp2b1/2b2 mRNA levels, and hepatocellular proliferation in the WT rats treated with amisulbrom or NaPB. In contrast, the treatment of the CAR KO rats with amisulbrom or NaPB did not cause these changes. In the experiment 2, chimeric mice with humanized liver (estimated more than 90% of the liver replaced with human hepatocytes) and severe combined immunodeficiency (SCID) mice, which were not transplanted human hepatocytes, received amisulbrom at 8,000 ppm via diet for 7 days. There were increased body weight relative liver weights, liver CYP2B6/Cyp2b10 mRNA levels and PROD activity in the chimeric mice and the SCID mice treated with amisulbrom. Increased hepatocellular proliferation was observed only in the SCID mice treated with amisulbrom, but not in the chimeric mice. These results suggest that amisulbrom has a potential for CAR activation in rodents and humans, and the amisulbrom-induced liver tumors in rodents is due to CAR activation and subsequent hepatocellular proliferation. In contrast, amisulbrom fails to induce proliferation of human hepatocytes in the chimeric mice with humanized liver. In conclusion, it is suggested that amisulbrom-induced liver tumors are mediated by CAR activation and the MoA for rodent liver tumors formation is not relevant to humans.

P05-011
Triorganotin derivatives: time-dependent expression of Vimentin, Annexin A5 and selected nuclear receptors mRNA in MDA-MB-231 breast cancer cells

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Trirganotin compounds are typical environmental contaminants that are used as biocides, agricultural fungicides, wood preservatives, and special paints for marine ships and endocrine disrupters [1,2]. A remarkable breakthrough in this field has been found that the triorganotin compounds are agonists of the RXR nuclear receptor subtypes [3]. Vimentin plays a very important role in the process of metastasis and its expression is typical for neoplastic cells with metastatic properties. The observed protein is a key element regulating the expression of the EMT-related transcription factors and thus it is associated with the metastatic spread of cancer. In addition, overexpression of Vimentin indicates the aggressive and invasive type of breast cancer [4]. Annexin 5 is the protein playing an anti-apoptotic role, promoting metastatic process and progression of breast cancer [5]. In this study, *in vitro* the effects of triorganotin ligands of nuclear retinoid X receptors in human MDA-MB-231 breast cancer cells were analysed. The cells were exposed to tributyltin and triphenyltin derivatives (TBT-Cl, TPT-Cl, TBT-ITC, TPT-ITC), 9-*cis* retinoic acid (9cRA) (100 nM) and/or *all-trans* retinoic acid (ATRA) (1 μM) for 6, 12, 24 and 48 hours. Expression of mRNA genes for Vimentin, Annexin A5 and selected nuclear receptors was analyzed by semi-quantitative real-time PCR. ATRA, 9cRA and tributyltin derivatives alone or in combination with ATRA, was found to significantly induce the expression of Vimentin and Annexin A5 mRNA after 6 h. However, after 12 h and 24 h the expression was decreased and these effects were fully manifested after 48 hours of cultivation with the substances. In the case of RARbeta receptors, the action of triorganotin derivatives resulted in increased gene expression after all time ranges, with the combined effect with ATRA resulting in a synergistic enhancement of expression. Expression of RARgamma mRNA was found significantly increased after 6 hours, but after 24 and 48 hours the increase was diminished. After 6 hours after administration of retinoic acids and triorganotin derivatives, expression of RXRalpha was increased, but a decrease in expression was observed after 48 hours in cells treated with TBT-Cl and ATRA alone and with combination of ATRA with triorganotin derivatives. Given the role of vimentin in the epithelial-mesenchymal transition process and annexin A5 in the membrane repair process, the synergistic effect of triorganotin compounds, and the ATRA mediated by RXR/RAR heterodimer could represent a promising opportunity to inhibit metastasis of aggressive forms of hormone-resistant tumours. *This project has been supported by the grants APVV-15-0372 and Vega 2/0171/17.*

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P05-012
Genotoxicity, homocysteine, dietary micronutrients and MTHFR gene polymorphisms in psoriatic patients treated by Goeckerman regimen

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Background: Goeckerman regimen (GR) of psoriasis vulgaris is a therapeutic combination of crude coal tar application and ultraviolet irradiation. Both these agents could induce genotoxic effects. Insufficient amounts of micronutrients including vitamin B12 and folic acid lead to genomic instability and possibly intensified genotoxic effects of GT.

Objective: We examined DNA damage, serum homocysteine, vitamin B12, folic acid, and two polymorphisms (C677T and A1298C) in the MTHFR gene in patients with exacerbated psoriasis vulgaris treated by GR.

Methods: Study group consisted of thirty-five patients classified according to PASI score. Genotoxicity was evaluated by the number of micronucleated binucleated cells (MNBC). Serum homocysteine, vitamin B12 and folic acid were determined immunochemically. DNA analysis was performed via real-time PCR.

Results: The median of PASI score decreased from 19.2 to 4.9, MNBC increased from 10 to 18‰ after GR ($P < 0.001$ in both cases). Homocysteine, vitamin B12 and folic acid were not changed significantly by the therapy. Correlations of MNBC with homocysteine and vitamin B12 before the regimen were observed. Hyperhomocysteinemia was an independent predictor of genotoxicity (OR 9.91; 95% CI, 2.09–55.67; $P = 0.003$). There was found a significantly higher MNBC In CC homozygous patients (A1298C polymorphism), than in AC heterozygotes and AA homozygotes.

Conclusion: Homocysteine is engaged in the pathogenesis of psoriasis vulgaris. Its serum levels correlating with MNBC enabled prediction of DNA damage induced by Goeckerman regimen. A potential link between the MTHFR C1298A polymorphism and genotoxic effects of this therapy was found. Both micronutrients status and homocysteine metabolic pathway contribute to genotoxicity of Goeckerman regimen.

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P05-013
Genotoxic effects of different technical products of dimethoate

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Technical products of generic pesticides may have dissimilar toxicological profiles due to different levels of impurities. Dimethoate is an organophosphate insecticide and acaricide. The data on its genotoxicity are controversial.

The aim of this study was to assess and compare genotoxic potentials of two technical grade active ingredients (TGAI) of dimethoate from different manufacturers.

The Ames test and mammalian erythrocyte micronucleus test were used for this study. It was shown that TGAIs of dimethoate induced reverse gene mutations in *Salmonella typhimurium* both in the presence and in the absence of S9 mix. However, the effect levels were different. TGAI1 shown statistically significant genotoxic effects only

in two strains. At high concentration (5 mg/plate) fold-increase in the number of revertant colonies per plate relative to vehicle was 1.7/1.9 (TA-100/TA-102; -S9) and 1.7/2.0 (TA-100/TA-102; +S9). TGAI2 induced the higher levels of mutations in 3 strains: 2.4/2.7/2.8 (TA-97/TA-100/TA-102; -S9) and 2.2/3.3/2.3 (TA-97/TA-100/TA-102; +S9) at concentration 5 mg/plate and the effects were dose-dependent. TGAI1 was non-genotoxic in micronucleus test *in vivo*, whereas after administration of TGAI2 to CD-1 mice (oral gavage; three doses) we observed the statistically significant dose-dependent increase in the incidence of micronucleated polychromatic erythrocytes (mPCE) in bone marrow (2.1-fold relative to negative control at high dose). However, the incidence of mPCE was slightly beyond the upper Poisson-based 95% control limit for the historical negative control. 95% Wald confidence intervals for the mean of mPCE were [0.09;0.11] and [0.17; 0.30] for vehicle and dimethoate at high dose (60 mg/kg b.w.), respectively.

The observed difference between two technical products probably is due to the quality and quantity of impurities. Dimethoate may contain omethoate and isodimethoate as the relevant impurities. According to FAO specification, the maximum levels of these impurities must not exceed 2 g/kg and 3 g/kg, respectively. Genotoxicity of omethoate was shown in some research [1].

Therefore, our data indicate that dimethoate has a weak genotoxic potential and the different technical products of the same pesticide active ingredient may reveal dissimilar genotoxicity level.

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P05-014
Cylindrospermopsin induces genotoxic damage in rats by the comet and micronucleus tests

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Nowadays, as a result of climate changes and water eutrophication an increase in the production of toxic cyanotoxins is happening. Cylindrospermopsin (CYN) is a cyanotoxin produced by different species with cytotoxic and hepatotoxic effects. Humans can be in contact with CYN by different routes, being the oral intake the main one. Among the toxic effects of CYN its genotoxicity is a keypoint. Previous studies pointed out that CYN is pro-genotoxic *in vitro*. Therefore, in accordance with the European Food Safety Authority (EFSA), it is needed to assess its genotoxicity *in vivo.* In this work, the genotoxic potential of CYN in Wistar rats was evaluated in liver, stomach and blood by the standard comet assay (OECD 489) and on bone marrow by the micronucleus test (MN, OECD 474). Moreover, the enzime-modified comet assay (Endonuclease III (Endo III) and Formamido pyrimidine glycosylase (FPG)) was used to assess oxidative DNA damage. Animals were exposed to CYN by oral gavage (7.5, 23.7, and 75 µg/kg body weight). Results for the standard comet assay showed no significant increase in DNA strand breaks at any dose assayed. However, after the post-treatment with Endo III a significant increase in the % of DNA in tail was observed in liver and blood cells exposed to the highest dose. Additionally, oxidative damage was observed in blood cells in presence of FPG after exposure to 23.7 and 75 µg/kg. Moreover, results obtained for the MN test showed CYN genotoxicity at any dose tested with increase in the % MN in immature erythrocytes. Therefore, these results show that CYN is genotoxic *in vivo* providing important data for a better risk assessment of this biotoxin.

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P05-015
Genotoxic and genoprotective effects induced by a stilbene extract in HepG2 cells

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The addition of sulfur dioxide seems to be essential during several processes involved in winemaking and ageing wine. However, due to health concerns and a recent growing consumer interest in wines containing less additives, its use has been a field of discussion in wine industry. Stilbenes are candidates of great interest for this purpose, not only because of their antioxidant and antimicrobial activities, but also because they are naturally found in the grapevine. In the present study, the *in vitro* genotoxicity and genoprotective effects of an extract from grapevine shoot, with a stilbene richness of 86%, was investigated. The exposure concentrations were selected based on previous studies in which the EC_{50} was determined in HepG2 cells. The standard and the FPG-modified version of the comet assay, after 24 h or 48 h of exposure to the extract, did not show genotoxicity at any of the studied concentrations. In order to evaluate the protection ability of our extract against DNA induced damage, HepG2 were pretreated with Ro19-8022 and then exposed to the extract during 24 h or 48 h. There were significantly lower levels of DNA breaks compared with control in cells preincubated with 15.95 µg/mL and 31.90 µg/mL for 24 h and with 22.30 µg/mL for 48 h, indicating an enhanced antioxidant defense. Similarly, incubation of Ro19-8022-treated cells with our extract led to a concentration-related decreased in induced DNA damage. Significant changes respect the control were shown from 31.90 µg/mL and 11.15 µg/mL after 24 h and 48 h of exposure respectively. In summary, our extract showed no genotoxic effects at any concentration tested. In addition, the extract presents interesting antioxidant abilities *in vitro*. Therefore, considering its promising usefulness as additive in wines, further studies are required in order to confirm its suitability and safety for this purpose.

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P05-016
A proposed workflow, considering non-testing methods, to qualify non-genotoxic impurities

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A reflection paper has been recently published by EMA addressing open issues in the qualification approach of non-genotoxic impurities (NGI) in chemically synthesized pharmaceuticals according to the ICH Q3A/Q3B guidelines. As highlighted in the EMA document, little guidance is provided on which criteria and methods should be applied to qualify NGIs, and concerns are expressed from a scientific and 3R's perspective on the current approach. When qualification of NGIs is required and data from the regular (non-)clinical development with the API batches is not considered sufficient, a recommendation to consider non-animal testing strategies, including both *in silico* and *in vitro* approaches, to evaluate the toxicity of individual NGIs, is discussed.

In the present poster we propose a workflow for the assessment of toxicity profile of individual NGIs based on non-animal testing strategies, with a particular emphasize on QSAR and read-across methodologies. A preliminary case-by-case analysis is performed considering the use and route of administration of the API as well as information on similar structures in order to define the set of endpoints to be investigated, and the most suitable *in silico* approaches to apply. The workflow can be represented as a decision tree, including the following key steps (each to be considered in a tier approach): 1) Evaluation of the applicability of the TTC approach for the target NGI. 2) Structural comparison with the API. 3a) NGI core structure is shared with the API: analysis of structural differences between the NGI and the API, including the assessment of any toxicologically relevant alert found in the NGI and not in the API. QSAR predictions are generated for selected endpoints to integrate alert analysis. 3b) NGI structure is not related to the API: QSAR predictions are generated for selected endpoints. 4) Search for structural analogues (including the API, if relevant) with experimental data on critical endpoints and/or endpoints not covered by the QSAR screening. 5) Evaluation of the feasibility of read-across. The proposed workflow offers the possibility to acquire impurity-specific data, also if testing is not feasible, and to decide on further *in vitro* testing, besides meeting 3R's principles.

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P05-017
Estragole DNA adduct formation in different liver cell models

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Estragole, one of the food-borne alkenylbenzenes, can naturally occur in a variety of herbs and spices such as sweet basil, fennel, star anise and essential oils. Estragole is of concern because of its genotoxicity and carcinogenicity, induced via DNA adduct formation after bioactivation [Miller *et al*.,1983]. In previous *in vitro* studies, alkenylbenzene DNA adduct formation was studied upon exposure of HepG2 cells to 1'-hydroxy metabolites instead of to the parent compounds, because of the limited P450 enzyme activities present in the HepG2 cells [Jeurissen, *et al*., 2008; Alhusainy, et al. 2013]. Use of 1'-OH estragole to induce DNA adduct formation may however result in levels of DNA adducts overwhelming DNA repair and thus will not provide a suitable *in vitro* model to study repair of alkenylbenzene DNA adducts. Therefore, this study was designed to define the most suitable cell model(s) to form a detectable level of DNA adducts upon exposure to the parent compound. HepG2 and HepaRG cells and primary rat hepatocytes were pretreated with or without CYP inducers and incubated with estragole or 1'-OH estragole. The DNA adduct formation upon exposure of the cells to both 1'-OH estragole or estragole increased in the order HepG2 cells < HepaRG cells < primary rat hepatocytes with levels upon exposure to estragole being 30 to 40 fold lower than upon exposure to 1'-OH estragole. The DNA adduct levels were not significantly affected by the CYP inducers.

It was concluded that non-induced HepaRG cells and primary hepatocytes exposed to estragole provide the most suitable *in vitro* models to study DNA adduct formation and subsequent repair.

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P05-018

This abstract has been withdrawn.

P05-019
Rhus coriaria extract: protective effect against UVA irradiation in human dermal microvascular endothelial cells (HMEC-1)

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Rhus coriaria L. (sumac) is a plant traditionally used in some countries (Turkey, Iran) as an anti- microbial and anti-inflammatory agent in skin disorders [1]. Its fruits are rich in tannins, anthocyanins, flavonoids and terpenoids and gallic acid derivatives [2]. Recently, different polyphenol-enriched extracts have been proposed for the prevention of UV-mediated skin damage. The extract was obtained by ethanol maceration for 48h of dried fruits. Then, the mixture was filtered and taken to dryness under reduced pressure and freezed dried. The extract (mERC) has been characterized in its components gallotannins and flavonols. UV-A rays can penetrate up to the dermal layer also affecting the microvascular endothelial cells of the papillary dermis, determining inflammation and photoaging, associated with redox imbalance. Treatment with the extract (10 and 25 µg/ml, 1h in serum-free media) was followed by the exposure to UV-A (10-15-20 J/cm^2) radiation in PBS on HMEC-1 cells. Cytotoxicity (MTT and apoptosis) and DNA damage (alkaline comet test, γH2AX foci formation) have been evaluated at T0 and T24 after exposure. The mechanisms of UV-A mediated DNA damage on this cell line have been also studied in order to highlight the ability of the extract components to interfere with oxidative damage (oxidized base formations, SBBs, apurinic sites), but also with the formation of cyclobutane pyrimidine dimers (CPDs) by comet modified with enzymes and immunohistochemistry assays.

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P05-020
UV-B damage: a potential molecular play of Vitis vinifera L. extract

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Ultraviolet component of sunlight UV-B (280-315 nm) is one of the major cause of skin damage. UV-B radiations have a low wavelength being absorbed almost completely by the epidermis; however, they interact directly with DNA causing molecular rearrangements. Photoaging and the development of skin cancer are of increasing relevance since lifestyle changes have led to an increase in the individual UV doses. Therefore, new prevention strategies have to be developed in order to reduce UV damage and delay photoaging process. In this context, different polyphenol-enriched botanicals have been proposed for the prevention of UV-mediated skin damage. Here it has been evaluated the antioxidant and DNA protective potential of an aqueous extracts of *Vitis vinifera L.*, validated for the contents of anthocyanins, flavonoids and caffeic acid, against UV-B radiation in human keratinocyte (HaCaT) cell line. Treatment with the extract (100 µg/ml, 1h in serum-free media), was followed by the exposure to UV-B (20-30-40-80-160-320-640 mJ/cm^2) radiation in PBS. The extract protects from the direct DNA damage induced by UV-B exposure even at doses where no oxidative damage was observed, as indicated by the alkaline comet and γH2AX tests, evaluated at T0 after the exposure. Moreover, it seems that the extract maintains the protective apoptotic pathway induced by UV-B. All these evidences led us to study the effect of the extract on DNA damage at molecular level, looking at the expression of genes involved in several pathways of DNA damage recognition, repair and apoptosis induction. Interestingly, the extract is able to modulate the expression of several genes involved both in damage signalling and NER (Nucleotide excision repair) pathway, mostly at 40 mJ/cm^2 dose. The most important effect of the extract is on GADD45α especially at 40 and 80 mJ/cm^2. This gene is pivotal in DNA repair pathway induced by UVR exposure, acting also on negative growth control. All together these findings suggest a potential interesting play of the extract also at the molecular level, modulating the expression of key genes involved in DNA damage signalling and repair.

P05-021
Difenoconazole – liver tumour mode of action and human relevance assessment

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Dietary administration of CD-1 mice for up to 18 months with difenoconazole (DFZ) resulted in toxicity in excess of a maximum tolerated dose (MTD) from 2500–4500ppm. Gavage pharmacokinetic studies demonstrate that the systemic exposure of the doses used in this study are not dose-proportional and therefore these mice were dosed in excess of a kinetically-limited top dose. At these excessive doses, liver tumor incidence was statistically increased in males at 4500 ppm and both sexes at 2500 ppm. We show that DFZ causes activation of the constitutive androstane receptor (CAR), which leads to increased expression of CAR-responsive pro-proliferative and anti-apoptotic genes and transient increases in hepatocellular proliferation. Associative events that are mediated by CAR activation include increased CYP enzyme expression and activity (primarily CYP2B and CYP3A), hepatocellular hypertrophy, and increased liver weight. These key and associative events are absent in CAR-knockout mice, demonstrating their CAR-dependency. The prolonged CAR-dependent liver weight increase at doses of 2500 ppm and above, result after several months of dosing, in fatty change, bile stasis, and ultimately necrosis of hepatocytes and is clearly in excess of the MTD. This effect serves as a late-acting, modulatory factor for the CAR-dependent alteration in proliferation which is the initial and main driver for the carcinogenic process. Furthermore we demonstrate that DFZ is not an activator of human CAR in a transactivation reporter assay, under conditions where it is a highly effective activator of an equivalent mouse CAR reporter. In an *in vitro* study with human hepatocytes, moderate increases in CYP2B and CYP3A activity by DFZ indicate some potential for CAR activation in human liver cells. However, DFZ does not stimulate the key event of cell proliferation in human hepatocytes *in vitro*, whereas it does in mouse hepatocytes *in vitro*. This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MOA for DFZ between mice and humans. consequently the data support the conclusion that DFZ does not pose a carcinogenic hazard to humans.

P05-022
A novel extension of the ToxTracker genotoxicity assay identifies aneugenic and clastogenic properties of chemicals

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ToxTracker® is a mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways upon chemical exposure. ToxTracker contains six different GFP-tagged reporter cell lines that together allow the discrimination between induction of DNA damage, oxidative stress and/or protein damage in a single test. Genotoxicity is detected by the Bscl2-GFP reporter for promutagenic DNA lesions and DNA replication stress, and the Rtkn-GFP reporter for DNA double strand breaks.

Here we investigated whether the ToxTracker assay could be adapted to allow the discrimination between clastogenic and aneugenic compounds. We included a DNA stain in the ToxTracker assay for cell cycle analyses and the detection of aneuploidy. Clastogenic compounds can cause cell cycle arrest, but generally do not cause aneuploidy. Aneugenic compounds, on the other hand, cause a cell cycle arrest in G2/M phase and aneuploidy. To validate the assay, we tested 4 clastogenic, 7 aneugenic, 2 pro-mutagenic and 3 non-genotoxic compounds in ToxTracker ACE (aneugen clastogen evaluation). As expected, the clastogenic, and pro-genotoxic compounds (with metabolic activation) activated the genotoxicity reporters in ToxTracker, but did not cause aneuploidy. The aneugenic microtubule disruptors and aurora kinase inhibitors arrested the cells in G2/M phase and caused an increase in aneuploidy.

Together, the differential activation of the ToxTracker genotoxicity reporters, in combination with the cell cycle analysis and polyploidy detection, allows for rapid identification of clastogens and aneugens and can further discriminate between microtubule poisons and kinase inhibitors.

P05-023

***In vitro* treatment of DMBA to murine mammary tissue-derived organoids induced adenocarcinomas/squamous cell carcinomas after their subcutaneous injection to nude mice**

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We previously reported that single administration of 7,12-dimethylbenz[*a*]anthracene (DMBA, 50 mg/kg body weight) by gavage induced mammary adenocarcinomas with or without adenosquamous characteristics with an incidence of over 70% after more than 13 weeks of DMBA administration in BALB/c-*Trp53*$^{+/-}$ mice. In addition, point mutations of *Hras* gene were frequently detected in the induced carcinomas. However, it could not be clarified whether the *Hras* mutation was an initial event or other molecular events by DMBA administration first occurred before the *Hras* mutations in the *in vivo* model. The purpose of the present study is to establishment of a simple model for evaluation of early molecular events of DMBA-induced mammary carcinogenesis, in which actual tumor development could be confirmed as its end point. We therefore here examined whether a short-term treatment of DMBA to normal mammary tissue-derived organoids of BALB/c-*Trp53*$^{+/-}$ mice *in vitro* would induce carcinomas after their subcutaneous injection to nude mice or not. [Materials and methods] Treatments with DMBA at concentrations of 0, 0.2 and 0.6 μM plus S9 mix to mammary organoids of BALB/c-*Trp53*$^{+/-}$ mice for 24 hours were repeated three times of passages of the organoids, followed by their subcutaneous injection to nude mice. [Results] The mammary organoids treated *in vitro* with 0.6 μM DMBA developed to adenocarcinomas and/or squamous cell carcinomas with the incidence of 4 of 4 but not in organoids treated with 0 and 0.2 μM DMBA (p=0.012) after 8 weeks of their subcutaneous injection to nude mice. [Conclusion and future plan] It was demonstrated that carcinogenic alterations of mouse mammary tissue-derived organoids treated with DMBA *in vitro* were induced in the nude mouse subcutis. We are now investigating whole exome sequence analyses of the DMBA-treaetd organoids before subcutaneous injection, to clarify the early genetic mutations in this carcinogenesis model.

P05-024

Generating a historical control database for the comet assay of mouse testes

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The OECD comet assay test guideline (TG489, 2016) requires that prior to using any organ in the assay the laboratory should build a historical database to establish positive and negative control ranges and distributions for relevant tissues and species. Different tissues and different species, as well as different vehicles and routes of administrations, may give different negative control % tail DNA values. It is therefore important to establish negative control ranges for each tissue and species. To build a historical database for mouse testes, male Hsd:ICR (CD-1) mice were dosed with vehicle (saline) or the positive control methyl methanesulfonate (MMS) at 40 mg/kg/day via oral gavage once per day for three consecutive days. Initial experiments were performed with one, two, or three days of dose administration. The one and two day dose administration regimens did not result in a significant increase in DNA damage with MMS. The three day dose administration was successful. In order to build a database, five independent experiments, with two groups of five animals each, were conducted with saline and MMS. On study day 3, animals were euthanized, testes collected, single cell suspensions prepared, and cell suspensions processed for the comet assay, per the OECD Guideline. The comet assay results from these five experiments demonstrated that oral dosing of MMS for three days results in significant DNA damage in mouse testes (% tail DNA range: 7.38 to 9.03) and the response was significantly higher than the vehicle control (% tail DNA range: 0.55 to 1.25).

P05-025

Exosome-mediated horizontal gene transfer: a possible new risk for genome editing

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The CRISPR/Cas system allows the introduction of double strand breaks (DSBs) at particular loci in the genome. DSBs are subsequently repaired through non-homologous end joining (NHEJ), or homologous recombination (HR).

We showed that DSBs introduced into the mice zygote by the CRISPR/Cas system are repaired by the capture of unintentional sequences, including retrotransposons, mRNA, and CRISPR-Cas9 vector sequences. This DSB repair mechanism with the capture of unintentional sequences were partially mediated by reverse transcription (RT), because the captured sequences were apparently derived from RT-mediated spliced mRNAs [Ono *et al*. 2015].

Therefore, it is possible that unintentional insertions associated with DSB repair represent a potential risk for human genome editing gene therapies. To address this possibility, comprehensive sequencing of DSB sites was performed *in vivo* and *in vitro* (mouse) by deep sequencing of PCR products amplified with two primers across the target DSB site. Although most of the unintentional insertion sequences were derived from plasmid vectors, 0,27% of the unintentional insertions were derived from bovine DNA fragments [Ono *et al*. 2019].

To determine the origin of bovine DNA fragments, we used goat serum, rabbit serum, and exosome-free FBS instead of FBS in the cell culture medium. Goat BovB, and rabbit LINE1 sequences were horizontally transferred to DSB sites by using goat and rabbit serum, respectively, however, almost no bovine DNA sequences were captured by using exosome-free FBS, suggesting that these horizontal gene transfers were mediated by exosomes.

We demonstrated that horizontal gene transfer assisted by CRISPR-Cas9 occurs in NIH-3T3 cells and mouse embryos, suggesting that exosome-mediated horizontal gene transfer is the driving force behind mammalian genome evolution. The findings of this study also highlight an emerging new risk for this leading-edge technology.

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P05-026
Mixture effects on BP-dependent AhR activation, metabolite and DNA adduct formation

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The prototypical carcinogen benzo[a]pyrene (BP), but also other non-carcinogenic polycyclic aromatic hydrocarbons (PAH) like pyrene (PYR) and fluoranthene (FA), are frequently found as contaminants in the diet. To identify mixture effects of BP, PYR and FA in proportions as occurring in grilled meat, the activation of the nuclear receptors AHR and CAR and their target gene expression (*CYP1A1*, *CYP2B6*) were investigated in human HepaRG hepatocarcinoma cells. To also examine important downstream key events, BP metabolites and the BP-dependent formation of DNA adducts were investigated under the influence of mixtures.

In contrast to the well-known AHR agonist BP, PYR and FA activated AHR only weakly and binary/ternary mixtures were less efficient than BP alone. However, analysis of *CYP1A1* gene expression showed synergistic effects after PAH co-exposure in HepaRG cells. By contrast, PYR and FA were strong CAR agonists, whereas BP was less potent. Mixtures containing BP caused a strong decrease of CAR transactivation in line with a lower *CYP2B6* expression level. Analyzing BP metabolites and the BP-dependent formation of DNA adduct levels revealed higher levels of detoxified mutagenic BP-diol epoxide with simultaneous lower DNA adduct levels after treatment of HepaRG cells with binary and ternary BP mixtures with FA and PYR.

PAH mixtures can modulate the induction of gene expression which may result in higher detoxification or bioactivation of carcinogenic xenobiotics like BP itself. The study supports the general concept that for the risk assessment of complex mixtures, alterations of molecular key events such as the activation of multiple receptors must be taken into account.

P05-027
Preclinical safety assessment of aqueous fern extracts

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Ferns and preparations thereof have been used in traditional medicine for a long time to treat a variety of ailments. However, there is also a hazard emerging from ferns, since poisonings have been reported in literature. Besides acute toxicity, mutagenicity is known for some fern species like *Pteridium aquilinum* (L.) Kuhn (*P. aquilinum*). Hence, a preclinical safety assessment is required for herbal medicines containing ferns. In the present work, the toxicological potential of two aqueous extracts from *P. aquilinum* and *Dryopteris filix-mas* (L.) Schott (*D. filix-mas*) ferns used as active substances in medicinal products was investigated. Both extracts were produced according to officially accepted pharmaceutical standards and evaluated in bacterial reverse mutation assays with and without exogenic metabolic activation following OECD standards. The aqueous extract of *D. filix-mas* was additionally tested in an *in vitro* cytotoxicity assay using mammalian cells performed in accordance with OECD and ISO standards. Moreover, the concentration of the mutagenic constituent ptaquiloside of *P. aquilinum* was determined in the aqueous *P. aquilinum* extract by a validated HPLC procedure. The aqueous extract of *D. filix-mas* exhibited cytotoxicity only at the highest concentration applied (50 mg/mL) and no evidence for mutagenicity was revealed. The aqueous extract of *P. aquilinum* was inconspicuous in the mutation assay with a ptaquiloside level below the limit of detection (0.1 mg/L). According to the results obtained, the two aqueous fern extracts do not exhibit any mutagenic potential. However, the cytotoxicity of the aqueous extract of *D. filix-mas* needs to be considered when used in herbal medicine at high concentrations. The data presented contribute to the preclinical safety assessment of aqueous fern extracts and herbal products derived therefrom.

P05-028
Characterization of enzymes oxidizing the tyrosine kinase inhibitor vandetanib and elucidation of the high efficiency of cytochrome P450 3A4 to generate *N*-desmethylvandetanib

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Metabolism affects the pharmacological efficiency of the tyrosine kinase inhibitor vandetanib. Here, we investigated the *in vitro* metabolism of vandetanib using (*i*) hepatic subcellular systems rich in drug-metabolizing enzymes (microsomes) isolated from livers of humans and several animal models, and (ii) human and/or rat recombinant biotransformation enzymes such as cytochromes P450 (CYPs) and flavin-containing monooxygenases (FMOs). In addition to the structural characterization of vandetanib metabolites, individual human and rat enzymes capable of oxidizing this drug were identified. Two vandetanib metabolites, *N*-desmethylvandetanib and vandetanib *N*-oxide, were formed in incubations with hepatic microsomes. The generation of *N*-desmethylvandetanib was attenuated by inhibitors of CYP3A and 2C subfamilies in both human and rat microsomes, while an inhibitor of CYP2D6 only decreased formation of this metabolite in human microsomes. The FMO inhibitor methimazol decreased the formation of vandetanib *N*-oxide in both rat and human microsomes. These results indicated that in the microsomal systems studied, CYP3A, 2C and/or 2D are mainly responsible for the formation of *N*-desmethylvandetanib and FMO1 and 3 mainly for the generation of vandetanib *N*-oxide. Human recombinant CYP3A4>>>2D6, 3A5, 1A1 and 2C8 oxidized vandetanib to *N*-desmethylvandetanib, while rat recombinant CYP2C11>>3A1>3A2>1A1>1A2>2D1>2D2 were effective in catalyzing this reaction. Cytochrome b_5 which serves as electron donor to CYP enzymes influenced the CYP-catalyzed formation of *N*-desmethylvandetanib; CYP3A4 was most affected. Human CYP3A4 is not only the most efficient enzyme oxidizing vandetanib to *N*-desmethylvandetanib, but also most important due to its high expression in human liver. Molecular modeling indicated that binding of more than one molecule of vandetanib into the CYP3A4-active center can be responsible for the high efficiency of CYP3A4 *N*-demethylating vandetanib to *N*-desmethylvandetanib. Indeed, the CYP3A4-mediated reaction was allosterically modulated exhibiting kinetics of positive cooperativity, which corresponds well to the *in-silico* docking model, where two bound vandetanib molecules were found in the active center of CYP3A4.

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P05-029

Migration and invasion of human renal cancer cells are impaired upon treatment with thymoquinone

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Thymoquinone (TQ), 2-methyl-5-isopropyl-1,4-benzoquinone, is a monoterpene isolated from the oil of *Nigella sativa* seeds, also known as black seed. The cytotoxic effects of TQ have been reported in different cancer cell lines. Nevertheless, the impact of this bioactive compound on the metastatic properties of cancer cells should be further studied, particularly in the context of renal clear cell carcinoma. The aim of this work was to evaluate the effects of TQ on the migration and invasion of human renal cancer cells (786-O cells) resorting to complementary cell-based methodologies. Firstly, noncytotoxic concentrations of TQ were determined using the crystal violet (CV) assay. TQ significantly decreased the collective migration of 786-O cells, according to the wound healing assay, although no effect was observed in terms of chemotactic migration (transwell assay, using FBS as the chemoattractant). Furthermore, TQ significantly reduced the invasiveness potential of 786-O cells, when compared with non-treated cells, using a transwell cell invasion assay. Overall, these results suggest that TQ has an important potential to counteract renal cancer cell migration/invasion anticipating further studies to unveil the mechanisms involved.

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P05-030

Uterine adenocarcinomas in isopyrazam-treated rats occur via a mode of action that has no relevance to humans

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Female rats treated with 3000ppm (232.8 mg/kg BW/day) isopyrazam (IZM) for 2-years had a greater incidence of uterine adenocarcinomas, lesser incidence of pituitary and mammary tumors, and ~40% reduction in body weight (BW) gain; tumor incidence was comparable to control at 500ppm (34.9 mg/kg bw/day).

For this investigative study, female rats were treated with 0, 500 and 3000ppm IZM (28 and 194 mg/kg BW/day) for up to 18 months. Decreased fat pad weights and reduced BW gain followed a dose-responsive pattern. The high dose group cycled regularly for a longer period of time and had a greater percentage of rats in persistent estrus from weeks 52 to 80 compared to control. Decreased fat pad weights, increased liver weights and reduced BW gain followed a dose-responsive pattern. Prolactin and leptin levels were significantly reduced at 3000 ppm and dopamine signaling to the pituitary was greater than controls at ≥ 26 weeks.

A mode of action (MoA) has been established that begins with decreased food utilization, fat pad weight and BW gain, and consequently decreased leptin levels which caused subsequent alteration in hypothalamic signaling resulting in an altered pattern of transition into reproductive senescence. The greater time spent in persistent estrus and exposure to circulating estrogens leads to an increase in uterine adenocarcinomas. Onset of senescence in rats is due to altered signaling within the hypothalamus, while menopause and reproductive senescence in humans is driven by depletion of a predefined number of primordial follicles in the ovaries with aging. The fundamental physiological difference in control of the reproductive cycle and the transition into reproductive senescence between rats and humans indicate that the established MoA leading to uterine tumor formation in rats is not relevant to humans

P05-031

The study of the cytotoxicity of zoledronic acid in a chronic experiment

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The aim of the research was to study the cytogenetic effects of zoledronic acid under conditions of chronic inhalation exposure for 4 months in white rats. Cyclophosphamide was used as a positive control. In the experiment, concentrations of drugs at the level of 0.10, 0.05 and 0.01 mg/m^3 were studied. At the end of the experiment, cytogenetic damage to nuclear cells was taken into account in washes of the lungs using light microscopy.

In the experiment, it was found that after two months of zoledronic acid administration, cytogenetic disorders (micronuclei in mono and polynuclear cells, bridges) increase depending on the concentration, while the maximum concentration (0.10 mg/m^3) is 2 times higher than the control indicators ($p < 0.05$). In case of administration of cyclophosphamide, an increase in cytogenetic disorders is observed at all studied concentrations ($p < 0.05$), when exposed to a concentration of 0.10 mg/m^3, 4 times the control values. In the second month of exposure, a high level of cell death of various types (interphase and reproductive) is observed against the background of seasonal activation of proliferation.

In the group of animals treated with zoledronic acid, at the 4th month of the experiment, a decrease in the number of cells with cytogenetic damage was noted by 4–6 times to the control level, which is explained by a decrease in the intensity of proliferation during this observation period. When exposed to cyclophosphomide, the number of cells with cytogenetic damage also decreased by 2–3 times, but significantly exceeded the values in the control group.

2 months after cessation of exposure to drugs, compared with the control, a high level of cells with cytogenetic damage remained only at a concentration of 0.1 mg/m^3 cyclofasfamide ($p < 0.05$), while at a concentration of 0.01 mg/m^3 of zoledronic acid an increase was observed damaged cells ($p < 0.05$).

As a result of the experiment, it can be concluded that cellular cytogenetic disorders during chronic inhalation exposure to zoledronic acid and cyclophosphamide are the result of direct and distant mutagenic action, as well as the effects of seasonal and compensatory mechanisms of cell replacement repair.

P05-032

Improving the predictive performance of *in silico* aromatic amine mutagenicity alerts through the analysis of proprietary data

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Aromatic amines are frequently used as building blocks in the synthesis of pharmaceutical products. Unfortunately, metabolic activation to DNA-reactive species results in aromatic amines being potentially mutagenic, which is often demonstrated by a positive result in the Ames test. *In silico* tools can be utilised to predict the potential mutagenicity of a query structure; however, aromatic amines are notoriously difficult to develop alerts for due to a combination of their complex structure-activity relationships and poor reproducibility of experimental results. We describe how physicochemical property relationships can be incorporated within alerts to refine the specificity of *in silico* predictions.

Alerts for aromatic amines have been developed based principally on data from publicly available literature and refined further using proprietary data. Despite refinements, validation studies indicate that the alerts generate many false positive predictions. Analysis of the physicochemical properties for a public and proprietary data set has previously identified a number of parameters which appeared to be important for determining absolute mutagenic activity of aromatic amines in the Ames test.

Previous analysis led to an *in silico* model containing 4 structural alerts for aromatic amines with molecular size restrictions to exclude compounds with a heavy atom count of > 25. For a proprietary data set of 651 compounds, the predictive performance of the model is: specificity = 87.7%, sensitivity = 63.0% and accuracy = 82.2%, compared with the unrestricted model: specificity = 84.6%, sensitivity = 63.7% and accuracy = 79.9%. In this study we have re-analysed the 4 alerts to determine whether maintaining a size restriction is appropriate following modifications to the scope of the alerts. It was found that lowering the heavy atom count to exclude compounds with a heavy atom count > 24 further improved specificity to 88.3% and accuracy to 82.6% while maintaining sensitivity. Overall, incorporation of this restriction increases specificity by 3.7 percentage points, resulting in an additional 19 compounds predicted correctly as Ames negative. This increase in specificity comes at a cost of a concomitant reduction of 0.7 percentage points in sensitivity, creating 1 false negative compound. Overall, the accuracy of the model was increased by 2.7 percentage points.

P05-033

Genotoxicity of synthetic pyrethroids in bone marrow of mice micronucleus test and fluctuation of Ames assay

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Pyrethroids are analogues of natural pyrethrins, first isolated from plants of the genus *Pyréthrum*, a family of astroids known for their insecticidal properties. We know synthetic pyrethroids (SP) about 70 years. Nowadays, there are a large number of SP have different insecticidal activity, and also can be used in combination with other chemical compounds. Study of genotoxicity is a mandatory of the toxicological assessment to justifying their safe usage in Ukraine. The Mammalian Erythrocyte Micronucleus Test *in vivo* (MN) and Ames test are recognized as most sensitive, widely and frequently used tests to identify genotoxic features.

The aim of the studies was to research the genotoxicity of 5 SP via MN *in vivo* and Ames assay. The tests are conducted following the SOPs, comply with GLP.

The MN was used to study mutagenic activity of 5 SP: Cypermethrin 94.0% at doses 46.0, 9.2, 1.84 mg/kg body weight, 2 samples of Alpha-cypermethrin - 94.0 and 94.7% in doses of 20.0, 2.0, 0.2 mg/kg, and 2 samples of Lambda-cyhalothrin - 95.2 and 97.1% in doses of 5.0, 1.0, 0.2 mg/kg. Acclimatization took 5 days before dosing. One treatment by gavage. Exposure time – 24 hours. We counted micronuclei in polychromatophilic erythrocytes (MNPCE) of bone marrow of mice (OECD 474).

Study of mentioned above 5 SP in fluctuation Ames assay using *S.typhimurium* TA98, TA100 w/wo S9, preincubation was 90 min. Selection of concentrations in Ames test were based on preliminary experiment which was performed before the main test. In the absence of cytotoxicity and precipitation in preliminary experiment the following concentrations (2.5; 0.5; 0.1; 0.02; 0.004; 0.0008 mg/ml) were defined for all 5 SP.

As a result: obtained experimental data of positive and negative controls were ranged with own laboratories historical control. The results of experimental studies indicate that Cypermethrin in doses from 46.0 to 1.84 mg/kg body weight, 2 samples of Alpha-cypermethrin in doses from 20.0 to 0.2 mg/kg, and 2 samples of Lambda-cyhalothrin in doses of 1.0 and 0.2 mg/kg did not reveal a significant increase in the level of MNPCE. Both samples of Lambda-cyhalothrin in doses: 5.0 mg/kg b.w. induces statistically significant excess of the spontaneous frequency of MNPCE ($p \leq 0.05$). Ames assay results showed statistically significant absence of the mutagenic effect.

P05-034

DNA damage signaling and genotoxic effects induced by complex mixtures of PAHs generated by biomass burning air particulate matter in human lung cells

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Most research concerning the effects of air pollutants on human health focuses on urban centers and on the role of vehicular and industrial emissions as major sources of pollution. However, approximately 3 billion people world-wide are exposed to air pollution from biomass burning [1]. Herein, particulate matter (PM) emitted from artisanal cashew nut roasting, an important economic and social activity worldwide [2,3], was investigated. This study focused on: *i*) chemical characterization of polycyclic aromatic hydrocarbons (PAHs) and their oxy-PAH derivatives; *ii*) time-dependent activation of DNA damage signaling and genotoxic effects, and *iii*) differential expression of genes involved in xenobiotic metabolism, inflammation, cell cycle arrest and DNA repair using A549 lung cells. Among the PAHs, chrysene, benzo[*a*]pyrene (B[*a*]P), benzo[*b*]fluoranthene, and benz[*a*]anthracene showed the highest concentrations (7.8-10 ng/m^3), while among oxy-PAHs, benzanthrone and 9,10-anthraquinone were the most abundant. Testing of PM extracts was based on B[*a*]P equivalent doses (B[*a*]P_{eq}). IC_{50} values for viability was 5.7 and 3.0 nM B[*a*]P_{eq} at 24 h and 48 h, respectively. Based on this, all other experiments were conducted at doses up to 2 nM B[*a*]P_{eq}. At these low doses, we observed a dose-dependent activation of DNA damage

signaling (phosphorylation of Chk1) and genotoxicity (double strand breaks). In comparison, effects of B[*a*]P alone was observed at micromolar range. To our knowledge, no other study has demonstrated an activation of pChk1, a biomarker used to estimate the carcinogenic potency of PAHs *in vitro* [4], in lung cells exposed to biomass burning extracts. Persistent increased gene expression of several important stress response mediators of xenobiotic metabolism (*CYP1A1, CYP1B1*), inflammation (*IL-8, TNF-α*), cell cycle arrest (*CDKN1A*), and DNA repair (*DDB2*) was also identified. In conclusion, our data show high potency of biomass burning PM to induce cellular stress including genotoxicity, and more potently so when compared to B[*a*]P alone. Our study provides new data that will help elucidate the mechanism of lung cancer development associated with biomass burning. In addition, the results of this study support the establishment of new guidelines for human health protection in regions strongly impacted by biomass burning.

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P05-035
Genotoxic safety evaluation of termite mushroom *Termitomyces Albuminosus*

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Termitomyces albuminosus, also known as Termite mushroom, has pharmacological effects such promotion of neuronal growth and prevention of age-related diseases, leading to its use as functional food or supplements. Despite its increasing consumption, there is a lack of comprehensive information regarding its safety. In this study, we conducted three genotoxicity tests to evaluate the safety of *Termitomyces albuminosus* powder (TAP): Ames test (OECD TG 471) using 4 strains of *Salmonella typhimurium* and 1 strain of *Escherichia coli* and chromosome aberration test (OECD TG 473) using Chinese Hamster Lung fibroblast (CHL) cell line for evaluation of clastogenic potential, and *In vivo* micronucleus test using ICR mice (OECD TG 474). TAP did not induce reverse mutations in all the bacterial strains used in this study nor chromosomal aberrations in CHL fibroblasts at concentrations up to 5000 μg/plate regardless of S-9-mediated metabolic activation. In ICR mice, no changes were noted in clinical signs and body weight in the groups treated up to 3000 mg/kg of TAP. There was no statistically significant increase in the frequencies of micronucleated polychromatic erythrocytes at any TAP-treated groups compared with the negative control group (6.0 ± 1.58 for TAP vs 5.2 ± 0.84 for the vehicle control, $p > 0.05$) and in the ratio of polychromatic erythrocyte to total erythrocytes (53.9 ± 6.48% for TAP and 47.0 ± 5.24% for the vehicle control, $p > 0.05$). In conclusion, our data from the standard battery of genetotoxicity tests demonstrated that TAP has no genetotoxic potential under the conditions of this study. These findings, together with the recent subchronic toxicity test results, suggest that *Termitomyces Albuminosus* is a safe and nontoxic for human consumption when appropriately used.

P05-036
Development of a biomolecular data computing environment for computer predicted adverse outcome pathways: benzene as a case study

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Purpose: A Biomolecular Data Computing infrastructure (BDC) is being developed to enable a top down toxicoinformatics modelling approach that is data driven from external exposure to molecular pathways to health effects. Here, the application is demonstrated to propose adverse outcome pathways invoked by benzene, known to cause acute myeloid leukemia (AML).

Methods: BDC integrates biomolecular computing tools (e.g. omics data integration tools), access to chemical-disease databases (e.g. Comparative Toxicogenomics Database (CTD)), pathway databases (e.g. KEGG, AOP wiki), and other software & hardware environments like KNIME & HADOOP for work-flow management. At present, the data content, tools and workflows within BDC mainly allow for proposing qualitative exposure response relationships, e.g. linking gene symbols to exposure to adverse outcomes and health effects**.**

Results: A BDC:KNIME workflow towards creation of computer predicted adverse outcome pathways (cpAOP tool) was realized with benzene (CAS 71-43-2) as the start (exposure), and chromosome aberrations (CA) (MeSH D002869) as intermediate biomarker end-point towards AML. Functional sub-blocks within the KNIME computational work-flow initially retrieved from CTD 16 unique genes relating benzene with CA disease mechanisms (from 'benzene' + 'CA': 8 genes; 'Micronuclei, chromosome defective' (= CA descendant term) + 'benzene': 14 genes). Further into the workflow, additional genes functionally related to these 16 genes were collected from GeneMANIA (http://genemania.org), eventually totaling to a set of 19 genes. Next, 4698 GO biological processes terms which were related to the 19 genes were further identified by accessing the CTD batch query tool within the KNIME flow. These GO terms were then compared with 28 benzene related phenotypes (with phenotype referring to a non-disease biological event; including GO biological processes terms) separately collected from CTD. This comparison yielded 5 common GO terms: telomere maintenance, DNA modification, cell proliferation, hematopoietic stem cell proliferation, glucose homeostasis. These could be reflective as AOP key events at inter-cellular, cellular, and system level, respectively and together may represent a plausible cpAOP candidate for benzene-induced AML.

P05-037
Combined *Pig-a*, micronucleus and comet: an *in vivo* genotoxicity assessment of benzene and cyproterone acetate using a triple endpoint approach

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Cyproterone Acetate (CYP) is a synthetic sex steroid derived from hydroxyprogesterone and known carcinogen. Existing data [1,2] have shown it induces Liver DNA adducts, gene mutation and tumours (at high dose levels only, predominantly female rats), negative for mouse micronucleus (single dose regimen), negative *in vitro* for HPRT, Ames and chromosome aberrations. Currently no *in vivo* gene mutation data

exists for extended treatments.

Benzene (BZN) is a genotoxic carcinogen. Genotoxicity data indicate that BZN (including metabolites) are Ames negative but clastogenic and aneugenic, producing micronuclei, chromosomal aberrations, sister chromatid exchanges and DNA strand breaks [3].

Using a 28 day dosing regimen, female rats were treated with CYP (50, 100 & 200 mg/kg/day) and male rats treated with BZN (500, 1000 & 2000 mg/kg/day) and tested for induction of gene mutations (*Pig-a* assay*)*, micronucleus (peripheral blood) and gross DNA damage in the Liver (Comet assay). Groups of 5 animals were dosed with vehicle, test article (see table). A mutagen control (ENU @ 20 mg/kg/day) was included. Blood was collected Days -1, 15, 29 (*Pig-a*) and Day 28 (micronucleus). Liver was sampled for Comet 3 hours post the last administration.

Results showed that CYP was negative for the two Pig-a endpoints (RBCs and RETs), positive for micronucleus and that the Comet endpoint was unscorable (due to high proportion of diffused cells). BZN was negative for the two Pig-a endpoints (RBCs and RETs), positive for micronucleus and weakly positive for the Comet endpoint. ENU was positive for all endpoints analysed.

The CYP pig-a mutation data was negative following dosing to 200 mg/kg/day (a dose that showed extensive toxicity to the liver). Concentrations selected for testing were based on published data for MN and mutation data from acute dosing studies [1] and a true MTD not achieved.

BZN a known genotoxic carcinogen produced the same profile as that for CYP, providing support for existing data that CYP may be a genotoxic carcinogen. Further testing at higher doses in the pig-a assay may be required to elucidate the true mutagenic profile.

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P05-038

This abstract has been withdrawn.

P05-039

Use of (Q)SAR models to investigate potential CMR properties of e-liquid ingredients

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Electronic cigarettes (e-cigs) are designed to heat and aerosolized mixtures of propylene glycol, glycerol, flavorings, humectants and, optionally, nicotine. Unlike cigarettes, the process involves no tobacco and no combustion; however, the inhalation and exhalation of vapour is reminiscent of smoking.

In this context, the use of these devices, might play an important role in smoking cessation and reduction; however, there is still a lack of international consensus over the public health role of the e-cig.

Despite the large use of e-cigs, still few toxicological studies are available on the potential long term effects of inhaled of many characterizing flavors used in e-cig products.

For instance, the FDA GRAS (Generally Recognized As Safe) designation for some flavorings compounds and for propylene glycol, does not apply to inhalation, and currently, there are no controlled long-term studies of the effects of inhaling heated aerosolized mixture in humans.

Thus, there is legitimate concern over the health effects of chronically inhaling these substances and the lack of toxicological studies.

In this respect, the aim of this study was to determine potential Cancerogenic, Mutagenic and Reprotoxic (CMR) properties of several e-liquid ingredients by means of *in silico* methods.

With reference to our e-liquid ingredients and CMR effects, we first conducted an in depth screening, through the literature reviews; and we found experimental data gap for all the three categories.

Specifically, for the investigated e-liquid ingredients, we observed 35%, 85% and 70% of experimental data gap for Cancerogenicity, Mutagenicity and Reprotoxicity effects, respectively.

By following a battery approach, almost all data gaps were successfully filled using Quantitative Structure-Activity Relationship (Q) SAR methods. The predictions were performed using several open source software (VEGA, Toxtree, ToxRead and T.E.S.T.) and the results were combined to obtain the highest possible prediction accuracy (consensus approach).

This *in silico* study is a part of a broader integrated approach (literature research, in chemico, *in vitro* and computational analysis) specifically designed to assess the potential risk associated with characterizing flavors and e-liquid ingredients.

P05-040

This abstract has been withdrawn.

P05-041

This abstract has been withdrawn.

P05-042

Evaluation of the MultiFlow platform to supplement the *in vitro* micronucleus test with genotoxic mode of action-based information

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The identification of genotoxic hazard is part of the routine test battery in pharmaceutical development. The objective is to identify direct DNA-damaging agents as well as those with different modes of action (MoA) like aneugens. The *in vitro* micronucleus (MN) test is routinely used to screen compounds for the induction of *in vitro* chromosomal damage. However, MN induction is not always indicative of a DNA reactive MoA, and determining the causal molecular pathway can be a relevant driver to mitigate the genotoxic flag. This MoA information is also highly useful when managing potential risk in supporting the Discovery teams.

Here we describe the evaluation of the MultiFlow (MF) platform, a DNA damage-based assay which multiplexes p53, γH2AX, phosphohistone H3, and polyploidization biomarkers into a single flow cytometric analysis. Human TK6 cells were exposed to 50 pharmaceutical internal test compounds (containing DNA reactive clastogens, tubulin poisons, kinase inhibitors, and non-genotoxicants) for 24 hr over a range of concentrations. Cell aliquots were removed at 4 and 24 hr for analysis and multiplexed response data were evaluated using 3 machine learning-based models which classified each compound as clastogenic, aneugenic or non-genotoxic. In addition, fold increases in biomarkers against global evaluation factors (GEFs) were also considered.

The obtained classifications were compared with the outcome of the *in vitro* MN test in TK6 cells. Of the 50 compounds tested, 24/26 and 18/24 showed comparable positive and negative outcomes respectively. The real value of the MF approach was demonstrated by the fact that the MoA-based classifications (signatures) correspond with the known profiles of the selected compounds. A detailed analysis of the MF responses often indicated atypical profiles for those compounds not inducing MN; observations that trigger additional work to explore deeper levels of information including aneugen molecular initiating events, discriminating primary vs. secondary DNA damaging agents, and the need for extended recovery designs in the MN test.

Overall, the obtained data show the strategic importance of understanding the underlying MoA driving MN induction, information which is needed to prioritize and select the most promising drug candidates in Discovery.

P05-043
Validation of the *in vivo* comet assay in various organs of Wistar rats

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The *in vivo* alkaline Comet Assay (OECD Test Guideline 489) also known as single cell gel electrophoresis assay is a sensitive method for the detection of DNA single and double-strand breaks as well as alkali labile sites in different organ tissues. It can be performed as part of the standard testing battery for pharmaceuticals integrated in repeated dose studies or as a follow-up to a positive result in an *in vitro* clastogenicity test for industrial chemicals.

According to the validation trial from 2006–2012 coordinated by the Japanese Center for the Validation of Alternative Methods (JaCVAM), historic control data were only obtained for the two organs liver and stomach. As also different tissues can be of interest concerning the mode of action of substances, we established this technique 2017 in our lab and did an internal validation study investigating the dose-dependent effects of ethyl methanesulfonate (EMS) as positive control at three concentrations in several tissues like lung, glandular stomach, liver, small intestine (jejunum), colon, kidney and spleen.

The test groups consisted of 3 male rats. As negative control 0.9% physiological saline was applied and for the positive control 100, 200 and 300 mg EMS per kg body weight (bw) were selected as appropriate concentrations. 4 h after the application, the animals were sacrificed one by one and the organs of interest were removed. Each organ was processed independently by three people to investigate the inter-analyst variability. The single cells were embedded in agarose and lysed overnight to remove cell membranes. After an unwinding step of the DNA in a high alkaline solution, electrophoresis was performed. The slides were dried in ice-cold ethanol and stained with GelRed® solution. 50 cells per slide were evaluated from two different scorers. Overall, 504 slides were analysed obtained from 3 different analysts, which processed 7 organs and prepared two slides for each of it from 12 animals in total.

P05-044
Applicability of *in-silico* tools to predict mutagenic activity of pesticides

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In-silico studies based on computer-aided prediction models or (Q) SAR tools are increasingly submitted for the purpose of authorisation and/or approval of pesticides. For regulatory authorities, it is thus indispensable to develop a detailed understanding of the potential and the limitations of such *in-silico* tools. The correct output of (Q) SAR tools depends on model assumptions and on the underlying data. Whereas chemical structures of pharmaceuticals are already implemented in the training datasets, molecular structures of pesticides and their metabolites are thought to be underrepresented, raising questions about the regulatory readiness of the respective (Q)SAR tools.

This project focussed on mutagenicity in bacteria, a relevant endpoint in pesticide assessment. A curated test data set was built from 200 studies on pesticides and their metabolites. Only valid studies performed according to OECD test guideline 471 and under GLP were included. *In-silico* predictions were performed for all structures in the dataset using freely available as well as commercial software. The latter included different versions of Derek Nexus and Sarah Nexus as predictions generated with these tools have been increasingly submitted over recent years. Derek is expert rule-based and covers numerous endpoints including bacterial mutagenicity. In contrast, Sarah is a statistical-based system and makes predictions only for bacterial mutagenicity.

In a first step, Sarah versions 1.2.0 (2016) and 3.0.0 (2018) as well as Derek versions 4.1.0 (2016) and 6.0.1 (2018) were evaluated against a balanced subset of 15 mutagenic and 15 non-mutagenic structures in order to examine whether the predictions had improved with version updates. Predictions with Derek remained unchanged. However, perfomance had improved in the later version of Sarah. This was attributed mainly to the inclusion of additional experimental data into the Sarah training data set. Secondly, as recommended by OECD and ECHA guidance, the combination of predictions obtained with the rule-based tool Derek and the statistical tool Sarah was explored. True balanced accuracy reached approx. 60–70%. Finally, the extended but unbalanced dataset comprising 200 studies was used for evaluation and further tools such as Toxtree and TEST were included in the analysis.

P05-045
Expression of γ-Glutamyltransferase in rats' hepatocytes after carbendazim exposure

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It was shown that carbendazim (methyl 1H-benzimidazole-2-yl carbamate (CAS)) leads to pathological changes in the liver, including neoplastic. Disturbance of metabolic processes in cells occurring in the process of carcinogenesis revealed by changes of enzymes activity that can be considered as preneoplastic and tumor markers.

γ-Glutamyltransferase (γ-GGT) enzyme is one of the classical markers of preneoplastic changes in population of hepatocytes. According to data obtained in chronic experiments carbendazim classified as a carcinogen of the C group (a possible carcinogen for a human) that causes a hepatocarcinogenic effect.

The aim of this study was to investigate the expression of γ-GGT in hepatocytes of rats under the action of a generic 98% carbendazim.

The experiment was done using the "NDEA-hepatectomy" model proposed by N. Ito *et al*. which is based on the initiation of hepatocytes by a single intraperitoneally injection of N-nitrozodiethylamine (NDEA) at a dose of 200 mg/kg associated with partial hepatectomy. The study was conducted on 75 male Wistar rats of 200–250 g b.w., 15 rats for each group: 1 – negative control, water; 2 – positive control, phenobarbital in the dose 37.5 mg/kg; 3, 4, and 5 – carbendazim at doses of 25, 75 and 300 mg/kg. The test substance was administered to animals by oral gavage for 8 weeks. After the end of the exposure period, all rats were sacrificed and liver slices are fixed in ice-cold acetone for examination of GGTase.

The results of the study showed that carbendazim in a dose of 25 mg/kg caused no effect; in a dose of 75 mg/kg – the number of γ-GGT positive foci significantly increased per slide unit area (cm^2). Also, the tendency to increase of the area γ-GGT positive foci were noted ($p = 0.06$). At dose 300 mg/kg (1/5 LD50) the number and area of foci did not differ from the negative control, this may indicate the activation of compensatory mechanisms of body self-regulation. Consequently, the dynamics of the increase in the number of hepatocytes expressing γ-GGT is paradoxical in terms of the effect in small doses.

P05-046
In vivo evaluation of glyphosate genotoxicity

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Background: Glyphosate is the active substance of the widely used herbicide Roundup®. Because of its ubiquity in the environment, humans get exposed to glyphosate daily in low or high doses, posing a real life risk for human health.

Aim: In this study, we aim to determine the genotoxic effects of glyphosate, in pure and commercial form, after long term exposure.

Methods: Twelve rabbits divided into 3 groups were used in this research. The 1st group (control) consumed normal diet, while the 2nd and 3rd groups were administered high dose of pure and commercial glyphosate, respectively. The administered doses were 10 x ADI, still much lower than the established NOAEL.The cytokinesis-block micronucleus (CBMN) assay was applied to lymphocytesin order to determine the genotoxic effects of glyphosate.

Results: Significant differences were observed between control and exposed groups in a dose response manner.

Conclusion: The purpose of the current study is to establish relationship between exposure (pesticide doses and dose duration) and genotoxicity (viability, number of mutation). Last but not least, the creation, establishment and validation of an appropriate index for genotoxicity GPI (Genotoxicity Potency Index) is an open area for scientific investigation and discussion.

P05-047
Prognostic efficacy of cellular test-models in assessing the mutagenicity of chemicals

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Research on the mutagenic properties of chemical compounds in cell cultures is a promising alternative to replace animal testing. But at this time there is a problem of insufficient reliability of the results. The mutagenic properties found in *in vitro* tests are not always confirmed in animal experiments.

The purpose of this work was to establish the cell lines that are most sensitive to the action of mutagens, to study their prognostic efficiency for the subsequent classification of the tested compounds.

In the work, about 20 chemical compounds belonging to different classes of mutagenic activity (mitomycin C, methylnitrosurea, cyclophosphamide, heavy metal salts, polyaromatic hydrocarbons, nitrosamines, etc.) were used as reference mutagens in various concentrations. The studies were carried out on cell cultures: primary cultures of laboratory animals and humans (dermal-muscular embryonic fibroblasts of mice of various lines, Chinese hamster, peripheral blood lymphocytes), as well as A549, HeLa, CHO cell cultures, etc.

To establish the mutagenic properties of chemical compounds, a micronucleus test (cytofluorimetric detection method), a comet test, a test for the induction of chromosomal aberrations was carried out (microscopic analysis). Different sensitivity of cell cultures to mutagens has been established. It was noted that the sensitivity had a direct correlation with the rate of cell division in culture. Prognostic efficiency, in turn, has no correlation with the sensitivity of cell cultures to compounds with mutagenic properties.

P06 – *In vitro* testing

P06-001
Cytoprotective autophagy protects cardiac cells from Dichlorvos-induced ER stress and necroptosis

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Dichlorvos (O,O-dimethyl-2,2-dichlorovinyl phosphate; DDVP) is an organophosphate pesticide (OP) that is widely used for the control of agriculture and animals pests. In the present study, we investigated the underlying mechanism of DDVP-induced toxicity in cardiac cells.

We show that 24h treatment with DDVP inhibits the growth of cells by inducing necroptosis. In fact, DDVP treatment upregulated RIP1 expression and chemical inhibition of RIP1 kinase activity by necrostatin-1 (Nec-1) protected the cells from death. After a short-time of treatment (6h) with DDVP, an increase in the level of Beclin-1 and LC3-II and an accumulation of the CytoID® autophagy detection probe were observed. In addition, inhibition of autophagy by chloroquine (CQ) significantly increases DDVP-induced necroptosis, suggesting that activation of autophagy is a cardioprotective mechanism against the toxicity induced by this OP.

We also demonstrate that inhibition (EX527) or knockdown (siRNA) of the deacetylase sirtuin 1 (SIRT1) significantly increases necroptosis induced by DDVP, whereas SIRT1 activation by resveratrol (RSV) greatly prevents the cytotoxic effects of this pesticide. When autophagy was inhibited by CQ, the protective effect of RSV against the cardiotoxicity of DDVP was not observed. Altogether, our results suggest that activation of SIRT1 protects cardiac cells from the toxicity of DDVP by an autophagy-dependent pathway.

P06-002
Dried blood spots combined to an UPLC–MS/MS method for thesimultaneous determination of antihypertensive drugs in forensic toxicology

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A method for the simultaneous determination of 4 antihypertensive drugs(Nimodipine, Valsartan, Arotinolol, Indapamide), using the dried blood spot (DBS) sampling technique combined with the UPLC–MS/MS technology was developed to study its applicability within the forensic toxicology. The DBS samples, prepared from a blood volume of 50 mL and using the bloodstain cards, were extracted with a methanol/acetonitrile mixture. The chromatographic separation was performed using an ACQUITY®HSS C_{18}(50 mm×2.1 mm, 1.8 µm) and an acetonitrile/Water (0.1% formic acid) gradient. The detection was accomplished with a TQ Detector, operating in the ESI+ and MRM modes. The method was validated in terms of selectivity, matrix effect, extraction recovery (46%–106%), carryover, LOD and LOQ (0.2–0.8 ng/mL and 1–4 ng/mL, respectively), linearity (LOQ to 100 ng/mL), intraday and interday precision (2.6–13% and 4.1–14%, respectively), accuracy (2.3% to 4.8%) and dilution integrity. An eight months stability study at room temperature, 2–8°C and −10°C, was also performed, with the best results obtained at −10°C.

The procedure was applied to 50 real samples. The results were compared with the methodologies routinely applied in the laboratory and the statistical analysis allowed to establish an acceptable correlation. This study permitted to determine that the DBS can represent an alternative or a complement to conventional analytical and sampling techniques, responding to some of the present issues concerning the different forensic toxicology applications.

P06-003
Assay of staphylococcal enterotoxin B by a QCM biosensor

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Staphylococcus aureus is a causative agent of infectious diseases and a producer of highly toxic proteins called Staphylococcal enterotoxins. In the current analytical praxis, presence of staphylococcal enterotoxins is assayed by standard immunochemical techniques or by instrumental methods based typically on the combination of chromatography and mass spectrometry. Despite availability of the current methods, they are not fully suitable for a simple, label-free, mode of use by an unskilled worker. In this study, a biosensor based on quartz crystal microbalance (QCM) sensor is performed as a tool for simple and reliable determination of Staphylococcal Enterotoxin B (SEB) which is one of the most important staphylococcal enterotoxins.

QCM sensors with 10 MHz basic frequency of oscillations and gold electrodes were used in this study for biosensor construction. The biosensor contained antibody against SEB immobilized through protein A on the QCM sensors and the interdigitated layer was stabilized by iron nanoparticles. The assay worked as a label-free and sample was applied without any pretreatment. Enzyme-Linked Immunosorbent Assay (ELISA) served for validation purpose.

The biosensors were tested for standard solution of SEB and calibration curve was prepared. In the assay, limit of detection 113 µg/ml was reached for a sample sized 50 µl. The assay by biosensors was compared to standard ELISA and correlation between the two methods was done resulting in coefficient of determination 0.993. Long term stability was also tested for an interval 90 days. In the end of the interval, the biosensors were able to provide approximately 85% of the initial sensitivity.

In a conclusion, the biosensors for SEB assay appears as a simple but reliable tool to be performed in field conditions, protection against warfare use of toxins etc. The facts that the assay is cheap and label free without any necessity to apply any reagents are significant advantages.

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P06-004
Solid-phase microextraction as a universal tool for quantitative *in vitro*-to-*in vivo* extrapolation studies

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Reducing and eventually replacing animal tests by *in vitro* bioassays requires the quantitative extrapolation of effect data generated with *in vitro* test systems to whole organisms (quantitative *in vitro*-to-*in vivo* extrapolation, QIVIVE). QIVIVE models usually compare the nominal effect concentrations of the chemicals in the *in vitro* bioassays with total plasma concentrations *in vivo*. However, other dose metrics have been suggested that account for differences in bioavailability of the chemicals *in vitro* and *in vivo* due to different composition of e.g., cell culture media and human plasma. A better comparison is possible if freely dissolved concentrations in the assay medium ($C_{free,medium}$) and in plasma ($C_{free,plasma}$) are used. In this study we want to demonstrate that solid-phase microextraction (SPME), a widely used sample preparation technique, can support QIVIVE studies in many different aspects. SPME has been applied in previous studies to determine partitioning in diverse biological phases from bovine serum albumin and phospholipid liposomes to complex matrices like cell culture media and plasma. We demonstrated that SPME cannot only generate partitioning data that are required as input parameters for mass balance models used to predict $C_{free,medium}$ and $C_{free,plasma}$ but can also be used for the time-resolved experimental quantification of $C_{free,medium}$ in cell-based *in vitro* bioassays and to determine $C_{free,plasma}$ in plasma samples from different species. We found that $C_{free,medium}$ in *in vitro* test systems can be several orders of magnitude lower than the nominal concentration (C_{nom}) and was not necessarily linearly related to C_{nom}. In human plasma $C_{free,plasma}$ was lower than $C_{free,medium}$ at the same C_{nom}, which can be explained by the fact that human plasma has more proteins and lipids than commonly used cell culture media. By comparing $C_{free,plasma}$ determined in human and trout plasma we found similar values for neutral and basic chemicals, but differences of several orders of magnitude for several acidic chemicals. These results emphasise the need to account for bioavailability for successful QIVIVE and that SPME may be used as a universal experimental tool that improves our understanding on how chemicals distribute *in vitro* and *in vivo*.

P06-005
Generation of proliferating mouse hepatocytes (upcyte® mouse hepatocytes)

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Introduction: The concern about the use of laboratory animals is increasing and leads to the support of alternative methods. Laboratory mice are frequently used for gene knockout studies *in vivo*. Additionally, isolated mouse cells are an appropriate tool for gene knockout studies on a cellular level. However, the use of primary mouse cells is hampered by e.g. short culture longevity, the limited quantity of cells that can be isolated from one mouse and the lack of proliferation capacity.

Since we have successfully generated several human upcyte® cells (e.g. upcyte® hepatocytes), the feasibility of the upcyte® technology on other species is of interest. Here, we show for the first time that the transduction of proliferation-inducing genes could extend the lifespan of primary mouse hepatocytes without losing their primary characteristics. For this purpose, primary mouse hepatocytes from three wildtype (WT) and three knockout (KO) C57BL/6 mice were isolated and subsequently transduced with upcyte® proliferation genes.

Methods: Murine hepatocytes were isolated from three wildtype (mouse donor number 16, 21 and 22) and three knockout (mouse donor number 17, 23 and 24) C57/BL6 mice using a two-step collagenase perfusion technique. Primary cells were transduced and cells were monitored for proliferating spots of hepatocytes.

Results: After 13 days proliferating cells were visible, whereas only senescent cells where found in untransduced control wells. For all six mice proliferating upcyte® cells were found. All six upcyte® mouse hepatocytes were analysed for their morphology and for the expression of mouse hepatocyte marker proteins.

Conclusion: In conclusion, the upcyte® technology can be used to generate proliferating mouse hepatocytes from wildtype and knockout mice, while retaining their phenotype. The resulting cells called "upcyte® mouse hepatocytes" express hepatocyte markers such as CK8, CK18 and MSA. Thus, the upcyte® technology can contribute to the 3Rs concept and provide a suitable tool for knockout studies on a cellular level.

P06-006
Analysis of hepatotoxic mixture effects of pesticides *in vitro*

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Consumers are exposed to mixtures of different contaminants and pesticide residues via the diet. This raises questions with respect to potential adverse health effects, especially when several compounds of a mixture exert toxicity by a similar mode of action. Efficient testing strategies for the nearly infinite number of combinations of chemicals are lacking. In addition, reduction of animal testing in toxicological risk assessment is a societal need. Consequently, it is important to establish *in vitro* tools to assess possible mixture effects.

A mixture testing strategy for different chemical groups of pesticides was developed by compiling an *in vitro* assay toolbox of different cellular hepatotoxic effect markers, together with PCR array-based expression analysis of hepatotoxicity-related transcripts and mass spectrometry-based determination of changes in protein expression. Human HepaRG liver cells were used as a metabolically competent *in vitro* system of human liver.

Following initial screening analyses with individual compounds, mRNA and protein hepatotoxicity marker patterns were determined for approximately 30 different pesticidal active compounds. Bioinformatic data evaluation was applied to deduce the hepatotoxic similarities as well as individual potencies of the compounds. The correlation between mRNA and protein marker deregulation and cellular triglyceride accumulation was established. Based on these results, effects of equipotent mixtures of compounds with similar or dissimilar modes of action were measured over a broad concentration range. In summary, we describe an *in vitro* toolbox for pesticidal active compounds in human cells for a testing of hepatotoxic mixture effects.

P06-007
Evaluation of primary human corneal epithelial cell lines from three different suppliers for use in *in vitro* mechanistic studies

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An antibody drug conjugate (ADC), in development within GSK as an anti-cancer therapeutic, is currently undergoing clinical trials. Preliminary results have shown adverse ocular events involving the cornea, an effect reported with similar ADCs. Primary human corneal epithelial (PHCE) cells from three suppliers (ScienCell, MatTek and American Type Culture Collection (ATCC)) were evaluated as potential *in vitro* models to investigate the mechanism of toxicity.

Characterisation experiments were performed on the PHCE cells from each supplier, applying a range of endpoints: phenotypic markers for corneal epithelial and stem cells (by immunocytochemistry (ICC) and quantitative polymerase chain reaction (QPCR)), electron microscopy (EM) and assessment of doubling times. Optimisation/standardisation of culture conditions (e.g. seeding density, culture vessels, extracellular matrix (ECM)) was also conducted. To standardise laboratory methods, one dissociation method was optimised and seeding densities for sub-culturing were standardised to approximately 4000–6000 cells/cm^2.

The characterisation studies confirmed the PHCE cells evaluated expressed a range of phenotypic markers, consistent with corneal epithelium markers reported in literature (e.g. positive expression via ICC of cytokeratin 3 and 19 and positive gene expression (QPCR) of Involucrin, Integrin a9, ABCG2 and p63). A 48-hour doubling time was defined for PHCEs from all suppliers. Culture on Thermanox coverslips coated with a range of ECMs (e.g. Matrigel, laminin, poly-l-lysine) showed no overt morphological differences, or changes in growth dynamics, when compared to those cultured on uncoated coverslips. ScienCell cultures sporadically developed a spindle-like phenotype (potentially batch related), which did not express any corneal epithelial markers, therefore further characterisation work with these cells was not pursued. Although the three suppliers recommended slightly different methodology, we are now using consistent culture conditions and have initiated the mechanistic work using the MatTek and ATCC-sourced PHCEs.

P06-008

***In vitro* assays in a high-throughput screening platform – strengths and challenges**

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The US Tox21 (Toxicology in the 21st century) effort represents a paradigm shift in toxicity testing of chemical compounds from traditional *in vivo* animal tests to less expensive and higher throughput *in vitro* assays that are based on target-specific mechanisms and biological observations. To assess the toxicological effects of hundreds of thousands of the environmental chemicals, a quantitative high-throughput screening platform has been utilized to profile hundreds of thousands of environmental chemicals using a battery of *in vitro* cell-based assays. The millions of data points generated from the primary screening were made publicly accessible. These rich datasets provide researchers with opportunities for further data mining, understanding compound action, and prioritizing compound for further in-depth studies. One of the critical components of screening in toxicological research is data quality and reproducibility. This presentation will focus on assay optimization, validation, and screening performance. The screening technology and case study will be presented to illustrate the screening strengths and weaknesses. The challenge of developing *in vitro* assays with more physiological relevance will also be addressed and discussed.

P06-009

Two read-across case studies using IATA approach focused on biological similarity

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Purpose: Integrated Approaches to Testing and Assessment (IATA) is one of the appropriate approaches to establish reasonable read-across strategy. However, it is still unclear how the biological similarity considering adverse outcome pathway (AOP) affects the prediction of toxicity. Therefore, we verified the usefulness of considering the AOP-related biological similarity by using IATA-based read-across.

Method: p-Alkylphenols and Chlorobenzenes were chosen as the target categories in this study. First, ADME and putative AOP for the category members were organized by *in silico* simulation or the published information. Next, AOP-related biological endpoint were examined by in chemico or *in vitro* tests. In case of p-Alkylphenols, chemicals were applied for GSH trapping test with metabolic reaction and cytotoxicity assay. In case of Chlorobenzenes, chemicals were applied for microarray to know the intracellular responses broadly. And then NO(A)ELs of target chemicals were decided by considering the similarity of biological responses.

Result: Regarding p-Alkylphenols, reactive metabolite quinone methides (QMs)-induced hepatotoxicity is hypothesized as a major toxicological effect. As a result of the metabolite prediction by *in silico* tools, there was the difference of QMs production among the 4-position's complexity. Additionally, the p-Alkylphenols with complicated structure at 4-position produced lower amount of GSH adducts with metabolite and showed high cell viability. These results suggested that the potency of hepatotoxicity depended on the metabolic reaction of 4-alkyl structures in p-Alkylphenols, though all the members were structurally similar. Regarding Chlorobenzenes, parent chemicals and their metabolites were known to cause hepatotoxicity through several stress responses. As a result of microarray, a part of category chemicals induced similar biological responses including β-oxidation, mitochondrial disorder. in conclusion, it is suggested that AOP-based biological response could support prediction of the tendency and similarity of toxicity, which could not be inferred just from chemical/physical similarities.

P06-010

Toxicological comparison of cigarette smoke and next generation product aerosol bubbled extracts using high content screening

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Smoking is a cause of serious disease in smokers. Tobacco-based and tobacco-free next generation products (NGPs) are understood to be a less harmful alternative to cigarettes, thereby creating a huge global public health opportunity if significant numbers of adult smokers fully switch.

The objective of this study was to compare the *in vitro* biological response of Phosphate Buffered Saline (PBS) which had either cigarette smoke or a selection of NGP aerosols bubbled through it. The *in vitro* response of each extract was determined in Normal Human Bronchial Epithelial cells using high content screening after 4 and 24 hours exposures. Products investigated were the Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HYB) and a *my*blu™ e-cigarette (Tobacco Flavour 1.6% Nicotine).

The 3R4F and THP were smoked using the Health Canada Intense method. HYB and *my*blu were vaped according to CORESTA Recommended Method N°81. The smoke and aerosols were bubbled through a series of impingers containing PBS. For every test day, fresh PBS solutions with 1.8 puffs/ml and 4 puffs/ml for the 3R4F and NGP samples respectively were produced. Chemical analysis of the 3R4F PBS solutions detected nicotine with an average of 86 ± 12µg/ml. The three NGP solutions contained nicotine levels from 70 ± 1µg/ml (HYB), over 150 ± 17µg/ml (THP) to 175 ± 17µg//ml (*my*blu).

The 3R4F bubbled PBS caused a significant dose dependent decrease in cell count and significantly altered y-H2AX, NfKB, p-c-Jun and cell count endpoints (at concentrations > 1%). A partial overlap with endpoints induced by the THP solution was observed at concentrations considerably higher than 3R4F. By contrast, *my*blu and HYB extracts did not induce any significant activity in all the parameters tested at the maximum use concentration (10%).

This data suggests that the extracts from NGPs elicit little to no *in vitro* biological activity, even at higher exposure concentrations, compared to combustible cigarettes under the conditions tested.

P06-011

Evaluation of the biological effects of tobacco vapor and cigarette smoke using three-dimensional-reconstructed tissue from human airways

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The popularity of e-cigarettes and heated tobacco products is increasing worldwide. Our novel tobacco vapor product (NTV) is one of these heated tobacco products, and studies on NTV vapor have shown that their vapor has a greatly reduced level of several harmful and potentially harmful constituents (HPHCs) compared with that from cigarette smoke (CS), with approximately 99% reduction. The objective of

this study was to evaluate the biological effects of NTV vapor on three-dimensional reconstructed human airway tissue in comparison with that elicited by CS. The tissue was primary human bronchial epithelial cells differentiated at an air–liquid interface and exposed to NTV vapor or CS from a 3R4F reference cigarette using a whole-smoke exposure system. The HPHC level in NTV vapor was much lower than that in CS, so the tissue was exposed to undiluted NTV vapor to achieve the highest concentration of exposure. Conversely, CS was diluted with clean air for exposure to match the concentration of total particulate matter in CS with the concentration of aerosol-collected mass in NTV vapor. The biological effects on the tissue were assessed by measurement of cytotoxicity (assays of adenylate kinase and lactate dehydrogenase), inflammation (interleukin-8 level) and tissue-barrier function (transepithelial electrical resistance) 48 h post-exposure. The tissue exposed to 45 puffs of CS showed significant changes in all analyzed endpoints compared with the air-exposure control. Significant changes were not observed in tissues exposed to ≤840 puffs of NTV vapor compared with the air-exposure control. The number of NTV vapor puffs required for induction of significant changes in the tissue was 1,120 puffs. This exposure condition (i.e., continuous exposure to ≥1,000 puffs of undiluted vapor) differs markedly from actual exposure conditions in humans, but enables detection of the biological effect of NTV vapor and its comparison with that of CS. Our results suggested that NTV vapor had reduced biological effects on a model of human airways *in vitro*. The number of puffs necessary for induction of a significant effect using NTV vapor was approximately 25-fold that required using CS.

P06-012
Assessment of skin sensitising potential of agrochemical formulations using OECD accepted *in vitro* test methods

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Assessment of skin sensitising potential of plant protection products represents a critical component of the safety evaluation process for agrochemicals. Skin sensitisation assessment has traditionally used animal tests, but OECD accepted *in vitro* assays now exist: Direct Peptide Reactivity Assay (DPRA; OECD TG442C), KeratinoSens™ (OECD TG442D) and human Cell Line Activation Test (h-CLAT; OCD TG442E). However, these assays currently lack validation for their use in the evaluation of complex mixtures. As such their regulatory use for complex mixtures is currently not common place, with more evidence required to establish their suitability for mixture testing. Here we investigate the applicability of these 3 assays for evaluating skin sensitisation potential of 10 agrochemical formulations: 4 suspension concentrates [SC], 2 emulsifiable concentrates [EC], 2 flowable concentrates for seed treatment [FS], and 2 water dispersibles [WG]. Of these, 6 were previously positive in *in vivo* skin sensitisation tests. The testing approach calculated an average molecular weight (MW) for each formulation by considering the MWs and individual proportions of each component, resulting in average MWs for testing ranging from 177 to 2259 kDa. For each assay, the procedure for solvent selection and solubilisation was performed according to the respective DB-ALM protocols. For DPRA, 5/10 formulations (2 FS; 3 SC) were compatible and solubilised successfully in dimethyl sulphoxide (DMSO):acetonitrile (1:1) with concentrations ranging from 25mM to 100mM. For KeratinoSens™, all formulations were soluble in DMSO, however, observed cytotoxicity resulted in testing of most formulations at dose ranges lower than OECD recommendations. Preliminary data from DPRA and/or KeratinoSens™ revealed a 'sensitiser' prediction that correlated with *in vivo* methods for 5/6 formulations. Confirming a negative result for the formulations with *in vivo* 'non-sensitiser' predictions was more problematic due to solubility/cytotoxicity profiles impacting assay performance to recommended guidelines, currently leading to their inconclusive interpretation. This is an ongoing study, with performance of all 3 OECD accepted assays being investigated, together with the hypothesis that individual co-formulants may be influencing assay results.

P06-013
Application of adverse outcome pathways and quantitative *in vitro-in vivo* extrapolation (QIVIVE) modelling for risk assessment based on *in vitro* data

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The Adverse outcome pathway (AOP) framework has been adopted as a valuable tool to support transition of drug/chemical safety testing from animal studies to more mechanism-based *in vitro* endpoints. Systematic mapping of AOPs and identification of key events (KEs) for a given hazard endpoint may serve as a basis for the development of suitable *in vitro* assays that can be integrated with quantitative *in vitro-in vivo* extrapolation (QIVIVE) modelling to estimate risk based on *in vitro* data.

The aim of this work was to provide a proof-of-concept for this approach using kidney toxicity as an exemplary area of repeated dose systemic toxicity. AOPs for kidney injury due to (a) receptor mediated endocytosis leading to lysosomal overload and (b) inhibition of mitochondrial DNA polymerase g were mapped and critically evaluated in terms of biological plausibility, essentiality of KEs, dose-response and temporal concordance using both literature data and novel data generated by *in vitro* assays reflecting the KEs in each of the AOPs. Quantitative response-response relationships between KEs established using model stressors for each AOP (a: polymyxin antibiotics, b: acyclic nucleoside phosphonate antiviral drugs) were used to predict downstream KEs based on *in vitro* data reflecting early KEs. Finally, toxicokinetic models comprising a proximal tubule cell compartment were established and utilized to transform *in vitro* effect concentrations to a human equivalent dose. Model outcomes for selected nephrotoxic drugs were compared to human clinical data to assess the performance of the *in vitro* approach to risk assessment. Using polymyxin B as an example, we show that predicted *in vivo* effective doses are in the range of therapeutic doses known to be associated with a risk for nephrotoxicity. While these data support the overall approach of the project, there are yet limitations and sources of uncertainty that remain to be addressed in the future.

P06-014

The kinetic Direct Peptide Reactivity Assay (kDPRA): an in chemico method to characterize the skin sensitization potency of chemicals

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While the skin sensitization hazard of substances can readily be identified using non-animal methods, the classification of potency into UN GHS sub-categories 1A and 1B remains challenging. The kinetic direct peptide reactivity assay (kDPRA) is a modification of the DPRA (OECD TG 442C) wherein the reaction kinetics of a test substance towards a synthetic cysteine-containing peptide is evaluated. For this purpose, several concentrations of the test substance are incubated with the synthetic peptide for several incubation times at 25°C. After the respective incubation time, the reaction is stopped by addition of the fluorescent dye monobromobimane (mBBr). The highly reactive and non-fluorescent mBBr rapidly reacts with unbound cysteine moieties of the model peptide to form a fluorescent complex. The remaining non-depleted peptide concentration is determined thereafter by fluorescence measurement at precisely defined time points. Kinetic rates of peptide depletion are then used to distinguish between two levels of skin sensitization potency, i.e. to discriminate between CLP/UN GHS sub-categories 1A and 1B. During an in house validation [Wareing *et al*., 2017] 35 of 38 substances with LLNA-based sensitizing potency were correctly assigned to the potency sub-categories, and the predictivity for 14 human data was similarly high. These results warranted the kDPRA for further validation. Here we present the results of a ring trial testing 24 blind-coded chemicals in seven labs [1]. In parallel we present the extension of the kDPRA database to further assess the predictive capacity of the assay. Eventually the kDPRA should be used as a part of defined approach(es) with a quantitative data integration procedure for skin sensitization potency assessment.

[1] Upon the abstract submission deadline the ring trial was still in progress and the substance identities remained blind coded. Therefore no results could be presented in the abstract.

P06-015

Improving the prediction of hepatotoxicity: impact of protein binding in the generation of *in vivo* relevant intracellular concentrations

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Purpose: The unbound intracellular concentration (ICC) is the driving force for processes that occur inside the hepatocyte, including metabolism, induction (metabolic and transporter), efflux based drug interactions, and hepatotoxicity. In sandwich-cultured hepatocytes, the intracellular milieu contains the components for drug binding, and in concert with hepatic uptake and efflux (basolateral and canicular) transporters, drug metabolizing enzymes, and key regulatory pathways allow generation of *in vivo* relevant unbound intracellular concentrations. Protein on the outside of the cell can also limit hepatic uptake of a drug. If hepatic uptake and intracellular concentration are dependent on the free concentration, then, parameters generated from experiments performed in the absence and presence of protein should be equal when normalized for protein binding.

Methods: Sandwich-cultured rat hepatocytes and B-CLEAR® technology were used to determine the ICC for 10 compounds (taurocholate, telmisartan, methotrexate, valsartan, DPDPE (1 and 10µM), digoxin, pitavastatin, rosuvastatin, and pravastatin) in the presence and absence of a physiological concentration of bovine serum albumin (4% BSA). IC_{50} values for inhibition of CYP2C9 and CYP3A4 metabolism by fluconazole and ketoconazole, respectively, were determined in the presence and absence of 4% BSA using Transporter Certified™ human hepatocytes in sandwich culture. The fraction unbound (f_u) in 4% BSA was determined in a separate experiment using equilibrium dialysis. The extent of protein binding was used to normalize the values obtained in the absence of protein (Predicted value), and compared to the value obtained in the presence of protein (Observed value) and the fold change was calculated.

Results: The ICC was over predicted for valsartan (2.1X), and underpredicted for pitavastatin (3.2X), rosuvastatin (2.2X), and telmisartan (90X). For the other compounds evaluated (pravastatin, digoxin, DPDPE - 10µM, and taurocholate), predicted values were not different from the observed values. Normalization of the IC_{50} values for CYP2C9 by fluconazole were over predicted by 1.9X, while the IC_{50} values for inhibition of CYP3A4 by ketoconazole were under predicted by 257X. *In vivo* it is the rate limiting step – the slowest process that determines the overall rate. The lack of agreement between observed and predicted ICC values may be due to measurement of the extent and not the affinity of the protein binding. If the dissociation rate of the drug off of the protein is much greater than the uptake rate, protein binding may not be a limiting factor in drug uptake.

Conclusions: If active transport processes are involved in hepatic uptake, the slowest process will limit the hepatic uptake. Addition of physiologic protein concentrations to *in vitro* systems may improve predictions of intracellular drug exposure and effects.

P06-016

Safety testing of cosmetics for eye irritation *in vitro*: evaluation of results from over 40 studies

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Cosmetics safety assessment typically involves: 1) theoretical evaluation of ingredients and raw materials to determine local and systemic toxicity potential, and 2) testing to confirm local toxicity effects of final formulations. Interactions among ingredients are not easily predicted by single-ingredient theoretical evaluations and this is where safety testing is critical. Given the momentum regarding refinement, reduction, or replacement (3Rs) of animal-based tests with *in vitro* methods, it is important to define testing strategies that meet those requirements and provide confident safety assurance for each toxicity endpoint (e.g., eye irritation potential). The Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability (BCOP) assays, when used in combination, have demonstrated to be relevant and reliable methods to predict eye irritation potential of cosmetics. These assays predict the possible effects of mixtures in the human eye fairly well partly because, together, they represent relevant eye areas (i.e., conjunctiva and cornea). In the BCOP assay, there are currently two suggested classifications at the low end of the eye irritation spectrum: 1) GHS (no category)

and 2) Gautheron (mild irritant). Results from our 40+ test batteries have been used to establish a prediction model for safety assessment, which can be used to guide decision-making regarding cometics warning labels. According to our data analyses, a formulation could be confidently predicted as "practically non-irritating" to the human eye when the CAMVA RC_{50} is >70% and the BCOP *in vitro* irritancy score (IVIS) is ≤3. Comparing *in vitro* test results predictions with post-marketing surveillance analyses allows evaluation of the accuracy of the two-test battery prediction model, and confirms the effectiveness of safety evaluation recommendations for product market release to better ensure consumer safety.

P06-017
Donor-to-donor variability of reconstructed human airway tissues in response to cigarette smoke

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Reconstructed human airway (RHuA) tissue is considered a reliable *in vitro* model for inhalation toxicity testing with airborne materials such as cigarette smoke (CS) because of its resemblance to *in vivo* tissues. However, it potentially possesses donor specific characteristics related to variations of genotype, resulting in differences of responder responses to toxicants. Therefore, it is important to understand and biologically interpret such variabilities when assessing toxicities of interest. In this study, we exposed commercially available RHuA (MucilAir) derived from three different donors to an aqueous extract (AqE) of CS. Tissues were exposed to three different concentrations of CS-AqEs. Tissues incubated with fresh medium alone were used as untreated controls. After 24 h of exposure, cytotoxicity (secreted adenylate kinase; AK), ciliary functions (beating frequency and area), IL-8 secretion and global gene expression profiles were analyzed. AK secretion increased by CS-AqEs exposure differed among donors, suggesting that reactivity to toxicants was different. Consistent with this result, dose-response curves of IL-8 secretion and ciliary beating area differed although all assays used in this study showed some reaction to CS. Furthermore, microarray analysis revealed that genes that had statistically significant altered expression levels related to CS exposure only had a 13.6% overlap, implying that the other differentially expressed genes (DEGs) responded dependent upon the donor origin. However, canonical pathway analysis with the 13.6% of overlapping DEGs showed that these genes were related to oxidative stress responses including NRF2 mediated oxidative stress, a well-known biological event elicited by cigarette smoking. Taken together, donor dependent characteristics were observed in RHuA tissues in response to CS, but the key responses were well conserved. In conclusion, we demonstrated the potential of donor-to-donor variability in an advanced *in vitro* test using RHuA, which might cause misleading results if there are no consensus targeted endpoints or appropriate dose settings. Therefore, transcriptomic data will be a useful tool to complement these results and investigate the key biological effects of test substances that might highlight fit-for-purpose endpoints.

P06-018
hTERT immortalized adult dermal melanocytes: an *in vitro* cell model for the study of skin pigmentation

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Skin pigmentation is a complex process; melanocytes produce melanin and package it into melanosomes that are in turn exocytosed into the surrounding extracellular matrix. Numerous genes play roles in controlling pigmentation at various levels of melanin production. Mutations in these genes are characteristic of multiple skin disorders, including hyperpigmentation, hypopigmentation, and mixed hyper-/hypopigmentation. Additionally, extrinsic factors secreted by the surrounding resident cell types also regulate the melanin expression in adult melanocytes. Human primary cells can be a useful model for elucidating melanocyte biology. However, primary cells have their limitations such as donor variability and limited lifespan. Consequently, a need exists for a more robust human cell model system for the study of skin pigmentation.

In this study, we immortalized primary dermal melanocytes by expressing human telomerase reverse transcriptase (hTERT) in cells that were isolated from an adult donor. The immortalized primary melanocytes were cultured continuously for more than 40 population doublings without any signs of replicative senescence, yet retained melanin production. The immortalized primary melanocytes maintained a consistent expression of the melanocyte-specific marker TRP-1, and lacked expression of the fibroblast-specific marker TE7. In addition, we demonstrate the capability of these immortalized primary melanocytes to transfer melanosomes to keratinocytes, the ability to modulate melanogenesis with stimulators and inhibitors, and their capacity to incorporate into a functional 3D human dermal organotypic culture. Taken together, the hTERT immortalized primary melanocytes described here provide a versatile *in vitro* cell model for the study of melanin production and melanocyte:keratinocyte interactions in the dermal environment.

P06-019
Screening of the cytotoxic, genotoxic, apoptotic and cell cycle effects of *Rubus rosaefolius* (Rosaceae) leaf extract on human HepG2/C3A cells.

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The *Rubus rosaefolius* plant recently have been some of its therapeutic properties confirmed by scientific analysis, among them the analgesic, antimicrobial, antihypertensive, antioxidant, antiproliferative effects in tumor cells, diuretic, gastroprotective and antidepressant. Such confirmation makes this plant of great interest to the pharmaceutical industry. However, before the commercial exploitation of *R. rosaefolius* as a herbal remedy, it is necessary to carry out tests evaluating the biosafety of the use of this plant by humans, ascertaining if it is free of cellular and genetic toxicity. In view of the above context, this research aimed to analyse the action of *R. rosaefolius* leaf extract on human hepatoma (HepG2/C3A) cells, regarding its cytotoxic, genotoxic, apoptosis induction, and cell cycle effects. The cytotoxicity of the extract after 24, 48 and 72 h exposure in a concentration spectrum of 0.01 to 100 µg/mL was evaluated by the MTT test. Results showed absence of cytotoxic effect of the extract in MTT test on HepG2/C3A cells at all concentrations and exposure time tested. The *in vitro* comet assay showed an increase in the DNA damage (class 1) at 0.1 µg/mL concentration and above. Micronucleus test evidenced no clastogenic/aneugenic effects. Flow cytometry analysis showed significant increase in the number of the apoptotic cells at 10, 20 and 100 µg/mL concentrations, and interference of the extract

in the cell cycle with increase number of the cells arrested in the S phase (only at 100 µg/mL). Under our experimental conditions, this preliminary *in vitro* biosafety evaluation showed that leaf extract of the medicinal plant *R. rosaefolius* presented some toxic effects on HepG2/C3A human cultured cells. Complementary studies are being performed to better determine the risk of this plant extract to the humam cells.

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P06-020

The THP-1 toolbox: a new method that integrates the 4 key events of skin sensitization

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Allergic contact dermatitis (ACD) is an adverse health effect that develops following repeated exposure to skin sensitizers. In the European Union, an animal testing ban has been applied under the Cosmetics Regulation, leading to development of reliably predictive non-animal methods. An adverse outcome pathways (AOP) for chemical induced skin sensitization has been already proposed in 2012 by OECD. AOPs outline causally linked key steps between a direct initiating event leading to an adverse health outcome. Four different key events (KE) have been identified and associated in the AOP for skin sensitization: (1) protein-binding reactions, reactivity, and metabolism, (2) epidermal inflammatory response, (3) DC activation and (4) T-cell proliferation. Different *in vitro* chemistry-based assays have been developed and allow the evaluation of sensitization hazards.

Since DC play a key role in the skin sensitization phase leading to the development of ACD, we propose to combine different tests covering all KE defined by AOP in a same cell line,the THP-1 cell, acting as a DC.

We decided to study the ROS production and GSH depletion as cellular oxidative stress for KE1, Nrf2 activation pathway and gene expressions for KE2, phenotype modifications as cell-surface markers and cytokines production for KE3, and T cell proliferation for KE4. All of those measurements were performed on the THP-1 cell-line, after exposure to a variety of chemicals, including irritants, non-sensitizers and allergens (pro/prehaptens).

Results showed early ROS production and reduction of intracellular glutathione are correlated with the potency of the chemicals such as cinnamaldehyde or methylisothiazolinone. Those chemicals as well as antioxidants specifically activate the Nrf2-Keap1 pathway, which were measured by western blot and a Nrf2 DNA-binding ELISA. They also strongly induced phenotype maturation of THP-1 cell-line with CD54 and CD86 expression at cell-surface and specific cytokine production such as IL-8, IL-18. All sensitizers were able to induce the T cell proliferation while non-sensitizers and irritants did not.

In the present study, we have demonstrated that the three main KE of skin sensitization AOP can be addressed in a same cell line as well as lymphocyte activation.

P06-021

Application of the Human Cell Line Activation Test to predict the skin sensitization potential of DDAC, PHMG, troclosan and propylene glycol

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The Human Cell Line Activation Test (h-CLAT) is an alternative *in vitro* test method using dendritic cells for prediction of skin sensitization and adopted as OECD TG 442E. The h-CLAT method was used to predict skin sensitization potential of didecyldimethylammonium chloride (DDAC), polyhexamethylene guanidine (PHMG), 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan), which often serves as biocidal agents. Propylene glycol (PG) was also tested, which is used to dissolve the active ingredients. The skin sensitization (SS) potentials of each of these substances are still being debated. All the experimental procedures were undertaken following the OECD TG. Proficiency testing of the nine coded substances listed in OECD Test Guideline 442E correctly detected all sensitizers and non-sensitizers. On 3 independent runs, DDAC, PHMG and triclosan were predicted to be sensitizers (in 2 of 2 or 2 of 3 runs, the CD86 RFI is ≥ 150% and/or the CD54 RFI is ≥ 200% at any tested concentration). In terms of DDAC, the CD54 RFI% exceeded 200% in all 3 runs and the CD86 RFI % exceeded 150% in 1 run. Concerning on PHMG and triclosan, the CD54 RFI % exceeded 200% and the CD86 RFI % exceeded 150% in consecutive 2 runs for both substances. Meanwhile, PG was also classified into a sensitizer, in that the CD54 RFI % exceeded 200% in 2 runs. Since humans can be occupationally or environmentally exposed to those biocidal or excipient chemicals, the present study could help regulatory bodies in their assessment of the skin sensitization potency of DDAC, PHMG, triclosan and PG. [supported by Korea National Research Foundation, Project no. 2017R1D1A3B03032723].

P06-022

Investigation of the genotoxic potential of green smoothies *in silico* and *in vitro*

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Green smoothies contain raw green vegetables or herbs blended together with fruits. Due to their high amount of phytochemicals, they are advertised as a healthy addition to the normal diet. However, recent studies indicate that the excessive consumption of raw vegetables and leafy greens may not be as healthy as advertised and it is recommended to rotate the green ingredients in the smoothie to avoid toxic side effects. Moreover, blending of vegetables leads to a ruptured cell wall, thus a higher number and a different spectrum of phytochemicals are available for absorption. In this project, an *in silico* genotoxicity assessment, as well as a dietary risk assessment was performed on green smoothies. This included a database search for the phytochemical content and associated concentration values in common smoothie ingredients, followed by the prediction of their genotoxic potential by three different QSAR models. Thereby, endpoints regarding the Ames test, micronucleus test, and chromosome aberration were chosen. Compounds of toxicological concern were added to the dietary risk assessment and their concentration values

in one smoothie were calculated and compared against the threshold of toxicological concern (TTC) for mutagenic compounds. HPLC-MS was performed to evaluate the number of phytochemicals in a sterile-filtered smoothie supernatant, showing the presence of thousands of compounds. In contrast to that, database search yielded a maximum of 384 known phytochemicals for a whole smoothie. Also, not for all phytochemicals a concentration value was available. QSAR analysis showed a genotoxic potential for multiple phytochemicals, but for several others a prediction was not possible due to missing data. Furthermore, the QSAR analysis showed high variations of the datasets between different models, resulting in different outcomes for the same endpoint. For a reliable dietary risk assessment too many data were missing. All in all, this project shows that there is need to define a safe exposure level also for plant-based compounds. For this, however, the databases for the chemical content in plants, as well as the datasets embedded in QSAR models do not provide sufficient information. This means, that more toxicological data are needed, and further research is necessary regarding the chemical content in plants.

P06-023
Vascularised cardiac tissue model for the assessment of efficacy and cardio toxicity

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Background: Cardiotoxic effects are among the most common reasons for discontinuation of development of a novel drug candidate at the Phase I or later. A commonly used test system is human induced pluripotent stem cell (hiPSC) derived cardiomyocyte (CM) monoculture. Monoculture lacks the 3D-tissue structure and phenotype of adult CMs and resemble closely the foetal one. As the microenvironment is essential for the full maturation of the cells, in our model hiPSC-CMs are cultured with human cellular vasculature. We hypothesized that using these more mature CM, would improve the predictability of the effective physiological concentration of a drug compared to monocultured CM.

Materials and methods: Vasculature was formed from Human adipose stromal cells (hASC) and human umbilical cord vascular endothelial cells (HUVEC) as described earlier. hiPSC-CM (iCell CM2, Cellular dynamics) were seeded on top of the vasculature. CM were let to mature for 8 days on top of the vasculature after which the cardiovascular model was used for chemical testing. For the assessment of the validity of our model in assessment of cardiac effects we used multi electrode array (MEA) measurements, and compared the published data obtained from monocultured CM. Set of 30 drugs with known effects on human heart functions and three negative controls were studied. This set of drugs included AR-agonists, AR-antagonist and various ion channel blockers.

Results and conclusion: The used negative controls did not significantly alter field potential duration or beat rate. The results with known drugs showed that majority of the effects in our model were obtained with concentrations closer to clinically observed C_{max} values compared to monocultured CM. These data suggest that culturing CM together with vasculature increases the level of maturation and provides more physiologically relevant model for assessing the cardiac effects of different compounds.

P06-024
In vitro viability tests to evaluate Fe_3O_4NPs cytotoxicity in human mesenchymal stem cells

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The emerging applications of superparamagnetic iron oxide nanoparticles (SPIONs), including magnetite (Fe_3O_4NPs), in life sciences and industrial and biomedical (diagnosis and therapy) fields, raise public health and scientific concerns over possible environmental and human health implications since Fe_3O_4NP toxicity is not yet fully understood.

In this study, *in vitro* tests, neutral red uptake (NRU), MTT tests and trypan blue assay (TB), were applied to evaluate cytotoxicity of Fe_3O_4NPs (1-300 mg/ml) after short-term exposure (24-48 h) using human mesenchymal stem cell derived from umbilical cord lining (CL-hMSCs), as an innovative and alternative cell model.

NRU results showed a concentration-dependent absorbance increase. Apparently, an enhancement of cell viability from 18 to 260% at 10–300 mg/ml Fe_3O_4NP was observed. Similar data were also obtained from MTT test (high cell viability) after NP exposure compared to control.

This absorbance enhancement was not supported by the evidence obtained with morphological analysis by phase-contrast microscopy. Cellular visual inspection, at both time points, showed cell density decrease and loss of the monolayer features at ≥ 50 μg/ml, and morphological alterations (large/flat cells, debris) at ≥ 150 μg/ml. Notably, the cell morphology changes paralleled with the results obtained with TB. The latter showed a cell death (35%) at ≥ 10 μg/ml, with maximum effect (65%) at 300 mg/ml Fe_3O_4NPs after 48 h.

Experiments carried out in a cell-free system confirmed that Fe_3O_4NPs interfered with the enzymatic activity of MTT and NRU assays (20–50% and 50–450% absorbance increase, respectively).

Altogether our data suggest that the agglomeration and settling of Fe_3O_4NPs in the specific medium used for CL-hMSC cultures, associated to the difficulty to remove them (by washing) from this cell type, appeared to be linked to light absorbance interference leading to overestimation (false) viability. On the contrary, in this culture conditions, TB seemed a suitable test to determine cell viability compared to MTT and NRU.

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P06-025
Contraction properties of human *in vitro* cardiac tissue model

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Background: Cardiotoxicity is one of the major causes for drug attrition and withdrawal during drug development process and post approval. Contraction force is an essential part of heart function and drugs affecting the contraction of cardiomyocytes can potentially cause severe cardiac risks. Human induced pluripotent stem cell -derived cardiomyocytes can be used for cardiotoxicity testing *in vitro*. They are commonly used as a cardiomyocyte monoculture in which they, however, remain immature phenotype resembling more fetal than adult cardiomyocytes.

Materials and methods: In this study, contraction properties of vascularized cardiac tissue models were studied. The cardiac tissue models were constructed by first co-culturing human adipose stromal cells (hASCs) and human umbilical vein endothelial cells (HUVECs) to produce vascular-like networks and then seeding cardiomyocytes on top of the vascular structures. The contraction force of cardiac tissue models was measured using in-house developed piezoelectric cantilever sensor. The contracting structures of cardiomyocytes in the cardiac tissue models were characterized using electron microscopy techniques and immunofluorescence imaging and compared to cardiomyocytes cultured in monoculture.

Results: Contraction forces of 7.2 to 16.6 µN were measured from the cardiac tissue models. The cardiomyocytes in the cardiac tissue models had more mature morphology compared to cardiomyocytes in monocultures.

P06-026
Identifying and characterising stress pathways of concern for consumer safety risk assessments

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As recently outlined by the International Cooperation on Cosmetics Regulation (ICCR) [1], key principles of modern non-animal cosmetic safety risk assessments are that they should be exposure-led, hypothesis driven, use a tiered and iterative approach and adopt robust methods for which sources of uncertainty are characterised and documented. In particular, many compounds for which consumer safety risk assessments need to be conducted are not associated with specific toxicity modes of action, but rather exhibit non-specific toxicity leading to cell stress. In this work, a cellular stress panel is described, consisting of forty biomarkers representing nine stress pathways and cell health markers, including oxidative stress, mitochondrial toxicity and ER-stress, measured predominantly using high content imaging. To evaluate the panel, data were generated using HepG2 cells for fifteen compounds at typical human exposure levels. The compounds were selected either because they are known to either be toxic to humans at such levels (and therefore present a 'high risk' from a consumer safety perspective) and have a mode-of-action associated with cellular stress (e.g. doxorubicin, troglitazone, diclofenac), or compounds widely regarded as innocuous (i.e. 'low risk') to humans (e.g. caffeine, niacinamide and phenoxyethanol). A key metric here is the point-of-departure (POD), the lowest concentration at which biological responses can be detected within a given set of assays. For each compound, dose response data (eight concentration points) were generated for each biomarker at three timepoints. A Bayesian model was then developed to quantify the evidence that, for a given set of dose-response data, a biological response does occur, and if so, a credibility range for the estimated POD. These PODs are then compared to Cmax estimates calculated using physiologically-based kinetic models. Results from the panel indicate a clear differentiation between the 'low-risk' and 'high-risk' compounds at typical human exposure levels, with low risk ones triggering significantly fewer stress pathways, and at higher concentrations, than the high-risk compounds. Comparisons to analogous stress panel data generated using other cell models (HepaRG and NHEK) will also be presented. Overall, the results provide a strong indication that the panel could serve as an assay for identifying and characterising stress pathways of concern, as part of a weight of evidence-based risk assessment that follows the ICCR principles.

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P06-027
Three-dimensional *in vitro* co-culture model of adipocytes and endothelial cells using magnetic levitation: toxicological evaluation of caffeine

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To evaluate new active substances and formulations for the treatment of cellulite and adipocyte dysfunctions, conventional two-dimensional (2D) cell cultures are usually employed. However, 2D culture models do not mimic the complexity of the adipose tissue. The interactions between the cells and their microenvironment govern various processes, such as cell differentiation, proliferation, and gene expressions. In this context, we are proposing a three-dimension new levitation cell culture system based on magnetic particles to assess the toxicological aspects of caffeine, as a model drug of lipogenic activity. The goal of this study was to compare cytotoxicity in 2D cell culture models based on OECD/GD 129 and the 3D models. The 3T3-L1 preadipocyte cells and rat endothelial cells were cultured in DMEM 10% FBS. Three-dimensional levitation cell cultures were based on previously established methodology and were set up using 96-well Bio-Assembler™kit (Nano3D Biosciences™Inc.) consisting of nanoshuttle (NS) solution and a plate magnetic drive. 3D cultures levitated for 1 day were induced for adipogenic differentiation (0.5 mM isobutylmethyxanthine, 1µM dexamethasone, 1.7 mM insulin in DMEM 10% FBS) for 72 h. After this, the induction medium was replaced with DMEM 10% FBS containing 1.7mM insulin. Caffeine 7.0 mM was added after 72h and maintained for 8 days. The 2D cultures were not submitted to adipogenic differentiation. Both cultures – 2D and 3D – were evaluated using PrestoBlue™ viability dye. The results of cell viability for 2D cultures were 14.96% for caffeine and 3.43% for DMSO meanwhile for 3D were 13.01% for caffeine and 8.7% for DMSO. The major difference was observed only for the positive control, as the undifferentiated cells also did not present difference in the cell viability in both systems. In conclusion, it will be possible to come up with a 3D *in vitro* model to evaluated new adipogenic actives in research and development of new cosmetic products.

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P06-028
Prediction of skin sensitization potency for risk assessment using noble biomarkers IL-1β and iNOS

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Biomarkers related to skin sensitization were analyzed in THP-1 human monocytic leukemia cells to predict skin sensitization potency for risk assessment, as an alternative animal tests. Cell viabilities of 90% (CV90) and 75% (CV75) were determined by WST-1 assay to establish the comparative conditions of 24 selected test materials. In addition, biomarkers related to skin sensitization were analyzed by western blotting under equivalent comparative conditions. In biomarker analyses, IL-1β, iNOS, IL-1β + iNOS, and THP-1 IL-1β + Raw 264.7 IL-1β were found to be suitable biomarkers for the prediction of skin sensitization potency following their classification as either skin sensitizers or non-sensitizers (accuracies of 91.7%, 87.5%, 83.3%, and 82.6%, respectively). In addition, a high positive correlation was found between these biomarkers and skin sensitization potency, with a correlation coefficient (R) of 0.7 or more (correlation coefficients of 0.77, 0.72, 0.70, and 0.84, respectively). Finally, the skin sensitization potency EC3 (%) was predicted using a biomarker correlation equation, with resulting prediction accuracy for the EC3 value (%) obtained from animal data was calculated as 83.3%, 79.2%, 79.2%, and 73.9%, respectively. These results suggest that biomarker analysis using IL-1β and iNOS in human THP-1 cells can be alternatively used to predict skin sensitization potency for risk assessment.

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P06-029
Mechanism-based alternative method for developmental toxicity testing in zebrafish embryos

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These days it is required to establish alternative testing methods for safety assessments. However, alternative methods for developmental toxicity tests have not been well developed because of its complicated toxicological responses. Zebrafish early embryos are non-protected animals (eg. Directive 2010/63/EU) and considered to be one of the promising models for screening of common birth defects owing to the conserved developmental program, low experimental costs, rapid development and transparency. Although conserved toxicity endpoints are necessary for accurate prediction of developmental toxicity in mammals, there is little information about cross-species conservation of teratogenic responses between mammals and fishes. We focused on 5 major targets of congenital birth defects (cranium, palate, nervous system, heart and musculoskeletal systems) and analyzed morphological, cellular and molecular responses to teratogens. In the present study, we investigated the conserved mechanisms of palate malformation between mammals and zebrafish.

Zebrafish embryos were exposed to 12 chemical compounds (valproic acid, warfarin, caffeine, imatinib, retinoic acid, salicylic acid, 5-fluorouracil, methotrexate, thalidomide, hydroxyurea, phenytoin and dexamethasone), which induce cleft palate in human or rodents. Palatal morphology and the number of proliferative cells and apoptotic cells were examined in zebrafish palate at 96 hpf using immunofluorescence staining and confocal microscopy. Also, we investigated the involvement of the canonical Wnt signaling pathway, which is one of the key contributors to orofacial clefts. Chemical rescue of the cleft palate were performed by simultaneous treatment with Wnt agonists (BIO, CHIR99021, and WAY262611) and specific teratogens (warfarin and valproic acid).

All 12 teratogens induced palatal defects in zebrafish embryos which showed decreased proliferation and increased apoptosis in the palate. These phenotypes were rescued at the cellular and molecular levels by the treatment with the Wnt agonists.

We showed the conserved responses to the teratogens between mammals and zebrafish: malformation of palate and regulation of proliferation/apoptosis via the Wnt signaling pathway. Thus, our results suggest that zebrafish early embryo assay would be a suitable model for assessing chemical-induced cleft palate as well as being a screening tool for prediction of cleft palate in mammals. We will confirm the key endpoints based on conserved molecular mechanisms by a comprehensive analysis as a next step for accurate prediction of teratogenicity in mammals.

P06-030
Data sharing on the INTERVALS platform and meta-analysis of *in vitro* toxicology assessment of diverse e-liquid and heat-not-burn products

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Extensive scientific studies are conducted to assess the relative risks of various candidate modified risk tobacco products compared with those of smoking cigarettes. As the scientific community conducts such assessments for diverse products and in a variety of laboratory models, knowledge on toxicity is spread across numerous scientific articles. We believe that by fostering the consolidation of data and knowledge gained from studies assessing novel tobacco/nicotine delivery products on a community platform, new hypotheses may be generated, and the weight of evidence may be increased. Therefore, we have created and are further developing INTERVALS (www.intervals.science), an online platform supporting independent, third-party collaboration by proactively sharing detailed protocols, tools, and data from assessment studies. Data files are accompanied by relevant information to foster reproducible research and encourage data reanalysis.

We will present a meta-analysis of *in vitro* toxicology assessment studies, including aerosol characterization, neutral red uptake assay, and mouse lymphoma assay, for various e-liquid and heat-not-burn platforms compared with the 3R4F reference cigarette. These studies have been conducted by multiple organizations using different methods and models. The content of the separate publications has been curated and included in INTERVALS in an interoperable format so that a meta-analysis of results can be performed.

The direct comparison of the platforms tested in separate studies with different study designs (e.g., different lists of chemicals quantified in the aerosols) makes it difficult to compare every single result across all individual studies. However, the overall result is consistent in that all of the studies included in this analysis demonstrate the reduction of harmful or potentially harmful chemicals and of toxicity assessed *in vitro* for the tested platforms compared with cigarettes. As the scientific community integrates more studies and datasets into INTERVALS, it will become easier to conduct such meta-analyses and review results obtained across institutions, models, and platforms.

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P06-031

The molecular basis for a functional dermal barrier in two biotechnologically produced human skin equivalents: the Phenion® FT Skin model and the OS-REp model

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Biotechnologically produced 3D skin equivalents are the state-of-the-art tools to study human skin physiology and pathology under standardized conditions *in vitro* and to replace animal experiments in the toxicological assessment of chemicals. In healthy human skin a functional and selective barrier, mainly located in the stratum corneum, discriminates between chemicals which penetrate the skin and subsequently reach the deeper tissue layers, or which remain on the tissue surface without any effect on the skin. Thus, lipid composition and structure of the dermal barrier are crucial for the access of chemicals into the skin and subsequently influence all downstream reactions, both *in vivo* and in 3D tissue models. The barrier lipid composition of 2 skin equivalents, the Phenion® Full-Thickness (FT) Skin Model and the Phenion Open Source Reconstructed Epidermis (OS-REp), was analyzed chromatographically. Ceramides, cholesterol and cholesterol derivatives, triglycerides and phosphatidyl choline were identified in all samples tested, although in slightly differing quantities. The lipid profiles of both 3D skin models closely matched the profile of native human foreskin tissue, the source for keratinocytes and fibroblasts which give rise to the tissue equivalents. Major enzymes of the epidermal lipid metabolism, e.g. ceramidases and serine palmitoyltransferase, were expressed in keratinocytes in monolayer culture and/or in the epidermis of the FT- and OS-REp models, as demonstrated by immunofluorescence and RT-PCR. Barrier integrity was analyzed by TEER value evaluation during the whole tissue culture period. The similarity of the lipid pattern in the 3D skin models with intact human skin, together with the presence of key enzymes of barrier lipid synthesis, provides strong evidence for a physiological barrier function. This is a key prerequisite for using the skin models in the toxicological assessment of substances, e. g. in *in vitro* skin irritation or corrosion tests or in dermal absorption studies. Thus, both the Phenion® FT Skin Model and the OS-REp model are well-suited to be used as *in vitro* surrogates for native human skin, or epidermis, respectively, in experiments which require a barrier function.

P06-032

Using 3D human liver microtissues to model NASH progression *in vitro* for drug discovery and safety testing

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent type of liver disease and currently affects ~30% of the population. With progression to non-alcoholic steatohepatitis (NASH), this disease can eventually lead to liver cirrhosis and failure. To date, there are no approved drugs for NASH treatment and drug development has been impeded by the lack of predictive *in vitro* models reflecting the complex pathology of NASH. Here, we present a human *in vitro* NASH model based on 3D microtissue technology. Engineered to incorporate the primary human hepatocytes, hepatic stellate cells, Kupffer cells (KCs) and liver endothelial cells (LECs), this model includes all the liver cell types that play a crucial role in disease initiation and progression. Upon treatment with free fatty acids and LPS in diabetic medium these microtissues showed key physiological aspects of NASH. The lipotoxic NASH stimuli increased the lipid accumulation within the hepatocytes as well as the tissue secretion of pro-inflammatory markers, such as TNF-a, IL-6, IL-8, MCP-1, MIP-1a and IP-10. Furthermore, lipotoxic stress stimuli increased the expression of pro-fibrotic markers such as collagen type I and III and the release of pro-collagen type I.

In summary, we present a human 3D NASH model that recapitulates key biological aspects of the NAFLD spectrum of diseases, including inflammation, steatosis and fibrosis. Compatible with high-throughput screening approaches, this model is a powerful tool for assessing efficacy of anti-NASH drugs.

P06-033

The mixture of persistent organic pollutants present in human follicular fluid stimulates the estradiol secretion by adult granulosa tumor spheroids via the classic and non-classic estrogen receptors.

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Epidemiological studies have found that women have detectable levels of organic pollutants such as hexachlorobenzene (HCB), 2,2-dichlorodiphenyldichloroethylene (p,p'-DDE), polychlorinated biphenyl 153 (PCB153), perfluorooctanoate (PFOA), and perfluorooctane sulfonate (PFOS) in their follicular fluid [1,2]. Thus, these chemicals may act on ovarian tissue in a paracrine manner. Our goal was to elucidate the effects of the mixture of these compounds, similar to the profile found in human follicular fluid, on 17β-estradiol (E2) secretion by KGN cell spheroids, which represent adult granulosa tumor subtype.

In this study KGN cells (RBRC-RCB1154, Riken Cell Bank, Ibaraki, Japan; after approval from Drs. Yoshiro Nishi and Toshihiko Yanase) were cultured using a three-dimensional (3D) model to reflect tumor microenvironment. Spheroids were cultured in DMEM/F12 medium containing 10% FBS with the mixtures of the test compounds, as follows, Mix 1 (2 ng/ml PFOA, 8 ng/ml PFOS, 50 pg/ml HCB, 1 ng/ml p,p'-DDE, and 100 pg/ml PCB153), Mix 10 (10-times concentrate), and Mix 0.1 (10-times diluted compare with Mix 1) with testosterone (100nM) as a substrate. Secretion of E2 was determined by ELISA kits (DRG Instruments GmbH, Marburg, Germany) and the expression of aromatase was evaluated by real-time PCR (Hs00240671_m1, Applied Biosystems/ThermoFisher Scientific) and confirmed by western blot (ab39742, Abcam). In addition, caspase activity was detected using a Caspase-Glo® 3/7 assay kit (Promega, France). Statistical analysis was performed using one-way ANOVA (Tukey's test, $P<0.05$).

We found that all of the mixtures stimulated E2 secretion and that this effect was independent of apoptosis. Moreover, a mixture of the five compounds does not affect aromatase expression. To investigate the mechanism by which the mixtures stimulate E2 secretion, we used pharmacological inhibitors and found that the mixtures acted through the classic estrogen receptors ERα and ERβ as well as the non-classical GPR30 pathway. Taken together, our results demonstrate for the first time that mixtures of persistent organic pollutants present in follicular fluids may stimulate E2 secretion through the classic and non-classic estrogen receptors pathways in granulosa tumor cells.

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P06-034
Characterization of a human proximal tubule epithelial cell/fibroblast transwell co-culture system

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Background: Predicting compound mediated nephrotoxicity in humans is still problematic irrespective of recent advancements in *in silico*, *in vitro* and *in vivo* approaches. Moreover current systems are geared toward identification of single compound toxicity in a single cell type and not optimized to reflect the physiological reality in the patient e.g. polypharmacy of elderly patients with impaired renal function. Indeed, the majority of *in vitro* approaches address renal proximal tubule toxicity using primary human proximal tubule epithelial cells (hRPTEC), transformed hRPTEC or transformed animal RPTEC in 2D plastic dishes cultured at 21% O_2 and high glucose (20 mM). While continuous comparability and quality of primary cells cannot be guaranteed, transformed cells present with reduced functionality due to their transformation. Moreover, primary and transformed renal epithelial cells will provide for falsified readings due to their 2D monoculture, abnormal microenvironment, i.e. missing physiological signaling crosstalk with other cell types, and hyperoxic (21% O_2) and hyperglucose (20 mM) conditions.

Approach: To overcome the latter obstacles, we are developing a transwell-based co-culture system encompassing human hRPTEC/TERT1 and human fibroblasts (fHDF/TERT166), cultured at physiological glucose (5 mM). Culturing at routine non-physiological O_2 (21%) levels were compared to physiological O_2 (5%). Co-cultures were characterized with regard to gene expression (mRNA and protein level) and physiological functionality (transepithelial electrical resistance (TEER), lactate:glucose ratio, viability, vectorial anion and cation transport). To determine and to compare the sensitivity of the co-culture systems, single and repeated treatments with clinically relevant cisplatin concentrations were initiated.

Results: Preliminary data at 21% O_2 suggest a tight barrier, enabling active vectorial transport of +/- charged molecules in the co-culture systems. Single exposures to cisplatin at concentrations ≤ 10 µM had no impact on TEER whereas higher concentrations severely diminished TEER within 48h (50 µM) or 24h (100 µM) of exposure. Concurrent analyses of co-cultures at 5% O_2, as well as repeated cisplatin treatments of co-cultures at 5% and 21% O_2 are ongoing.

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P06-035
Problem with incorrect classification of substances in terms of irritation or serious eye damage using Short Time Exposure test method

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Short time exposure (STE) test method is a cytotoxicity-based *in vitro* assay. After exposure to a test item, the cytotoxicity is quantitatively measured as the relative viability of SIRC (Statens Serum-institut Rabbit Cornea) cells. Decreased cell viability is used to predict potential adverse effects leading to ocular damage. The test items can be classified as chemicals inducing serious eye damage (category 1 in UN GHS) or as chemicals not requiring classification for eye irritation or serious eye damage (no category).

The study was performed according to OECD TG no. 491 (2018) [1] to confirm correct classification.

In this study 37 random substances were checked and the results were compared with the ECHA database [2]. There were 3 independent runs with 3 repetitions each.

The confluent monolayer SIRC cells (ECACC 89090404) were treated with two concentrations of the test items (5% and 0,05% w/w) for 5 minutes. After washing the test items with DPBS, the medium MEMα (ThermoFisher Scientific) with MTT (0,5 mg/ml, Merck) was added and cells were incubated for 2 hours (37 ± 1°C, 5 ± 1% CO2, 90 ± 10% RH). The extraction of formazan was performed with 0.04 N hydrochloric acid in isopropanol. Next, the absorbance was measured (FLUOStar Omega) at 570nm with reference wavelength (690 nm). The obtained cell viability is compared to the solvent control (saline or mineral oil) and used to estimate the potential eye hazard of the test chemical.

There were 18 correct (48.6%) and 11 incorrect (29.7%) classifications. 8 substances (21.6%) were not classified ("no prediction can be made") and they require further studies. Importantly, in substances belong to category 1 (according to ECHA database) 3 test items (of 9) were classified as "no category". What is more, 7 of 14 substances that cause eye irritation (category 2) were also incorrect classified as chemicals not requiring classification. This situation is very dangerous for health, because 27% of substances were assigned to a safer category.

In conclusion, the STE test method needs to be changed to reduce the number of incorrect categorizations. To improve this method, we strongly recommend adding overnight post-incubation of cells before performing MTT test to exclude the delayed effect of the test item on the cells.

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[2] https://echa.europa.eu/pl/information-on-chemicals

P06-036
Integration of extracellular metabolomics and intracellular transcriptomics to unravel the mechanisms of 5-FU-induced gastrointestinal toxicity

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5-fluorouracil (5-FU) is a chemotherapy agent whose use in the clinic has been hampered by reports of gastrointestinal (GI) adverse drug reactions (ADRs, i.e., mucositis, diarrhea). Despite that, there still exists a gap in the knowledge of the molecular mechanisms leading to toxicity in these target organs, and how these changes can be quantitatively linked to observed ADRs. Therefore, the aim of the current work was to investigate molecular-level alterations induced by 5-FU in cultures of human intestinal organoids derived from colon and small intestine (SI). For this, alterations in the transcriptome and metabolome in the media were quantified in organoids following exposure to three doses (10, 100 and 1000 mM) over a time course (24, 48 and 72h). Alterations at the transcriptome level were first evaluated by generating co-expression networks using the complete set for each

organ. Analyses of the modules revealed large global effects induced 5-FU, and investigation of underlying cellular processes yielded a number of modules with equivalent biological responses across colon and SI experimental models. These processes comprised, for instance, cell cycle, mitochondrial-related processes such as the TCA cycle and electron transport chain, as well as transcription and translation-related processes. Pathways involved in inflammation such as TNFaand TGFbwere also found to be enriched across modules. In both cases, the eigengenes (vectors summarizing expression) for each dose/time point pair of such modules were found to vary in a dose-dependent manner, with the highest dose often showing the largest variation when compared to lower doses. To investigate the relationship between gene expression and extracellular metabolomics, correlation analyses were conducted between modules' eigengenes and levels of metabolites measured in the media. A number of metabolites were significantly correlated to changes at the transcriptome level and an integrated pathway analysis using both transcriptome and metabolome revealed the alteration of metabolic pathways related to metabolism of nucleotides, in particular purines and pyrimidines, as well as urea cycle and metabolism of amino acids. In summary, the results from this work identified relevant co-expression networks embedded with relevant biological information that can be related to the toxic effects induced by 5-FU. Furthermore, integration with metabolomics highlighted specific sections of metabolic networks linking intracellular changes to external traits. Taken together, these findings may serve as basis for further investigations targeting the quantitative modeling of these pathways/networks in drug-induced GI toxicity.

P06-037
The role of bile salts in cholestatic injury and fibrosis using a human 3D *in vitro* model

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Bile formation and secretion is fundamental for the successful intestinal absorption of lipids and fat-soluble vitamins. On the other hand, a disrupted regulation of bile acid modulation and accumulation in the liver can contribute towards progressive liver damage and fibrosis. The molecular mechanisms of this process are still unclear; therefore a physiologically relevant human *in vitro* model would be beneficial to elucidate the role of bile in the onset of fibrosis. A multicellular, 3D liver microtissue comprising HepaRG, THP-1 and hTERT-HSC has been shown to recapitulate key fibrotic events elicited by known pro-fibrotics such as methotrexate. In this work, we aimed at utilising this 3D human *in vitro* model to investigate the role of bile salts in the progression of cholestatic injury and fibrosis. Furthermore, we investigated the direct effects bile salts may have on the three separate cell types using standard 2D monolayer culture.

We exposed both the 2D monocultures and 3D co-culture systems to bile salts treatment (50% cholic acid & 50% deoxycholic acid) for 7-14 days. Bile salts had a minimal effect on the 2D culture of both hTERT-HSC and differentiated THP-1. Treatment on 2D hTERT-HSC showed no cytotoxicity and no increase in activation markers (α-SMA, Collagens I and IV). The 2D differentiated THP-1 had a slight decrease in viability and no significant increase in TNF-α and TGF-β1 expression. Bile salts had a more pronounced effect on the 2D HepaRG, leading to a decrease in viability and increased miR-122, -192 and -34a release at 24 hours. In comparison in the 3D co-culture of the three cell types, there was an increase in expression of the key fibrotic markers α-SMA, Collagens I and IV, vimentin and the pro-inflammatory markers IL-6, TNF-α and TGF-β1. Exposure to bile salts also led to miRNA release (miR-122, -192 and -34a) and a decrease in albumin production, indicating hepatocellular damage in the 3D culture system. Finally, the expression of CYP7A1, a key regulatory cytochrome in bile acid synthesis, which is known to be inhibited by bile acid accumulation, was also decreased in the microtissue model.

In conclusion, we have demonstrated that the hepatocellular damage occurs in both 2D and 3D culture systems, suggesting that HepaRG are an appropriate cell type for studying bile-induced hepatocellular damage. This is also confirmed by the expected decrease in CYP7A1. We also demonstrated that the bile salt treatment does not result in activation of stellate cells or elicit an inflammatory response in the THP-1 in 2D monolayer culture, which suggests that the hepatocellular damage and the cellular interplay is required for eliciting a fibrotic response. These results suggest that the 3D multicellular microtissue model can recapitulate liver damage caused by bile acids and can therefore be used to elucidate the processes involved during the onset of fibrosis by cholestatic injury.

P06-038
Effects of electrospun nanofiber curcumin on bisphenol A exposed Caco-2 cells

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Purpose: Curcumin is the major polyphenolic compound of curcuminoids, extracted from *Curcuma longa* L. (turmeric). Curcumin gained increasing interest for its anti-inflammatory, anti-diabetic, anti-carcinogenic and anti-rheumatic properties with good tolerability and safety. However, several problems prevent marketing of curcumin as a drug such as the poor aqueous solubility, intense staining color, and extremely low oral bioavailability. In order to enhance the solubility, curcumin loaded polyvinylpyrrolidone (PVP) K90 nanofibers were prepared using electrospinning method and physicochemical properties of nanofibers were characterized. Bisphenol A (BPA), the major endocrine disruptor chemical, which stimulate estrogen receptors at very low concentrations, induce estrogen related carcinogenesis inducing proliferation in colon. Therefore, the aim of this study was to determine the effects of electrospun nanofiber curcumin on Bisphenol A treated human colorectal adenocarcinoma cells (Caco-2) *in vitro*.

Methods: Electrospinning solution; consisted of PVP 12% and curcumin (10mg) was prepared in ethanol. The mixture was stirred for 2 h at room temperature to obtain homogeneous solution and used for electrospinning. Caco-2 cells (ATCC HTB-37,USA) were seeded at 80% confluence; where curcumin nanofiber at concentration of 2.7, 6.4 and 12,8 µg/ml were coincubated with BPA at 2nM-2µM. Following 24h coexposure, MTT assay along with standard trypan blue technique by JuLI Br Counting starter kit (NanoEnTek Inc, Seoul, South Korea) were used.

Results: BPA induced proliferation in the cells at 8 nM. Viability of the cells compared to untreated control against curcumin nanofibers were 67,64 ± 1,06 for 2.7 µg/ml, 55.12 ± 1.12 for 6.4 µg/ml, 50,88 ± 3.03 for 12.8 µg/ml; while BPA at 8 nM were 85.97 ± 8.11. A significant difference were observed for curcumin nanofibers compared to BPA only control ($p<0.05$); while between 6.4 and 12.8 µg/ml, no difference were observed ($p>0.05$). The current study supports the enhanced cytotoxic potential of curcumin nanofiber effective at 6.4 µg/ml concentration on Caco-2 colon cancer cells; where antiproliferative effects on cell proliferation induced by the environmental carcinogen Bisphenol A were found *in vitro*.

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P06-039
In vitro modelling of the GFB – characterization of a podocyte/endothelial cell co-culture system

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Purpose: The kidney is responsible for the excretion of xenobiotics and continuously exposed to drugs. The filtration units of the kidney, the glomeruli, are bundles of capillaries functioning as size-selective glomerular filtration barrier (GFB). The GFB results from the tight interaction of endothelial cells, forming the capillaries, and podocytes, specialized cells that cover the capillaries with interdigitating foot processes. Destruction of the GFB by nephrotoxins or disease, e.g. diabetes, results in glomerulosclerosis, proteinuria and end-stage renal disease. Consequently, to better understand and detect glomerulotoxicity in humans there is a need for a suitable *in vitro* model system. Obviously, the predictivity of *in vitro* model systems will be improved by mimicking the normal physiological environment as closely as possible. We addressed this issue by *i.)* co-cultivating podocytes and endothelial cells, thereby enabling cell-cell interactions and the development of an *in vitro* GBM, and *ii.)* cultivating the cells at physiological oxygen levels (10%).

Methods: Cells (PODO/TERT256 & HUVEC/TERT2) were cultivated under 10% and 21% O_2. Expression levels of podocyte markers were analyzed at the mRNA (RT-qPCR) and protein level (ICC). Barrier permeability was investigated using fluorescently labelled dextrans of different molecular sizes. Cytotoxicity of known glomerulotoxins was analyzed via LDH leakage.

Results: PODO/TERT256, showing tight cell-cell confluency with elongated flat cells, expressed several podocyte specific markers and reacted to known glomerulotoxins when cultivated at 21% O_2. Cultivation at 10% O_2, resulted in characteristics typical of primary podocytes, i.e. individualized cells with a more rounded morphology and cytoplasmic protrusions (filipodia). This resembled a more *in vivo* like phenotype in relation to their functional tasks. Analysis of podocyte specific markers and sensitivity to toxins is ongoing. Concurrently, assessment of barrier permeability demonstrated the ability of PODO/TERT256 to form a size-selective filtration barrier. Preliminary results suggested an increased size-selectivity when PODO/TERT256 were co-cultivated with endothelial cells. Analysis of podocyte specific markers and sensitivity to toxins in the co-culture is ongoing.

P06-040
The development of a generic physiologically based kinetic model to predict *in vivo* endocrine activity in rats based on *in vitro* bioassays

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The development of non-animal based testing strategies of chemicals is important in current human safety testing. Many efforts focus on the development and standardization of *in vitro* models that provide concentration-response data. However, concentration-response data obtained from *in vitro* models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the *in vitro* concentration-response data should be translated to *in vivo* dose-response data to obtain points of departure (PODs) to set safe human exposure levels. It has been proven that *in vivo* dose-response data can be predicted by *in vitro* concentration-response data using physiologically based kinetic (PBK) modelling-based reverse dosimetry, thus enabling the use of *in vitro* toxicity data for risk assessment and prioritization. Given the definition of the PBK model, it can be resource and time consuming to develop the model for the individual compound, efforts should be directed at the development of generic PBK models for large groups of compounds. The present study assessed the potential of the generic PBK model to predict the *in vivo* endocrine activities in rats for a series of compounds. PBK models for these compounds were developed using a generic approach and *in vitro* concentration-response data from the MCF-7/BOS proliferation assay and the yeast estrogen/androgen screening (YES/YAS) assay were translated into *in vivo* dose-response data. The benchmark dose (BMD) values derived from the predicted dose-response data were compared with the BMD values obtained from the *in vivo* uterotrophic assay or *in vivo* hershberger assay to evaluate the model predictions. The discrepancy in the ability of the *in vitro* assays to predict the *in vivo* toxicity may be related to the fact that the variation between the *in vitro* data of one compound obtained in the same assay could be up to 2 orders of magnitude in terms of EC50. Taken the large variation within the *in vitro* assay data into account, the predictions are reasonable. The current study indicates the feasibility of using the combination of *in vitro* toxicity data and a generic PBK model to predict *in vivo* endocrine activities for groups of endocrine disruptors. Further studies can expand the current approach for other *in vivo* endpoints.

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P06-041

Particles from different pyrotechnic smokes induced anti-oxidant and inflammatory responses in primary pulmonary cells after air-liquid interface exposure

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Smokes are widely used for military or civilian applications: obscuring, signaling, security or festivity. Smokes generate short-lived aerosol clouds which increase atmospheric particulate matters. However, there is a lack of data on the biological effects of such emissions. Moreover, there is a multiple type of smokes, characterized by various compositions and it is not excluded that they generate particles with different toxicological properties.

Consequently, the aim of this study was to develop an *in vitro* methodology using primary human pulmonary cells (SAEC) to assess toxicological effects of particles obtained from combustion of three different smokes: a red signaling smoke (F1) and two obscurant ones (F3 and F4). Cells were exposed at the Air-Liquid Interface, using a novel approach for preparation of standardized particle suspensions. Cytotoxicity (MTT), gene expression (RT-qPCR) and cytokine secretion (ELISA) were explored after 24h exposure.

Results show that particles did not induce cytotoxicity but altered genes expression that was dependent on particles type. Particles from F1 significantly induced superoxide dismutase 2 (SOD2), NADPH quinone oxidoreductase-1 (NQO-1) and heme oxygenase-1 (HO-1) expressions whereas particles from F3 moderately increased the expression of these genes. HO-1 expression was not modified after exposure with particles from F4 whereas SOD2 and NQO-1 expressions were significantly increased. In addition, particles from F3 and F4 decreased catalase expression. Concerning inflammatory response, particles from the 3 smokes induced IL-8 gene expression (F1 > F2 = F3). TNF-alpha expression was moderately induced after exposure of particles and only particles from F4 induced IL-6 expression and secretion.

These results showed that all particles types induce an anti-oxidant response as well as an inflammatory response. However, different response profiles were observed, which might depend on the different composition of particles. In conclusion, the methodology used in this study is applicable to the toxicological evaluation of particles produced by different smokes and obscurants and could be useful to assess human health risk.

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P06-042

Optimization of Spectrophotometric Direct peptide reactivity assay for skin sensitization

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The chemical hapten bind to cellular protein, called haptenation, is considered essential process in skin sensitization. Thus, examination of reactivity of chemicals with peptides or proteins has been considered as candidate animal alternatives for identifying skin sensitization potential. Direct peptide reactivity assay monitored the reactivity of peptides with haptens by using chromatographic methods such as HPLC and adopted OECD TG. Globally, there have been many attempts to develop an easy and accurate *in chemico* tool with same KE (chemical-peptide reactivity) as DPRA. In previous study, we developed the convenient detecting method using spectrophotometric analysis for the monitoring peptide reactivity with haptens and identified the possibility as a new animal alternative. However, our model has a relatively low accuracy in the lysine peptide single predictive model (50% accuracy), so that some sensitizers couldn't be predicted as sensitizer. Thus we performed the optimization studies to achieve higher accuracy of lysine peptide reactivity in this study. Lysine (Ac-RWAAKAA-COOH) was used as model peptides and these peptides were reacted with 23 chemicals (19 sensitizers, 4 non sensitizer) that is used as proficiency chemical in animal alternative studies of sensitization at various peptide-chemical reaction ratios. And non-reacted peptides were monitored by the fluorometer using fluorescamine as a detection reagent for free amine group, respectively. The condition of 1:20 peptide-chemical reaction ratio (Lysine peptide 100uM: chemical 2mM) and 15% depletion cut off –lysine model showed higher accuracy (above 80%) than previous our model. From these results, we were able to confirm the possibility of a lysine peptide reactivity assay as a single prediction model with high accuracy.

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P06-043

Toxicological risk assessment of pyrrolizidine alkaloids – Investigations of the hepatotoxic and genotoxic potential

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Background: Pyrrolizidine alkaloids (PAs) are a large group of natural toxins synthesized as secondary metabolites by different plant species. To date, approximately 600 different PAs are known [1]. PAs can be found as contaminants in foods like teas, herbs and honey [2]. They are generally considered acutely and chronically hepatotoxic, genotoxic and carcinogenic [3].

Objectives: There is a lack of data concerning *in vitro* cytotoxicity and genotoxicity of food-relevant individual PAs. For this reason, we want to assess potential risks and confirm the influence of PA structures on their *in vitro* toxicity.

Methods: Genotoxicity of these selected PA congeners was determined in HepG2-CYP3A4 clone 9 cells [4] by the micronucleus test: monocrotaline, echimidine, europine, heliotrine, indicine, lasiocarpine, lycopsamine, retrorsine, senecionine and seneciphylline. Cytotoxicity of PAs was tested in incubations of primary rat hepatocytes, HepG2 cells and HepG2-CYP3A4 clone 9 cells. They were tested at concentrations ranging from 1 to 300 µM. The cell viability was measured using the Alamar blue assay after 24 h and 48 h of incubation.

Results: Dose-dependent increases in micronuclei were observed in most of the PAs. In the Alamar blue assay in primary rat hepatocytes lasiocarpine (open-chained di-ester, 7S-structure) was the most cytotoxic congener, followed by the di-esters echimidine, retrorsine,

seneciphylline and senecionine. The mono-esters heliotrine, indicine, europine and lycopsamine and the di-ester monocrotaline were much less cytotoxic. Similar cytotoxic effects were observed in Hep-G2-CYP3A4 clone 9 cells. In Hep-G2 cells none of the selected PAs showed cytotoxicity in the concentration range tested. It is possible that the absence of substantial CYPs activity is the reason for this.

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P06-044
Electrophysiological evaluation of LUHMES cells as model of human dopaminergic neurons

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The loss of dopaminergic neurons in the substantia nigra plays an important role in the development of the Parkinson's disease. The symptoms of this disease typically occur after around 80% of these neurons degenerated. This cell decay can be caused or promoted by genetic defects or environmental factors including chemical compounds like pesticides. For a proper testing of neurotoxic effects on these neurons as well as for the development of neuroprotective drugs, assays based on animal primary cells lack predictivity since the correlation between animal and human data is weak in some cases. Therefore, models based on human neuronal cells have the potential to overcome the limitations of animal models. One interesting neuronal cell line is the LUHMES (Lund human mesencephalic) line, which consists of immortalized fetal human mesencephalic precursor cells that can be differentiated into fully post-mitotic dopaminergic neurons within one week.

We currently investigate the electrophysiological properties of these neurons using manual and automated patch clamp as well as high-throughput calcium imaging for a functional characterization on both single cell and network level.

LUHMES neurons were capable to generate spontaneous and stimulated action potentials. The underlying Na^+ channels were TTX-sensitive. Biophysical and pharmacological tests indicate the presence of the Nav 1.2 subtype.

Furthermore, we checked for the presence of neurotransmitter receptors and compared them to data obtained by mRNA analysis from these cells. We found that several key receptor subtypes were expressed functionally in the cells, including dopamine, serotonin and acetylcholine receptors. Next, we investigated whether the neurons were capable of forming functional neuronal networks using a high-throughput calcium imaging system. While at rest cells were quiescent, oscillatory network activitiy was visible in the presence of neurotransmitter receptor agonists like serotonin and norepinephrine as well as by modulating the extracellular K^+ and Ca_2^+ concentration. These oscillations were sensitive to modulators like Haloperidol or the anticonvulsive drug Phenytoin dose dependently.

The results show that differentiated cells derived from LUHMES cells express electrophysiologically neuronal characteristics and form functional networks. The capability of using increased throughput techniques including automated patch clamp and HTS Ca imaging makes this cells attractive for neurotox experiments at industrial relevant scales.

P06-045
New-tiered approach to *in vitro* predictive toxicity screening using retrospective analyses

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Background: Product safety is a major question to address during the agrochemical development process to ensure that products do not pose adverse effects to human. One of the greatest challenges is accurately predicting unanticipated adverse effects in animal toxicity studies that can result in late stage failure of promising new candidates. Early *in vitro* screening of new candidates is therefore essential to improve the selection process, as well as to minimize and refine animal use.

Objective: Our goal is to build a tiered toxicity screening toolbox with assays that allow a robust translation of *in vitro* toxicity data into meaningful prediction of potential *in vivo* effects.

Methods: We have incorporated a battery of assays that provides predictive indicators for endocrine disruption (ED), genotoxicity, carcinogenicity and developmental toxicity. Pharmacokinetic profiling is conducted in parallel using *in vitro* ADME assays and *in vivo* kinetics in a minimal number of animals. This combination gives a view of the potential cellular activity and exposure.

Results: The screening battery was tested on candidates from a promising chemical class. The approach was first evaluated through a retrospective analysis. We used a former candidate from the same class with late stage development failure, partly due to ED alerts observed in rodents. The experimental data (short-term and developmental toxicity study) were compared to the *in vitro* systems, to evaluate the predictiveness and accuracy of the *in vitro* data. We observed robust correlations, thus validating the screening approach for a comprehensive interpretation of data for new candidates. We showed that they display a much-improved toxicity profile compared to the reference compound with a remarkably lower bioavailability, a low ED risks based on safety margin and a lower teratogenicity index using zebrafish embryos.

Conclusions & perspectives: Retrospective weight of evidence validated our new-tiered toxicity screening. Only the two safest candidates identified were promoted for further toxicity studies. Using the same conceptual approach, we are currently conducting data analysis of at least 100 fully developed molecules in order to learn about the correlation or gaps of our screening strategy.

P06-046
Using the real architecture ror 3D tissue (3D RAFT™) system as a versatile tool to build *in vitro* models relevant for toxicity testing

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Conventional *in vitro* assays are based on cells grown on two-dimensional (2D) substrates, which are not representative for the true *in vivo* cell environment. In tissue environments, cells interact with neighboring cells and with the extracellular matrix (ECM). Three-dimen-

sional (3D) cell culture methods mimic these interactions and allow cells to grow in structures resembling more the *in vivo* environment.

The RAFT™ 3D Culture System uses a collagen matrix at physiologically relevant concentrations. Cells and neutralized collagen are mixed and dispensed into wells of standard cell culture plates or transwell inserts, and subsequently incubated at 37°C to allow the formation of a hydrogel. Specialized RAFT™ Absorbers are placed on top of the hydrogels. These absorbers gently remove abundant medium and compact the hydrogel to a layer approximately 100 µm thick. The cultures are then ready to use, but additional epithelial or endothelial cells may be added on top.

The resulting models provide valuable tools to investigate tissues in an *in vivo*-like micro-environment, potentially for use in pre-clinical efficacy and safety testing. This presentation focuses on skin, lung and liver models.

A full-thickness skin model was generated by embedding primary human dermal fibroblasts within the RAFT™ Collagen and seeding and differentiating human primary keratinocytes on top of the air-lifted cultures. Histological and immuno-histochemical evaluation confirmed the resemblance to native skin.

A RAFT™ 3D lung co-culture model containing normal or asthmatic bronchial epithelial and smooth muscle cells was compared to 2D cultures with respect to cell proliferation and morphology as well as growth factor and cytokine secretion.

We also demonstrate the feasibility of using the RAFT™ 3D System to create a robust model for the long-term maintenance of primary human liver cells. We compared the viability and morphology of primary human hepatocytes and the maintenance of Cytochrome P450 activity grown in the traditional Sandwich Model with that of cell cultured in the RAFT™ 3D System. Hepatocyte metabolism is stabilized in the RAFT™ 3D Cell Culture System for up to 17 days in culture, which enables long-term toxicity analysis using primary hepatocytes.

P06-047
Implementation of a mucus containing advanced *in vitro* model of the human intestinal barrier for a more predictive evaluation of food grade nanomaterials

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Nanotechnology provides many benefits to the food industry due to their versatile properties. Engineered nanomaterials (ENM) deliver for example new tastes, antimicrobial properties or improve the nutritional value of food (novel food). However, the impact of ENMs on the gut epithelium and their translocation through the intestinal barrier is still poorly investigated and understood. Mechanistic insights required for the safe design and use of ENMs in food applications can be obtained from advanced human *in vitro* models of the intestinal barrier that contain mucus and different cell types of the intestine (e.g. enterocytes, goblet cells and M cells). The mucus layer as a physical barrier is particularly important to achieve predictive results, however, it interferes with many conventional assays.

Here, we aimed to establish an *in vitro* platform comprised of an advanced human *in vitro* intestinal co-culture model and a set of mucus-compatible assays for the toxicity assessment of food-relevant nanomaterials. We successfully implemented co-cultures of enterocytes (Caco-2), goblet cells (HT-29-MTX) and M cells (differentiated from Caco-2 cells in presence of Raji B-lymphocytes) with a continuous mucus layer. Different cell seeding numbers were exploited to achieve an *in vivo* relevant continuous mucus layer and the formation of a tight barrier. Moreover we have identified assays that are suitable to investigate ENM impact on cell viability, production of reactive oxygen species, cytokine release, mucus coverage, barrier integrity, microvilli function and relevant physiological endpoints (e.g. iron, glucose or lipid transport) in the mucus-containing intestinal co-cultures.

In future studies, we will use this platform to investigate the interaction of nanostructured food grade synthetic amorphous silica (SAS, E551) with the mucosal lining and distinct cell types of the intestinal barrier. A panel of four different SAS products, which differ in size, surface area and production route will be assessed to identify potential structure-activity relationships.

P06-048
In vitro toxic assessment of pyrotechnic red signaling smoke particles

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Pyrotechnic smokes are widely used for military operations such as obscuring or signaling purposes as well as for civilian applications such as security or festivity. Smokes generate short-lived aerosol clouds which increase atmospheric particulate matters. Because of the recognized health risks for military and civilians exposed to old smokes, a variety of alternative pyrotechnic smokes have been developed. However, there is a lack of data on the biological effects of emissions produced by these alternative pyrotechnic smokes.

In this study, we examined the toxicity of particles obtained from combustion of a red signaling smoke (RSS) on human alveolar cells (A549), and studied the anti-oxidant and inflammatory responses by RT-qPCR. Cytotoxicity (MTT, trypan blue tests), cell cycle distribution and gene expressions were assessed after 24h and 48h exposure to particles collected by an impactor placed to the smoke source and suspended in the culture medium. In parallel, mutagenicity of organic extract prepared from RSS was evaluated using the bacterial Ames assay.

Particles significantly decreased cell viability (trypan blue) at 0.25 mg/mL whereas mitochondrial activity (MTT) was unaltered at this concentration. Exposure to 0.25 mg/mL of particles significantly increased cells in the sub-G1 phase and decreased cells in the G0/G1 phase. At this concentration, particles induced superoxide dismutase 2, heme oxygenase-1, NADPH quinone oxidoreductase-1 as well as IL-6 and IL-8 expressions. In parallel, Ames test showed significant response in *Salmonella* typhimurium tester strains TA98 + S9mix at 12.5 µg/plate (≈ 0.06 mg/mL), and in a larger extent at 2 µg/plate (≈ 0.009 mg/mL) in YG1041 + S9mix, a strain highly sensitive to aromatic amines.

Results showed that it is relevant to analyze multiple biomarkers to evaluate effect of particles. In this study, particles from RSS induced antioxidant and inflammatory responses as well as mutagenicity. These effects are likely due to the chemical composition of particles that contained numerous compounds (aromatic amines, quinones, naphthalene derivatives, azoic dye derivatives and metals). This study outlined the requirement of improving the knowledge of the toxicity of pyrotechnic mixtures like smoke particles in the context of protection of the human health.

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P06-049
Quantification of seizurogenic activity with multiwell microeletrode array technology for proconvulsant risk assessment

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The lack of advancement in anti-epileptic drugs (AEDs) over the last 30 years, along with the continued need for improved proconvulsant screening in drug safety, motivates the need for new assays of seizurogenic neural activity. Previous work has established an *in vitro* approach for detecting and quantifying seizurogenic activity using multiwell microelectrode array (MEA) technology, providing a predictive and high-throughput avenue for the evaluation of the efficacy of AEDs and the proconvulsant risk of other drug candidates. Here, we present an updated assay of seizurogenic activity based upon guidelines developed in the Translational Biomarkers of Neurotoxicity (NeuTox) Committee of Health and Environmental Science Institute (HESI), a consortium of academic, commercial and pharmaceutical representatives working towards the development of *in vitro* assessment of proconvulsant risk. We used previously published metrics for detection of burst spiking events and the quantification of synchronisation across a neural population, in spontaneous and evoked conditions. Data are included from cryopreserved rat cortical neurons evaluated with the 10 compounds selected by NeuTox consortium, which include reference compounds with known proconvulsant risk via multiple mechanisms and negative control compounds. Our results support the combined use of spontaneous and evoked neural activity, collected using multiwell MEA technology, for the high throughput evaluation of complex neuronal networks *in vitro* to quantify the proconvulsant risk of candidate pharmaceuticals in a pre-clinical setting.

P06-050
Prediction of human cardiotoxicity of methadone by a combined *in vitro* – physiologically based kinetic (PBK) modelling- based reverse dosimetry approach

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Cardiotoxicity is a leading cause of drug failure during development and an adequate preclinical strategy that predicts *in vivo* human cardiotoxicity would thus be of great value. The aim of the present study was to develop an integrated animal alternative testing strategy to predict human cardiotoxicity of chemicals. We have shown before that the combination of an *in vitro* assay and PBK modelling-based reverse dosimetry can be very powerful to predict *in vivo* dose-response curves for different toxicological endpoints. In the present study we provide data extending this principle to cardiotoxicity in humans. Methadone was used as a model compound as several human case studies report cardiotoxic side effects in clinical settings. Here we assessed the effect of methadone on cardiac electrophysiology using the multi-electrode array (MEA) combined with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). The PBK model was developed based on metabolic parameters obtained from *in vitro* liver microsomal incubations and parameters derived from *in silico* simulation and the literature. Using PBK modelling-based reverse dosimetry, the *in vitro* concentration dependent prolongation of field potential duration was translated into *in vivo* dose dependent prolonged QT interval. The *in vitro* effective concentration was corrected for protein binding (unbound fraction) to extrapolate to the real-life conditions in humans. The predicted *in vivo* 10% effective dose was used as point of departure (PoD) to evaluate the *in vitro* PBK modelling-based reverse dosimetry approach. Our results show that the PoDs derived from our *in vitro* studies were comparable with the PoDs derived from published clinical studies with less than a 4.3-fold difference. We also found that protein binding in plasma is an influential factor in the adverse cardiac effects of methadone. Therefore the individual variation in plasma binding might provide an important factor in a personalized prediction of undesirable side effects of the clinical treatment. The results provide a proof of principle that PBK modelling-based reverse dosimetry of *in vitro* data obtained using the MEA and hiPSC-CM can well predict the electrophysiological cardiotoxicity in humans and provide a promising tool for detecting cardiac safety liabilities during drug development.

P06-051

This abstract has been withdrawn.

P06-052
Hyperoxia reduces benzo[a]pyrene-induced toxicity by increasing the activation of Nrf2 in HaCat cell

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Benzo(a)pyrene (BaP) can be exposed to skin via environment, soil, air, fire, tyres, automobile exhaust, and so on and can therefore cause skin damage including skin cancer and aging. The main factor for reducing skin damage is antioxidant and detoxifying enzymes, which are regulated by Nrf2. Hyperoxia means a situation higher than the concentration of atmospheric oxygen, and is being used in various medical fields. In this study, hyperoxia was investigated in reducing the toxicity caused by BaP in the skin. Under the condition of hyperoxia, HaCat cells treated with BaP increased Nrf2 mediated by NF-κB, GSK-3β, p38 MAPK and PPARα activities. Hyperoxia also increased the expressions of HO-1, SOD2, GPX-1/2 that reduced toxicity by BaP. Thus, hyperoxia may regulate the enzymes involved in detoxification by promoting the activity of Nrf2 in HaCat cells.

P06-053
High content *in vitro* assessing of cardiotoxic risk and adjuvant chemotherapy effects in breast cancer

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New anticancer agents have led to higher life expectancy for cancer patients. However, treatment related morbidity factors such as cardiac toxicity have become important issues for long-term cancer survivors. Cardiotoxic side effects such as arrhythmia, thromboembolism and myocardial ischemia are common with anti-cancer drugs. This led to the development of cardio-oncology field, to promote cardiovascular health while providing the best cancer therapy.

Using change in impedance, we monitored breast cancer cell regrowth after chemotherapy treatment *in vitro*, coupled with the acute and chronic effects of this treatment on human stem cell derived cardiomyocytes (hsc-CMs). One of the standard clinical regimens for breast cancer is a combination of cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF). Even though initially successful, tumor recurrence after this therapy remains a major cause of mortality in breast cancer patients. We investigated responses from murine H8N8 (immortal mammary carcinoma cell line with tumor stem cell properties) and H8N8 T3.2 (once-treated recurrent tumor variant) cells, to single and recurrent CAF treatment. Changes in impedance and confluency of these cells were used as a measure of toxicity, with cell viability monitored under physiological conditions for 500h. Dose- and treatment dependent effects of CAF clinical treatment on cycle- regrowth of tumor cells were observed.

We further investigated putative cardiovascular side effects of CAF mix and paclitaxel (acute and chronic) on hsc-CMs viability. We observed the cardiotoxic effects of paclitaxel (a microtuble stabilizing drug approved for the treatment of breast, ovarian and lung cancer). Paclitaxel and CAF also induced negative changes in cell contraction properties. hsc-CMs' viability and beating patterns were monitored over 190 h. Paclitaxel showed a time and dose dependent decrease in base impedance and impedance amplitude, cyclophosphamide and 5-fluouracil shown no or small effect, while doxorubicin shown significant toxic effects in all combinations.

In summary, long-term high-resolution impedance monitoring provides amenable insights into dynamics of cell proliferation and contraction, for *in vitro* investigations of adjuvant chemotherapy in both cancer and cardio-oncology fields.

P06-054
In vitro skin sensitization testing of Medical Devices using GARD®

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Allergic contact dermatitis can severely affect quality of life and is induced by certain substances, referred to as sensitizers. In order to prevent individuals from exposure, chemicals and materials in contact with patients are required to be safety tested. Skin sensitization is included in the Biological Evaluation of Medical Devices (ISO 10993-1:2018) as one of three biological endpoints to be evaluated for all Medical Devices. This test commonly involves animal testing (ISO 10993-10:2010), but there are growing regulatory, economic and public interests for animal-free test methods.

The GARD platform is an *in vitro* state-of-the-art assay developed for the identification of sensitizers. The assay is based on a human dendritic-like cell line, SenzaCells, and analysis of gene expression using pattern recognition post stimulation.

The guidance document describing preparation of sample extracts of medical devices (ISO 10993-12:2012) requires the use of both polar and non-polar extraction vehicles (typically saline and an oil); the latter a challenge for many *in vitro* assays. Previously, we have shown that GARD is compatible with both saline and oil.

In this study, polymers spiked with strong, moderate or weak sensitizers and non-spiked material were used as model materials (produced by Research Institute of Sweden) to develop the GARD assay for testing of Medical Devices. Additionally, commercially available medical grade materials (supplied by Medizintechnik Promedt) were used as control materials.

Extracts from the materials were prepared according to ISO 10993-12:2012; in saline or Super Refined Olive Oil. The SenzaCells were incubated with the extracts and isolated RNA analysed with the GARDskin prediction model.

All the polymers used as model materials were correctly classified; spiked material as sensitizers, and non-spiked materials as non-sensitizers. The commercial medical grade materials were all classified as non-sensitizers.

Here, we show the results from the in-house validation, confirming the extended applicability domain for GARD to facilitate testing of polar and non-polar extraction vehicles and detection of leachables in extracts from solid materials. Thus, GARD can be used as an *in vitro* alternative for assessment of skin sensitization for Medical Devices and medical grade materials.

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P06-055
Efficient transfection and sustained long term functionality of primary human hepatocytes

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Purpose: Primary Human Hepatocytes (PHH) are the state-of-the art *in vitro* human liver model system in the field of toxicology. Just as virtually all non-dividing primary cells, PHH are difficult to transfect. Furthermore, PHH tend to lose their typical liver functions rapidly in culture. In this study, we optimized the thawing, transfection and culture procedure for cryopreserved PHH. Transfection efficiency and hepatocyte functionality were analyzed over 7 days.

Method: Lonza's cryopreserved plateable human hepatocytes were transfected using the 4D-Nucleofector™ System: Cryopreserved PHH were gently thawed and resuspended in P3 Nucleofector™ Solution. Following transfection using program EX-147 or DS-150, PHH were plated on collagen-coated cell culture vessels in Matrigel™ (Corning) sandwich culture. We characterized specific hepatocyte functions of the resulting transfected sandwich cultures for up to 7 days. Transfection efficiency of both pmaxGFP™ plasmid DNA and Cleancap® mCherry RNA (TriLink) was assessed by fluorescence microscopy. PHH were analyzed for cell viability, bile canaliculi formation, albumin secretion and CYP3A4, CYP1A2 and CYP2B6 metabolite formation.

Results: With program EX-147, DNA transfection efficiencies of up to 68% were observed 24 hours post transfection. The results were identical in the 100 µL Nucleocuvette™ Vessel and 20 µl Nucleocuvette™ Strip. Bile canaliculi formation was unaffected for up to 7 days. Albumin secretion and CYP activity were also clearly detectable. Following transfection with program DS-150, efficiencies of up to 20% for DNA and up to 85% for mRNA were achieved and sustained for the complete culture period. Viability and albumin secretion at 24h after transfection were slightly reduced, but recovering over time. In comparison to control cultures, initial CYP1A2 and CYP2B6 activity was ~60% and CYP3A4 ~80% and restored after one week of culture. Transfected PHH formed complex, branched bile canaliculi network.

Conclusion: We present reliable protocols for efficient DNA and mRNA expression in cryopreserved PHH. We demonstrate highly preserved functionality of transfected hepatocytes for 7 days when using program DS-150. Our protocols enable transfection of human

hepatocytes for generation of more sophisticated long-term *in vitro* liver models.

P06-056
Evaluation of an *in vitro* assay for skin sensitization of medical devices

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Purpose: Skin sensitization, one of three biocompatibility tests recommended for all medical devices is still based on *in vivo* approaches (ISO 10993-10). Yet, the recent validation of *in vitro* skin irritation test of medical device extracts demonstrated the added value of reconstructed human models such as SkinEthic RHE, in the context of medical devices (ISO DIS 10993-23). The goal of this study was to evaluate the capacity of SENS-IS assay, a quantitative analysis of specific genes expressed in Episkin or SkinEthic RHE models, to predict *in vitro* skin sensitization potential of medical device extracts.

Method: After optimization of the original protocol used for neat chemicals, the capacity of this assay to detect sensitizing medical devices has been assessed with two approaches: 1) using polar (NaCl) and nonpolar (sesame oil) extracts of non-sensitizing medical devices (MED-2000 silicone) spiked with known concentrations of sensitizing chemicals. 2) using polar and nonpolar extracts of polymer preimpregnated with sensitizers (10% W/W): 1-phenyl-1,2 propanedione, 1-Chroro-2,4-dinitrobenzene, Diethyl maleate, p-Benzoquinone, Propyl gallate and Phenyl Benzoate.

Results: In the first approach, all the spiked extracts were successfully classified with the SENS-IS assay. In the second approach, the polymers impregnated with known sensitizers were correctly classified. The silicone spiked with Phenyl benzoate, a weak sensitizer, was classified as non-sensitizer. This is in accordance with the calculated maximum quantity in the extract leading to an exposure situation of the RhE under the NESIL value.

The performance of this assay was evaluated after transferring the method to a naive laboratory (RISE, Sweden) who successfully classified the extracts of blind-coded impregnated polymers.

Conclusion: These preliminary results show that the SENS-IS assay performed with EpiSkin or SkinEthic-RHE is able to detect the sensitizing potential of medical device extracts. The transferability of the method, already demonstrated for neat chemicals, was confirmed for medical device extracts. Further studies are engaged with a more comprehensive set of test items, but this work paves the way for a broader multicenter study to validate the integration of SENS-IS in future *in vitro* testing strategies to address the sensitizing potential of medical devices.

P06-057
Study of the effect of quaternary ammoniums on dendritic cells *in vitro*

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Neuromuscular blocking agents (NMBA) are the first incriminated molecules in intraoperative anaphylaxis, leading to high mortality risk. These reactions often occur during the first contact with the drug, suggesting that patients have previously been sensitized by exposure to molecules with structures common to NMBA. Pholcodine, a morphine-derived molecule used for its antitussive properties, has already been suggested as a potential sensitizer for NMBA allergic patients. Indeed, after withdrowal of pholcodine in Norway, patients were clinically more tolerant to NMBA. However, despite pholcodine withdrawal, IgE sensitization to NMBA remains high, suggesting other compounds might be involved. Structure-activity studies and epidemiological analysis have suggested that quaternary ammonium compounds (QA) may play this role, but the immunological mechanism remains unknown. Moreover, QA are presents in many daily use products (cosmetics, detergents, disinfectants ...).

This work aims to document the involvement of eight commonly used molecules containing a QA and of pholcodine (tertiary amine) in the immunization towards NMBA. Since dendritic cells (DC) are essential in the initiation of the immune response, we studied the DC activating ability of these molecules using two *in vitro* models: DC derived from fresh human monocytes (MoDC) and THP-1 cell line considered as DC-like.

The results showed that hexadecyltrimethylammonium bromide (CTAB), ethylhexadecyldimethylammonium bromide (EHD), polyquaternium-7, polyquaternium-10 and pholcodine, induce an increased expression of activation markers CD54 and CD86 on THP-1. Moreover, CTAB and EHD also increase the expression of CXCR4 on MoDCs. We also found an induction of pro-inflammatory cytokine production (IL-8, TNFα) and an activation of MAP kinases and NFκB intracellular pathways by these MoDCs exposed to QA.

These results suggest that some molecules with a QA can induce DC maturation and thus potentially initiate a specific immune response. The following part of this study will investigate the effect of QA-activated DCs on the activation and polarization of T lymphocytes. In addition, *in vivo* exposure models will allow us to confirm these data and understand the mechanisms involved.

P06-058
Human-based primary neural progenitor cells as a 3D *in vitro* model to investigate neurodevelopmental toxicity of Chinese herbal medicines

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Traditional Chinese Medicine (TCM) has been applied for thousands of years to treat or prevent all kinds of health problems. Specifically Chinese Herbal Medicines (CHMs) have been widely used during pregnancy to promote the health of mothers and fetuses. However, information on toxicities of most CHMs that are being used during pregnancy is sparse. Considering the fact that the nervous system, especially during development, is a sensitive target, it is essential to assess if CHMs taken during pregnancy might exert adverse effects on brain development. Currently, developmental neurotoxicity (DNT) testing is performed according to *in vivo* guideline studies, which is resource-intensive with regards to number of animals, time and costs and bears the issue of species extrapolation.

We have developed a 3D neurosphere *in vitro* model based on human primary neural progenitor cells (NPC), which mimics a variety of neurodevelopmental processes, i.e. key events (KEs), like NPC proliferation, migration and differentiation into neural effector cells

(astrocytes, neurons and oligodendrocytes). Using this model we analysed the effects of selected CHMs (Tian Ma (TM) and Lei Gong Teng (LGT)) on these endpoints. According to *in vivo* toxicity studies, TM is classified as non-toxic, while LGT exerts toxicity to the central nervous and cardiovascular systems and is thus classified as a strong toxic CHM. Based on the results of the "Neurosphere Assay" we observe that TM does not affect any of the analysed endpoint, while LGT reduces NPC migration and differentiation.

This pilot study indicates that testing CHM with the *in vitro* "Neurosphere Assay" might be helpful for the assessment of CHM safety. More data are needed to substantiate these findings and in the end more tests covering a broader variety of neurodevelopmental endpoints should be performed.

P06-059
Comparison of the transport of sulfated and non-sulfated bile salts by rat and human Mrp2/MRP2 and Bsep/BSEP transporters

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The aim of the study was to investigate the transport of chenodeoxycholate (CDC), its glycine-conjugated form (GCDC), and the sulfated forms of both (3S-CDC and 3S-GCDC) by rat and human Mrp2/MRP2 and Bsep/BSEP to map similarities between rat and human transporter affinities. In addition, CDC, GCDC, 3S-CDC, and 3S-GCDC transport was compared to taurocholic acid (TCA) transport. Vesicular transport assay allows the investigation of efflux transporters *in vitro*. Plasma membrane prepared from rat and human Mrp2/MRP2 and Bsep/BSEP overexpressing human embryonic kidney 293 cells form inside-out vesicles, that enables efflux transporters to pump their substrates into the vesicle. Bile salt export pump (BSEP) is the most important transporter of bile acids across the canalicular membrane of hepatocytes and, because of this, the functional deficiency of BSEP transporter resulting from BSEP mutations leads to progressive familiar intrahepatic cholestasis type 2 or type 2 benign intrahepatic cholestasis. Similar to BSEP, multidrug resistance-associated protein 2 (MRP2) is also localized in the canalicular membrane of hepatocytes but it is also expressed in renal proximal tubule cells, enterocytes (luminal side) and solid tumors as well. MRP2 is responsible for the transport of conjugated bilirubin and divalent bile salts from the hepatocytes. MRP2 mutation in human causes Dubin-Johnson syndrome, which involves chronic conjugated hyperbilirubinemia. Rat Mrp2 and Bsep transporter genes correspond to 88% and 91% to human MRP2 and BSEP genes, respectively, although there might be dissimilarities in their substrate affinity/specificity. To obtain the best prediction of the function of bile acid transporters of human based on animal experiments, differences in substrate affinity need to be mapped. Despite the thorough preclinical testing, drug-induced cholestasis is still frequent in humans. Currently, the most commonly used substrate for examining bile acid transporters is TCA, however, TCA is not the most relevant bile acid in human. Focusing on a bile acid that is more specific to human and transported with similar affinity on rat and human Bsep/BSEP or Mrp2/MRP2 could be more predictive than examining TCA. The results (K_M, μM) showed no significant CDC transport on any of the transporters. K_M of GCDC transport on rat and human Bsep/BSEP is similar (Bsep: 2.506; BSEP: 2.652), while human BSEP shows more than 25 times higher affinity for TCA than rat Bsep (Bsep: 40.71; BSEP: 1.460). Human BSEP and MRP2 also have high affinity for 3S-CDC (BSEP: 10.38; MRP2: 14.67). In rat, the transport of 3S-CDC and 3S-GCDC was only significant on Mrp2 with K_M = 47.67 and 14.48, respectively. Human BSEP and MRP2 also transported 3S-GCDC with K_M = 8.716 and 13.61. In summary, both rat and human Mrp2/MRP2 transported only the sulfated forms with similar affinity, while rat and human Bsep/BSEP showed significant difference in substrate specificity.

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P06-060
ALT4EI: Evaluation of eye irritating potential of 59 chemicals using EpiOcular™ time-to-toxicity (EpiOcular ET-50) neat and dilution protocols

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Evaluation of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The objective of the ALT4EI (ALTernatives for Eye Irritation) project was to confirm the testing strategy developed in the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project. These projects focussed on the development of tiered testing strategies for eye irritation assessment for all drivers of classification and evaluation of whether the test methods can discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling for Category 1 (Cat 1) and Category 2 (Cat 2).

A new set of 59 chemicals (41 liquids: (un)diluted, and 18 solids) was tested using the reconstructed human cornea-like epithelium (RhCE), EpiOcular, in two EpiOcular time-to-toxicity Tests (Neat and

Dilution ET-50 protocols). The set of chemicals contained 32 chemicals not requiring classification (No Cat) and 27 chemicals requiring classification (16 Cat 2 and 11 Cat 1). The chemicals were tested blinded in two independent runs by MatTek *In Vitro* Life Science Laboratories. In this study, a testing strategy to achieve optimal prediction for all three classes that was developed in CON4EI project (which combines the most predictive time-points of both protocols and which tests liquids and solids separately) was used.

Using the CON4EI testing strategy, we were able to identify correctly 63,6% of the Cat 1 chemicals, 56,6% of the Cat 2, and 76,6% of No Cat chemicals. Reproducibility between both runs was 88,7%. The combination of the EpiOcular ET-50 neat and dilution protocols seem to be promising in an integrated testing strategy (ITS) for eye irritation assessment.

P06-061
Mitochondrial impairment and oxidative stress play an important role in the toxicity of synthetic cathinones to dopaminergic SH-SY5Y cells

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β-keto amphetamines, widely used as alternatives to amphetamines, with which they share the phenethylamine backbone, have been shown to display neurotoxic properties. In this study, the mechanisms by which two synthetic cathinones, 3,4-dimethylmethcatinone (3,4-DMMC) and 4-methylmethcathinone (4-MMC), exert their toxicity *in vitro* were evaluated, using methamphetamine (METH) as comparative agent, in differentiated SH-SY5Y cells.

The dopaminergic phenotype was achieved by treatment of SH-SY5Y cells with retinoic acid and 12-*O*-tetradecanoyl-phorbol-13-acetate. These differentiated cells were exposed to 0-5 mM 3,4-DMMC, 4-MMC or METH, for 6, 12 or 24 h. In addition, cells were pre-treated with 100 nM clorgyline, rasagiline or selegiline, 1mM NAC, or 1 μM trolox, 30 min prior to their exposure to the tested drugs, in neuroprotection experiments. The production of reactive oxygen and nitrogen species (ROS/RNS) was measured, as well as total gluthatione (tGSH) intracellular levels. Mitochondrial membrane potential and ATP intracellular levels, as well as caspase 3 activity, were also assessed.

Cytotoxicity was observed for cathinones and METH in a concentration- and time-dependent manner, both in MTT reduction and NR uptake assays. At 24 h of exposure, and according to MTT reduction assay, the following order of toxic potencies was 3,4-DMMC > 4-MMC > METH. The decrease in intracellular tGSH levels elicited by 3,4-DMMC and 4-MMC, in addition to an increase in ROS/RNS production induced by of these two cathinones confirmed the oxidative stress elicited by these drugs. Clorgyline, rasagiline, selegiline and trolox provided partial protection for all tested drugs, while NAC only prevented the toxicity induced by cathinones, in the MTT reduction assay. The significant increase in ROS/RNS production elicited by cathinones was lessened by the putative protectors, with NAC totally preventing it. Both cathinones and METH caused mitochondrial dysfunction due to mitochondrial membrane depolarization and depletion of ATP intracellular levels. Moreover, caspase-3 activation was triggered by cathinones and METH.

In conclusion, under the present experimental conditions, mitochondrial activity appeared to be a main target for the toxicity of the studied cathinones, leading to cell death.

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P06-062
Enantioselective absorption of cathinones by intestinal ephitelial: studies in Caco-2 cells

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The *in vitro* model of the intestinal barrier, Caco-2, has been frequently used to evaluate drug permeability [1]. This model represents a relatively reproducible and inexpensive tool and shows a good relation to *in vivo* data [2]. Synthetic cathinones are psychoactive substances derivatives of cathinone, a naturally occurring β-ketone amphetamine found in Catha *edulis* (khat) [3–5]. Being chiral molecules, each enantiomer of cathinone derivatives may have different binding to proteins, or other chiral biomolecules, leading to many kinetic or dynamic variations [6,7]. The absorption of these compounds occurs mainly through the oral mucosa and the second route takes place in the stomach and small intestine [8]. The most common gastrointestinal effects reported by the consumers of "bath salts" are abdominal pain, nausea and liver failure [8–11]. However, the level to which these compounds cross the intestinal barrier have not yet been determined, and as chiral molecules, it could be expected a differentiated permeability between enantiomers. The present study aimed to develop and validate an HPLC-UV method for the determination and quantification of racemic form and enantiomers of pentedrone and methylone to study the intestinal permeability of these drugs. Both cathinones were efficiently separated and determined with a single 7 minutes chromatographic run-time. The method was validated concerning selectivity, linearity (coefficients always > 0.999), accuracy (88.62-106.48%), inter-day and intra-day precisions (always below 10%), limits of detection and quantification and stability. In Caco-2 cell line, the kinetic studies were performed to evaluate the ability of pentedrone and methylone (racemate and enantiomers) to pass across the intestinal barrier model. Pentedrone and methylone enantiomers were obtained by our group with a chiral semi-preparative liquid chromatography method [12]. During the experience, the cells were incubated with 500 μM of pentedrone and methylone and 200 μL were collected at 7 time points. It was possible to observe a differentiated passage of the cathinones enantiomers through intestinal membrane. For pentedrone, this difference is observed after the first hour, being *R*-(-)-pentedrone the most permeable compound. Concerning methylone, the difference is noted after the fourth hour, with *R*-(+)-methylone being the most absorbed. In conclusion, we developed and fully validated a method that allowed the identification and

quantification of pentedrone and methylone. The method was successfully applied for the analysis of Caco-2 cell samples, which allowed to discover the enantioselectivity of these cathinones in intestinal permeability.

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P06-063
In vitro toxicity assessment of toxic cyanobacteria as an emerging environmental risk in Europe

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Toxigenic Cyanobacteria is one of the main health risks associated with European water resources. According to the European Safety Authority and Food (EFSA) and Agriculture Organization of the United Nation (FAO), cyanobacterial blooms are classified as an emerging risk.

The aim of the study is to select *in vitro* methods for characterization of toxic microalgae in Bulgarian dams, which are used for drinking purposes. The presence of cyanobacteria in Bulgaria is monitored every year. In 2004, the first study, which presents the results of HPLC analysis for microcystins content in Bulgarian water bodies was conducted. In a scientific paper M. P. Stoyneva-Gärtner at all [1] summarized the results of the studies cart out during the 15 years' period (2000–2015) in Bulgaria.

In this study to assess the presence of Cyanobacteria, RealTime PCR analyses are used. For identifying the toxic species Microcystis aeruginosa are selected the following genetic markers of the gene cluster mcy: mcyA, mcyB, cya359. In most of the samples, Cyanobacteria was detected. In some of them (two of them are drinking-water reservoirs) Microcystis was found.

Some of these Cyanobacteria produced cyanotoxins, which are neurotoxic- for example, anatoxin-a. *In vitro* experiments (on neuroblastoma cell line SH-SY5Y and isolated rat brain synaptosomes) proved that anatoxin-a is not toxic on SH-SY5Y cell line, but revealed statistically significant neurotoxicity on isolated brain synaptosomes, at concentration 500 μM, compared to the control (non-treated cells and synaptosomes).

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P06-064
This abstract has been withdrawn.

P06-065
Co-culture model Caco-2/HT29-MTX: a promising tool for toxicity investigation of phycotoxins on the intestinal barrier

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Lipophilic phycotoxins produced by marine microalgae can accumulate in edible shellfish. Some of them are documented to affect the gastrointestinal tract provoking acute intoxications in humans. However, for some toxins, the absence of proven human intoxications makes it difficult by public health authorities to estimate the risk for humans following acute exposure. Investigation of toxins toxicity through both *in vitro* and *in vivo* studies can provide key information. In fact, several phycotoxins have been shown *in vivo* to induce toxic effects on the intestinal epithelium such as cell detachment, fluid accumulation and villous erosion. Nevertheless, most of the toxicity data have been obtained *in vitro* on intestinal epithelial cell monolayers with a single cell type. Recently, co-culture models have been developed to mimic more closely the human intestinal barrier and are expected to improve evaluation of the toxicity of ingested compounds. Using such relevant co-culture model with enterocytic Caco-2 cells and HT29-MTX goblet cells, we investigated the effects of four phycotoxins (okadaic acid (OA), yessotoxin (YTX), pectenotoxin-2 (PTX2) and azaspiracid-1 (AZA1)). Cell viability, permeability, production of mucus and inflammation were evaluated using various approaches such as TEER, ELISA, histology and High Content Analysis. Our results showed that OA and PTX2 affected the monolayer permeability and that YTX and AZA1 increased the mucus layer through histological analysis. Only OA seems to induce inflammation through IL8 cytokine release. Additional results using RT-PCRq will highlight the pathways and genes affected by these toxins on the investigated processes. This co-culture model appears to be a promising tool to evaluate and compare the effects of phycotoxins on the human intestinal barrier.

P06-066

Aerosol bubbled extracts of next generation products show significantly reduced toxicity compared to cigarettes in a series of *in vitro* assays.

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To assess the potential harm reduction of tobacco-based and tobacco-free Next Generation Products (NGPs), three different products were compared to conventional cigarettes (3R4F) in a series of *in vitro* assays. The trapping of smoke/aerosols in phosphate buffered saline (PBS) was used, to enable the use of *in vitro* systems where direct exposure to smoke/aerosol is not possible. The objective of this study was to assess the smoke chemistry and *in vitro* biological activity of PBS which had either cigarette smoke or a selection of NGP aerosols bubbled through it. The products investigated were the Kentucky reference cigarette (3R4F, 1.8 puffs/ml), a tobacco heating product (THP), a hybrid product (HYB) and a *my*blu™ e-cigarette (Tobacco Flavour 1.6% Nicotine) all at 4 puffs/ml of PBS. The 3R4F and THP were smoked using the HCI Intense smoking regime, with HYB and *my*blu™ vaped according to CORESTA Recommended Method N°81. The cigarette smoke and NGP aerosols were bubbled through a series of three impingers (10mls each) containing PBS and combined to form a mixed sample.

Chemical analysis of the 3R4F stock solution, quantified nicotine at 64 µg/ml and a selection of carbonyls ranging between 5.9–157 µg/ml. The three NGP stock solutions contained nicotine levels ranging from 46–169 µg/ml and had marked reductions in carbonyls when compared to 3R4F (*my*blu™ had no detectable carbonyls present).

3R4F extract was cytotoxic in the Neutral Red Uptake assay and mutagenic in the Ames assay with both strains TA100 and TA98 with S9 activation. The THP extract was less cytotoxic than 3R4F extract, with only a weak positive response observed in the Ames test with TA100+S9. The HYB and *my*blu™ extracts were both non-cytotoxic and myblu™ non-mutagenic at the maximum tested concentration of 10% in PBS, under the conditions of test. None of the PBS samples were active in the *in vitro* micronucleus assay. Only the 3R4F extract was classified as having tumour promoting activity in the Cellular Transformation Assay (Bhas 42 strain).

Both the HYB and *my*blu™ extracts were non-cytotoxic and *my*blu™ non-mutagenic under the conditions of this study. Using a core battery of *in vitro* tests, *my*blu™ demonstrated the lowest biological response compared to 3R4F and the other NGPs tested.

P06-067

Development of an *in vitro* photosensitization assay using reconstituted 3D human epidermis and a genomic signature: the PhotoSENS-IS assay.

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Chemical photosensitivity can be elicited by exposure of the skin to various pharmaceutical substances, foods, cosmetics and other environmental chemicals, followed by exposure to sunlight. In order to develop an *in vitro* test for photosensitization, we decided to use the advantages of the SENS-IS assay in terms of chemical sensitization potency measurement and skin metabolism capabilities. After optimizing the time of contact between the chemical product and the 3D human epidermis (Large Episkin model) and the intensity of UV irradiation using 6-methyl coumarin as a prototypical photosensitizer, we have tested 8 phototoxic or photosensitizer chemicals. All chemicals were tested at 50% or 10% in DMSO except for Chlopromazine which was tested diluted in PBS. 2-ethylhexyl-p-methoxycinnamate, 2-tert-butyl-4,6-dinitro-5-methylanisole, para-amino-benzoic acid, bithionol and 6-methyl coumarin used as a positive control, were all detected as moderate photosensitizer, i.e. positive at up to 10%. Bithionol was also detected as an irritant and para-amino-benzoic acid as a weak sensitizer without UV irradiation. Sulphanilamide was measured as a weak photosensitizer, i.e. positive only at 50%. Carprofin and Chlopromazine were too irritant and could not be analyzed at the concentrations tested. These very encouraging preliminary results suggest that the PhotoSENS-IS assay may be a promising *in vitro* tool for the assessment of potential photosensitizers.

P06-068

Dosing considerations for *in vitro* inhalation testing of VOCs in air-lifted Interphase (ALI) cultures

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VOCs are difficult to dose in submerged *in vitro* systems, since evaporation may lead to rapid changes in the available concentration. Moreover, due to high vapor pressures, inhalation is an important route of exposure and, thus, ALI exposure of cells from the respiratory tract seems to be a highly relevant setup. In an ALI setup, it is reasonable to assume that the applied dose (D) is defined by the general equation $D = c \times t \times Q$, with the concentration c, the exposure time t and the exposure volume flow Q, which quantifies the flow that is conducted over the surface of the exposed cells or tissues. This study aimed at the identification of an experimental window within which this correlation is valid. Formaldehyde was chosen as a hydrophilic, relatively high toxic model compound with a high vapor pressure and low boiling point. Human lung epithelial A549 cells were exposed to formaldehyde vapor at ALI conditions using the P.R.I.T.®ExpoCube® device. This unique exposure device provides a highly efficient exposure situation by preventing contact between the test compound and the culture medium. Test atmospheres were generated by evaporation of the volatile test compounds and diluted in clean air. FT-IR spectroscopy enabled online analysis of the exposure concentration. Dose-response curves were obtained using WST as readout for cytotoxicity and compared to a comprehensive historical data set available at ITEM. Clear and conclusive results were evaluated leading to a simple definition of an experimental window within which the above named general formula for calculation of the dosage was valid. It is defined by

$8\ \text{min} \leq t \leq 60\ \text{min}$ and $3\ \text{ml/min/cm}^2 \leq Q \leq 10\ \text{ml/min/cm}^2$

The efficiencies of low exposure flows (1.5 to 2.5 ml/min) were clearly lower as during historical experiments. The reason for this may be a lack of gas phase concentration equilibration or the stability of fluid dynamical boundary layers such as the Prandtl's boundary layer over the cellular surface.

In summary, in conclusive explorative experiments it could be shown that the general formula for the dosage D as defined above was valid in the experimental setup used and in an experimental window defined in this study.

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P06-069

Differentiation and freeze-thawing of human iPS cell-derived brain microvascular endothelial cells

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Purpose: The blood-brain barrier (BBB) is composed of brain microvascular endothelial cells (BMECs) that are surrounded by pericytes, astrocytes and neurons. The BMECs, which are characterized by robust tight junctions and the enrichment of efflux transporters, have an essential biological barrier function to protect the brain from toxic factors and pathogens. In drug development, accurate evaluation of BBB permeability is required to predict not only efficacy but safety of drugs. Although BBB permeability has been evaluated by experimental animals, accurate prediction in human is difficult because of species differences. Therefore, a human induced pluripotent stem (iPS) cell-derived BBB model has been developed for preclinical drug screening. However, in previous study, human iPS cell-derived BMECs (iBMECs) express the low levels of endothelial cell markers and are difficult to maintain the barrier function after freeze-thawing. In this study, we attempted to promote differentiation of iBMECs and investigated the effect on freeze-thawing by compounds X.

Methods: Differentiation to iBMECs was performed with reference to previous report [Lippmann *et al*., 2012] and compounds X were added to the differentiated media for appropriate period.

Results: As the results of immunofluorescence staining and tube formation assay, compounds X remarkably increased the protein expression level of vascular endothelial cell marker and enhanced the ability of blood vessel-like structure formation. Moreover, transendothelial electrical resistance (TEER) values of iBMECs were significantly increased by compounds X. Although TEER values were significantly decreased in frozen cells without compounds X, we succeeded in maintaining TEER values in frozen cells by compounds X.

Conclusion: We have succeeded in discovering compounds X, which enhanced the barrier functions of iBMECs and suppressed the cell damage by freeze-thawing. We concluded that compounds X would be useful for developing *in vitro* BBB models from iPS cells.

P06-070

Development of *in vitro* cholestatic drug-induced liver injury evaluation system using HepG2-hNTCP-C4 cells with sandwich culture

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Introduction and Purpose: Toxicological approaches for screening of drug candidates causing drug-induced liver injury (DILI) during early stage of drug development studies are needed to reduce risk and cost. It is thought that some kinds of cholestatic DILI cases are caused by the accumulation of bile acids (BAs) in hepatocytes due to inhibition of transporters. Then, the expression of Na^+-taurocholate cotransporting polypeptide (NTCP), which incorporates BAs into hepatocytes, is essential for properly constructing cholestatic DILI evaluation systems.

We investigated whether sandwich-cultured HepG2-hNTCP-C4 (SCHepG2-hNTCP-C4) cells were available as the evaluation of cholestatic DILI.

Methods: We evaluated the expression of mRNA and protein and functions in SCHepG2-hNTCP-C4 cells. We also exposed 22 compounds, whose clinical DILI risks are known, under the optimal conditions.

Results: In SCHepG2-hNTCP-C4 cells, the gene expression levels of NTCP and MRP2/4 were comparable to those in human primary hepatocytes, although BSEP expression was low. The correct cellular localization of NTCP, breast cancer resistance protein (BCRP), multidrug resistance-associated protein 2 (MRP2), and F-actin was also observed. In addition, tauro-nor-THCA-24-DBD, which is a fluorescent substrate of NTCP, was incorporated into hepatocytes, and CDF, which is a substrate of MRP2/3, was excreted into the bile canaliculi. When 22 compounds were exposed with BAs to evaluate cholestatic DILI, most of compounds showed cytotoxicity in the presence of the 25-fold concentration of BAs.

Conclusions: These results concluded that SCHepG2-hNTCP-C4 cells might be a useful preclinical screening tool to predict cholestatic DILI risk in liver-on-a-chip etc. However, we thought that the accurate prediction of the risk of DILI would be inadequate in the SCHepG2-hNTCP-C4 cells. Therefore, further studies are needed to address these issues.

P06-071

Differentiation of human iPS cell-derived endothelial progenitor cells into brain microvascular endothelial cells

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Purpose: Brain microvascular endothelial cells (BMECs), which are one of the constituents of the blood brain barrier (BBB), inhibit the non-specific entry of substances into the brain parenchyma through strong intercellular adhesions and expression of multidrug efflux transporters. Recently, human induced pluripotent stem (hiPS) cell-derived BMECs (iBMECs) were developed as new resources for the human BBB models. However, in iBMECs previously reported, the expression levels of endothelial markers are low. Previously, we succeeded in differentiation, expansion and cryopreservation of human iPS cell-derived endothelial progenitor cells (iEPCs). Therefore, the aim of this study was establishment of original method for differentiation of iBMECs with endothelial phenotypes from cryopreserved iEPCs.

Methods: The iEPCs were differentiated and cryopreserved. Further, the cryopreserved iEPCs were thawed and differentiated into iBMECs. Expression of genes and proteins were determined by RT-qPCR and immunofluorescence analysis, respectively. Furthermore, transendothelial electrical resistance (TEER) values, which represent the intensity of tight junction, were measured in the iEPCs and iBMECs.

Results and Discussion: The iEPC-derived cells strongly expressed endothelial markers. Further, they also expressed multidrug efflux transporters and tight junction makers. Besides, iEPC-derived cells showed higher TEER values and lower permeability of FITC-Dextran 4,000, which is index of paracellular pathway, than iEPCs. These results suggest that iEPC-derived cells have a stronger barrier function than the iEPCs. Thus, we conclude that iEPC-derived cells have features similar to those of BMECs *in vivo* and the iBMECs can be differentiated from cryopreserved iEPCs.

Conclusion: We succeeded in differentiation of cryopreserved iEPCs into iBMECs with endothelial phenotypes.

P06-072
Effect of glyphosate at low concentrations on chromosome missegregation and aneuploidy induction in human peripheral blood lymphocytes *in vitro*

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Aneuploidy, a state of imbalance in chromosome numbers implicated in cancer development and progression, arises after erroneous chromosome missegregation during anaphase. Glyphosate is the world's most commonly used herbicide, frequently examined for its potential toxicity in non-target organisms. To assess whether exposure to glyphosate might affect chromosome segregation fidelity in human cells, we have treated human whole peripheral blood with solutions of glyphosate *in vitro* for 24 h at final concentrations equivalent to acceptable daily intake (ADI; 0,5 µg/mL) and acceptable operator exposure level (AOEL; 3,5 µg/mL). After processing whole blood cultures according to cytokinesis-block micronucleus assay, we have performed fluorescence *in situ* hybridization with directly labeled pancentromeric probes for chromosomes 18, 9, X and Y. We have noticed a significant increase in chromosome loss in binucleate lymphocytes at both concentrations for chromosomes 18, 9, and X as well as for chromosome Y at AOEL. We conclude that glyphosate exposure affects chromosome segregation, potentially increasing the risk of malign transformation. These results will be useful for future risk assessments of this herbicide.

P06-073
Identification of key transcription factor networks mediating valproic acid-induced mitochondrial dysfunctioning in primary human hepatocytes

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Valproic acid (VPA) is one of the most frequently prescribed antiepileptic drugs worldwide. Besides its pharmacological activity, it may cause liver toxicity and steatosis through mitochondrial dysfunctioning. Nevertheless the mechanisms underlying these adverse effects are incompletely understood. We and others have previously studied the effect of VPA on gene expression and DNA methylation profiles in primary human hepatocytes (PHH). In the current study we aimed to link changes in gene expression to mitochondrial dysfunctioning by performing an RNA interference (RNAi) screening strategy. For this we predicted which transcription factors needed to be activated in order to explain the VPA-induced expression profiles. This resulted in the identification of a panel of 18 TFs that were constitutively activated during 3 days of repetitive VPA exposure. Next, we applied RNAi, using lentiviral-based shRNAs, to knockdown the expression of 16/18 TFs identified and determine their effect on mitochondrial dysfunctioning. For each of the knockdown models we determine oxygen consumption rates (OCR) using Seahorse technology. Importantly we found a dose-dependent decrease in OCR in PHH after 24, 48 and 72 hours of repetitive VPA administration. There was no acute effect of VPA administration on OCR, indicating the need for prolonged exposure. Knockdown of several TFs modified the response of PHH to VPA treatment after 72 hours. CEBPA was one of the most prominent TFs of which the expression affected the cellular response to VPA. Knockdown of CEBPA increased basal and maximal respiration rates in PHH upon VPA exposure compared to control shRNAs. In order to identify CEBPA-dependent gene expression, RNA-seq was performed on CEBPA knockdown and control cells under both control conditions and after VPA exposure. Out of the 27 transcriptional targets initially used to identify CEBPA activation we could confirm 23. Four of the previously identified transcriptional targets of CEBPA did not respond to CEBPA depletion, suggesting that these targets are not regulated by CEBPA in the cellular context of the PHH we used. Altogether, our study demonstrates that VPA-induced changes in gene expression can be causally linked to mitochondrial dysfunctioning in PHH.

P06-074
Investigation into the cross-species sensitivity of erythrocytes *in vitro* to hydroxylamine-mediated stress and cytotoxicity

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Rheumatoid arthritis (RA) is a chronic autoimmune condition which causes inflammation and destruction of the diarthrodial joints of the hands and feet. The pathogenesis of RA involves complex interactions between environmental and genetic factors, leading to the permeation and aggregation of immune cells and macrophages in the synovium. Infiltrating macrophages produce a variety of pro-inflammatory cytokines that contribute to the destruction of cartilage and bone. RA is the most common inflammatory arthritis, affecting around 400 000 adults in the UK.

GSK *Compound X* has the potential to be an effective agent in the treatment of RA. However, the production of the carboxylic acid of the hydrolysed hydroxamic acid of *Compound X* was identified in rat, monkey and human hepatocytes, indicating the potential formation of hydroxylamine (HA). HA is associated with haematotoxicity *in vivo*, methaemoglobinaemia, haemolytic anaemia and haemosiderosis. It also a potent non-genotoxic rodent carcinogen with the potential to cause splenic haemangiosarcomas (HS) in male rats, as described in the adverse outcome pathway.

To determine whether there are species differences in the sensitivity of erythrocytes *in vitro* to HA-mediated cytotoxicity, a series of erythrotoxicity and viability studies were conducted. These included measurement of glutathione/oxidised glutathione (GSH/GSSG), observation of Heinz bodies and intracellular reactive oxygen species (ROS) detection via flow cytometry.

Results suggest rat red blood cells are most sensitive to HA-mediated toxicity followed by human, then dog. A favourable ranking of human versus other species (particularly the rat and dog) could be of value in defining the relevance of any eventual findings in the 2-year rodent carcinogenicity studies. The data generated was integrated into the assessment of the translational risk of HA-mediated haematotoxicity and haemangiosarcoma after chronic exposure to *Compound X* in humans. Overall, the data added to the weight of evidence that the risk in humans is likely to be lower than what has been observed in the rat following HA exposure.

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P06-075

Induced pluripotent stem cell-derived human retinal model containing microglial cells as a platform for toxicology studies

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Microglia are the primary tissue resident immune cells in the retina. They co-exist in close interaction with Müller glial cells and are essential for normal development by regulating neuronal survival and synaptic pruning. In the adult retina, they regulate homeostasis by maintaining synaptic structure and function. Under pathological conditions, microglia-Müller cell signalling can mediate adaptive responses within the retina following injury. In addition, microglia can trigger neurodegeneration within the retina exacerbating the effect of the disease making it a potential therapeutic target. Retinal organoids derived from human induced pluripotent stem cells (hiPSC) provide a human physiologically relevant platform to study retinal development, disease modelling and compound screening. However, due to the differences in their developmental origins, microglia and retina do not arise under the same differentiation conditions. Therefore, to enhance the current retinal model, a co-culture approach is needed. We developed a differentiation protocol for deriving microglia from hiPSCs. The cells expressed key developmental markers, including CD14, CX3CR1 and IBA1. In addition, they were functional as was shown by their ability to phagocytose fluorescent beads. In parallel, we differentiated hiPSCs to retinal organoids using our established protocols. We assessed their development by confirming the expression of key markers, including RECOVERIN, HUC/D, AP2α, and PROX1. To enhance our retinal model, we incorporated hiPSC-derived microglia and tested their retinal invasion capacity and function in response to agents causing retinal degeneration. Our *in vitro* retinal model which incorporates immune cells represents a tissue structure with greater physiological relevance to the *in vivo* human retina and provides a platform for compound screening and disease modelling.

P06-076

Simultaneous real-time monitoring of cytotoxicity and stress response pathway by means of dual color luciferase monitoring system

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Recently, luciferase reporter assay has become one of the conventional methods for cytotoxicity evaluation. Typically, cells are destroyed at a particular time point, called the endpoint assay, enabling conventional and high-throughput assay. On the other hand, luciferases are also used in the longitudinal monitoring of such cellular events *in cellulo*. In addition, recent advances in luciferase technology allow us to monitor the expression of multiple genes simultaneously when luciferases are used that induce differently colored emission spectra, namely, green-emitting and red-emitting beetle luciferases that act on a single bioluminescent substrate [1]. To improve cytotoxicity evaluation using luciferase, in this study, we developed real-time multicolor luciferase measurement method to simultaneously monitor dynamics of expression of cellular toxicity and activation of stress response pathway, including Keap1-Nrf2 pathway.

First, we generated HepG2 cells harboring mouse artificial chromosome (MAC) vector [2]. Next, an internal control reporter plasmid, in which green-emitting luciferase is expressed under the control of TK promoter, was inserted into R4 site of the MAC vector. Finally, test reporter plasmid, in which red-emitting luciferase is expressed under the control of antioxidant response element (ARE), was inserted into φC31 site of the MAC vector.

To verify the monitoring system, luciferase-expressing HepG2 cells seeded into 96-well plates were treated with representative Nrf2 activators, including tertiary butylhydroquinone, dimethylfumarate and sulforaphane. Bioluminescence was measured for 72 h at 37°C in 5% CO_2 atmosphere under saturated humidity. As expected, luminescence intensity of green-emitting luciferase (internal control reporter) was dose and time dependently decreased. In contrast, luminescence intensity of red-emitting luciferase (ARE-dependent expression) was significantly increased. These results clearly demonstrated that the system successfully monitor dynamics of activation of Keap1-Nrf2 pathway accompanying increase of cytotoxicity. Thus, real-time monitoring system developed in this study would be useful for mechanism-based cytotoxicity assays by directly monitoring both events.

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P06-077

Retinal organoids derived from human induced pluripotent stem cells as an *in vitro* model for toxicological studies for new retinal disease treatments

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Vision is one of our most important sensory senses and diseases such as age related macular degeneration (AMD) and retinitis pigmentosa (RP) cause the loss or dysfunction of retinal photoreceptors and/or the underlying retinal pigment epithelium (RPE). This results either in irreversible impairment of vision or complete vision loss as the retina is unable to regenerate. To date there are no effective treatments to halt progression of retinal degeneration. The generation of 3D laminated retina derived from human pluripotent stem cells (hPSCs) provides a suitable platform and offers the opportunity to develop new strategies for treatment of these conditions, drug screening and repurposing and diseases modelling. Recent research demonstrated that retinal organoids derived from hPSCs contain all retinal cell types which are capable of forming synapses. Moreover, these organoids are physiologically functional, meaning they are responsive to light. This study investigated the applicability of human induced pluripotent stem cell (hiPSCs) derived retinal organoids as a human *in vitro* model for toxicological studies. Therefore retinal organoids, which are aged around 5 months and resemble the normal retina, were used to test different drugs: Digoxin, Thioridazine and Methanol are drugs with well-known side effect on the retina and Flubiprofen and Ketorolac are drugs without any reported effects serving as a control in this study. Retinal organoids were exposed to the drugs for 24 hours and subsequently analysed by Lactate Dehydrogenase (LDH) assay to determine cytotoxicity and by immunohistochemistry to evaluate possible structural and functional changes within the retina. Our results, so far, indicated that Digoxin, Thioridazine and Methanol affected the retinal structure and the control drugs (Flubiprofen and

Ketorolac) showed no effects on the retinal structure and/or physiological functionality regardless of the concentration (200ug/ml – 2mg/ml). This study is a proof of principle and demonstrates that the retinal organoids derived from hiPSCs represent a good human *in vitro* model, which can be used for drug screening to develop new treatments for retinal diseases.

P06-078
Protective and reparative effects of fluconazole on neuronal differentiation altered by 5HT

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Micromass test involves exposing undifferentiated rat embryo midbrain cells put in culture at high density to test compounds and observing the subsequent effect on cell differentiation. Within these high-density cell colonies, cells that are destined to differentiate move together and form distinct foci (tridimensional aggregation of cell bodies) interconnected by bundles (aggregation of neuronal processes, differentiated foci) which can be distinguished from foci without bundles (undifferentiated foci). Recently, a pro-differentiating effect in oligodendrocyte precursors (OPC) has been demonstrated for miconazole, beside its antifungal activity. In order to test whether other antifungal azoles show the same activity on cell maturation, micromasses were incubated during the whole culture period with a known promoter of differentiation (RA), with a known differentiation inhibitor (5HT) or with azoles in clinical use. Fluconazole, itraconazole and miconazole, whose differentiative potential was first confirmed in OPCs cultured both alone and with neurons, were selected based on *in silico* prioritization. Results confirmed 5HT inhibitory effects at 50-100 mM. Among RA and the selected azoles, the most promising molecule in our model was fluconazole, tested at 5-100 mM. In order to test protective effects of fluconazole on 5HT inhibition, we co-exposed micromasses to both molecules during the whole culture period. Results show that co-exposed groups displayed parameters comparable to controls, suggesting a protective effect of fluconazole. A second set of experiments were devoted to the evaluation of a fluconazole-related reparative effect. Cultures were exposed during the first day to 5HT alone and during the remaining culture days to fluconazole alone. Results show that the one-day 5HT exposure affected development while after the post-exposure to fluconazole a reparative effect was evident.

P06-079
Co-exposure to preadipocytes and TCDD increase breast cancer cells aggressiveness and leads resistance to chemotherapy

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Breast cancer is an important disease with high incidence as well as mortality among women. In the past 50 years, it has become a major health problem with over 2 million new cases diagnosed in 2018. This represents about 12% of all new cancer cases, 25% of all cancers in women and more than 600.000 cases of deaths worldwide in 2018. Metastatic process, or the spread of tumor cells throughout the body, is responsible for about 90% of cancer patient deaths and represents the central clinical challenge of solid tumor oncology.

The development and progression of breast cancer are complex processes that involve hormonal factors as well as numerous genetic and epigenetic alterations. During the past 10 years, many studies have focused on the role of the tumor microenvironment and the peritumoral stromal fraction. During tumor progression, cancer cells modify their microenvironment which in return will promote the growth and dissemination of the tumor. Adipose tissue, consisting of mainly mature adipocytes and progenitors, is the most abundant component surrounding breast cancer cells. It exerts a major endocrine and secretory role, and represents then an essential actor in the remodeling responses of the extracellular matrix, which influences tumor behavior. Recent studies have shown that pre-adipocytes/adipocytes may promote migration and invasion of breast cancer cells. Adipocyte cells are also responsible for the storage of persistent organic pollutants (POPs) for long periods of time. POPs are a major public health concern due to their toxic effects and their persistence in the environment and organisms.

Here, we studied the paracrine role of pre-adipocytes during metastasis in mammary cancer epithelial cells and the aggravating role of TCDD exposition. We set up an original *in vitro*co-culture model using mainly human mammary tumor cells (MCF-7 and MDA-MB-231) and human pre-adipose cells (hMADS, human multipotent adipose-derived stem cells), exposed or not to TCDD. The first part of this study reports the effect of the co-culture and TCDD on the phenotypical characteristics of MCF-7 cells. Next, we performed a large-scale proteomics-based experiment and identified specifically in the co-exposure condition, a stem-cell biomarker, the Aldehyde dehydrogenase 1 family, member A3 (ALDH1A3). We then monitored assays of sphere formation and studied their cancer stem-cell (CSC) like properties of MCF-7 cells. To ensure that these aggressive features were also observed *in vivo*, we demonstrate the ability of co-exposed cancer cells to metastasize *in vivo in* a Zebrafish model. We then examined if these cells display specific cellular characteristics and found that the co-exposure leads to the generation of giant polynuclear cells (PGCCs), a cancer sub-population strongly associated with aggressiveness and chemoresistance in cancer

P06-080
Response of MCF7 cells to Vincristine in presence of BPA and DEHP

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Aim: Among phthalates DEHP (Bis(2-ethylhexyl) phthalate) and Bisphenol A (BPA) are estrogen active compounds. Eventhough its ban in toys and catheters, there are still various possible exposure scenarios. In estrogen receptor (ER)-positive breast cancers, environmental estrogen active compounds pose a risk for prominent treatment. In this study, we searched the efficacy of vincristine and tamoxifen, in presence of the endocrine active compounds DEHP and BPA, in ER+ mammary tumor cancer cell line MCF7 (human breast adenocarcinoma).

Material method: DEHP and BPA at 0.1–100 nM concentrations alone and in combination with Tamoxifen (at cytotoxic concentration of 9 nM) and Estradiol (E2) (at proliferative concentration of 1 nM) were applied to MCF7 cells. Vincristine was applied at its cytotoxic concentration (IC50 = 5.45 nM) following preincubation (4 hrs) with the same protocol of the tested estrogen active compounds. Cell viabilities were evaluated by MTT assay.

Results: Preincubation with DEHP and BPA at 1 nM were found to decrease the cytotoxicity of Vincristine by 18.71 (±5.43) and 31.63% (±1.93). These estrogen active compounds were found to decrease

the cytotoxicity of Tamoxifen by 34.27% (±3.53) for DEHP and 39.94% (±1.55) for BPA; while Tamoxifen-Vincristine combination were decreased by 8.43% (±2.65) and 14.92% (±0.56). In presence of the standart proliferative ligand E2, the cytotoxic effect of vincristine were also decreased by 59.42% (±6.32) for DEHP and 60.20% (±4.87) for BPA. The results indicate that the cytotoxic effect of vincristine, a vinca alkaloid drug used in metastatic breast cancer chemotherapy regimes in combination with tamoxifen were found to have decreased in presence of the environmental contaminant estrogen active compounds, DEHP and BPA, in *in vitro*. The preliminary results indicate that presence of low-dose exposure to these pollutants, might lead a decrease of the cytotoxic potency of the chemotherapeutics; where studies should be repeated with more other estrogen active compounds in real life exposure doses and in mixtures.

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P06-081
Establishment of a human embryonic stem cell test with hiPSC derived cardiomyocytes for developmental toxicity testing

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Developmental toxicity testing is performed with animal testing according to several guidelines like OECD or U.S. EPA. Due to the resource-intensity, testing of all registered chemicals using OECD guidelines is logistically and financially not feasible. As an alternative method for embryotoxicity testing, the European center for validation of alternative methods (ECVAM) validated the mouse embryonic stem cell test. This test is based on a permanent mouse embryonic stem cell line, which is differentiated into cardiomyocytes. Hence, the test assesses the effects of chemicals onto the early developmental aspect of cardiomyocyte differentiation. However, this test is based on mouse cells and therefore, bears the issue of species extrapolation to humans. To overcome this issue, we established a human cell based embryonic stem cell test (hEST) using human induced pluripotent stem cells (hiPSC). These hiPSC originate from somatic cells e.g. fibroblasts, which were reprogrammed into stem cells that cover all the characteristics of embryonic stem cells like self-renewal and pluripotency yet does not bear any ethical concerns. By modulation of the Wnt and BMP pathways, cardiomyocyte differentiation is induced. We show characterization of the cell culture by assessment of beating cells, as well as marker expression by PCR, FACS and immunocytochemistry. In a next step, these markers have to be established as test methods with the final goal of testing compounds for their ability to interfere with hiPSC differentiation into cardiomyocytes.

P06-082
Characterization of fresh hepatocytes isolated from TK-NOG chimeric mice with humanized livers

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Purpose: Human hepatocytes are an important tool for drug development and *in vitro* toxicity studies. We developed a TK-NOG transgenic mouse which can be used for the expansion of human hepatocytes within the host liver tissue. The purpose of the present study was to evaluate hepatocytes isolated from TK-NOG chimeric mice with humanized livers(Hu-liver), and determinewhether these hepatocytes could be used as an alternative to primary human hepatocytes for *in vitro* toxicity studies.

Methods: Hepatocytes were harvested from chimeric mice with Hu-liverusing a two-step collagenase perfusion method. The purity and viability of the prepared hepatocytes was analyzed using flow cytometry. The expression levels of drug-mediated cytochrome P450 genes were determined using real-time polymerase chain reaction analysis with gene-specific primers. The ability of CYP3A to induce enzymatic activity was evaluated using testosterone as a CYP3A probe and rifampicin as CYP3A inducer.

Results and Discussion: Up to 98% of the Hu-liver cells stained positive for human leukocyte antigen, with a mean viability exceeding 87% (n=29). Monolayer-cultured Hu-liver cells were binucleated and displayed a cobblestone cell morphology. A good correlation was observed between the mRNA expression levels of 16 P450 forms belonging to P450 subfamilies 1–4 in the Hu-liver cells and those in human donor hepatocytes. Similar to the observations made using donor human hepatocytes, omeprazole/β-naphthoflavone and rifampicin/phenobarbital treatment caused a more than two-fold induction of CYP1A2 and CYP3A4 mRNA in the Hu-liver cells. We also confirmed a significant increase in the production of 6β-hydroxytestosterone in the Hu-liver cells after treatment with rifampicin, a CYP3A inducer. In long-term cultures of Hu-liver cells, both the rate of human albumin production and the expression levels of CYP3A4 mRNA were maintained for up to 4 weeks in commercially available medium. These results suggest that Hu-liver cells have characteristics similar to those of human hepatocytes. Hu-liver cells can therefore potentially be used for *in vitro* toxicity studies.

P06-083
MAKE people BETTER scientists in the lab: Altertox Vision

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Altertox Academy connects international experts to provide hands-on-training (HOT) in human-relevant alternative methods and technologies for toxicologists of all levels of experience, from entry level technician to laboratory or department manager. The participants will become familiar with new technologies and their critical steps.

Like “a picture is worth a thousand words”, a HOT is a perfect way to quickly approach a method. The training allows not only to understand methods that researchers want to set up, but also the data analysis and interpretation that could be a critical step when generating results.

In the past three years, Altertox Academy has organized more than 30 HOT with a format allowing a detailed and practical description of the methods (20% lectures and 80% HOT or case studies throughout two days).

With a maximum of 15-20 participants, divided in small groups for the practical component of the training, this format allows networking and connects experts to people that will daily use their method. After one of our training a participant said: "*The group size was also very good to stimulate discussion and work in focused groups on the case studies. There was also sufficient time spent on the sessions to allow discussion and interaction.* "

Focused on alternatives to animal testing, the topics covered by our trainings are: *in silico* methods (endocrine disrupting compounds, *in silico* models for cosmetics) and *in vitro* methods (lung inhalation, skin sensitization, hepatotoxicity, proarrhytmia cardiac assay and more).

Promoting education and training brings improvement in scientists' day-to-day work and can also have a positive impact on the general scientific community. Participating in our trainings will improve your skills for a specific method, showing each method's limitations, and provide you with the capacity to challenge the tests and interpret data.

Altertox Academy follows also the vision to **MAKE scientists BETTER citizens,** by offering a Skills4Science training for young researchers. The primary focus of "Skills4Science" is to tackle topics that do not emerge during conventional scientific congresses and to empower in particular young researchers on understanding social media influence on researchers' actitvity, gender inequality, scientometrics and scientific collaboration.

P06-084
A battery of animal-free *in vitro* assays for evaluating prenatal developmental toxicity potency of highly complex petroleum substances

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Given that i) prenatal developmental toxicity (PDT) testing is one of the most complex, and animal- and resource-intensive regulatory requirements for substances produced at >100 tonnes/year, and that ii) petroleum substances (PS) are UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials), the development of alternative non-animal based testing strategies for PS poses huge challenges. Some PS contain high concentrations of polycyclic aromatic hydrocarbons (PAHs) and we hypothesize that PDT as observed for some PS is caused by certain types of PAH present in these products. To this purpose, DMSO-extracts of 9 PS (varying in PAH content; from 5 different PS categories) and 2 gas-to-liquid (GTL) products (devoid of PAHs) were tested in a battery of *in vitro* assays, including the embryonic stem cell test (EST), the zebrafish embryotoxicity test (ZET), and the aryl hydrocarbon (AhR) CALUX assay. All DMSO-extracts of the PS, but not of the GTL, induced concentration-dependent PDT as quantified in the EST and ZET, with their potency being proportional to their 3- to 7-ring PAH content. Moreover, all PS extracts also showed AhR-mediated activity in the AhR CALUX assay, suggesting a role of the AhR in mediating the observed PDT by these substances. Combining the results of the EST, ZET, AhR CALUX assay, and the PAH content, ranked and clustered the test compounds in line with their *in vivo* PDT potencies, from the most potent PS category under study, heavy fuel oil, to the test compounds showing no effect at all in any assays of the test battery, the GTL products. In conclusion, our battery of *in vitro* assays, consisting of the EST, ZET and AhR CALUX assay, is able to evaluate and differentiate the PDT potency of highly complex PS, within and among categories. The results are also in concordance with our hypothesis on the role of PAHs present in some PS for the observed PDT induced by these substances. This may assist in the grouping of PS, based on their bioactivities and chemical compositions, allowing us to identify the worst-case representatives per PS category for further *in vivo* testing where needed as a last resort to fill data gaps. Thus, such an intelligent testing strategy will ultimately reduce animal testing and resources needed to study PDT potency of PS UVCBs.

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P06-085
Gaining insights into mechanism of mitochondrial toxicity using a comprehensive approach of *in vitro* assays.

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Mitochondrial dysfunction has been implicated in numerous drug induced adverse events, such as liver failure and cardiac toxicity. The detection of potential mitochondrial toxicants can be determined using numerous *in vitro* approaches. Firstly, comparing the increase in cytotoxicity of compounds in galactose compared to glucose media (Glu/Gal assay). Alternatively, the use of a mitochondrial stress test (XF^e assay) measuring cellular oxygen consumption rate (OCR), reserve capacity (RC) and extracellular acidification rate (ECAR). The third approach utilises fluorescent dyes to measure changes in mitochondrial membrane potential (MMP) by high content imaging compared to ATP depletion and cell loss.

Mechanistic understanding can be gained from the XF^e assay, identifying uncouplers from inhibitors of the electron transport chain (ETC) or ATP synthase. Additionally, utilising permeabilised cells and the XF^e analyser, we can identify the individual complexes of the ETC, providing more detailed information. Seventy compounds (known mitochondrial toxicants with varying mechanisms and compounds with no mitochondrial effect) were screened through the Glu/Gal assay, the MMP/cytotoxicity assay, and the XF^e assay, in HepG2 cells.

The XF^e assay showed a sensitivity, specificity and accuracy of 78, 100 and 90% respectively, the Glu/Gal assay showed 41,100 and 73% respectively, whereas the MMP assay was 83, 76 and 79% respectively, additionally a combination of all three approaches improved overall accuracy. Comparing the data showed there were a number of toxins, e.g rosiglitazone, flagged as positive in the XF^e assay and the MMP and cytotoxicity assay but not detected in the Glu/Gal assay.

Assessment of positive compounds in permeabilised cells identified compounds with differing mechanisms of action, e.g. rotenone as a complex I inhibitor and sodium azide as a complex IV inhibitor.

In summary, determining cellular OCR, RC and ECAR provides a predictive and sensitive measure of mitochondrial toxicity whilst providing basic understanding of potential mechanisms of action when compared to the Glu/Gal and MMP assay. The MMP assay provides a more sensitive alternative to the Glu/Gal assay, whilst having a higher throughput than the XF^e assay. Further insight into the mode of action of mitochondrial toxicants was provided using permeabilised cells.

P06-086
Evaluation of dermal absorption in micro-pig dermal tissue model for prediction of bifenthrin residues.

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It has been widely known that the skin can be an important route of absorption for pesticides. As the guidelines of *in vitro* skin absorption studies have been drafted by OECD to predict skin absorption rate in human, we intended to measure the *in vitro* absorption rate of bifenthrin, a widely used pesticide in laying hen farms (but banned from used on chickens in South Korea), to predict the pesticides residues in laying hens. 2000ppm, 1000 ppm and 100 ppm of bifenthrin were applied to the micro pig's skins mounted on franz diffusion cells. 50% ethanol-PBS solution in receptor fluid was taken after 1, 2, 4, 8, 12, 24, 48 and 72 hours of application and dermal tissues, tape strips, washing solutions and washing swabs were taken for analysis at 72 hours after application. In the 100 ppm treated group, total absorption rate was 37.7 ± 13.9%. 28.7%, 9.02% and 31.6% of applied dose were found in receptor fluids, dermal tissues, and washing materials, respectively. In the 1000 ppm treated group, total absorption rate was 11.8 ± 6.60%. 4.43%, 7.33% and 54.3% of applied dose were found in receptor fluids, dermal tissues, and washing materials, respectively. In the 2000ppm group, total absorption rate was 10.4 ± 3.77% and 57.8% of applied bifenthrin did not penetrate into the skin and washed out after 72 hours. The sum amount recovered in all analyzed samples was 68.1 ± 10.7% in this study. In this study, the absorbed bifenthrin was found mostly in skins after 72 hours of application. Although further studies are needed to improve the recoveries, these results could support to drafting regulations on food safety for using bifenthrin in laying hen farms.

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P06-087
Tyrosine kinase inhibitor Dasatinib as reversal agents for anthracycline resistance mediated by carbonyl reducing enzyme 1B10

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Targeted therapy that has been approved lately is directed against cancer-specific molecules and signaling pathways and thus has more limited nonspecific toxicities. Tyrosine kinases are significant targets playing an important role in the modulation of growth factor signaling. Several tyrosine kinase inhibitors (TKi) have been found to have effective antitumor activity and have been approved or are under clinical trials [1]. Recent studies show that some TKi are able to enhance the cytotoxicity of anthracyclines [2]. Combination strategy of the anthracyclines together with TKi may therefore minimize the adverse effects of each individual drug, enhance the effectiveness of the treatment and allow its prolonged continuity.

Dasatinib, currently in Phase III clinical trials, is an orally administered, small molecule inhibitor of multiple tyrosine kinases that blocks the function of the Bcr-Abl protein that signals cancer cells to multiply. Targeted therapy of dasatinib used to treat mostly cases of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) in patients who are positive for the Philadelphia chromosome [3]. Additional use is for the treatment of patients with non-small cell lung cancer, metastatic breast carcinoma and advanced solid tumors.

AKR1B10 has been recently found to be overexpressed in certain types of cancers, including hepatocellular carcinoma and lung cancer associated with tobacco smoking, also aimed as a therapeutic target for the prevention and treatment of several types of cancer [4].

The aim of our research is to explore whether the chemo-sensitising properties of TKi are connected with the inhibition towards anthracycline reductases.

Dasatinib exhibited significant inhibitory effect on recombinant AKR1B10, with a half-maximal inhibitory concentration (IC50) of 0.8 μM. Its inhibition constant Ki was found to be 0.4 μM, and the inhibition data best fitted a mixed-type mode with α=1.7. In conclusion, based on our results, dasatinib may affect the therapeutic efficacy of anthracyclines by preventing anthracycline resistance and reducing their adverse effects.

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P06-088
aProximate™ as a novel, predictive model of aminoglycoside-induced nephrotoxicity

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Aminoglycosides are a class of antibiotics favoured for their bactericidal activity and their cost effective route of production. However, these compounds are known to be toxic in a range of organ systems, including the kidney. The aProximate™ model can be utilized to give an indication of the nephrotoxicity of a compound, using the FDA approved, clinically relevant biomarkers: NGAL, clusterin and KIM-1. Here we demonstrate this using a panel of aminoglycosides, which are known to induce nephrotoxicity to varying degrees, *in vivo*.

aProximate™ monolayers were generated by isolating human proximal tubule cells (hPTCs) followed by culture on Transwell filter inserts. The monolayers were grown to confluency before being challenged with a range of aminoglycosides (gentamicin, streptomycin, tobramycin, amikacin and neomycin) at 0–3000 μM for up to 96 hours. Monolayer integrity was assessed by via of trans-epithelial electrical resistance (TEER) and cell viability via the LDH and ATP assays. Biomarker generation was assessed by ELISA at the protein level, using a multiplex ELISA system.

Exposure of the aProximate™ monolayers to the aminoglycosides resulted in significant decrease in TEERs, along with a decrease in cell viability. The amount of KIM-1, NGAL and clusterin secreted by the monolayers were significantly more when compared to untreat-

ed monolayers when exposed to, for instance, neomycin, tobramycin and gentamicin (e.g. clusterin production was 5.3-fold, 5.1-fold, 4.9-fold above control levels). Interestingly, pretreatment of monolayers with receptor associated protein (RAP), an antagonist of endocytosis-mediating receptors megalin/cubilin, decreased the production of biomarkers. TEERs and cell viability were also improved with the pretreatment with RAP, which suggests uptake and corresponding toxicity may be megalin/cubilin mediated.

In summary, these data suggest that aProximate™ hPTC monolayers express clinically relevant biomarkers of nephrotoxicity and their apical release is induced by aminoglycoside challenge. The model was able to detect varying levels of toxicity between compounds of this class, mirroring what is reported *in vivo*, demonstrating their potential as a predictive *in vitro* model for toxicity screening in pharmaceutical development.

P06-089
How to assess a phototoxicity risk related to topical exposure by using the *in vitro* SkinEthic RHE model

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The skin exposure to photoreactive chemicals may produce abnormal skin reaction, an acute light-induced phototoxic response, which occurs when photoreactive chemicals are activated by solar lights and transformed into products cytotoxic against the skin cells.

It is therefore essential to ensure the photosafety of chemicals when there are probabilities of human exposure as can be clearly exemplified by pharmaceutical or cosmetic ingredients.

To evaluate the potential of phototoxicity of a chemical, various test methods that range from *in silico* to *in vitro* assays have been introduced. *In vitro* test methods include the 3T3 NRU-PT assay, a test method officially been endorsed as OECD TG 432 but also human 3-dimensional (3D) epidermis methods that might overcome some limitations of the 2D 3T3-NRU-PT.

The aim of the study was to investigate the ability of human reconstructed epidermis SkinEthic RHE to identify the phototoxic potential of topically applied chemicals.

Eight chemicals including some challenging ones in terms of solubility assessment were tested. Following topical application for 18 hours, tissues were exposed to non-cytotoxic doses (range finding-test) of 6 J/cm2 UVA. After rinsing and post-incubation steps, the cell viability was measured using MTT.

Our results show that the phototoxic potential of chemicals can be determined using cell viability. Moreover, the method was able to discriminate efficiently between phototoxic and non-phototoxic products being compared to the existing *in vivo* and 3T3 NRU-PT data.

Taken into account these promising results, further investigations are needed using an extending chemicals set to confirm its integration into decision-making processes of phototoxicity assessment.

P06-090
Cyclophosphamide metabolites 4-hydroxycyclophosphamide and acrolein exert strenuous cardiotoxicity in AC16 human cardiomyocytes, at clinical relevant concentrations

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Cyclophosphamide is a widely used drug in immunosuppressive and anticancer therapies, but cardiotoxicity often limits its use after high doses. Although there is no complete understanding on the mechanism of cyclophosphamide-induced cardiotoxicity, the involvement of metabolism is considered likely. The aim of this study was to assess the cytotoxicity of cyclophosphamide (100 to 10000 µM) and two of its main metabolites, 4-hydroxycyclophosphamide (1 to 25 µM) and acrolein (5 to 100 µM) in a human cardiomyocyte cell line, AC16 cells. Experiments were performed in AC16 cells maintained in DMEM/F12 medium, at two states of differentiation: (i) differentiated (with 2% horse serum) and (ii) proliferative cells (with 12.5% fetal bovine serum). Two cytotoxicity assays, the tetrazolium-based colorimetric (MTT) and the neutral red (NR) uptake assay were performed at different time-points. Regarding cyclophosphamide, in differentiated cells, only concentrations higher than 7500 µM caused cytotoxicity at 24h, whereas in proliferative cells, cyclophosphamide induced a time-dependent toxicity above 5000 µM. For 4-hydroxycyclophosphamide, in differentiated cells, cytotoxicity was observed already at 1 µM (only in the MTT assay). At the proliferative state, this metabolite had a time-dependent toxic profile in both tests, above 5 µM. Regarding acrolein, differentiated cells were also highly sensitive to its effects, since significant cytotoxicity was observed at 15 µM. Atconcentrations higher than 25 µM, acrolein was cytotoxic to proliferative cells as early as 24h (toxicity was not time-dependent). Thus, for the two metabolites tested, the differentiated cells were more susceptible to toxicity. With this study, we observed that cyclophosphamide *per se* only exerts cardiotoxic effect at high concentrations, while its metabolites were cytotoxic at clinical relevant range of concentrations.

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P06-091
A quantitative adverse outcome pathway for hepatic steatosis combined with *in vitro* kinetics using HepaRG cells

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Adverse outcome pathways (AOPs) are tools in toxicology to link molecular initiating events (MIEs) to key events and adverse outcomes. Quantitative AOPs describe this relationship in a quantitative way and is a more advanced stage of an AOP, which shows how severe the adverse outcome is at a certain perturbation of the MIE. The aim of this study is to show how *in vitro* methods can be used to build qAOPs. It specifically investigates the role of *in vitro* kinetics in building qAOPs. Two test compounds that are known to perturbate the MIE of the hepatic steatosis AOP, amiodarone (AMI) and valproic acid (VPA), are used to illustrate this role. The human hepatoma cell line, HepaRG, was exposed to different concentrations of AMI or VPA and effects were measured over time. We used qPCR to quantify mRNA expression levels of genes involved in steatosis. The AdipoRed™ assay and High Content Imaging (HCI) were used to quantify triglyceride accumulation and fatty liver cells, respectively. Moreover, *in vitro* concentrations of AMI and VPA were determined in medium, cells and plastic over time. The results show that using a series of *in vitro* assays, an AOP can be described quantitatively by deriving dose-response and response-response relationships. The AdipoRed™ assay

showed an increase in triglyceride accumulation and HCI experiments showed an increase in fatty liver cells after exposure. Effects of AMI and VPA on SREBF1, SCD and other genes were also observed. Moreover, *in vitro* kinetics are important to consider, as binding to plastic and the time it takes to reach the cellular target influence the results of the *in vitro* assays.

P06-092
Long term *in vitro* hepatocyte toxicity screen of a panel of perfluoroalkyl substances using 3D culture system

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Per- and poly-fluoroalkyl substances (PFAS) are wildly used in industry for different purposes, such as water/oil resistance coating for clothing/food contact paper and formulating firefighting foams. Plenty of researches indicate the health concerns of PFAS, especially perfluorooctanoic acid (PFOA). These substances have been proved to be associated with proerty of endocrine disrupting and effects on the immune system. Although replacement chemicals of PFAS have been manufactured, health concerns of new chemicals, especially about the hepatocyte toxicity, still remain. The task, how to evaluate their toxicity precisely and quickly, has a high priority at both regulation and industry site. Using *in vitro* screen system could short the evaluation period compared to animal experiments, however, long-term culture and whether *in vitro* could mimic *in vivo* conditions are still the questionable. Keeping culture cells in artificial 3D structure could prolong their survival. In this study, we used a new type of 3D culture 96 well-plate for long term hepatocyte culture. The plate, named as Cell-able™, contains 800 circle areas for cell culture in single well. A developed human hepatoma cell line, HepaRG cell, was seeded to wells at cell density of 2.0×10^5/ml without support cells. HepaRG cells made the spheroid after one-week and remained stable for additional 28 days. Cell viability was assessed by measuring the intracellular ATP content, function of hepatocyte was evaluated by expression of Glutathione-SH (GSH). Cell viability did not significantly change until 35 days. Expression level of GSH became stable after one-week culture and also did not show significant decrease at the end of culture. A concentration series (1 ~ 1000ppm) of PFAS were added to culture system at one week after seeding. Toxicity was assessed by observing morphology of spheroids and the level of intracellular ATP content and GSH. One thousand ppm PFOA induced collapse of spheroid structure at 3 days after stimulation. Concentration over 300 ppm significantly reduced the level of intracellular ATP and GSH at 3 days after stimulation. On the contrast, concentration lower than 100 ppm could not induce morphology change of spheroid, level of intracellular ATP and GSH. In addition, other PFAS also showed different level of hepatotoxicity. The results are comparable with that of animal chronical experiments. Our results demonstrate the promise of this system for *in vitro* hepatocyte toxicity testing.

P06-093
Studies of cadmium-induced cytotoxicity: From the perspective of oxidative stress, ER stress and autophagy

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Cadmium (Cd) is an environmental pollutant that affects various cellular processes, including cell proliferation, differentiation, and survival. This study investigated the mechanisms of cd induced autophagy and ER stress in the relationship between autophagy and cell survival. Cd treatment significantly increased autophagy in human prostate epithelial cells. Cd induced expression of ER stress regulators chop, eIf2α, IRE and ATF4, and activated autophagy as evidenced by increased LC3. Treatment with Azoramide suppressed Cd- induced chop protein and autophagic vacuoles. Finally, our findings suggest that cadmium induced oxidative stress trigger ER stress and autophagy in human epithelial cells.

P06-094
Online aerosol monitoring for *in vitro* toxicological studies using single-photoionization mass spectrometry

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Chemical and physical characterization of transported evolving aerosols in *in vitro* systems present a series of challenges. These span from appropriate sampling ability of delivered aerosols through measurement capabilities of their properties to possibilities of performing online measurements of the compounds of interest in the flowing aerosol during exposure. A single-photoionization time-of-flight mass spectrometer (SPI-TOF-MS) together with a VITROCELL® 24/48 exposure system was used to measure the chemical composition of the e-cigarette aerosol. The initial e-cigarette test liquid (e-liquid) was composed of propylene glycol and glycerol (80%), nicotine (1.6%), and water. Measurements were performed by sampling undiluted and diluted test aerosol from the VITROCELL® system by mixing it with pure air. The chemical composition of the test aerosol was measured with a time resolution of one second with the SPI-TOF-MS. The measurements with the SPI-TOF-MS showed the concentration of the main aerosol compounds with low fragmentation. The time series of aerosol flowing puff by-puff after dilution will be presented for various dilutions (e.g., 82% and 45%). As an example, the nicotine, propylene glycol, and glycerol average puff concentrations were 93, 1900, and 2148 ppm, respectively, at 82%. The applied technique opens the possibility not only to quantify compounds of interest during exposure but also to perform detailed time-resolution of delivered aerosol on a puff-by-puff basis. The system can be applied to monitor targeted compounds in a specific mass-to-charge range (40 to 200 m/z). Complementary measurements are ongoing to further investigate the application of SPI-TOF-MS for online monitoring of *in vitro* exposures.

P06-095
A cold-hearted guinea pig: cardiovascular toxicity elucidated using *in vivo* and *ex vivo* models

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We studied the cardiovascular safety of a novel small molecule (Cmp A) before selection as a candidate for further development. *In silico* testing (DEREK, Lhasa) indicated that the structure contained a hERG pharmacophore. However, *in vitro* off target testing did not reveal any effect on cardiac ion channels. The compound was tested *in vivo* by oral administration to conscious guinea pigs instrumented with telemetry implants enabling the measurement of blood pressure, ECG parameters, and core body temperature. The guinea pigs were dosed with 80, 200, and 500 mg/kg Cmp A, resulting in exposure 20-, 30-, and 120-fold above the predicted free human therapeutic doses.

The *in vivo* study revealed a dose dependent, significant and long-lasting decrease in core body temperature and heart rate, significant

effects on ECG parameters (including a substantial QTc prolongation and decrease in PR interval), and a decrease in systolic pressure following dosing with Cmp A.

Changes in core body temperature are known to affect a number of cardiovascular parameters such as the ECG, heart rate, and blood pressure.

To investigate whether the effects by Cmp A were driven by the decrease in temperature and/or by a direct cardiovascular effect of the test compound, the compound was subsequently tested in the *ex vivo* Langendorff retrograde perfused guinea pig heart model. The temperature was either kept constant at 37 °C, or decreased to reflect the temperature changes observed in the *in vivo* study. The concentrations used for perfusion reflected the free concentrations measured in the *in vivo* study.

The *ex vivo* study revealed that at 37°C, Cmp A decreased the left ventricular pressure, decreased heart rate, and increased the PR interval and the coronary flow. There were no effects on QTc interval length.

When the temperature was decreased, a temperature dependent increase in QTc and PR interval was observed together with a decrease in heart rate.

In conclusion, the *ex vivo* study established that the QTc prolongation observed *in vivo* likely was induced by the decrease in temperature, whereas the decrease in heart rate and increase in PR interval may be caused directly by Cmp A.

P06-096
Deep learning methods to translate gene expression changes induced *in vitro* in rat hepatocytes to human *in vivo*

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In the pharmaceutical drug development process, *in vitro* cell lines and animal models are often used to evaluate the toxicity of a novel compound before progressing to human trials. However, relating the results of animal and *in vitro* model exposures to the human *in vivo* state presents a challenge. In previous work we demonstrated the ability of simple deep learning architectures (artificial neural networks, convolutional neural networks) to predict time series of human *in vitro* gene expression given rat *in vitro* gene expression following an exposure to a previously unseen compound. In this study, we leverage the relative abundance of rat *in vitro* and *in vivo* data to train a predictor model of human *in vivo* gene expression given human *in vitro* gene expression using Unsupervised Domain Adaptation (UDA).

For this study we used a subset of 45 compounds of the TG-GATEs database for which multiple time points and dosages (low, medium, and high) are available in all three domains (rat *in vivo*, human *in vitro*, and rat *in vitro*). Making use of replicates and controls, 720 learning examples were generated. Given the relatively limited number of learning examples, we identified four subsets of genes (consisting of 22 to 77 genes) reported in literature as being genomic fingerprints of relevant toxicological outcomes, such as genotoxicity. To maximise the number of learning examples available for training model performance is assessed using leave one out cross validation.

Following optimisation of the network architecture and training procedure, UDA predictions of rat *in vivo* gene expression consistently outperform predictions made by previously validated deep learning architectures and more traditional machine learning methods, such as k-nearest neighbours and random regression forest, for our identified gene sets. Moreover, the UDA model provides a prediction of time series of human *in vivo* gene expression given human *in vitro* following exposure to a novel compound. Cursory exploration of the latent spaces generated by these neural networks also suggests a promising new method for the classification of compounds by toxicity.

P06-097
Advancing human relevant solutions in science; The Lush Prize in 2020 and beyond

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The Lush Prize is pleased to present its innovative science and policy award strategy at EUROTOX 2019 and invites delegates to find out how their research could be eligible for future funding.

To support and sustain the activities of the very best in toxicology, R&D, policy and regulatory advancement, each Lush Prize year awards up to €400,000 across five key categories; Science, Young Researchers, Training, Lobbying, and Public Awareness. There is also a special sixth category – the Black Box Prize, which may award a further major prize of €290,000 (£250,000) for a breakthrough achievement in human-relevant toxicology.

The Lush Prize was established to address an urgently needed shift towards more 'fit for purpose' human-relevant toxicity testing methods, in order to meet the increasing demands of high throughput, chemical safety assessment. Another key aim of the Prize is to overcome the limitations of current preclinical regulatory requirements, which are of great concern with regard to their ability to predict human safety and disease pathogenesis, as well as being resource-intensive in terms of costs, time and animal use. This results in multiple scientific and ethical issues.

To address this, the prize continues to fund innovative new research worldwide and plays its part in the drive for the next generation of cutting edge technologies, especially with the success of its Young Researcher initiative across Europe, Asia and the Rest of the World, often providing bursaries to early career scientists who might otherwise face financial, political or social challenges in continuing their work.

Since 2012, the Prize has provided €2.5 million to a portfolio of outstanding achievements, including many *in vitro, in chemico* and *in silico* approaches in the field. Just a few examples include Multi Organ Chips (MOC), 3D Bioprinting, Adverse Outcome Pathways (AOPs), advanced evidence-based data strategies, AI based platforms for toxicity testing and new approaches in cancer drug screening. These technologies provide new direction to a future based on high quality, human based safety testing and disease research, having established a wealth of evidence- as well as attracting increasing regulatory interest – to date.

The presentation will provide an overview of the Prize and the success of more than 100 prize winning projects to date. **Nominations are now open for the 2020 Lush Prize.** Find out more, including how to submit a nomination at www.lushprize.org.

P06-098
Role of GSH as first line of defense against oxidative stress-induced cytotoxicity in SH-SY5Y cells exposed to sterigmatocystin

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Sterigmatocystin (STE) is a mycotoxin that has been shown to have a significant impact on human and animal health. Considering the limited number of study about the effect of STE on neuronal system and the impact of oxidative stress in the development of neurodegenerative disorders, the present study investigates the role of oxidative stress and intracellular defense mechanisms in human neuroblastoma (SH-SY5Y) cells after exposure to STE for 24 h. Our previously results demonstrated that in SH-SY5Y cells exposed to STE at different concentration (from 0.19 to 25 µM) for 24, 48 and 72 h, cell viability was reduced in a time and concentration dependent manner, showing a decrease ranging from 14% to 91% respect to the control. In the present study, increased reactive oxygen species (1.37- to 1.59-fold) were observed after 24 h of STE exposure at all concentrations tested (0.78, 1.56 and 3.12 µM). Sterigmatocystin exposure for 24 h resulted also in a depletion of intracellular reduced glutathione (GSH) levels (from 47% to 61%) and a decrease in GSH/GSSG ratio (from 75% to 85%) at the highest concentrations tested of STE. To determine the role of GSH in the protection against STE cytotoxicity, the effects of BSO and NAC pre-treatments were assessed. Pre-treatment with 60 µM BSO induced a decrease in GSH levels (from 41% to 79%) respect to no BSO pre-treated cells exposed to STE at all concentrations tested for 24 h. Additionally, pre-treatment with 1 mM NAC increased GSH levels (from 25% to 51%) respect to no NAC pre-treated cells after 24 h of exposure at STE 0.78 and 1.56 µM. No significant increase was observed at the highest concentration (3.12 µM), suggesting an inhibition of NAC effect. The decrease effect of BSO pre-treatment (from 20% to 73%) and the increase effect of NAC pre-treatment (from 25% to 83%) on GSH/GSSG ratio was observed in cells exposed to 1.56 and 3.12 µM STE. Our results suggest that STE could injure SH-SY5Y cells via oxidative stress and highlight the antioxidant role of the glutathione system. Moreover, BSO enhances the oxidative damage caused by STE while NAC shows an effective scavenger activity. However, further investigations about the effect of STE exposure on the antioxidant enzyme systems are needed.

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P06-099
Testing strategies for detection of endocrine disrupting potential

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Detection of endocrine disrupting chemicals has become a recent topic with a permanent increasing relevance. A battery of validated *in vitro* and *in vivo* test methods for the detection of endocrine disrupting potential is available and, furthermore, there is a steady effort to update the existing test methods or develop new methods to include new scientific approaches. Despite the volume and complexity of existing test methods, there is no single assay that is capable of determining endocrine activity or adversity. With the OECD 150 guidance document a helpful tool for interpreting the outcome of individual tests and compiling evidence on whether or not a substance may be an endocrine disruptor is given. Within the scope of the 3R principle, Eurofins BioPharma Product Testing Munich offers the whole battery of available validated *in vitro* studies which are described in the Conceptual Framework Level 2 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. Based on the OECD 150 guidance document, Eurofins Munich developed an *in vitro* testing strategy for the stepwise investigation of the endocrine disrupting potential for test chemical. The applicability of the developed test strategy was examined exemplarily using a test chemical. It could be shown that this testing strategy is a reliable and useful tool to step-wise approach test substances with unknown ED potential.

P06-100
Extended solvent selection for *in vitro* sensitization testing using GARD®

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The prevalence of allergies has increased since the industrial use of chemicals has grown. Certain substances, sensitizers, can induce allergic contact dermatitis which provoke symptoms that severely affect life quality. To prevent individuals from harmful exposure, chemicals are required to be safety tested. Historically, those tests have included animals but regulatory authorities, economic and public interests demand animal free methods.

The GARD platform is an *in vitro* state of the art assay developed for the prediction of sensitizers. It is based on a human dendritic-like cell line, SenzaCells, and analysis of gene expression post substance stimulation using machine learning based on pattern recognition. During the development of the GARD platform, two solvents were used; DMSO and Water. However, there is a wide range of substances, including complex mixtures, that are not soluble in either of those. Therefore, it is of high interest to increase the selectable array of solvents compatible with GARD.

A variety of solvents with the capability to dissolve polar and non-polar test items were selected. SenzaCells were stimulated with the pure solvents in a concentration range (0.1–5%). Cells exposed to solvent concentrations that did not generate cytotoxicity or changed the cell phenotype were further analysed by the GARD prediction model to ensure assay compatibility. To prove that the test substances dissolved in the solvents were bioavailable to the cells, and correctly classified, a known sensitizer was dissolved in the respectively solvent and used as a positive control.

We found a range of both polar and non-polar solvents that did not affect the cells, were predicted as non-sensitizers and where the positive control was predicted correctly. Hence, GARD is compatible with a wider range of solvents thus extending the applicability domain to include test items with low solubility in aqueous solution opening for testing of difficult to test substances like UVBCs and other mixture types.

P06-101
Primary human hepatocytes production for pharmacology, toxicology and basic research: four years experience

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Introduction: Primary human hepatocytes (PHH) *in vitro* models are the gold standard for a wide range of applications in biomedical research such as biological, pharmacological and toxicological studies. Researchers face many challenges in this area as complex ethical and legal framework, poor quality tissue and scarce tissue source for fully characterized specimens suitable for research.

Objectives: Consolidate a network that ensures a regular PHH supply aimed at research. Study the factors that may influence the obtainment and isolation of human liver tissues in order to increase the HPP quality and viability.

Materials & Methods: The program linked eight hospitals. The tissue was procured from patients undergoing planned liver resection surgeries due to primary or secondary tumours (living donors). The program follows the European legislation to ensure quality, safety and traceability of all the procedures.

PHH isolation was conducted through the two-step collagenase perfusion technique and the established in-house methods [1]. Cell viability, yield production, plateability and CYPs activity have been studied pre and post-thawing.

Results: Since 2015, 64 human liver tissues have been processed. In average, a PHH viability and cellular density of 84.22% and 14.05 M/gr were achieved respectively. 51% of cryo-preserved lots have been plateable (confluence >80%) with a viability post-thawing of 89,93% and 53,47% of recovery rate.

Statistical analysis concluded that items as age or chemotherapy dose were not relevant for the final cell viability or yield. The tissue weight and macroscopic aspect were extremely relevant to prevent any poor cell condition or further issues during PHH isolation and to improve the plateability after post-thawing. Therefore, donor acceptance criteria have been expanded to donors older than 65 years and limited to liver tissue weight bigger than 60gr.

Conclusions: PHH isolation is a complicated procedure which requires well-trained staff and a good cooperation with the surgical department performing the liver resections. Our team has developed a leading-edge technique for PHH isolation certified for induction, metabolism and transporters, highly qualified to be used in toxicology screening, hepatic genomics and proteomics, toxicokinetic and human metabolism.

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P06-102
Molecular docking and *in vitro* bioactivity of 5-fluoroindole derivatives on ER, aromatase and CYP1B1 activity in breast cancer cells

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Breast cancer is the second cause of death among female cancers and its incidence is increasing rapidly all over the world. Many studies have demonstrated that endogen estrogens and xenoestrogens play a role in the initiation and progression of breast cancer via two complementary pathways; by increasing cell proliferation via binding to estrogen receptor and by inducing oxidative DNA damage as a result of metabolism of estrogens to their reactive quinone metabolites. Melatonin, a natural indolic hormone, is known to be beneficial in breast cancer via modulation of estrogen receptor mediated effects as well as inhibiting local biosynthesis of estrogens by aromatase inhibition. Furthermore melatonin is reported to increase effectiveness and to reduce side effects of the conventional breast cancer therapy. The short half life as a consequence of rapid metabolic inactivation of melatonin has prompted investigators to synthesize novel melatonin analogues as therapeutic agents. The present study is aimed to investigate the potential effects of novel 5-fluoroindole derivatives on the selected targets that are involved in the initiation and progression phases of estrogen-induced breast carcinogenesis. Estrogen receptor (ER) agonist/antagonist effects were evaluated by proliferation assay in estrogen-dependent MCF-7. Inhibitory effects of the compounds on both aromatase, the enzyme responsible for local estrogen biosynthesis, and CYP1B1, the enzyme catalysing biotransformation of procarcinogens to their reactive metabolites, were investigated. Furthermore cytotoxicity of the compounds were evaluted in both non-tumorigenic breast epithelial cell line (MCF-10A) and two different breast cancer cell lines; in MCF-7 BUS estrogen-dependent and MDA-MB-231 estrogen-independent breast cancer cell lines. All of their effects were evaluated in comparison to melatonin activity. Possible binding of the compounds with selected targets is also explored by molecular modelling studies. Several of the compounds have ER antagonist, aromatase inhibitor and/or CYP1B1 inhibitory activitiy while some of them were effective against more than one target. Furthermore some of the compounds that were found to be effective on selected targets induced cytotoxicity in human breast cancer cell lines without having cytotoxic effect on non-tumorogenic breast cancer cell line. In conclusion, present data indicates that 5-flouroindol derivatives can be promising adjuvant agents for breast cancer therapy without causing cytotoxicity to the healthy cells. Further studies will be performed to draw the structure activity relation and to synthesize and evaluate the effectiveness as well as toxicity of the hit derivative(s).

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P06-103
Pre-clinical assessment of a dual-temperature operated heated tobacco product

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Heated Tobacco Products (HTPs) generate a nicotine-containing aerosol by heating a tobacco portion using an electrical device. As the tobacco is heated and not burned, the resulting aerosol is expected to contain substantially fewer toxicants at lower levels, as well as reduced *in vitro* toxicity compared to conventional cigarettes.

This study presents the pre-clinical assessment of a new HTP with two temperature settings ("eco", 315°C; "standard", 345°C) developed by Imperial Brands. The aerosol generated under both temperature modes was analysed and compared to 3R4F cigarette smoke under the ISO Intense machine-puffing regime. To demonstrate the tobacco is heated and not burned, the combustibility potential of the tobacco in the device was also assessed.

The HTP was found to produce lower levels of targeted cigarette smoke toxicants under both operating temperatures. The toxicant levels were substantially reduced across all chemical classes measured and were lower in the aerosol generated using the "eco" mode

than compared to the "standard" mode. In turn, the biological responses of cell cultures exposed to the HTP aerosol was substantially lower compared to 3R4F cigarette smoke under both HTP device operating temperature settings.

These results demonstrate the potential for the tested HTP to offer substantially reduced exposure to toxicants and biological toxicity compared to conventional cigarette.

P06-104
Comparison of *in vitro* and *in vivo* skin absorption rate of Spinosad product for veterinary use

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Spinosad, a pesticide derived by fermentation of the Actinomycete bacterium *Saccharopolyspora spinosa*, is a mixture of spinosyn A and spinosyn D. The product is used for the control of a variety of insect pests, such as poultry red mite in chicken farms (but do not permitted for use on food-producing animals in South Korea). Therefore, we intended to measure dermal absorption rate of Spinosad *in vitro* and compare with the *in vivo* dermal absorption rate for regulation's purpose. Spinosad was applied in label concentration (4114 ppm) on 500 μm of micro-pig' skins mounted on Franz diffusion cells. Receptor fluid (50% ethanol-PBS solutions) were taken after 1, 2, 4, 8, 12, 24 hours of application. Dermal tissues, tape strips, washing solutions and swabs were taken at 24 hours of application. All samples were analyzed using LC-MS/MS. 0.87 ± 0.76% of applied Spinosad was present in the receptor fluids and the residue in dermal tissue was 2.28 ± 0.66 ppm (0.48 ± 0.11%). 85.0 ± 5.23% of applied Spinosad did not penetrate into the skin within 24 hours and washed off. The total absorption rate of Spinosad was 1.35 ± 0.80% during 24 hours of application. The sum amount recovered in all analyzed samples was 86.69 ± 4.55% in this study. In *in vivo* study, Korean Hy-line brown layer chickens were treated with veterinary products containing Spinosad directly on the skin (Contact area: 60cm^2). After 24 hours of treatment, Spinosad concentration in the tissues was highest in the skin, followed by fat, feces, liver, and muscle. The estimated absorption rate of Spinosad in *in vivo* study was 33.58%. These results could support for drafting regulations for food safety on veterinary drugs and pesticides.

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P06-105
The GARD assay: a new *in vitro* testing strategy for skin sensitization

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The use of non-animal test methods, including *in vitro* studies, provides important tools to enhance our understanding of hazardous effects by chemicals and for predicting these effects on humans. The reduction of animals in toxicology research by encouragement of the development and validation of effective *in vitro* alternative methods or models is one of the outmost concerns at Eurofins BioPharma Product Testing Munich. In the last year several efforts have been made to develop alternative methods to assess the sensitizing potential of chemicals by addressing each single key event (KE) defined by the adverse outcome pathway (AOP). The first three KE are covered by the Direct Peptide Reactivity Assay (KE 1), the KeratinoSens™ (KE 2) and the human Cell Line Activation Test (KE 3), while KE 4 event is still only covered by the *in vivo* method LLNA (Local Lymph Node Assay). These three *in vitro* methods are a good way to qualitatively evaluate the sensitizing potential of a substance but they do not allow a potency assessment if the substance is a sensitiser. Therefore, a new testing method, the GARD™ (Genomic Allergen Rapid Detection) Assay, has been developed. The GARD™skin determines in the first step (input finder) the test substance concentration that gives 90% relative viability of the cells and then examines in three independent main stimulations this concentration. A set of 200 different markers is measured in a nanoString analysis and pattern recognition using Support Vector Machines is used to decide whether it is a sensitiser or not. In the event of a positive outcome, the potency of the sensitizing potential can be assessed in a second step using the GARD™potency assay. Besides the fact, that the GARD Assay provides more accurate, more sensitive and more specific results when compared to the established OECD test guideline methods, it was demonstrated that this method is a reliable asset and that it completes the lack of the current *in vitro* testing strategies for skin sensitization when it comes to potency assessment.

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P06-106
Development of an alternative method for the evaluation of the anti-pollution efficacy of cosmetic products using reconstructed human tissues

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Introduction: Air pollution caused by industries, vehicles, cigarettes smoke and other sources together with solar radiations (expecially in the UV range) is known to have an harmful effect on human health, in particular on human skin. These elements are implicated in several skin disorders and pathologies, but also in extrinsic ageing which leads to premature skin ageing due to a complex cascade of reactions initiated by the generation of reactive oxygen species (ROS). Daily application of specially formulated anti-pollution skin care products may improve skin barrier functions, thus providing a skin defense mechanism that can contribute to the slowing of both extrinsic ageing and other pollution-dependent skin damages.

Objectives: The aim of the study is to develop an *in vitro* test useful to predict the protective efficacy of a cosmetic product against pollution and environmental stresses.

Materials and methods: We used Reconstructed Human Epidermis (RHE) as a skin tissue model. In order to mimic the exposure to environmental pollution, we used a standard of urban pollution (urban dust) and irradiation with UV rays using a solar light simulator. A first set of tissues was treated for 1, 2 and 4 hours with urban dust (0.5 mg/ml). A second set of tissues was irradiated with UVA rays (315–400 nm) for 1, 2, 3, 4 and 5 minutes (irradiance 25 W/m^2). As negative control we used tissues kept in maintenance medium in the dark. As positive control we used ascorbic acid (0.2 mg/ml). As a marker of oxidative stress we measured the ROS production by oxidation of 2'-7'-dichloro-fluorescein diacetate to 2'-7'-dichloro-fluorescein.

Results: In irradiated tissues we observed a time-dependent increase in ROS production. In RHE tissues treated with urban dust we observed a significative increase of ROS production yet after 1 hour of contact (+165%). The effect is much more evident after 2 and after 4 hours (+333.6% and +390.8%, respectively). The effect is reversed by ascorbic acid.

Conclusions: RHE is a good model suitable for the study of anti-pollution efficacy of cosmetic products. The protocol developed on RHEs tissues stimulated with a combination of a urban pollution standard and UV rays can be easily applied to the study of the protective efficacy of a cosmetic product against the oxidative stress induced by environmental pollution.

P06-107
HPLC-MS/MS based DPRA passed OECD TG 442C requirements and extends the application domain of this assay to complex substances and mixtures

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The OECD Test Guideline N°442C, describes the Direct Peptide Reactivity Assay (DPRA) which addresses the human health hazard endpoint skin sensitisation, following exposure to a test chemical. While the HPLC-UV based DPRA identifies dermal sensitizers with approximately 80% accuracy, it displays limitations to accurately classify certain chemicals, and is inapplicable to assess complex mixtures.

After having demonstrated the advantage of HPLC-MS/MS over HPLC-UV for performing such an assay, we are now presenting the demonstration of the compliance of the HPLC-MS/MS methods for accurately assaying both reference peptides with respect to the OECD TG 442C requirements, then their application for performing the proficiency test requested to fully comply with the guideline.

Results of both Lysine-peptide depletion, on one hand and Cysteine-peptide depletion, on the other hand, by the 10 reference substances are online with the guideline requirements.

Furthermore, these 10 substances were accurately classified into sensitizers and non-sensitizers after application of the prediction model.

As a consequence, HPLC-MS/MS based DPRA considerably extends the field of application of the assay towards complex substances and mixtures.

P06-108
Use of organotypic small intestinal tissue model for drug induced gastrointestinal toxicity studies

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The objective of the study is to evaluate the utility of a 3D primary human cell based small intestinal tissues (SMI) as an investigational tool for drug induced gastrointestinal (GI) toxicity. GI toxicity often leads to late-stage drug attrition or chronic diseases. Animal models have been widely applied for assessing GI toxicity as preclinical test models, however, animal models are expensive, time consuming and less translatable to human conditions. Hence, *in vitro* models are needed to guide the design of molecules or dosing schedules that mitigate safety risks in humans. Here we tested a) N = 5 therapeutic compounds for which animal toxicity studies were not predictive of human toxicities. Drugs that were well tolerated (N = 3) or known to cause gut irritation/toxicity (N = 2) in humans were used as negative and positive controls, respectively. MTT viability and tissue barrier integrity as measured by transepithelial electrical resistance (TEER) were used as endpoints to monitor drug induced GI toxicity. The results showed that the SMI system detected drug induced disruption of intestinal barrier function (TEER) in 5/5 problematic drugs with human GI toxicity at concentrations within or below 30x clinical exposure levels. Importantly, the SMI system showed no effect within 1,000x clinical exposure levels for the three negative controls. We found that TEER measurement was more sensitive than the MTT viability assay. Using the TEER endpoint we also assessed and confirmed gut barrier dysfunction by two known GI toxicants SN38 (metabolite of Irinotecan) and Ibuprofen in a time and concentration dependent manner. Irinotecan, the parental drug for SN38 did not induce toxicity on the intestinal tissue model. Changes in barrier integrity as measured by TEER was found to be a valuable endpoint as a predictive tool to assess toxicity of clinically-relevant drug exposures. In conclusion, the *in vitro* human primary cell-based small intestinal tissue model may serve as a promising tool to predict GI toxicity in humans.

P06-109
Glutamate in the apical side was absorbed and metabolized but not passed to the basolateral side in polarized monolayer culture of human epithelial cell line

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Previous *in vivo* studies indicated that virtually all the dietary glutamate is metabolized in the gut and that glutamate intake does not increase circulating glutamate concentrations. However, it has not been clarified which kinds of cells in the gut contribute to the glutamate metabolisms. Present study hypothesized that intestinal epithelial cell layer metabolizes and controls entry of dietary glutamate into the body, since all the dietary glutamate has to pass this layer which possesses enzymes metabolizing glutamate. Therefore we investigated fate of dietary glutamate in polarized monolayer culture of human intestinal epithelial cell line Caco-2.

Caco-2 cells were cultured and differentiated to polarized cells in a dual cell culture system. [U-^{13}C]glutamate (300 μM) was added to inner chamber (apical side), and medium samples of inner and outer (basolateral side) chambers were collected after 1, 3, 6, 10, 24 h of culture. Concentrations and ^{13}C-enrichment of each amino acid were measured using amino acid analyzer and LC-MS/MS, respectively.

Apical [U-^{13}C]glutamate decreased to nearly zero (2% of initial value) after 24 h culture while only minor part (< 4% of added) was found in basolateral side, indicating almost all the apical glutamate-C taken up by the cells and metabolized. Indeed some proportions of ^{13}C were detected in alanine, aspartate and ornithine, which resembles the fate of dietary glutamate-C in the gut in *in vivo* studies. These results strongly suggest that intestinal epithelial cell layer limits entry of dietary glutamate into internal body. Thus, intestinal epithelial cells

would play roles to maintain glutamate homeostasis in spite of dynamic changes of glutamate entry from foods. This is the first *in vitro* evidence indicating roles of intestinal epithelial cells in metabolisms of dietary glutamate. Based on the finding of the present study, underlining mechanisms for safety of dietary glutamate will be discussed.

P06-110
U-SENS™: New perspective for the evaluation of chemicals interfering with FITC

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The U937 Cell Line Activation Test U-SENS™ is one of the *in vitro* OECD endorsed test method (TG442E) along the skin sensitization AOP (Adverse Outcome Pathway). It could be used for hazard identification, potency and risk assessment. Experimental procedure: the human myeloid U937 cell line (CRL-1593.2) is treated for 45 h with a dose-range of different substances. The CD86 (B70/B7-2) costimulatory molecule expression is then measured by flow cytometry using the FITC-coupled mouse anti-human CD86 monoclonal antibody. Propidium iodide is used as the viability marker to exclude dead cells from the analysis. Some substances such as well-known hair dyes increase the auto-fluorescence of cells in the FITC channel by flow cytometry, compromising the quantification of the CD86 induction. To avoid such interferences and biases, an alternative of the fluorophore FITC (excited at 488 nm), the APC (excited at 633 nm) has been evaluated. Based on the study of 4 interfering substances (1,4-phenylenediamine (pPD), 2-Methyl-p-phenylenediamine sulfate salt (pTD), 2,4,5,6-Tetraaminopyrimidine Sulfate (TAP), and 1,3-Phenylenediamine (mPD)), the non-specific fluorescence increase was drastically reduced with APC leading to clear dose response curves. The approach using an APC-coupled mouse anti-human CD86 monoclonal antibody was further validated on a set of 24 substances composed of an equal number of sensitizers and non-sensitizers. All acceptance criteria applied to the standard protocol were met (CV70, EC150, CD86 baseline expression, …). In conclusion, the use of the alternative APC coupled antibody opens new perspectives to evaluate skin sensitizers interfering with the fluorescence at 488 nm in the U-SENS™ test method (OECD 442E).

P06-111
Evaluation of renal and hepatic metabolism of short chain and long chain parabens in *in vitro* systems.

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Parabens are the most commonly used antimicrobial agents added in foods and cosmetics. They are odourless, colourless and inexpensive. These compounds have been widely used for decades but in the last 20 years suspicion has been raised regarding their potential endocrine disrupter activity. *In vitro* studies have reported that the main metabolic route of these compounds occur through hydrolysis into p-hydroxybenzoic acid by human carboxylesterases (hCES) in the liver. To better understand the effects of these chemicals and their metabolic rates, the four parabens, Methylparaben, Ethylparaben, Propylparaben, Butylparaben were studied in two hepatic *in vitro* systems, HepG2 and HepaRG cell lines and the renal proximal tubular cell line RPTEC/TERT1. The effect of CES inhibition was also studied using the CES inhibitor, Paraoxon-ethyl. Biotransformation overtime was assessed using HPLC and LC/MS time of flight. The potential effect on mitochondrial function was evaluated by the Seahorse bioanalyser. All four paraben were rapidly metabolised to p-hydroxybenzoic acid within four hours in HepaRG cells and exhibited an inverse correlation of metabolism time to sidechain length. Paraoxonethyl (0.5 µM) inhibited metabolism to p-hydroxybenzoic acid but promoted metabolism to glucuronidated metabolites. Glucorination was absent in HepG2 and RPTEC/TERT1 cells. Paraben metabolism to p-hydroxybenzoic acid was slower in HepG2. RPTEC/TERT1 cells were capable of long chain paraben metabolism, but exhibited a complete lack of methyl paraben metabolism. There was no evidence of (mito) toxicity of the parabens or their metabolites up to 200 µM for up to 5 days of exposure in HepaRG. The findings show that HepaRG and RPTEC/TERT1 exhibit the expected tissue type paraben metabolism, i.e. CES1 and CES2 in hepatocytes and CES2 only in RPTEC/TERT1. These results predict that short chain parabens entering the blood stream via oral or dermal exposure, would be rapidly metabolised via CES1 hepatic metabolism and via CES2 in several tissues to non-toxic metabolites.

P06-112
Demonstration of hepatocyte-targeted siRNA transfection and gene silencing in the micro-patterned hepatocyte co-culture system (HEPATOPAC®)

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The HEPATOPAC® model, an *in vitro* bioengineered co-culture of primary hepatocytes and fibroblasts, has demonstrated invaluable utility for liver-based safety, metabolism, and efficacy evaluation for small molecule drug candidates, due to its longevity and close resemblance to the *in vivo* liver. Here, we identify a method to specifically deliver small-interfering RNAs (siRNA) into the hepatocytes in the HEPATOPAC® co-cultures, by using a commercially available, non-liposomal transfection reagent that targets hepatocytes (PromoFectin-Hepatocyte). Upon the transfection of a fluorescent control siRNA, fluorescent signal was detected mainly in the hepatocyte islands, but not in the surrounding stromal cells. When siRNA targeting a cytochrome P450 enzyme was transfected in HEPATOPAC® cultures, a time-dependent reduction in the CYP activity following transfection was observed. The results provide a proof of concept that HEPATOPAC® platform is amenable to hepatocyte-specific siRNA transfection and siRNA-mediated gene knockdown, which can be useful in elucidating the hepatocellular mechanisms in various research areas, aiding in reaction phenotyping assessment, as well as *in vitro* safety and efficacy studies for novel RNA therapeutics.

P06-113
EU-ToxRisk knowledge infrastructure – effective sharing of data, results and knowledge

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EU-ToxRisk – An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century – is a European collaborative project funded by the EU Framework Programme for Research and Innovation, Horizon 2020. Its complex structure with almost 40 partners requires effective solution for sharing of data, knowledge and tools, first between consortium

partners and later with the complete scientific community. The Knowledge Infrastructure (KI) of EU-ToxRisk is designed as an one-stop shop effectively organising data and knowledge sharing and facilitate the usage of *in silico* prediction and risk assessment workflows. It builds the link between, on the one hand, the data and tool providers and, on the other hand, the read-across experts and risk assessors as the consumers of the data and users of the provided tools, in this way, supporting the ambition goal to generate new testing strategies and read-across applications for the assessment of human health risks that are based solely on *in vitro* and *in silico* new approach methods (NAMs).

To be able to serve the requirements of all stakeholders, the KI consists of different modules that include long-term data storage solutions, linked visualization and modelling tools, test methods and *in silico* methods repositories, case studies and AOP collaborative sections. One of its central components is the ToxDataExplorer, which makes project data centrally stored on BioStudies, the EU resource for data capturing and storing, available via advanced application programming interfaces (APIs). This technology allows customized searching and filtering even across different datasets and enables a direct access and use of the data in a wide range of analysis or modelling workflows (e.g. KNIME) and programming languages (e.g. R, Python).

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P06-114
The cytotoxic effect of irradiation on epidermal cells is only partially and temporary alleviated by sea buckthorn oil treatment

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Introduction: Sea buckthorn (*Elaeagnus rhamnoides L.*) is a unique medicinal and aromatic plant, rich in fatty acids and flavones. Sea buckthorn alcoholic extracts and seed oil were tested repeatedly for antioxidant, antitumor and regenerative properties.

Aim: Our study aimed to test whether cold pressed sea buckthorn oil had a protective effect against UVA radiation on human skin cells.

Materials and methods: Two epidermal cell lines (normal human epidermal keratinocytes –NHEK (Lonza) and dysplastic oral keratinocytes – DOK (ECACC) were irradiated with UVA for 30 minutes and tested for changes in cell adherence and proliferation in presence and absence of non-toxic doses of cold pressed sea buckthorn oil. Treatment dose of oil was selected by MTS and LDH assays. Oil uptake was assessed by OilRed staining. Cell adherence and proliferation were tested by real-time impedance reading and video microscopy.

Results: UVA radiation of both normal and dysplastic epidermal cells impaired both cell adhesion (during the first two hours of substrate attachment) and proliferation in both lines, but only transiently. During the 24 hours post UVA exposure, irradiated cells regained proliferative activity to match the non-irradiated ones. Treatment with cold pressed sea buckthorn oil further impaired the adhesion and proliferation in the irradiated group, but showed no effect on non-irradiated cells. 72 hours pretreatment of cells partially decreased the effect of UVA radiation, but in the long term it favored the proliferation of dysplastic cells.

Conclusion: Cold pressed sea buckthorn oil could have a protective effect against detrimental UVA irradiation, but only as a preventive treatment. In the long term, the oil treatment can enhance the proliferative abilities of dysplastic cells, although it shows no effect on normal cells. The benefits and disadvantages of skin applications should be carefully weighed.

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P06-115
Drug permeability and safety screening using a reproducible *in vitro* 3D-human small intestinal tissue model

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Reliable and highly reproducible *in vitro* models of small intestine are a paramount in prediction of safety and bioavailability of compounds intended for oral administration. Here we describe reproducible production of a recently developed *in vitro* 3D-human small intestinal (SMI) microtissue model and its use in predicting the drug absorption. Characterization of the microtissues included evaluation of structural features, barrier properties, and expression of drug transporters and drug metabolizing enzymes. The quality and reproducibility of tissue production was compared in two independent production facilities (MatTek, Ashland, MA, USA and IVLSL, Bratislava, Slovakia) by measuring TEER and Lucifer Yellow (LY) leakage. To evaluate the suitability of the microtissues for drug absorption, the apparent permeability coefficient (Papp) values for a panel of benchmark drugs with known human absorption values were measured. Drug-drug interactions were examined using drugs known to be substrates or inhibitors of efflux transporters. Results showed that tissues are highly reproducible with physiological TEER values averaging 146.4 ± 20.8 Ω^*cm^2 (% CV = 14.2%) in the USA (N = 128 lots) and 162.6 ± 10.2 Ω^*cm^2 in Slovakia (N = 60 lots) facilities. The real-time PCR analysis revealed that microtissues expressed all tested drug transporters and metabolizing enzymes known to be present *in vivo*. Drug permeation analysis with 18 selected drugs showed that the intestinal microtissues could discriminate between low and high permeability drugs with 94% accuracy. The *in vitro* Papp values correlated well with human absorption data ($r^2 = 0.91$), while correlation of CaCo2 results showed $r^2 = 0.71$. SMI microtissues show an active efflux transport as when exposed to substrates of ABC pumps, the resulting drug efflux ratios were > 2.0. Moreover, addition of efflux transporter inhibitors reduced the drug efflux ratio while increasing the bioavailability of the test drugs, providing further evidence of ABC transporter activity. In conclusion, the SMI microtissues appear to be a promising tool for predicting safety and bioavailability of orally administered drugs.

P06-116
Method for assessment of intracellular level of cadmium and thallium

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Cadmium (Cd) and thallium (Tl) are highly toxic metals. Their deleterious effect on human health is well established, and their mechanisms of toxicity are still explored on *in vitro* model. To accurately link specific change in the cell with the exposure to metals, the intracellular level of metal should be determined. Therefore the aim of this study was to develop method that will enable accurate determination of intracellular level of Cd and Tl of cells treated with these two metals. Cells (HepG2; at planting density of $2.5x10^5$ cells/mL) were treated with Cd or Tl (1 and 10 mg/L) for 24 h. Afterwards, cells were washed in PBS buffer and resuspended in 0.5 mL of PBS buffer. Cells were digested with equal volume of 2% HNO_3. For monitoring intracellular level of Cd and Tl, ICP-MS (ELAN DRC-e, Perkin Elmer, Singapore 2008) was applied. For validation experiments Cd and Tl standards as well as reference materials CRM DORMA 4 Fish protein (NRC Canada) for Cd and NCS ZC 73028 Rice (China National Analysis Center) for Tl were used. In concentration range tested (0.2–200 µg/L) developed method was linear and correlation coefficient (R^2) for Cd was 0.9999 with intercept 0.0732 while for Tl was 1.00009 with intercept - 0.1497. Limit of detection (LOD), repeatability and trueness for Cd were 0.020 µg/L, 2.9%, 96.6% and for Tl 0.097 µg/L, 3.7% and 102%, respectively. Developed method was applied to cells treated with Cd or Tl. The intracellular level of Cd and Tl was concentration-dependent. At higher concentration higher intracellular level of Cd and Tl were detected. However, higher uptake of Cd in comparison to Tl was observed (at concentration level 1 mg/L; 10% for Cd in comparison to 1% for Tl) that can explain higher toxicity of Cd to cells. In conclusion, validation parameters indicate that developed method is reliable and accurate for intracellular assessment of Cd and Tl and enabled following low levels of Cd and Tl within the cell. Furthermore, from obtained results it can be concluded that cells more readily accumulate Cd than Tl.

P06-117
Development of a novel human 3D *in vitro* model for evaluating new anti-fibrotic drugs

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Background: The discovery and development of anti-fibrotic therapies remains heavily reliant on animal testing. There is an urgent need to develop robust and relevant *in vitro* models to support the identification and preclinical evaluation of potential new anti-fibrotic drugs. To this end, we have developed and characterized a novel human 3D liver co-culture model using our proprietary ORGANDOT™ platform.

Methods: Liver ORGANDOT cultures (5mL comprising 50K hepatocytes, 1.5K Kupffers, 3K stellates) were created on 12mm MilliCell CM inserts and maintained at air-liquid-interface in hepatocyte maintenance medium (MM250) for up to 28 days. The viability of the cultures was measured using Promega's CellTiter-Glo® 3D cell viability assay and CYP3A4 activity was measured using Promega's P450-Glo™ CYP3A4 assay. To demonstrate Kupffer cell functionality, ORGANDOT cultures (± Kupffer cells) were treated with 10mg/mL LPS for 24 hours and IL-6 secretion measured by ELISA. For fibrosis induction experiments, ORGANDOT cultures (hepatocytes, Kupffers, stellates) were maintained for 3 days before being treated with 0.5ng/mL Transforming Growth Factor-b1 (TGFb1) for 4 consecutive days ± SB525334 (0.01mM, 0.1mM, 1mM, 10mM). On day 7, the cultures were either used for functional assays (ATP content, CYP3A4 activity, albumin secretion, or hyaluronic acid secretion), lysed for RNA extraction and qRT-PCR (COL1A1, ACTC2, OPN, TIMP2), or fixed and processed for immunostaining (collagen I and SMA).

Results: The liver ORGANDOT co-cultures not only maintained viability and functionality for up to 28 days in culture, but could also be treated with TGFb1 to induce a fibrotic phenotype. The TGFb1-treated ORGANDOT cultures showed a decrease in hepatocyte function and a concomitant increase in fibrogenic gene expression (COL1A1, ACTC2, OPN, TIMP2), hyaluronic acid secretion, and collagen I deposition. Furthermore, co-administration of an ALK5 inhibitor was able to completely prevent these fibrotic changes and rescue the functionality of the cultures.

Conclusion: These data demonstrate the potential utility of this novel *in vitro* model for for research into the mechanism of hepatic stellate cell activation and fibrosis induction / drug induced hepatotoxicity and for testing the efficacy of new anti-fibrotic drug candidates.

P06-118
Development of a subacute 28-day respiratory toxicity assay using an *in vitro* human airway model

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Knowledge of subacute (28-day) respiratory toxicity potential is an important component of establishing safe use of chemicals and consumer products. The current work describes efforts to develop an alternative, non-animal method for determining subacute respiratory toxicity using the EpiAirway™ *in vitro* human airway model. Initial acute toxicity experiments were conducted by exposing EpiAirway tissues to four concentrations of test chemicals via apical application using either aqueous or corn oil vehicles for three hours. After exposure, the test chemicals were rinsed off and the tissues were incubated for an additional 21 hours. An IC75 concentration (concentration required to reduce the endpoint value to 75% of vehicle exposed controls) was determined from the dose-response data using barrier function (determined by measuring transepithelial electrical resistance (TEER)) and tissue viability (MTT assay) as endpoints. Based on the determined acute IC75 value, EpiAirway tissues were exposed to additional serial dilutions of the test chemicals, using the IC75 as the baseline dose. Tissues were apically exposed for three hours, followed by rinsing, every Monday, Wednesday and Friday, with TEER measured prior to each dose application. Experiments were continued for at least 30 days to determine no-observed-adverse-effect level (NOAEL) doses. Rank ordering of NOAEL levels obtained for 8 chemicals was as follows: formaldehyde << butyl amine < oxalic acid << vinyl acetate < morpholine < methyl methacrylate << dimethylacetamide < ethanol. Expansion of the data set to include additional chemicals of different classes, chemical structures and physical properties is ongoing. These results indicate that *in vitro* airway tissue models using TEER as a convenient non-destructive endpoint are a promising alternative to animal tests for assessment of subacute 28-day respiratory toxicity and NOAELs. With further *in vivo* correlation and validation, this test may be a useful non-animal alternative for determining safe human subacute exposure levels for inhaled chemicals.

P06-119

This abstract has been withdrawn.

P06-120
Mechanical strain mimicking breathing influences nanoparticle induced effects on A549 cells

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The effects of engineered nanomaterials on human health are still intensively studied in order to facilitate their safe application. However, relatively little is known how mechanical strain as induced in alveolar epithelial cells by breathing movements modifies biological responses to nanoparticles. In a previous study, gene array data, qPCR, and ELISA revealed an amplified effect of 25 nm amorphous colloidal silica NPs when cells were mechanically stretched in order to model the physiological mechanical deformation during breathing. Gene expression alterations showed a surprising similarity to those known to be induced by TNFα. Nanoparticle uptake studies revealed that elevated intracellular NP accumulation was not responsible for the observed effect. [1] In this study, we aimed at further elucidating the mechanisms responsible for the modulation of the cellular response by mechanical strain. In order to get deeper insight into the question of the specificity of this response, A549 cells were exposed to various types of NPs under dynamic and static culture conditions. For a better understanding of the molecular mechanisms involved, the level of reactive oxygen species (ROS) production at the various conditions was measured. In addition, the translocation of RelA p65 and NF-κB2 p52 into the nucleus was analysed. Overall, the inclusion of mechanical strain into *in vitro* models of the human lung may have a strong influence on the test results.

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P06-121
The use of a 0.20μm particulate matter filter decreases cytotoxicity in lung epithelial cells following air-liquid interface exposure to motorcycle exhaust

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Our study was designed to establish and apply a dynamic *in vitro* model for direct exposure of human cells to gaseous contaminants to investigate the cellular responses to airborne chemical exposures and investigate whether the use of a 0.20 μm particulate matter (PM) filter reduced the cytotoxicity induced by motorcycle exhaust (ME), a mixture of gases and particles, in lung epithelial cells cultured in air-liquid interface (ALI) inserts. The concentrations of PM, carbon monoxide, carbon dioxide, total hydrocarbons (THC), total volatile organic compounds, and nitrogen oxides were measured. Lung epithelial cells were exposed to clean air, fME, or non-fME in the ALI chamber. Cell relative viabilities (CRV) and the reactive oxygen species (ROS) generation were determined. Our results revealed that PM and THC levels were significantly reduced, as compared with non-fME. When compared with the clean air exposed group, the CRV in both fME and non-fME-exposed group was significantly reduced ($p < 0.001$), while their ROS generation were markedly increased ($p < 0.001$). When compared with non-fME-exposed group, the CRV and ROS generation were significantly improved following fME exposure ($p < 0.05$). As a result, PM and THC concentrations were decreased approximately 90% and 22.71%, respectively, the CRV was improved from 40.4% (non-fME) to 55.7% (fME), and the increased ROS generation by non-fME was decreased about 51.6%. Our results provided evidence that levels of PM and THC in ME were significantly reduced, and oxidative stress were significantly improved after filtration as compared with non-fME.

P07 – Inflammation

P07-001
Comparative evaluation of hemantane and diclofenac topical formulations on complete Freund's adjuvant-induced inflammation in rats

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Hemantane (N-2(adamantyl)hexamethylenimine hydrochloride) demonstrates pronounced analgesic and anti-inflammatory effect with intraperitoneal (i.p.) administration in rodents.

The purpose of the study is to evaluate the effect of hemantane 5% topical gel formulation in comparison with diclofenac 1% topical gel in white outbred male rats with inflammation induced by complete Freund's adjuvant (CFA).

Methods and results: 5% hemantane gel was prepared by the experimental technology department of the Zakusov Institute of Pharmacology. 5% hemantane gel and 1% diclofenac gel (Hemofarm, Serbia) were applied locally to the left hind paw of rats weighting 220 to 285 g daily for two weeks, starting one day before the subplantar injection of 0.1 ml CFA (Sigma-Aldrich, USA) into the left hind paw. Rats of the control group received only the CFA injection. Edema of the metatarsus and ankle joint of the injuered hind paws was measured with a caliper on days 3, 6, 9 and 12 after the CFA injection.

5% hemantane gel reduced the diameter of the injured metatarsus by 50.0% and 20.8% on day 3 and 6 respectively, and the diameter of the ankle joint decreased by 50.0–71.4% on all days of the experiment, while edema reduction in rats receiving 1% diclofenac gel did not significantly differ from the control group. Compared with intact rats, thymus involution (by 26.7%) and spleen hypertrophy (by 15.5%) were registered in the control group of rats on day 14 after CFA injection. 5% hemantane gel and 1% diclofenac gel did not significantly influence the mass ratio (organ weight in mg / rat body weight in g) of thymus and spleen. Interestingly, the most pronounced spleen hypertrophy (+71.8% compared with intact rats) was recorded in animals that received 1% diclofenac gel.

5% hemantane gel administered topically to the hind leg of rats for two weeks exhibited no toxic effects; in contrast, the toxicity of 1% diclofenac topical gel was made evident by the death of 4 rats out of 11 in the group while no animals died in the control group of rats.

Conclusion: Hemantane 5% topical gel formulation was more effective than diclofenac 1% topical gel formulation in reducing the left hind paw CFA-induced edema in rats and, in contrast to 1% diclofenac gel, exhibited no toxic effects when administered daily for two weeks.

P07-002
Methotrexate-induced intestinal mucositis in the rat

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Methotrexate (MTX) is used for the treatment of many diseases, including psoriasis, rheumatoid arthritis and various neoplastic diseases. Nevertheless, among various adverse effects MTX causes intestinal mucositis and diarrhea. Mucositis incidence can vary with the drug regimen, and we therefore aimed to investigate the effects of MTX in rats following a single or a repeated treatment.

Male Wistar rats (n=8/group) were injected with vehicle (PBS), MTX (7 mg/kg, i.p.) for 3 consecutive days or MTX (25 mg/kg, i.p.) once. Body weight and diarrhea (4-scale score) were assessed over the test period, and inflammatory and histopathological responses were investigated 5 days after the first injection. The potential protective effects of sucralfate were then evaluated in a separate experiment.

In contrast to controls, no gain in body weight was observed in both MTX regimens. A single treatment at 25 mg/kg did not induce signs of mucositis, while MTX (7 mg/kg/day for 3 days) increased the diarrhea score and its incidence rate compared to controls (+1.4 and 62.5%, respectively, $p < 0.05$), increased the ileum MPO activity (+3640%, $p<0.05$), ileum TNF-α levels (+364%, $p < 0.001$), tended to reduce ileum GSH levels (-25%, $p = 0.09$) and induced a marked disruption of the intestinal architecture. The repeated administration of a 7 mg/kg MTX daily dose therefore induced a clear inflammatory response, a modification of mucosal integrity, and a moderate diarrhea, while a single administration of a higher MTX was inactive. Sucralfate (300 and 600 mg/kg, p.o. for 4 consecutive days), failed to reverse the effects of repeated MTX administrations.

These findings suggest that the repeated MTX treatment-induced gastrointestinal toxicity in rats shows similarities with clinical intestinal mucositis manifestations. This rat experimental mucositis model therefore offers a promising tool for evaluating the side effects of novel chemotherapeutic agents or the efficacy of potential treatments against chemotherapy-induced mucositis. The fact that sucralfate did not reverse MTX-induced mucositis is consistent with the absence of effective clinical treatment.

P07-003
Dietary advanced glycation endproducts and glucocorticoid resistance, are the two linked?

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Protein- and sugar-rich food products processed at high temperatures contain considerable amounts of dietary advanced glycation endproducts (dAGEs). The effect of dAGEs in humans, and especially in the human gastro-intestinal (GI) tract, is still relatively unclear. Our previous studies have shown that specifically protein-bound dAGEs induce a pro-inflammatory reaction in human macrophage-like cells and that protein-bound dAGEs are not hydrolysed in a human GI *in vitro* model [1]. Indicating that dAGEs enter the human GI tract with pro-inflammatory characteristics. People that could potentially suffer from these effects are inflammatory bowel diseases (IBD) patients. Glucocorticoids are very effective anti-inflammatory drugs and widely used in IBD patients. However, approximately 20% of IBD patients do not respond to glucocorticoids and the reason for this is largely unknown.

The present study aimed to investigate whether the dAGE-induced inflammation could be mitigated by glucocorticoids and what the possible underlying mechanism is of glucocorticoid resistance caused by dAGEs. Human macrophage-like cells were exposed to 10% (v/v) dAGEs or 3 µg/ml LPS with and without 3nM cortisol. This pro-inflammatory response was measured by IL-8 secretion and then modulated by adding various pharmacological compounds interfering in different steps of the anti-inflammatory mechanism of glucocorticoids: rapamycin, quercetin, and theophylline. Additionally, intracellular reactive oxygen species (ROS) were measured. The results show that dAGEs induced glucocorticoid resistance which could be mitigated by quercetin. Additionally, intracellular ROS formation was induced by dAGEs, which was diminished by quercetin. This indicates that dAGE-induced ROS is an underlying mechanism to dAGE-induced glucocorticoid resistance. Our findings indicate that food products with a high inflammatory potential can induce glucocorticoid resistance. This study shows for the first time the phenomenon of dietary AGE-induced glucocorticoid resistance due to the formation of ROS. The type of food that IBD patients eat may be of large importance to IBD patients suffering from glucocorticoid resistance. These results are part of a larger study on the health risk of dAGEs in processed food.

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P07-004
Immunomodulatory effects of *Alternaria alternata* mycotoxins: down-streaming effects from the cell membrane

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Purpose: *Alternaria alternata* fungi produce a wide variety of secondary metabolites. Among these, several are toxic for mammalian cells and can be classified as mycotoxins. In recent times, several mycotoxins were found to have an immunomodulatory potential and alternariol (AOH), one of the main *Alternaria* toxins, was described to modulate the lipopolysaccharide (LPS) induction of pro-inflammatory cytokine release in macrophages [1–2]. Even though these effects are robust and involve several classical inflammatory pathways, the molecular events triggering these responses are not completely elucidated.

Methods: In this study, we investigated the immunomodulatory mechanisms of two *Alternaria alternata* mycotoxins. In particular, we used the immunoactive AOH and compared it to the perylenequinone toxin altertoxin II (ATXII), both in a concentration range between 0.1 and 1 µM. For the study monocytes derived from acute monocytic leukemia cell line (THP-1) were used. Differentiation into macrophages was obtained incubating the monocytes with 10 ng/ml PMA for 72 h [2]. Recruitment of the Toll-like Receptor 4 (TLR4) was monitored via immunofluorescence, and membrane properties were characterized via membrane fluidity assay and live cell imaging.

Results: Both toxins actively modulated the morphology of the cell membrane, however the effect of AOH was strongly modulated by the concomitant presence of a pro-inflammatory stimulus (LPS). The effect of ATXII on the macrophages membrane appeared independent on the activation status. However, the incubation with ATXII altered the localization-recruitment of TLR4 thus suggesting that the immunomodulatory potential for *Alternaria* toxins might have to be extended from AOH to other molecules.

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P07-005

This abstract has been withdrawn.

P07-006

This abstract has been withdrawn.

P07-007

Sanguisorba minor subsp. Balearica inhibit production of cytokines in a chronic model of inflammation induced by complete Freund's adjuvant

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Rheumatoid arthritis is an idiopathic auto-immune disease characterized destruction of cartilage and synovial membrane. TNF-alpha, IL-12 and IL-1 are well known inflammatory mediators of "rheumatoid arthritis". These cytokines activate synoviocytes and chondrocytes and this results in the secretion of matrix metalloproteinase into the synovial fluid. Analgesic and anti-inflammatory drugs are used to suppress the symptoms. However, all these have several side effects. Recent research aims to discover long-acting anti-inflammatory drugs with minimal side effects. *Sanguisorba minor subsp. Balearica (Smb)* is known as a medicinal plant and has been used for treatment of various disorders. The usage of *Smb* for relieving of inflammation is noteworthy. This study aims to invastigate possible cytokine mediated anti-inflammatory effects of (*Smb)* on arthritic rats. Aerial parts of *Smb* were air dried and lyophilized. Powdered plant was subjected to maceration process. Phenolic compounds of *Smb* was identified by LC-MS method. LC-MS analysis revealed high amount of phenolic compounds of *Smb* extract. Complete Freund's Adjuvant induced arthritic rat was used as a model animal. Animals were orally administered *Smb* extract at a dose of 34 and 70mg/kg daily. Other experimental groups are vehicle, negative control, quercetin (25mg/kg) and diclofenac sodium (5mg/kg) administered groups.TNF-α and IL-12 were choosen as target inflammatory cytokines. Paraffin sections of rat paw tissues were dewaxed, rehydrated, and subjected to antigen retrieval. After blocking with 5% normal goat serum, sections were incubated with anti-TNF-α and anti-IL-12 antibodies for 1hr. at 4°C. Then incubated again for 30 sec. with secondary antibodies with streptavidin peroksidase enzyme. Staining step was done with AEC substrate chromogen and Mayer's hematoxylin. Signals were quantified using Leica DFC 420 camera with plug-in Olympus BX51 microscope. Finally, % immunopositive stained areas were evaluated by Leica QWin image analysis software.

The suppressing effect of *Smb* extract on chronic inflammation was characterized by examining the expression levels of TNF-α and IL-12 using immunohistochemistry. All obtained data were interpreted with % immunopositive stained areas. According to these data very low levels of TNF-α and IL-12 expressions were observed in vehicle group among all experimental groups. CFA induced negative control group has the highest TNF-α and IL-12 expression levels ($p < 0.005$) compared to other experimental groups. 34mg/kg *Smb* treated group has a reducing effect on both TNF-α and IL-12 levels ($p < 0.005$). Same result was also observed ($p < 0.005$) for positive control group. 70mg/kg *Smb* treated group has lower TNF-α and IL-12 expression level ($p < 0.05$) compared to 34mg/kg *Smb* treated and positive control group. Quercetin treated group has the lowest TNF-α and IL-12 levels ($p < 0.005$) among all experimental groups.

P07-008

Manganese enhances microglial activation in the substantia nigrain response to systemic infection with H1N1 Influenza Virus

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Background: Exposure to elevated levels of manganese (Mn) during juvenile development causes cognitive and motor disturbances that are associated with neuronal injury and glial activation in the cortex and basal midbrain. Glial inflammatory responses to Mn can exacerbate neuronal injury following secondary exposures to other neurotoxicants but whether Mn can also enhance the severity of neurological damage from encephalitic viral infections is not known. We therefore tested the hypothesis that mice exposed to Mn during juvenile development would display a greater neuroinflammatory and neuropathological response to infection with H1N1 influenza as adults than mice not receiving Mn during juvenile development.

Methods: C57Bl/6 mice were exposed to Mn in drinking water (50 mg/Kg) for 30 days from days 21–51 PN, then infected intranasally with H1N1 three weeks later. Control mice received only drinking water following by either mock infection or infection with H1N1. Stereological counts of dopaminergic neurons and microglia in the substantia nigrapars compacta (SNpc) were performed based upon immunohistochemical reactivity to tyrosine hydroxylase (TH) and ionized calcium-binding adapter molecule 1 (IBA-1), respectively. Total RNA was isolated from the SNpcand transcripts examined by RNA sequencing (RNAseq).

Results: Stereology results showed that although there was no significant loss of DA neurons within the SNpc, there was pronounced microglia activation in Mn+H1N1 treated mice relative to control and H1N1-only treatment groups. Activated microglial displayed a reactive, amoeboid phenotype and could be seen phagocytizing damaged dopaminergic neurons. Whole transcriptome analysis was performed by gene ontology term enrichment and overrepresentation analysis was applied to unique transcripts identified within each treatment group. These analyses revealed multiple genes involved in the immune response to stimulus were underrepresented within the Mn+H1N1 group, including genes relevant to PD susceptibility: Park 7(protein DJ-1), Lingo2(Leucine-rich repeat and immunoglobulin-like domain-containing nogoreceptor-interacting protein 2), Atg9a (Autophagy-related protein 9A) and Ligp1(Interferon-inducible GTPase 1).

Conclusions: Taken together, these results suggest exposure to elevated levels of Mn during juvenile development enhance neuroinflammatory damage to dopaminergic neurons after infection with H1N1 influenza virus later in life.

Programme | 10 September 2019

9:00 am – 6:00 pm
Veranda Lounge, Piazza

PV02 | Poster Viewing 2

P08 – Liver toxicology

P08-001

This abstract has been withdrawn.

P08-002
Connexin hemichannels and pannexin channels as drug targets in liver toxicity and disease

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Because of its unique function in the body, it is not surprising that the liver is a frequent target in toxicity and disease. Liver pathology is typically associated with cell death and inflammation. Connexin (Cx) hemichannels and pannexin (Panx) channels have emerged as key players in a plethora of tissues in both processes over the past few years. In this research, it was investigated whether this also holds true for hemichannels consisting of Cx32 and Cx43 as well as Panx1 channels in the liver. Focus was hereby put on acute liver failure, non-alcoholic steatohepatitis (NASH) and liver fibrosis. It was found that inhibition of Cx32 and Cx43 hemichannels by using TAT-Gap24 and TAT-Gap19 peptides, respectively, in acetaminophen-induced acute liver failure counteracts inflammation, while their co-administration leads to diminished liver damage in mice. Likewise, mice with diet-induced NASH treated with TAT-Gap19 or TAT-Gap24 peptides display less inflammation and steatosis. Along the same line, TAT-Gap19 peptide reduces scar formation in carbon tetrachloride-evoked liver fibrosis in mice. Furthermore, inhibition of Panx1 channels by ^{10}Panx1 peptide or genetic ablation of Panx1 alleviates liver damage, inflammation and oxidative stress in acetaminophen-intoxicated mice. Similar results are seen upon inducing NASH in whole body Panx1 knock-out mice, while these genetically modified animals respond in distinct ways to different experimental triggers of liver fibrosis. Collectively, these results suggest that Cx32 and Cx43 hemichannels as well as Panx1 channels may represent promising pharmacological targets for the treatment of acute and chronic liver toxicity and disease.

P08-003
Fine and ultrafine particles issued from oil fuels and second-generation biofuels combustion: a comparative study of the physico-chemical and *in vitro* toxicological characteristics

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Air pollution is a serious worldwide issue due to its health impacts. A correlation between air pollution and the development of respiratory and cardiovascular diseases has been demonstrated by numerous scientific studies. Particulate matter (PM) has drawn more attention by different aspects such as successive particulate air pollution episodes, physicochemical characteristics, and its pathological consequences. Depending on its size, PM is deposited at different levels of the respiratory system: coarse particles (PM_{10}) in upper airways, fine particles ($PM_{2,5}$) in the lower airways and ultrafine particles ($PM_{0,1}$) until the deeper airways (alveoli). In addition to size, the composition of particles leads to different toxicological responses. PM is constituted by organic, inorganic, and biological compounds that can alter several biological activities. In outside environment, the main source of PM comes from combustion of fossil fuels and biomass. Biofuels seem to be an alternative in particle pollution control, although new methods to evaluate health effects from particles, and especially ultrafine particles, are required to support biofuels development. Interestingly, *in vitro* toxicology approaches such as primary cultures of lung cells grown at the air-liquid interface depict a situation close to physiological conditions and allow estimating the toxicity of combustion particles. The present project evaluates the biological effects of fine and ultrafine particles produced during oil fuels and biofuels combustion. We develop an innovative protocol of primary lung cells to fine and ultrafine particles exposure in which generation, characterization and particle exposition are done simultaneously under controlled conditions. During exposure, particle generation is done by a miniCAST adapted to liquid fuels, cell particle exposure is made by a Vitrocell® system and aerosol particles is sampled for chemical analysis. This approach will lead us to investigate the influence of the physicochemical composition, on the response patterns after particles exposure.

P08-004
How similar among different toxicogenomics study designs for liver?

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Toxicogenomics (TGx) is an important tool to gain an enhanced understanding of toxicity at the molecular level. A broad ranges of TGx study design has been reported, some based on the existing animal models (e.g., one-day short-term assay or repeated dosing for 28 days) and the other applied *in vitro* systems (e.g., cell lines from rat, humans and cancer). A question is naturally rasied: how similar among different TGx study designs? In fact, this question can be asked in many different ways: (1) is a one-day *in vivo* short-term assay able to replace the 28-day standard and expensive toxicological assay? (2) are some biological processes more conservative across different preclinical testing systems than others? (3) do these preclinical testing systems have the similar resolution in differentiating drugs by their therapeutic uses? (4) Is it possible for *in vitro* to *in vivo* extrapolation? And (5) can genomic profiles from a cancel line predict drug-induced liver injury? In this presentation, these questions will be explored using several large genomics datasets including Open Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System (TG-GATEs) and L1000 for assesing drug-induced livery injury.

P08-005
Characterization of a human liver spheroid model comprised of HepaRG™ and hepatic stellate cells

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In vitro hepatotoxicity assessment of Philip Morris International's Reduced-Risk Products requires an *in vitro* liver model that closely mimics the human liver. Therefore, we have developed and characterized a spheroid model composed of HepaRG™ and human hepatic stellate cells for its morphology, metabolic capacity, viability, and functionality. Spheroids with HepaRG™ cells and stellate cells (mixed-cell) were compared for six weeks with spheroids composed only of HepaRG™ cells (HepaRG™-only).

Based on the spheroid aspects and staining of different cell types, both spheroid models had similar morphologies. ATP content and LDH secretion remained stable over the six weeks and were comparable between the two models. Given the essential function of the liver in metabolizing xenobiotics, the activities of CYP1A1/1B1, 1A2, and 3A4 were evaluated weekly, and the expression of phase 1 metabolism-associated genes was profiled. The metabolic capacity (based on the CYP inducibility after stimulation) was conserved at any time point tested; no differences were observed between the two models. Secretion of albumin, a marker of hepatic function, was slightly higher during the first two weeks in the mixed-cell model than in the HepaRG™-only model. Procollagen 1α1 levels in the medium surrounding the spheroids were greater in the mixed-cell spheroids regardless of treatment with TGFβ1, a known inducer of collagen production. A whole-human transcriptome analysis consistently showed an upregulation of genes associated with collagen secretion and an enrichment in senescence and apoptosis pathways in the mixed-cell spheroids when compared with HepaRG™-only spheroids.

In summary, the spheroid model composed of HepaRG™ and stellate cells demonstrated stable viability, functionality, and metabolic capacity over six weeks. The increased procollagen 1 secretion that was associated with an upregulation of genes involved in the secretion of collagen suggested that the cells were already fibrotic (or activated) at week 2 after the spheroid preparation. While the reason for stellate cell activation remains unclear, a non-adapted coculture medium could explain the results obtained. Given their fibrotic phenotype, the mixed-cell spheroid model needs to be optimized before being used for toxicological assessment.

P08-006
Correlation between cytochrome P450 enzyme induction and up-regulation of oxidative stress mediators by the pyrethroid insecticide lambda-cyhalothrin in rat liver

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Although cytochrome P450 (CYP) enzymes normally generate metabolites with diminished biologic activity and represent a defense for detoxifying the ROS entities $O_2^{\bullet}$ and $O_2^{2\bullet}$, there are numerous examples where these enzymes catalyze the metabolic activation of chemically inert agents to electrophiles. This study aimed (i) to examine if lambda-cyhalothrin interacts with microsomal CYP system and (ii) to analyze whether oxidative stress, proinflammatory and apoptosis mechanisms should be also co-affected by this pyrethroid. All experimental procedures were conducted with ethic requirements and authorized by the Institutional Animal Care and Use Committee of our University. It was evaluated in the liver of male Wistar rats following oral pyrethroid exposure (4 and 8 mg/kg bw in corn oil, 6 days): (1) CYP isoform activities (CYP1A1, CYP2B1/2, CYP3A1/2, CYP2A1, CYP2C11 and CYP2B1). (2) Oxidative stress markers (ROS, and enzymatic antioxidant activities). (3) Gene expression of proinflammatory (NFκB, IL-1β), oxidative stress and apoptosis (Nrf2, p53, caspase-3, Bax) mediators. Quantitative real-time PCR assays for rat CYP1A1, CYP1A2, CYP2A1, CYP2B1/2, CYP2E1, CYP3A1/2, and CYP4A1 mRNA were also performed. The results demonstrated: (1) Lambda-cyhalothrin exposure produced significant increase in CYP3A1/2, CYP2A1 and CYP2B1 activities. (2) Hepatic CYP1A1, CYP1A2, CYP2A1, CYP2E1, CYP3A1 and CYP3A2 gene expressions increased significantly in both groups treated with lambda-cyhalothrin. The major significant increase of mRNA levels of CYP isoforms was observed in CYP2B1 (1463% and 961%) and CYP2B2 (604% and 501%). Finally, IL-1β, NFκB, Nrf2, p53, Casp-3, and Bax mRNA levels were also significantly increased by lambda-cyhalothrin exposure. In conclusion, the present study demonstrates, in liver microsomes from rats treated orally with lambda-cyhalothrin, an induction of CYP1A, CYP2E, CYP2B and CYP3A subfamilies; results confirmed analyzing gene expression by real-time PCR. Our study also provides links between inflammation, oxidative stress, NFκB activation and CYP regulation in lambda-cyhalothrin toxicity. Work supported by Project Ref. S2013/ABI-2728 from Comunidad de Madrid, and Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

P08-007
Effects of 2-mercaptobenzimidazole and its methyl derivatives on liver drug- metabolizing enzyme system after repeated oral administration in rats

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Purpose: 2-Mercaptobenzimidazole (MBI) and its methyl derivative 4(5)-MeMBI (1:1 mixture of 4-MeMBI and 5-MeMBI) are widely used as rubber antioxidants. MBI was strongly thyrotoxic due to the thioureylene structure in repeated oral administration toxicity studies, causing marked enlargement of the thyroid, and decreased thyroid hormones. On the other hand, MeMBIs (4-MeMBI, 5-MeMBI, and 4(5)-MeMBI) were also thyrotoxic but to a lesser degree with smaller or no changes in thyroid hormones. In this study, we examined and compared the effects of MBI and MeMBIs on the drug-metabolizing activity in rat liver microsomes, since the differences in their thyrotoxicity seem to depend on their toxicokinetic profiles.

Methods: MBI (0.3 mmol/kg/day), 4-MeMBI (0.6), 5-MeMBI (0.6), and 4(5)-MeMBI (0.6, 1.2) were administrated orally once a day to male Wistar rats for 8 days. Microsomal pellets were obtained from rat liver and the contents of cytochrome P450 (CYP), and cytochrome b5 (CYB5) were measured. The activities of NADPH-cytochrome P450 reductase (POR), 7-ethoxycoumarin O-deethylation (ECOD), 7-ethoxyresorufin O-deethylation (EROD), and 7-pentoxyresorufin O-depentylation (PROD) were determined, and the amounts of microsomal CYPs were determined semi-quantified by western blot analysis.

Results and discussion: MBI and MeMBIs increased the weight of liver and thyroid; MBI was most potent, and there was no additive or synergistic effect between 4-MeMBI and 5-MeMBI. MBI decreased the CYP content, and the activities of POR and ECOD, but increased the PROD activity, suggesting overall inhibition of the drug-metabolizing activity with simultaneous induction of CYP2B activity. In contrast, 4-MeMBI, 5-MeMBI, and 4(5)-MeMBI increased the contents of CYP and CYB5, and the activities of ECOD, EROD, and PROD, indicating that MeMBIs mostly induce CYP activity. 5-MeMBI and 4(5)-MeMBI appeared inhibitory for CYPs 2C11 and 2C13. There was no additive or synergistic effect, but was counteraction, between 4-MeMBI and 5-MeMBI. These effects on the liver drug-metabolizing system seem to be related to the toxicological differences between MBI and MeMBIs.

P08-008
Dosing corrected for species differences in toxicokinetic s using PBPK modelling predicts equivalent reactive metabolite burden following acetaminophen overdose

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Species differences in metabolic pathways, arising from cofactor turnover and differences in drug metabolizing enzyme affinity and expression, and physiology effect the toxicokinetics of compounds. Differences in phase I and II metabolism of acetaminophen (APAP) between rat and mouse affect production of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is primarily detoxified through conjugation with the anti-oxidant glutathione (GSH). However, excess production of NAPQI results in depletion of GSH and subsequent formation of protein adducts, oxidative-stress and liver injury.

Based on literature and publically accessible data repositories, PBPK models were established describing the disposition of APAP in both species. Specifically, these models incorporated sulphation, glucuronidation and CYP-mediated hydroxylation of APAP. Using these models, oral equivalent doses (OEDs) of 300 mg/kg and 1000 mg/kg were predicted for mouse and rat, respectively. OEDs were defined as the oral APAP dose resulting in the same hepatic NAPQI burden in both species. These doses were subsequently used in a preclinical single dose study in both species. Following overnight withdrawal of food, animals were dosed via oral gavage and sacrificed at 0.5, 1, 3, 6, 9 and 24h after dosing (n = 7–14 per timepoint). APAP and conjugated metabolite concentrations in plasma (APAP-GSH, APAP-CYS, APAP-NAC), as well as hepatic GSH concentrations were determined at each timepoint. Total hepatic NAPQI was then calculated from the AUC of all conjugated APAP metabolites in mouse via mass-balance analysis; assuming no protein binding, active secretion or re-absorption, and corrected for biliary clearance. A second approach assumed 1:1 stoichiometry between GSH and NAPQI, and calculated total hepatic NAPQI burden from total hepatic GSH depletion in both rat and mouse. Our experimental results show a mouse:rat total hepatic NAPQI burden ratio, based on GSH depletion, was 1.4. The ratio calculated based on mass balance in mouse and GSH depletion in rat was 0.64. Thus, PBPK predicted OEDs resulted in cross-species total hepatic NAPQI burden within 1.6 fold. Biomarker results show the mouse is intrinsically more susceptible to hepatic injury following APAP overdose. Despite the similar hepatic NAPQI burden at the chosen doses, mean mouse ALT and AST levels increased by 67 and 44-fold, respectively, rat levels only increased 3.5 and 7-fold, respectively.

Preclinical study design informed by PBPK modelling and simulation facilitates robust cross-species comparison of intrinsic susceptibility to toxicological hazard. Such an approach can reduce the number of animals required, help to refine study design, and inform on species selection for safety testing to facilitate extrapolation of findings to humans as part of the risk assessment of compounds.

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P08-009
Comparable findings in the rat liver with a long-acting glucagon receptor agonist, SAR438544, and an 8-hour infusion of glucagon

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Background and Aims: Glucagon agonism using synthetic receptor agonists has potential for weight loss therapy. For further characterization of the safety profile of SAR438544, a long-acting glucagon receptor agonist, its effects on selected liver findings were assessed in rats following subcutaneous (SC) injection and were compared to effects of exposure profile matched (short acting) glucagon. Exposure profile machting was achieved by 8-h continuous intravenous (IV) infusion of (short acting) glucagon.

Materials and methods: Low (1 mg/kg/day) and high (10 mg/kg/day) doses of SAR438544 or low (0.146 mg/day) and high (1.46 mg/day) doses of (short acting) glucagon were administered daily for 7 days by SC injection or 8-h continuous intravenous (IV) infusion, respectively. Mortality, clinical signs, body weight, clinical chemistry,

and anatomic pathology were recorded in the main group of rats. In a toxicokinetics satellite group blood was collected on Days 1 and 7 of dosing.

Results: Daily SC administration of long-acting glucagon or IV infusion of (short acting) glucagon were well tolerated with limited clinical observations. No deaths or consistent trends in body weight or food consumption were noted with either compound. Toxicokinetic profiles were generally similar. Both compounds resulted in findings in the liver including increased organ weight and glycogen accumulation.

Conclusion: The incidence and severity of liver findings in rats dosed daily with long-acting glucagon were consistent with the effects of daily dosed and exposure profile matched (short acting) glucagon.

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P08-010
Relation between DMSO application and selected cytochromes P450 in developing liver

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Living organisms are exposed to a number of structurally different chemicals in their natural environment every day. In addition to the substances of natural origin, they also receive a large amount of synthetic foreign substances – xenobiotics. Xenobiotics cannot be used by organism, they are potentially harmful and therefore should be excluded from the body as soon as possible. The study evaluates the effect of dimethyl sulfoxide (DMSO) on the developing chicken liver after its application on the 4th embryonic day (ED4) in application dose 5, 10, 15, 20, 25, 30, 35 a 50 µl per egg. The liver was removed on ED9 for the gene expression analysis of selected cytochrome P450 genes (CYP1A5, CYP3A37 and CYP3A4).

Cytochrome activity and their gene expression is a major determinant of drug efficacy and toxicity, thereby determining the therapeutic outcome. In monitoring the activity of detoxifying enzymes in various poultry species great differences in enzyme kinetics were found. CYP3A37 and CYP3A4 isoforms belong to the cytochrome CYP3A group, mainly found in the liver and intestine. Their expression can be induced by a wide variety of compounds such as antibiotics, glucocorticoids or pesticides. DMSO has been shown to increase the expression of cytochrome family enzymes, especially the CYP3A family. The increased expression of the CYP1A5 gene after DMSO administration may be related to the fact that DMSO is capable of activating the aryl hydrocarbon receptor (AhR), which functions as a ligand-activated transcription factor regulating gene expression. AhR is also referred to as the regulator of xenobiotics metabolising enzymes. DMSO exposure activates AhR and induces AhR translocation to the nucleus and its binding to the target gene promoter, resulting in increased gene expression. When the ligand binds to the nuclear receptor, it causes nuclear receptor activation and subsequently it binds to the DNA promoter and induces gene transcription initiation.

In our study, gene expression in developing liver tissue isoforms of both cytochrome P450 families (CYP1A and CYP3A) was increased by DMSO administration. It could be concluded that gene expression increased in proportion to an increased application dose. The statistically significance increase of gene expression of isoforms CYP1A5 and CYP3A37 has been noticed in the application doses 15, 20, 25, 30, 35 and 50 ul. The isoform CYP3A4 has the similar results except the application dose 15 ul. Generally, the highest induction of gene expression by DMSO has been noticed for the isoform CYP1A5 in application dose 50 ul (8.83 times), followed by CYP3A37 (5.67 times). The lowest of gene expression induction has been noticed for isoform CYP3A4 (3.37 times).

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P08-011
Disruption of liver gene expression and ultrasonic vocalization of infant mouse offspring perinatally exposed to 2,3,7,8-tetrabromodibenzofuran

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Exposure to chlorinated dioxins/furans that activate aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, has been reported to induce a variety of toxicities, such as tumorigenesis and cognitive impairments, in humans and laboratory animals. We have shown that *in utero* and lactational exposure to 2,3,7,8-tetrachlorodibezo-*p*-dioxin induces expression of AhR-target genes in the liver and suppressed ultrasonic vocalization (USV) of infant mouse offspring. Since certain brominated analogs are also able to activate AhR, it is reasonable to consider that such brominated congeners have toxicities relevant to chlorinated congeners. In the present study, we studied effects of perinatal exposure to 2,3,7,8-tetrabromodibenzofuran (TBDF) on gene expression and USV in the mouse. Pregnant C57BL/6J mice were orally administered TBDF at a dose of 0, 9, or 45 µg/kg b.w. on gestational day 12.5 (hereafter, named as the control, TBDF-9, and TBDF-45 groups, respectively). USVs of offspring on postnatal days 3–9 were measured for 1 min in sound-attenuate chamber, and the USV frequencies of 60–100 kHz were analyzed. Liver and brain tissues on postnatal day 5 were collected, and mRNA expression between the control and TBDF-45 groups was analyzed by gene microarray. In addition, the expression of the top four mRNAs that significantly increased or decreased was confirmed by quantitative PCR. As a result, total USV duration in the TBDF-45 group, but not the TBDF-9 group, was significantly lower than that in the control group. Gene microarray revealed significant changes in expression of 1,181 genes in the liver between the control and TBDF-45 groups. Among them, *Cyp1a1*, *Cyp1a2*, *Fmo3*, and *Pnliprp1* mRNAs were significantly increased and *Tff3*, *Ocstamp*, *Kcnk16*, and *Lgals2* mRNAs were significantly decreased in the TBDF-9 and -45 groups compared to those in the control group. On the other hand, no significant difference in expression of *Cyp1a1* and *Tff3* mRNAs was observed in the brain between the control and TBDF-45 groups. Our findings show gene expression changes in the liver, but not the brain, of the TBDF-exposed mouse offspring, which suggests that suppressed USV might be partly caused by impairment of peripheral tissues, including the liver.

P08-012
Assessment of drug hepatotoxicity in 3D InSight™ Liver Microtissues with expanded panel of cytotoxicity markers (AST, LDH and ATP)

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3D InSight™ Human Liver Microtissues consisting of primary liver cells are an attractive tool for *in vitro* drug safety assessment due to preservation of liver specific functions and metabolic activity over a long culturing time. 3D InSight™ Human Liver Microtissues were previously shown to outperform 2D primary hepatocytes in prediction of hepatotoxicants causing drug-induced liver injury (DILI), based on cell viability assay.

In this study, we established a method to assess a clinically relevant marker for liver damage - aspartate aminotransferase (AST), and correlate AST levels with other cell viability and cytotoxicity markers (leakage of lactate dehydrogenase (LDH) and intracellular ATP). 3D InSight Human Liver Microtissues were exposed to a set of reference compounds with known DILI grade for 7 days. Measurement of all 3 markers was performed for a single microtissue, with LDH and AST measurements multiplexed in the same microtissue supernatant.

Cell viability profiles were able to discriminate between structurally related compounds manifesting different clinical toxicity grade, such as Troglitazone/Rosiglitazone and Tolcapone/Entacapone. Importantly, profiling of ATP levels together with AST and LDH leakage signal for DILI+ reference compounds showed either a strong correlation between decrease of ATP levels and increase of AST and LDH leakage signal or decrease of ATP levels with concomitant mild or no release of AST and LDH. Reduction of ATP levels with moderate-to-no leakage signal from LDH and AST can be an indicator of metabolic stress rather than necrosis as a cause of toxicity. Together, these results 1) prove feasibility of profiling of clinically relevant marker (AST) in human liver microtissues 2) expand cytotoxicity marker panel that can be measured in a single microtissue to ATP, AST and LDH 3) show application of complementary profiles of different toxicity markers in *in vitro* assessment of hepatotoxic compounds and their potential mechanism of toxicity.

We suggest this method as a promising approach for safety assessment in drug development with a direct correlation to DILI markers used in clinical trials.

P08-013
The method of spheroid formation for 3D cultures of primary hepatocytes influences hepatocellular functions and hepatotoxicity

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Primary hepatocytes of human and animal origin are the gold standard for all pharmacological-toxicological studies in drug development. They play a major role in eco-toxicological evaluation as well. Three-dimensional (3D) cultures became more popular in recent years since they might mimic the *in-vivo* cell morphology, polarity and cell-cell interactions better than traditional two-dimensional (2D) cultures. Here, two types of cell culture plates were used to generate 3D cultures with primary hepatocytes: the GravityPLUS Hanging Drop System with subsequent culture in Gravity TRAP plates in comparison to U-bottom ULA (ultra-low attachment) plates with cell repellent surfaces. Standard 2D cultures were performed as well.

Hepatocellular detoxification functions like urea release and CYP450 activity as well as the response to the hepatotoxin Diclofenac were analysed in these culture systems. The results were normalized to the corresponding volume of culture medium or to protein content.

The secretion of urea was improved and maintained at higher levels in U-bottom ULA plates compared to the Hanging Drop System. CYP1A activity was better inducible by ß-Naphthoflavone in U-bottom plates than in the Hanging Drop System at all 3 tested cell numbers. Basal Cytochrome P450 activities were higher in U-bottom plates and showed a better inducibility in these plates compared to the Gravity TRAP plates.

Diclofenac, a known and well-described hepatotoxic compound, did show similar effects on hepatocytes with regard to the ATP content in both 3D culture systems. Beside this, the decrease of ATP content due to Diclofenac treatment was higher in 2D culture than in the 3D culture systems.

In summary, our results indicate that major differences may exist between different 3D culture systems and in comparison to standard 2D culture methods. These differences may lead to different and conflicting results in the assessment of drug toxicity and drug-drug interaction.

P08-014
Development and characterisation of 3D liver models to investigate drug toxicity

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Drug induced liver injury (DILI) is a leading safety problem for the pharmaceutical industry and healthcare providers. Yet, many drugs are withdrawn from the market as causing human hepatotoxicity. Hence, identification of liver predictive models to maximize the amount of drug-candidate information is critical during preclinical stages of drug discovery. Work conducted by Meritxell Huch has shown that by isolating adult stem cells from a liver biopsy and culturing them in artificial ECM matrix and medium supplemented with specific growth factors, these cells naturally self-organize in fully functioning 3D liver structures defined as "organoids". However, using the liver organoid as model to detect hepatotoxicity is currently an open question. To address this, initial focus was directed towards the differentiation of liver organoids into hepatocytes like cells as these are the main epithelial cells involved in drug metabolism. I have developed a protocol for the differentiation of liver organoids in a stem cell like state into organoids expressing hepatocyte cells, characterised by a higher expression of hepatocyte markers like *ALBUMIN, and CYP3A4* genes. Transmission electron microscopy analysis showed cellular polarization marked by formation of bile canaliculi like structures in differentiated liver organoids. This led to the analysis of the transport activity of efflux transporters located at the apical membrane of hepatocytes. Immunostaining analysis showed expression of the bile salt export pump (BSEP), a bile canalicular transporter involved in secretion of bile and xenobiotics, but also an important target for drug toxicity. A transport assay using fluorescein diacetate confirmed the functional activity of BSEP to transport its substrate into the bile canaliculus. In summary, I have validated a method to differentiated organoids into mature hepatocytes and preliminarily investigated the structural functionality of this novel 3D model. Future work will aim to further characterise the metabolic activity and then investigate the toxicological predictivity of the model towards known human hepatotoxins.

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P08-015
Hepatic IGF signalling is dysregulated by *in utero* exposure to maternal smoking

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Background: Insulin-like growth factors (IGF-1 and IGF-2), acting through their receptors (IGF1R and IGF2R), are major regulators of fetal growth. IGF bioavailability is regulated by high-affinity binding proteins (IGFBPs). Fetal expression of IGFBPs is tissue-specific with high abundance in the liver. Small for gestational age (SGA) newborns exhibit reduced IGF and increased circulating IGFBP. Maternal smoking during pregnancy is associated with intrauterine growth restriction, but molecular mechanisms remain unclear. Our aim was to evaluate whether *in utero* exposure to cigarette smoke (containing more than 7000 pollutants) could affect the IGF axis in the human fetal liver.

Methods: 80 human fetal livers from elective terminations of normal pregnancies (12-19 gestation week), were collected (NHS Grampian Research Ethics Committees, REC 04/S0802/21). RNA was extracted and the Illumina NextSeq platform produced 76 bp single end RNA sequencing reads. These reads were quality controlled, aligned to the human reference genome and quantified at gene regions. Significant differentially expressed genes were identified using a generalised linear model with a three-way interaction model between fetal sex, fetal age and maternal smoking status.

Results: IGFBP1 and IGF2BP2 were significantly upregulated in the older male (>17 gestation week) smoke-exposed fetuses. The developmental increase in IGFBP4 and IGFBP7 expression was significantly accelerated only in males exposed to smoke. IGF2R decreased throughout gestation only in male fetuses not exposed to cigarette smoke.

Conclusions: The significant changes in key elements of the IGF axis indicate a general dysregulation of IGF signalling within the male fetal liver, with a striking sex-difference. Interestingly, in epidemiological studies, males exposed to maternal smoking are more susceptible to growth restriction. Increased transcript levels of IGFBPs may decrease IGF bioavailability, ultimately resulting in reduced IGF signalling and potentially impairing fetal growth. Investigation of protein and gene expression of IGF pathway members in the placenta and other fetal tissues will be performed.

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P08-016
Grayscale differential box counting as a measure of complexity of liver texture in common carp (*Cyprinus carpio*) sub-chronically exposed to perfluorooctanoic acid (PFOA)

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Perfluorooctanoic acid (PFOA), a perfluorinated alkylated substance (PFAS), poses a worldwide concern for its wide distribution, bioaccumulation in food webs, long half-life in organisms, and potential toxic, carcinogenic and endocrine disrupting effects on animals. Fish are excellent candidates in aquatic biomonitoring programs and toxicologic testing, frequently focusing on liver due to its pivotal role in the health of the whole organism and its highly sensitivity to contaminants. Histopathology can be useful in evaluating toxicological effects on fish health and texture analysis can represent an objective, replicable diagnostic tool, potentially free from operator-dependent bias. In the present survey, liver histological texture was comparatively assessed in specimens of common carp (*Cyprinus carpio*) subchronically exposed to PFOA. Twenty specimens were exposed to two PFOA dosages (10 exposed to 200 ng l^{-1}, 10 exposed to 2 mg l^{-1}) for 56 days and compared to other 10 unexposed fish. Grayscale differential box counting (fractal dimension and lacunarity) was evaluated on representative pictures taken from liver histological sections. Differential box counting was implemented by converting two-dimensional grayscale images into pseudo three-dimensional information. Hence, fractal dimension and lacunarity acted as a measure of the complexity and of the heterogeneity of the grayscale levels distribution, respectively. Redundancy Analysis (RDA) was performed on the obtained numerical data in order to summarize the part of grayscale differential box counting variation that is explained by the following biometric/experimental variables: PFOA liver concentration, liver mass, proliferating cell nuclear antigen (PCNA) positive nuclei, after removing the effects of fish total length. The t-values of the regression coefficients of liver PFOA concentration and of liver mass with both fractal dimension and lacunarity, and of PCNA positive nuclei with lacunarity, showed values larger than 2, while the t-value of the regression of PCNA positive nuclei with fractal dimension appeared to be close to 2. Considering the selected biometric/experimental variables, liver PFOA concentration correlated with PCNA positive nuclei but did not correlate with liver mass, whereas PCNA positive nuclei correlated with liver mass. Interestingly, fractal dimension contributed better than lacunarity in treatment groups ordination. Recently, fractal analysis has been adopted to estimate the complexity loss associated with pathological changes. In the present survey, contrary as expected, liver texture modification related to liver PFOA concentration increase was associated with a significant complexity increase, related to reversible changes (hydropic degeneration), possibly acting as an initially adaptive strategy, rather than representing mere degeneration, to cope with PFOA challenge. The possible occurrence of a hormetic response should be further investigated.

P08-017
Pluripotent stem cells differentiation towards definitive endoderm

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Hepatocyte-like cells generated from induced pluripotent stem cells (iPSC) represent a promising tool as a human liver cell model for different applications including drug toxicity screening. The main complexity of this approach is that obtaining of mature functional hepatocytes remains challenging therefore require detailed study of every step of the cells differentiation. Current research is dedicated to comparison of different methods of obtaining of definitive endoderm (DE) – the first stage of stem cells differentiation towards hepatic lineage. Aim of this study is to develop an effective method of hPSC differentiation to cells of DE in 2D conditions. We differentiated two cell lines using six conditions, contained of growth factors (Activin A (AA), Wnt3a), or small molecules (sodium butyrate (NaB), IDE1). We checked three NaB, which were purchased from three different suppliers to

test the affection of the product purity on cell viability. Change of cell morphology showed which condition facilitated faster cell differentiation and how different conditions influence cell viability. At four time points (day 0, 1, 4 and 6), we measured relative mRNA expression of gene markers for pluripotency, DE, hepatic, mesendoderm and ectoderm. We found that cells had different sensitivity to NaB obtained from different companies and one of them was excluded due to massive cell death. We observed effective DE formation with AA alone, with the combination of AA and Wnt-3A, and with the combination of AA and NaB obtained from two companies. NaB leads to the fastest differentiation, but it is toxic for cells, which restricts it's usage for obtaining of big amount of DE cells. Hierarchical cluster analysis showed similarities between different conditions and allowed us to divide them in two groups, based on affection on the gene expression; none of them included IDE1. Immunofluorescent method confirmed effectiveness on protein level of four conditions for DE formation. We showed that the IDE-1 at the tested concentrations is ineffective for DE formation. In conclusion, we obtained an effective protocol for obtaining DE cells for the further differentiation towards hepatic lineage.

P08-018
Versatile pro-fluorescent and fluorescent coumarin derivatives as substrates for different types of xenobiotic metabolizing enzymes

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Detailed knowledge of xenobiotic metabolizing pathways is essential for understanding toxicity of substances and for evaluation of their health risks. For small-molecule drugs, cytochrome P450 (CYP) enzymes catalyze most functionalizing reactions, with glucuronosyltransferases (UGT) and sulfotransferases (ST) mediating most conjugation reactions. 7-hydroxycoumarin and its substituents are usually strongly fluorescent, whereas the parent coumarins or their ether derivatives such as glucuronides are non-fluorescent. We have established novel metabolism reactions for CYPs, UGTs, STs and catechol-O-methyltransferase (COMT) using coumarin or its derivatives as probe substrates. In these reactions, coumarin derivatives are oxidized to corresponding fluorescent 7-hydroxycoumarins by different CYPs, and 7-hydroxycoumarins are conjugated to non-fluorescent metabolites. The change in fluorescence can be determined in simple and sensitive assays in a multiwell plate format by either kinetic or end-point measurements. Coumarin substituted with different types of phenyls at position 3 or with chlorine, methoxy or methyl at position 6 are oxidized to fluorescent 7-hydroxycoumarins by human CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C19 or CYP3A4 enzymes. Similar 7-hydroxycoumarins are glucuronidated or sulfonated by microsomal UGTs or sulfonated by cytosolic STs to non-fluorescent conjugates. The weakly fluorescent 6, 7-dihydroxcoumarin was methylated by cytosolic and microsomal COMT to strongly fluorescent 6-methoxy-7-hydroxycoumarin. The substrates were used to detect CYP and conjugating enzyme activities in the liver and intestine in humans and various preclinical animal species. Some of the substrates are selective for individual human CYP and UGT enzymes, and have facilitated evaluation of hepatic vs extrahepatic enzyme activities. In drug development, these new probe substrates can be used to study inhibitory potential of drug candidates towards specific enzymes in a high throughput format.

P08-019
CYP1A2 enzyme activity and protein abundance in normal and diseased pediatric livers

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CYP1A2 is a drug-metabolizing enzyme whose expression begins between birth and 4 weeks of age, and gradually increases to about half of the adult levels by 6 years of age. The enzyme constitutes 4–16% of the hepatic CYP pool, and is a major determinant of the biotransformation of ~9% of clinically used drugs. Interestingly, CYP1A2 activity decreases in adults with non-alcoholic fatty liver disease (NAFLD). Considering the increase in the number of medications given to children, as well as the rise in childhood obesity and NAFLD, this study aimed to determine whether donor age and health status influence CYP1A2 abundance, lobular localization and enzyme activity. Pediatric liver microsomes and a corresponding tissue microarray (TMA) were our test system. The TMA contained 25 tissues from donors aged 3 months to 18 years old and 5 adult controls. The donor demographics and health data were collected from interviews with next of kin and from hospital records. Three consecutively cut arrays were stained with hematoxylin and eosin (H&E) and Gömöri trichrome for a pathologist's determination of liver disease status or with anti-CYP1A2 Ab. Liver microsomes were prepared and CYP1A2 phenacetin *O*-dealkylase activity assayed according to published methods. CYP1A2 protein was detected in all tissues, except in one 4-month old, and it increased with age. In tissues judged to be normal or having minimal pathological findings, CYP1A2 protein was located in zone 3 and 2 hepatocytes. In 20 samples that were free of significant necrosis and NAFLD, the abundance of CYP1A2 enzyme correlated with the donor age ($R^2 = 0.28$). In microsomes prepared from these tissues, CYP1A2 enzyme activity was independent of donor age ($R^2 = 0.01$). A diffuse localization, associated with reduced CYP1A2 level, was seen in tissues affected by necrosis and ischemia. Pediatric NAFLD was associated with diminished CYP1A2 staining (3 donors, 10–14 years old, steatosis 50–80%, BMI 32.2–32.5) paralleling what is seen in adults. In conclusion, the pediatric liver TMA but not microsomes, was a useful tool to elucidate the ontogeny of CYP1A2 protein in healthy livers. Better preservation of immunoreactive CYP1A2 protein than its enzymatic activity in our samples may reflect the priority given to organ transplantation over utilization of donor tissue for research.

P08-020
Extracellular vesicles are involved in polycyclic aromatic hydrocarbon hepatotoxicity

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Purpose: Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that can be found in cigarette smoke and contaminated food, the main exposure for non-smokers, and that are considered for some of them as hepatotoxicants. Extracellular vesicles

(EVs) are membrane-surrounded nanostructures released by cells into the extracellular environment [1] that are now recognized as major actors of intercellular communication and in this context, as pathogenic mediators in several liver diseases [2]. Regarding xenobiotic liver injury, EVs emerge as potential actors of drug induced liver injury [3], yet nothing is known concerning toxicant-associated liver diseases. We previously demonstrated that three PAHs i.e benzo(a) pyrene (BP), dibenzo(a,h)anthracene (DBA) or pyrene (PYR), were able not only to increase the EV release by primary rat and WIF-B9 hepatocytes but also to modify EV composition. Therefore, the aim of this work was to study the impact of hepatocyte-derived EVs on target hepatocytes.

Methods: WIF-B9 and primary rat hepatocytes were treated by 100 nM BP, DBA or PYR. PAHs were selected based upon their various concentrations in common food and their various affinities for the AhR (Aryl hydrocarbon Receptor) as AhR mediates most of the biological effects of several PAHs by leading to the production of reactive oxygen species and metabolites [4]. Then, EVs were isolated from extracellular medium by differential ultracentrifugation and put in contact with non-treated hepatocytes.

Results: EVs released from PAH-treated hepatocytes (PAH-EVs) were more cytotoxic than control EVs, as they were able of causing an increase in apoptosis of target hepatocytes by activation of caspases. The triggering of apoptosis was dependent on an EV uptake by endocytosis. In line with this, PAH-EVs contained more pro-apoptotic components. In addition, greater levels of pro-oxidative components were found in PAH-EVs and PAH-EVs were capable of generating an oxidative stress in target hepatocytes. Finally, PAH-EVs were demonstrated to be able to reach the lysosomal compartment. As the expression of the iron storage protein, ferritin, was higher in PAH-EVs, it could be suggested that Fenton and Haber-Weiss reaction occurred in lysosomes leading to the production of the powerful oxidative species, hydroxyl radical. Thus, a lysosome membrane oxidative damage may explain the lysosome membrane permeabilization (LMP) found with PAH-EVs, that ultimately caused target hepatocyte death.

Conclusion: PAH-EVs are implicated in apoptosis of target hepatocytes suggesting a possible involvement of extracellular vesicles in PAH-induced liver injury.

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P08-021
A new strategy for exploring the role of hyperthermia in MDMA-induced toxicity in primary mouse hepatic cells using GC-MS-based metabolomics

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Hyperthermia is a life-threatening consequence of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) abuse. In this work, a metabolomics approach based on gas chromatography-mass spectrometry was used to investigate the hepatic metabolic changes caused by MDMA under normothermic and hyperthermic conditions. For this purpose, freshly isolated mouse hepatocytes were exposed to three concentrations of MDMA (corresponding to LC_{01}, $LC_{10}$and LC_{30}, as assessed by the MTT assay) for 24 h, at 37.0 °C or 40.5 °C, and the alterations on the intracellular metabolome were evaluated.The results obtained by the multivariate analysis showed that metabolic patterns of MDMA exposed cells are separated from control in a concentration-dependent manner, both in normothermic and hyperthermic conditions. Normothermic data revealed a significant alteration ($p < 0.05$) in the levels of malate, fumarate, 2-oxoglutarate, lactate, phosphoric acid, ornithine, glutamate, cysteine, aspartate, myo-inositol, gluconic acid, among others. This panel of discriminant metabolites are mostly involved in energetic metabolism, amino acids metabolism, urea cycle, ammonia recycling, fatty acid metabolism and antioxidant defenses. These metabolic disturbances were significantly more pronounced at 40.5°C. Importantly, our resultsalso demonstrated that hyperthermia *per se* induces significant alterations in levels of metabolites involved in the TCA cycle, amino acids metabolism and urea cycle. Taken together, these findings indicate that MDMA triggers significant metabolic alterations on hepatic cells and that these effects are clearly exacerbated under hyperthermic conditions, emphasizing the potential increased risks of MDMA abuse owing to the thermogenic action of the drug.

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P08-022
Proteomic analysis reveals perfluoro-(3,5,7,9-tetraoxadecanoic) acid (PFO4DA) induced hepatotoxicity via activation of PPARs on male mice

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Introduction: Perfluoro-(3,5,7,9-tetraoxadecanoic) acid (PFO4DA), an alternative to perfluorooctanoic acid (PFOA), has been detected in aqueous samples. However, its potential toxicities remain unclear.

Objectives: This study was designed to assess the hepatotoxicity of PFO4DA on male mice, especially its effects on liver lipid metabolism.

Methods: Male mice were exposed to 0, 0.4, 2, or 10 mg/kg/d PFO4DA for 28 d. We measured triglyceride (TG) and total cholesterol (TCHO) content in serum and liver. Differentially expressed proteins (DEPs) between the control and 10 mg/kg/d groups were identified using isobaric tags for relative and absolute quantification (iTRAQ). Bioinformatics analysis were used to identify the networks between key proteins and DEPs and enrich the regarding GO terms and KEGG pathways. Western blotting was performed to verify iTRAQ results and analyze the expression levels of key proteins and nuclear receptors.

Results: Compared to the control group, changes of liver injury index and serum lipid content in 0.4 and 2 mg/kg/d PFO4DA groups did not exceed 20%. After 10 mg/kg/d PFO4DA exposure, relative liver weight and liver injury index significantly increased, indicating the occurrence of acute liver injury in mice. In serum, we observed no change in TG and TCHO content, but in liver there were significantly decreased TG and TCHO levels. Exposure to 10 mg/kg/d of PFO4DA led to 198 differentially expressed liver genes (56 down-regulated, 142 up-regulated), mainly involved in fatty acid metabolism, oxidation-reduction process and transport. Metabolic pathways, fatty acid degradation, peroxisome, and PPAR signal pathway were enriched, highlighting the stimulation of lipid metabolism in mice liver. The

significantly increased PPARα and the downstream proteins after 10 mg/kg/d PFO4DA exposure might be mainly responsible for the decreased lipid content in liver.

Conclusion: This study concluded that PFO4DA exposure could cause hepatotoxicity, and decrease lipid content in mice liver. PPAR pathway activation in the mice liver may contribute to the observed toxic effects. As it has been detected in water samples with much higher concentrations than PFOA, efforts to remove or at least decrease its occurrence in drinking water should be made urgently.

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P08-023
Study of long term culture condition of hepatocytes for chronic toxicity test

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Purpose: At the early stage of drug development, drug-induced liver injury (DILI) is evaluated by *in vitro* assay using hepatocytes. However, hepatocytes are difficult to culture for a long time while maintaining their functions. Therefore, they are not suitable for evaluation of chronic toxicity. To solve this point, sandwich culture, 3D-bioreactor, and spheroid culture have been developed. By using such culture system, hepatocyte functions can be maintained for several weeks. However, these have not been widely used because they are complicated or require specific culture material and devices. Therefore, we examined simple method for long term culture of hepatocytes.

Methods: We cultured human cryo-preserved hepatocytes from vendor A and human iPS cell-derived hepatocytes (hiPSC-hep) from vendor B for 28 days in the long-term culture medium of hepatocytes from vendor C. This culture medium does not require sandwich culture, and can maintain hepatocyte functions with high survival rate for several weeks in 2D culture. Gene expressions of major *CYPs* and maturation makers were measured by qPCR and formations of bile canaliculi were observed.

Results: When human cryo-preserved hepatocytes were cultured in long-term culture medium, a part of cells are detached on day 28, but gene expressions of major *CYPs* were maintained. On the other hand, when hiPSC-hep were cultured in long-term culture medium, cells were not detached even on day 28 and gene expressions of major *CYPs* were increased. In addition, the expression of *ALB*, which is used as a mature hepatocyte marker, was increased, and the expression of *AFP*, which is used as an immature hepatocyte marker, was decreased. These results suggested that hiPSC-hep were matured by long-term culture. Furthermore, extension of the bile canaliculi, which is required for evaluation of biliary excretion, was observed in hiPSC-hep. From the above, it was demonstrated long-term culture medium from vendor C is effective for long term culture of hepatocytes.

P08-024
CYP-dependent destruction of hepatic sinusoidal endothelial cells and induction of cholestasis by the hepatotoxic pyrrolizidine alkaloid senecionine

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Pyrrolizidine alkaloids (PA) are phytotoxins that may cause severe liver damage. However, molecular mechanisms of PA hepatotoxicity are not well understood. Therefore, we investigated metabolism-dependent development of PA hepatotoxicity *in vivo*, using an acutely toxic dose of senecionine in mice. Analysis of the liver was performed by intravital two-photon microscopy, histology and clinical chemistry.

Pericentral liver sinusoidal endothelial cell (LSEC) necrosis was observed together with elevated sinusoidal marker proteins in the serum of senecionine-treated mice and increased platelet aggregation. *In vitro* experiments showed no cytotoxicity to the freshly isolated non-parenchymal cell fraction (predominantly LSECs) up to 500 µM senecionine. However, metabolic activation of senecionine by preincubation with primary mouse hepatocytes increased the cytotoxicity to cultivated LSECs. CYP-dependent bioactivation was confirmed in CYP reductase-deficient mice *in vivo*. Analysis of hepatic bile salt transport by intravital two-photon imaging revealed a delayed uptake of a fluorescent bile salt analogue from the hepatic sinusoids into hepatocytes and delayed elimination. This was accompanied by mRNA downregulation of hepatic bile salt transporters.

In conclusion, toxic metabolites are generated by hepatic CYPs during intoxication with senecionine that destroys LSECs in the pericentral region of the liver lobules. Together with the observation of compromised bile transport the results explain the observed cholestasis and the clinical symptoms of veno-occlusive disease due to platelet aggregation and LSEC destruction after PA intoxication.

P08-025
Special aspects of the hepatotoxic action of tetrachloromethane in rats of different ages

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Age-related changes cause shifts in the physiological and biochemical functions of the organism, the consequences of which may result the sensibility to the toxic effects of chemical agents.

Goal of the research was studying the special aspects in mechanism of carbon tetrachloride-induced hepatotoxicity in rats of different ages.

Materials and methods: The studies carried out on young rats (3 months) and older (18 months) male rats of *Wistar* line, divided into control and experimental groups. Liver pathology formed by daily *per os* of a 25% oil solution exposure of carbon tetrachloride for 4 days at 0.2 ml/100 g weight. In the blood were determined standard kits to study clinical diagnostic parameters: ALAT, ASAT, hydroperoxide (HP) and malonic dialdehyde (MDA). Statistical processing of results – according to the criterion of Student's test.

Results: By comparison of different age'sanimals reactions with each other showed, that the hepatotoxic effect of CCl_4 in age rats is

higher. This manifested itself by a relative ("Experience / Control") increase in the activities of hepatospecific enzymes – $ALAT_{elderly}$ = 10.42 ($p < 0.01$), $ALAT_{young}$ = 5.46 ($p < 0.001$); $ASAT_{elderly}$ = 6.1 ($p < 0.001$), $ASAT_{young}$ = 3.46 ($p < 0.001$). The de Ritis coefficient for the compared groups was: $RdR_{eld.}$ = 0.65 ($p < 0.05$), RdR_{young} = 0.69 ($p < 0.001$). The concentration of lipid peroxidation markers, reflecting the degree of toxic effects of CCl_4 on the organism, revealed that the conversion of xenobiotics in older rats was reduced relatively young ($HP_{eld.}$ = 3.16 ± 0.13 µmol/l; HP_{young} = 4.59 ± 0.12; $MDA_{eld.}$ = 38.29 ± 1.39 µmol/l, MDA_{young} = 41.8 ± 0.96).

Consequently, despite of the relative decrease in the activity of the cytochrome system in older rats, the toxic effect of CCl_4 in them is more pronounced, which may be based on the weakening of clearance and more longer contact of the body with toxic metabolites.

P08-026
Human non-parenchymal cells protect against acetaminophen hepatotoxicity in a co-culture spheroid model

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In addition to hepatocytes, the liver comprises a variety of non-parenchymal cells (NPCs) such as stellate, Kupffer and liver sinusoidal endothelial cells, which all have specialized functions. Incorporating NPCs into *in vitro* models may therefore provide a more physiologically relevant platform for studies of liver injury and/or disease.

In this study, cryopreserved primary human hepatocytes and mixed NPCs were co-cultured in 3D spheroids. The presence of each cell type was confirmed through immunohistochemistry for cellular markers such as CD68 (Kupffer cells) and CD31 (endothelial cells). Although faint staining for a-SMA and COL1A1 (markers of activated stellate cells) was observed in untreated co-culture spheroids, this increased significantly upon TGF-b treatment, indicating that the culture conditions were suitable for maintaining stellate cells in a quiescent state.

Interestingly, the addition of NPCs protected the spheroids from acetaminophen-induced toxicity, an effect which has previously been reported in animal models [Ju *et al*. 2002]. NPC-containing spheroids were less sensitive when considering all readouts examined (depletion of ATP and glutathione, and miR-122 release), particularly after repeated dosing. This effect was observed with multiple NPC donors. Despite all spheroids containing the same number of hepatocytes, mRNA expression of CYP1A2, CYP2E1 and CYP3A4 (enzymes responsible for the bioactivation of APAP) was lower in co-cultures and may therefore have contributed to the protective effect observed.

To understand whether the introduction of NPCs increased the physiological relevance of the model, the expression of a panel of miRNAs associated with APAP-toxicity in patients [Vliegenthart *et al*. 2015] was compared between mono- and co-cultures. Of the six miRNAs analysed, only miR-122 was readily detected in cell culture media following APAP treatment, reflecting the high levels present in the liver. In addition, increased cellular expression of miRNAs implicated in inflammation (miR-155) and liver regeneration (miR-382) was observed in co-culture spheroids.

This work highlights the importance of multiple cell types in the liver's response to toxic insult and suggests that the presence of non-parenchymal cells can significantly impact upon toxicity mechanisms.

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P08-027
A retrospective analysis of hepatocyte hypertrophy in repeated dose rat studies

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Hepatocyte hypertrophy is generally considered an adaptive change of the liver which reflects drug metabolism and hepatic enzyme induction, which sometime results in decreased systemic exposure. This is more often observed in nonclinical studies as high doses are administered to animals to investigate the toxicity of the test article. Extensive research has been conducted especially in rodents to address the significance of this change relative to drug metabolism and safety evaluation. The purpose of this investigation is to analyze the hepatocyte hypertrophy observed in rat studies conducted at the facility and provide references as background data. It was also intended to analyze if there are any relationships between hepatocyte hypertrophy and accumulation index of systemic exposure. A retrospective analysis was performed on approximately 150 repeated dose rat studies of 4-week to 26-week duration tested with non-biologics. The animals used on studies were Sprague Dawley and Wistar rats from BioLASCO Taiwan Co., Ltd., Sprague Dawley rats from Vital River Laboratory Animal Technology Co., Ltd. Beijing, and Wistar Han rats from Charles River Laboratories, USA. The results showed that hepatocyte hypertrophy was noted in approximately 14% studies and represents approximately 14% of total compounds tested. The hepatocyte hypertrophy was in centrilobular or diffuse pattern, of minimal to moderate severity, and was accompanied with increased relative liver weight (to body weight) by 12% to 98% relative to the concurrent control. There were no associated changes in ALT (Alanine Aminotransferase) or AST (Aspartate Aminotransferase) or the increases were of low magnitude and were not considered adverse. In 12 of the 21 studies, hepatocyte hypertrophy was the only test article-related change observed in the liver. Other liver changes included hepatocellular vacuolation and/or necrosis. The presence of hepatocyte hypertrophy does not appear to result in meaningful differences of accumulation index of systemic exposure. However, when this was observed along with follicular hypertrophy in the thyroid glands, which is another indicator of enzyme induction, these tend to be associated more with decreased systemic exposure and greater magnitude of liver weight increase. The hepatocyte hypertrophy was reversible in all studies analyzed expect that in 2 studies it was still noted as a minimal or mild change in one animal following a 2-week recovery or only in the high dose group following a 4-week recovery. For two compounds that both the IND enabling and longer term studies were conducted at WuXi, hepatocyte hypertrophy was noted in both the 4-week and 13- or 16-week studies. Among the 19 compounds that resulted in hepatocyte hypertrophy in rat studies, only three compounds also had similar reversible changes in the non-rodent species. In most studies, the hepatocyte hypertrophy by itself was not considered an adverse change

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P08-028
Development of high-throughput assays for the screening of drug-induced mitotoxicity (Glu/Gal assay) and membrane potential integrity (Mito-ID assay): workflow and software for efficient end-to-end accurate data delivery.

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Drug-induced hepatic injury is the most common reason cited for withdrawal of an approved drug and impaired mitochondrial function is increasingly implicated in this etiology. To shift early on in deselection of molecules with mitochondrial liabilities during the drug development process, the operational *in vitro* screening screenings assays needed to be tuned into high-throughput assay formats. We demonstrate that by implementation of modern HTS infrastructure and sophisticated data analysis software packages, throughput, efficiency, reproducibility and reliability could be optimized, essential for a robust fully integration of mitochondrial toxicity data compliant with the drug discovery data warehouse. The use of Echo 555 spotted plates, Genedata Screener, 3DX enabled the development of an end-to-end platform that was used to screen ~10,000 compounds in dose response and can be used in hit-to-lead compound DILI characterization in a streamlined and modern pharma research workflow.

P08-029
Effects of antipsychotic drugs on mitochondrial bioenergetics *in vitro*

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Mitochondria are the cellular organelles that generate 95% of the energy needed for a cell to remain viable. It has long been recognized that mitochondrial dysfunction can be the result of drug-induced toxicities and a major mechanism of hepatotoxicity and cardiotoxicity [1]. In fact, drug-induced liver injury is a major cause of safety-related drug-marketing withdrawals [2]. The structural and functional characteristics of mitochondria provide a number of off-targets for some pharmaceutical drugs that can ultimately lead to cellular bioenergetic deficit, increased free radical production, alterations in cell signalling pathways and even cell death. The aim of this study was to identify detailed mechanisms involved in the toxicity of three antipsychotic drugs: chlorpromazine, haloperidol and olanzapine. Here we investigated the *in vitro* effects of these neuroleptic drugs on bioenergetic functions of isolated rat liver mitochondria using high-resolution respirometry in combination with simultaneous evaluation of membrane potential. O_2 fluxes determined in the presence of 12.5 mM succinate and 1 µM rotenone supporting complex II-linked respiration showed that respiration was highly inhibited by chlorpromazine where as olanzapine and haloperidol showed slight effects. Furthermore, complete dissipation of mitochondrial membrane potential was observed in the presence of 200 µM chlorpromazine. MTT assays revealed the effects of these antipsychotics on the viability of HepG2 cells. Results showed that both chlorpromazine and haloperidol were cytotoxic at 15 µM (80% viability) and 50 µM (40% viability), respectively. Interestingly, 50 µM olanzapine, the highest concentration tested, showed no cytotoxic effects on HepG2 cells. On the contrary, it increased proliferation with respect to the control (140% viability). The relative potencies of these therapeutic agents as inhibitors of mitochondrial function are in accordance with the known risk of adverse effects. Our data agree with reports indicating that olanzapine, an atypical antipsychotic, is a safer drug than the typical antipsychotics chlorpromazine and haloperidol [3].

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P08-030
PXR activation dissociates hepatosteatosis from insulin resistance in obese mice

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Pregnane X receptor (PXR; NR1I2) is a xenobiotic sensing nuclear receptor identified as a regulator of glucose and lipid metabolism. Many drugs and environmental chemicals activate PXR causing impaired glucose tolerance and liver steatosis, which precede type 2 diabetes. Still, the significance of PXR in energy metabolism and in the onset of metabolic syndrome remains unclear. We aimed to investigate how PXR activation affects metabolic health in obese mice.

C57BL/6N mice were fed high-fat diet for 15 weeks and then treated with PXR activator (pregnenolone-16α-carbonitrile, PCN, 50mg/kg) for 4 days. PCN treatment dramatically potentiated hepatosteatosis in obese mice. Despite the drastic steatotic effect, PCN treatment improved glucose tolerance and HOMA-IR index, and a trend towards better insulin sensitivity was seen in insulin tolerance test. Hepatic gluconeogenesis was not affected in pyruvate tolerance test although gluconeogenic genes PEPCK1 and G6P were repressed.

A gene expression profiling study was performed to identify glucose responsive genes that may be modified by PXR activation in mouse liver. IGF-binding protein 2 (IGFBP-2) was the top hit among the glucose repressed, but PCN upregulated candidate genes. IGFBP-2 has been previously reported to improve glucose homeostasis through IGF-dependent and independent mechanisms. The upregulation of IGFBP-2 was confirmed with qPCR in PCN-treated mouse liver, while PXR knockout abolished the effect. PXR activation increased plasma soluble IGFBP-2 in chow-fed and high-fat diet-fed mice, and also in humans.

In summary, PXR-activation greatly potentiated high-fat diet-induced hepatosteatosis. Despite the aggravation of hepatosteatosis, PXR activation improved glucose tolerance and insulin sensitivity. This was associated with increased liver and circulating IGFBP-2, which may provide a mechanistic explanation for dissociation of hepatosteatosis from insulin resistance.

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P08-031
Effects of α-amanitin in HepG2 cells are not prevented by drugs used in *Amanita phalloides* intoxications

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Amatoxins, specially α-amanitin, are responsible for the major deleterious effects of *Amanita phalloides* mushrooms. However, until now, there is no clinical effective procedure or antidote for *Amanita phalloides* intoxications. The liver is a major target of α-amanitin toxicity, thus it is crucial to identify the mechanisms of α-amanitin hepatotoxicity and search for effective antidotes. The aim of this study was to evaluate the feasibility of HepG2 cells for this purpose.

α-Amanitin cytotoxicity was evaluated in HepG2 cells by MTT reduction and neutral red uptake assays, following exposure at different concentrations (0.1-20 µM) for 24 or 48 h. The effect of α-amanitin in nascent RNA synthesis, in total and reduced glutathione (GSH) levels, in mitochondrial membrane potential (MMP) and in ATP levels was assessed following exposure for 24h. Additionally, the influence of 1 µM oligomycin, an ATP synthesis inhibitor, and of 25 µM buthionine sulfoximine (BSO), an inhibitor of gamma-glutamyl-cysteine synthase, was evaluated towards the effects of α-amanitin following exposure 24h or 48h. Lastly, the influence of previously identified antidotes (1 mM N-acetylcysteine, 10 µM silibinin and 0.5 mM benzylpenicillin) but poorly effective for amatoxin-intoxications was evaluated towards the cytotoxic effects of α-amanitin 48h after exposure.

α-Amanitin caused a concentration- and time-dependent mitochondrial and lysosomal dysfunction. Additionally, α-amanitin produced a significant decrease in nascent RNA synthesis. While this amatoxin did not induce changes in MMP, it caused a significant increase in intracellular ATP levels, which was not prevent by incubation with oligomycin. α-Amanitin provoked a significant increase in total and reduced GSH levels that was abolished by pre-incubation with BSO. Notwithstanding, BSO provided partial protection towards the cytotoxic effects of α-amanitin. None of the clinically used antidotes conferred protection against α-amanitin cytotoxicity.

HepG2 cells have proven to be an interesting model for evaluating the mechanisms of α-amanitin hepatotoxicity. Nonetheless, lack of protection of the previously described antidotes for amatoxin poisoning towards α-amanitin cytotoxicity highlights the importance of the development of better antidotal strategies.

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P08-032
Computable biological network models for mechanistic 21st century toxicology

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Systems toxicology approaches with extensive molecular measurements complement apical endpoints in various areas of research, and new methods are needed to interpret these rich data and derive new hypotheses. Causal biological network models, scripted in the Biological Expression Language, facilitate the assembly of available biological knowledge in a structured format and, owing to their computability, offer mechanistic interpretation of molecular data in a well-defined biological context. The network model consists of biological entities (nodes) and relationships between the nodes (edges). Information regarding gene expression regulation by some of the nodes in the network backbone is employed to build a second, scorable layer to the network model. This layer is used to infer the activity of the backbone nodes from transcriptomic data, and the impact on the network as a whole can be assessed using the network perturbation amplitude algorithm.

Previously, we have constructed a suite of causal biological network models for the three phases of xenobiotic metabolism to better understand how toxicants are metabolized in the liver. In this work, we introduce a network model that was built to describe signaling pathways that contribute to biological processes involved in liver steatosis, involving hypoxia inducible factor 1 and 2a, sterol regulatory element binding protein, hepatocyte nuclear factors 1 and 4, and various nuclear receptors and molecules essential for lipid metabolism. Each statement (network edge) extracted from scientific literature was extensively annotated to trace back the source (PMID) as well as the biological context (i.e., species, tissue/cell type, and disease state).

When used in combination with transcriptomic data from relevant studies, the model provides mechanistic understanding and quantitative impact assessment on toxicant effects in the liver. These efforts are the beginning of the development of a suite of biological network models that can be used in the context of 21st Century Toxicology for a mechanistic and quantitative understanding of how toxic substances impact the biological system.

P08-033
Comparison of 2D and 3D cell-based models using human chemical derived hepatic progenitors to predict drug-induced liver injury

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Drug-induced liver injury (DILI) is one of the major cause of decline in new drug approval and withdrawal from the drug market. During drug development process, two-dimensional (2D) culture systems have been used to detect drug efficacy and toxicity. However, 2D systems have limitations to reflect the complexity of the liver microenvironment. In this study, we established 2D and 3D culture models using human chemically derived hepatic progenitors (hCdHs) which were reprogrammed from human primary hepatocytes, and also evaluated hepatotoxicity using traditional DILI drugs: diclofenac so-

dium (DF) and acetaminophen (APAP). For determining drug-induced liver injury, we analyzed multiple hepatotoxicity-related parameters: albumin, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and major cytochrome P450 levels (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4). After DF treatment, both 2D and 3D culture models showed increases of ALT and LDH levels in a dose-dependent manner but 3D models displayed approximately 15-fold higher compared to 2D models at the highest concentration (1000 μM). APAP has been known metabolized by CYP1A2 (contributed 30%–56%) and CYP2E1 (contributed 30%–78%). After APAP treatment, CYP1A2 and CYP2E1 mRNA levels were elevated in 3D models. Our current study presented alterations in hepatotoxicity-related parameters in both 2D and 3D hCdHs culture systems after treatment of two well-known DILI drugs. Further investigations on hepatotoxicity in hCdHs culture systems may provide valuable insights into new appropriate model for evaluation of DILI.

P08-034

Transcriptomic profiling of compound treated human liver spheroids to investigate the underlying mechanisms of drug induced liver injury observed in the clinic

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Drug-induced liver injury (DILI) is the most common cause for acute liver failure in the USA and Europe and is the leading cause of attrition of compounds in drug development. Although insights have been gained into the underlying mechanisms of idiosyncratic DILI, the prediction of human liver toxicity is still problematic. In this study, 3 compounds were selected which were active on the same therapeutic target and stopped in clinical development. Compound A and B induced clinical liver toxicity, while compound C did not.

To assess the underlying mechanisms of the clinical adverse effects, a mechanistic *in vitro* study was set-up to explore the transcriptomic profiles induced by these 3 compounds. In this proof-of-concept approach, different human liver models were used. Here, we will focus on the study performed with the 3D InSight™ Human Liver Microtissue model from InSphero.

Concentration selection was based on the clinical plasma concentrations (Cmax) and on the cytotoxicity profiles obtained in a range finder study. In total 336 RNA samples were isolated and microarray expression analysis was performed to assess the transcriptional response from >20,000 well-annotated genes, both after a 1- and 12-day exposure period. Differentially expressed genes and pathways were compared. In addition, the role of oxidative stress – cytotoxicity in the presence and absence of BSO – was assessed as possible initiator of hepatocellular necrosis.

This proof-of-concept study showed that the BSO-assay and transcriptional profiling in 3D human spheroids can discriminate between the 3 compounds in a way which is consistent with the observed clinical liver toxicities. Although this approach requires further validation (e.g. testing of high-quality annotated DILI reference compounds), it may eventually become a valuable prioritization screening tool in the selection process of safe NME candidates.

P08-035

(*S*-)Metolachlor – human relevance framework assessment of liver tumour induction in female rats

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A number of dose- and time-related key and associative events have been identified that characterise the hypothesised MOA for (*S*-)metolachlor-induced liver tumours in female rats. A non-genotoxic mode of action (MOA), initiated via activation of the constitutive androstane receptor (CAR) was demonstrated using *in vitro* and *in vivo* mechanistic studies. CAR activation was demonstrated using a CAR transactivation assay. In mechanistic studies of up to 60 days duration, (S-)metolachlor, at the tumorigenic dose of 3000ppm, increased hepatic PROD & BROD activity, liver weight, smooth endoplasmic reticulum proliferation and centrilobular hypertrophy. A key event in the CAR-mediated MOA, increased cell proliferation was maximal after 1–3 days of treatment, but not at later time points (≥7 days exposure). In contrast, the non tumourigenic dose levels (≤300 ppm) produced only minor changes in a small subset of the measured liver parameters.

The following alternative MOAs for tumour formation were evaluated and excluded with experimental data: genotoxicity, AhR activation, PPARα activation, cytotoxicity and subsequent regenerative growth and oestrogenicity.

The lack of human relevance for the proposed MOA was assessed by comparing the effects of (*S*-)metolachlor on primary cultures of rat and human hepatocytes. Treatment of rat hepatocytes resulted in CYP450 induction and replicative DNA synthesis. In contrast, treatment of human hepatocytes resulted in neither CYP450 induction, nor replicative DNA synthesis, indicating a qualitative species difference in response to CAR activation. The lack of proliferative response in human hepatocytes means it can be concluded that (S-)metolachlor does not pose a hepatocarcinogenic hazard to humans.

P08-036

An optimized process for medium chain fatty acid profiling or quantitation in com-plex matrices using derivatization and LC-MSMS analysis

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Free fatty acids (FFAs) are key molecules namely implicated in cell signaling and metabolism. The variation of the fatty acids profiles in liver cells are therefore relevant biomarkers of various toxicological effects, thus, fatty acid profiling may become a relevant endpoint for liver pathology evaluation.

Profiling and quantitation of these molecules is a powerful tool for the analysis of these molecules, however, their poor ionization efficiency under electrospray ionization mass spectrometry represent a clear limitation to the development of assays for such analysis.

In the present study, we describe the development of an optimized process for the profiling and quantification of free fatty acids by derivatization in complex matrixes such as liver cell homogenates.

Medium-chain fatty acids Pelargonic acid (C9), Capric acid (C10, Undecylic acid (C11), Lauric acid (C12), Tridecylic acid (13) and Myristic acid (C14) were analyzed using the same analysis method with clear separation and relative quantitation. Furthermore, absolute quantitation was achieved by comparison of the signal against a standard down to 50 ng/ml.

The same methodology can be anticipated as applicable to lower or higher free fatty acids chain length leading to cutting-edge technology for free fatty acids profiling in complex matrixes.

P08-037
Rifampicin induces the bone form of alkaline phosphatase (ALP) in humans

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Nuclear receptor Pregnane X receptor (PXR) is a xenobiotic-sensing nuclear receptor that regulates expression of drug metabolizing enzymes and drug transporters mainly in the liver and intestine [1]. Recently, PXR activation has been shown to have affect also other tissues including bone, but these effects are still poorly understood [1,2]. PXR knockout mice display osteopenia and reduced bone formation [2].

We performed a clinical trial on healthy volunteers to discover novel functions of PXR activation. Rifampicin, a well establish ligand for human PXR, was used as a study compound. The design of the study was randomized, single-blind, placebo-controlled and crossover. Rifampicin 600 mg a day or placebo was dosed on each arm for a week. Rifampicin induced alkaline phosphatase (ALP) blood level in healthy volunteers. Further analysis indicated that this represent the bone form of ALP.

To investigate the mechanism(s) involved, we used human osteoblast lineage differentiated from bone marrow-derived mesenchymal stromal cells. The differentiated cells were treated with different concentration of two PXR ligands i.e., rifampicin and hyperforin. Both compounds induced the mRNA level of bone biomarker genes (ALP, MGP, OPN, OPG). We also measured the ALP activity from the treated cells, and it was significantly increased. PXR expression was detected in the cells, however, the expression was very low compared with the human liver.

To further investigate potential role of PXR in the observed ALP induction by rifampicin, we treated mice and rats with a rodent PXR ligand PCN. However, PCN did not increase plasma ALP indicating that the rifampicin effect in humans is either species specific, or alternatively is not mediated by PXR.

In conclusion, we showed that rifampicin treatment induces the bone form ALP in the plasma of human volunteers. Further studies are required to establish the mechanism more precisely.

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P08-038
Study of the hepatic metabolic effects induced by PFOA exposure using a multiplatform metabolomics approach

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Perfluorialkylated substances (PFAS) are used for a wide range of industrial applications, including the manufacturing of anti-adhesive cookware coatings and waterproof clothing textiles. PFOA (perfluorooctanoic acid), one of the most studied PFAS, has raised major concerns regarding public health over the last years. Although its use is decreasing in the industry, consumers are still exposed to PFOA, which is extremely persistent and bioaccumulates in the environment. Dietary intake is reported as the main source of human exposure, especially through seafood and freshwater. Epidemiological surveys and *in vivo* studies in rodents suggest that PFAS can induce metabolic effects, especially on lipid metabolism, and might be involved in hepatotoxicity. However, the metabolic pathways affected by these compounds and the underlying mechanisms for the observed alterations remain to be characterized.

In this study, we aim at assessing the metabolic effects of acute and sub-chronic exposures to different concentrations of PFOA, focusing on the liver as the main metabolic target. We performed *in vitro* studies using the human hepatic cell line HepaRG, which constitutes a particularly relevant model for studying the long-term effects of low doses of xenobiotics and is recommended by OECD, as well as the American Tox 21 program. HepaRG cells were exposed to three concentrations of PFOA (0.001 µM, 0.1 µM and 10 µM) and during two exposure durations (24h and 7 days). Both extracellular and intracellular samples were collected to have access to the intracellular metabolic content and extracellular fluxes (intake and secretion of metabolites by cells). Three complementary approaches (untargeted NMR, targeted LC-HRMS and IC-HRMS) were performed on all samples to increase the coverage of the metabolome. The first results show a discrimination between exposed and non-exposed cells, as well as between the different doses of exposure, with distinct metabolomic patterns, suggesting the involvement of specific and distinct metabolic pathways. Further analysis of these fingerprints, in the context of the human genome-scale metabolic network, will allow getting a more comprehensive picture of the metabolic modulations induced by PFAS and improving the understanding of their Modes of Action at different doses.

P08-039
What can an animal tell the toxicologist? Concordance of toxic effects to liver and kidney between rats and humans

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Aim: This study was designed to test the hypothesis that rat models allow an accurate prediction of liver and kidney toxicity in humans. Specifically, we investigated the predictivity of common liver and kidney toxicity related endpoints from subchronic and chronic rat studies in relation to liver and kidney toxicity found in clinical trials.

Background: Many drug discovery and development (DDD) projects face severe adverse effects of the active ingredient as late as in the clinical phase or even in the post-marketing phase [1]. In many of these cases liver or kidney toxicity plays a major role. Previously, the power of animal tests to predict clinical trial outcomes has already been elucidated for large sets of substances [2,3]. However, analyses did not differentiate between different types of animal studies. Another obstacle in previous studies was the lack of reliable data on predicted negative, so a lack of detailed knowledge about the study scopes of the evaluated animal studies. Therefore the present study can facilitate animal model and lead selection during DDD as well as

uncertainty assessment in chemical risk assessments.

Methods: Observations related to liver and kidney toxicity in subchronic and chronic preclinical safety studies in rats from the eTOX and RepDose databases have been curated. In total they cover more than 100 unique chemical entities. Based on a standardized effect terminology these observations were compared to clinical trial outcomes reported in the PharmaPendium database for the same substances.

Expected results: The predictivity of liver and kidney effect-related preclinical endpoints for related clinical trial outcomes is presented in terms of accuracy as well as positive and negative likelihood ratio (LR+/-). Conclusions will be drawn on strengths and weaknesses of different animal test parameters as markers of liver and kidney toxicity in humans.

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P08-040
Development of MS-based immunoassays for quantification of drug-induced liver injury candidate biomarkers across species

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Drug-induced liver injury (DILI) shows various stages from mild to severe liver injury of which an adequate prognosis for liver damage is particularly difficult to determine. Currently, the golden standard to diagnose DILI is determining alanine transaminase and bilirubin levels, however, these markers lack of specificity and capability for an early onset diagnosis. Thus, DILI is one of the major reasons for withdrawals of approved drugs. Moreover, idiosyncratic factors prevent an effective safety toxicity assessment so far.

Within the recent years a set of proteins was identified by large consortia as the Safer and Faster Evidence-based Translation Consortium (SAFE-T) and the Predictive Safety Consortium as potential biomarkers to indicate DILI and provide insight into DILI mechanism.

Here, we aimed on establishing immunoaffinity mass spectrometry (MS)-based assays to accurately quantify these potential DILI biomarkers in plasma or serum of patients and animal models. In our approach, plasma or serum proteins are proteolytically cleaved and a unique peptide per candidate is captured by antibody-based immune precipitation. The peptides are then quantified by parallel reaction monitoring mass spectrometry in reference to a corresponding stable isotopic synthetic labelled peptide. Targeting conserved sequences in proteotypic peptides enabled assay development for several species with antibodies comprising the same epitope.

Results from assay development addressing DILI biomarkers across species such as rodents, canines, and humans will be presented and discussed. Protein biomarker levels are compared among the stated species.

Quantification of candidate biomarkers for DILI in animal models corresponding to the human biomarkers may facilitate drug development by identification of harmful drugs rather in preclinical studies than after drug approval.

P08-041
Changes in bile acid profiles induced by cholestatic drugs in HepaRG hepatocytes cultured in bile acid-enriched medium

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The two primary bile acids (BAs), cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized in the liver, and their secretion into bile is facilitated by conjugation to taurine (T) or glycine (G). In the gut they are deconjugated and dehydroxylated to form secondary BAs, mainly lithocholic acid (LCA) and deoxycholic acid (DCA). Intrahepatic cholestatic diseases of various etiologies are characterized by accumulation of BAs in the liver. Many drugs can induce cholestasis in humans but limited information exists on associated-changes in serum and liver BA profiles. The aim of this work was to analyze changes in BA profiles induced by various cholestatic and non-cholestatic compounds in HepaRG hepatocytes cultured in presence of a cocktail of nine major BAs either at physiologic (1x) or 60-fold higher (60x) concentrations for 24h, following 24h pre-incubation with the BAs only. BAs were measured by HPLC-MS/MS. Whatever the condition, no marked effects on BA profiles were observed with the non-cholestatic drugs compared to untreated cultures. In the presence of 1xBAs the main changes were observed with major cholestatic drugs, such as cyclosporine A, troglitazone, bosentan, fasudil and chlorpromazine; they were typified by increased (CDCA) or detectable (LCA and DCA) amounts in both supernatants and cell layers (intracellular + bile canaliculi). With 60xBAs, CDCA, CA and DCA conjugates and sulfated LCA were decreased while their unconjugated forms were increased, in supernatants. Most conjugates, including sulfated LCA (LCA-S3 and TLCA-S3), and strikingly unconjugated CDCA, LCA, DCA and CA were markedly increased in cell layers. These data demonstrate that in presence of exogenous BAs cholestatic drugs can alter *in vitro* BA profiles in both supernatants and cell layers, particularly by causing preferential cellular accumulation of unconjugated toxic hydrophobic BAs.

P11 – Nanotoxicology

P11-001
Lipidomic analysis of PLHC-1 topminnow liver cells exposed to bisphenol F and bisphenol A diglycidyl ether

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Plasticizers are widespread environmental contaminants that have been described as obesogens in terrestrial vertebrates. However, there are currently no equivalent in-vitro methods to investigate their mode of action in fish. This work explores the use of PLHC-1 cells as an alternative model to assess the alteration of hepatic lipids after 24 h of exposure to bisphenol F (BPF) and a chlorinated derivative of bisphenol A diglycidyl ether (BADGE·2HCl). PLHC-1 lipid extracts were analyzed by flow injection coupled to high resolution mass spectrometry (FIA-ESI(+/-)-Orbitrap-Exactive). The analysis of the intracellular concentration of the chemicals revealed the highest bioconcentration of BADGE·2HCl, which in turn induced a significant depletion of triacylglycerides (TGs) in PLHC-1 cells at internal concentrations close to those described in the liver of marine mammals. Exposure to BPF induced the generation of reactive oxygen species and a lipidic profile

characterized by (a) a significant decrease in phosphatidylcholine (PC)- and phosphatidylethanolamine (PE)-plasmalogens, which are the lipids more sensitive to oxidative damage, and (b) hydrolysis of TGs, particularly of those enriched in polyunsaturated fatty acids. Changes in the lipidic profile occurred at concentrations well below the cytotoxic effect of the chemicals, and provided evidence of the different modes of action of BPF and BADGE·2HCl. Overall, the use of topminnow liver cells in lipidomic studies is a powerful tool to evaluate the bioconcentration and metabolic/ lipidic responses to plastic additives in fish.

P11-002

Cadmium telluride quantum dots induced the histopathological changes of livers and kidneys in mice via elevating hydroxyl radicals and decreasing antioxidant capacities

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Although quantum dot (QD)-induced toxicity occurs due to free radicals, generation of oxidative stress mediated by ROS formation is considered an important mechanism. However, free-radical mechanisms are essentially difficult to elucidate at the molecular level because most biologically relevant free radicals are highly reactive and short-lived, making them difficult to directly detect, especially *in vivo*. Antioxidants play an important role in preventing or, in most cases, limiting the damage caused by ROS. Healthy people and animals possess many endogenous antioxidative substances that scavenge free radicals *in vivo* to maintain the redox balance and genome integrity. The antioxidant capacity of an organism is highly important but seldom studied. In this study, male ICR mice were administered a single intravenous dose (1.5 μmol/kg) of CdTe QDs, and liver and kidney function and morphology were subsequently examined at 1, 7, 14, and 28 days. Furthermore, ·OH production in the tissue was quantified by trapping ·OH with salicylic acid (SA) as 2,3-dihydroxybenzoic acid (DHBA) and detecting it using a high-performance liquid chromatography fluorescence method. The antioxidant capacities of the liver and kidneys were investigated using the EPR spin tapping technique. We found that the QD-induced histopathological changes were time-dependent with elevated ·OH and decreased antioxidant capacity, and could recover after a period of time. The ·OH exhibited delayed effects in terms of histopathological abnormalities. QD-induced antioxidant efficiency reduction was time dependent with GSH decrease. These experimental results offer new information on QD toxicity *in vivo*. Specifically, CdTe QDs can elevate ·OH and deplete GSH to reduce the elimination ability of liver and kidneys for ·OH and $\cdot O_2^-$, thus inducing oxidative damage to tissues.

P11-003

***In vivo* toxicological evaluation of natural repellent in nanotechnological matrix**

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It is well known that millions of people around the world are affected every year by diseases transmitted by several mosquitoes, among them Aedes aegypti, which transmits diseases such as dengue, chikungunya and Zika. Vector control and personal protection, such as the use of repellent, are important to minimize the onset of disease. For this reason, safer alternatives with an effective and lasting impact against various insects is necessary. Many essential oils have been labelled with repellent properties, such as citronella oil, in addition to their terpene alcohols. The encapsulation of citronella oil and / or association with essential oils or vegetable oils, is expected to increase repellent action time and decrease its characteristic odor. Lipid nanoparticulate systems (LNS) are underexplored and are highly promising for the delivery of bioactive substances. In order to optimize their properties and application, it was proposed in this project to carry out safety studies for solid colloidal carriers of lipid base associated with vegetable oils. The Fluorescence Lifetime Imaging Microscopy (FLIM) method, which is predominantly used to evaluate the metabolic state of the tissue in response to a change in the microenvironment by monitoring changes in the fluorescence lifetime of endogenous fluorophores such as NAD(P)H and FAD. Metabolic changes in tissues can be measured as changes in NAD(P)H or FAD fluorescence lifetime, and / or as changes in the redox ratio or proportion of free and to protein-bound NAD(P)H (α1/α2). FLIM images were acquired with the DermaInspect Multiphoton Microscope (JenLab GmbH) equipped with a TCSPC830 detection module (Becker & Hickl GmbH). *In vivo* experiments were conducted on three participants. We found that there were no significant changes in NAD(P)H lifetime and α1/α2 ratio in the viable epidermis *in vivo* following topical application of LNS formulations containing Citronella Oil. This suggests that LNS formulations are safe for application for use on human skin and do not cause any measurable changes in the metabolic activity of the viable epidermis.

P11-004

Evaluation of the effect of cellulose nanofibers on skin irritation using a 3D *in vitro* reconstructed human epidermis model

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Cellulose nanofibers (CNFs) are new nanomaterials with a potential to be used in various applications. However, to accelerate the practical use of CNFs in society, hazard assessment of CNFs needs to be performed. With regard to dermal exposure to chemical substances, evaluation of permeability of chemical substances and the consequent skin irritation is an important part of hazard assessment. For this, tests using animal skin have been conducted so far. However, for animal protection in Europe, animal testing for safety evaluation of cosmetics and their raw materials has been banned. In recent years, as a substitute for animal skin testing, three-dimensional (3D) *in vitro* reconstructed human epidermis (RHE) models have been gaining popularity. Unlike animal skin, human cells do not pose interspecies difference challenges. In addition, there are fewer variations between batches, leading to high reproducibility across test results. In view of these circumstances, our research project was aimed at developing a method for testing skin penetration of CNFs, aiming at support of voluntary safety management of business operators. There have been no reports of studies on dermal toxicity and skin permeability of CNFs using a 3D RHE model. Thus, we propose that an appropriate skin penetration test is required, as CNF has an intermediate property between gel and sol, and its viscosity changes with time and shear stress (thixotropy). CNF demonstrates diverse physical properties, such as fiber diameter, fiber length, morphology, functional group, and impurities, depending upon the type of raw material and the method of chemical treatment/defibration treatment

used. Therefore, TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical)-oxidized CNFs (TOCN) were selected as a test material from representative CNFs developed in Japan. In this study, we analyzed CNF sample preparation conditions, and employed an *in vitro* skin irritation test using two 3D RHE models (EpiDerm™ (EPI-200SIT) and SkinEthic™ RHE), according to the OECD test guideline for the Testing of Chemicals 439. This study was supported by the New Energy and Industrial Technology Development Organization (NEDO), Japan.

P11-005

Amorphous silica nanoparticles trigger human dendritic cell maturation *in vitro* and provoke CD4+T Cell proliferation

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Danger signals activate dendritic cells (DCs) stimulating both the innate and adaptive immune responses. DCs could sense nanomaterials, considered as NAMPs (nanoparticles-associated molecular patterns) and undergo a maturation process enabling them to migrate to regional lymph nodes and to activate naive T-lymphocytes. Amorphous silica nanoparticles (aSNPs) are widely used in dietary supplements, biomedical applications, cosmetics or construction materials. General population is probably more exposed than initially anticipated and occupational exposure, particularly via the inhalation route, should be better evaluated. Indeed, despite generally presented as highly biocompatible as compared to their crystalline counterparts, aSNPs could contribute to or exacerbate the onset of allergic airway disease.

The aim of this work was to evaluate the effects of aSNPs on human DCs *in vitro*. Human monocyte-derived DCs were exposed for 16 hours to final concentrations of 12,5 and 25 µg/ml of fumed silica nanoparticles. We measured cell viability, phenotypical changes, cytokines production and allogenic CD4+ T cells proliferation upon NP treatment. Endotoxin levels were unlikely to have any effect on DCs since no activity was found in the media.

Results showed that the aSNP significantly upregulated the CD86 costimulatory molecule, as well as the CD83 maturation marker and the CXCR4 chemokine receptor surface expressions. Secretions of inflammatory cytokines such as IL-1b, IL-6, IL-8 or TNF-a were significantly enhanced in a dose-dependent manner in the DC culture supernatants. To evaluate whether aSNPs could induce DC to become functionally mature, we assessed their capacity to activate allogeneic T cells. Results showed that the increase in T-lymphocytes proliferation in presence of aSNP-treated moDCs was statistically significant for all tested DC/T ratios compared to uncharged DCs. Moreover, analysis of the co-culture supernatants for the production of T cell-derived cytokines showed a significant increase of IL-9 and IL-17A and F, and an upregulation of IL-5, consistent with the pro-inflammatory phenotype of DCs described above.

Taken together, these results suggest that aSNPs are able to induce functional DCs maturation and could act as adjuvants of the immune system.

P11-006

Evaluation of DNA damage in the rat lung after inhalation exposure to TiO_2 and SiO_2 nanoparticles

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Titanium dioxide (TiO_2 NPs) and silica (SiO_2 NPs) nanoparticles are widely used for several applications, increasing the concern about the possible risks they may pose to human health. Inhalation is a major route of exposure for these nanoparticles. This study aimed at evaluating the potential DNA damage in the lungs of male Sprague Dawley rats(n = 5/per group) exposed for 5 days, 6h/day by whole-body inhalation to aerosolised TiO_2 NPs (0.5 to 10 mg/m^3) and SiO_2 NPs (0.5 to 5 mg/m^3) at 5 days(nonrecovery group) and 21 days (recovery group) after the initial exposure. Rats administered (i.p.) with methyl methanesulfonate (MMS) at a dose of 100 mg/kg were used as positive controls (n = 3). Lung cells were isolated by mechanical disruption and primary and oxidative DNA damage assessed by the alkaline and FPG-modified Comet assay versions, respectively. At least 100 cells/tissue (50 in each replicate gel) were scored using the Comet Assay IV software (Perceptive Instruments, Suffolk, UK) and the mean of the percentage of DNA in the comet tail (% tail intensity) was used as DNA damage descriptor.

Exposure to all tested TiO_2 NPs doses did not induce significant primary DNA damage in the lung tissue in the nonrecovery (5.60 ± 1.84 *vs* 5.47 ± 2.03% tail intensity;10 mg/m^3) and recovery animal groups (9.81 ± 4.51 *vs* 8.06 ± 1.93; 10 mg/m^3) compared to the respective controls. Similar findings were observed in the lung of aerosolised SiO_2 NPs exposed rats either in the nonrecovery (14.87 ± 3.19 *vs* 15.84 ± 2.47% tail intensity; 5 mg/m^3) or recovery group (8.87 ± 1.41 *vs* 6.36 ± 1.17% tail intensity; 5 mg/m^3) compared to the controls. In addition, exposure to both types of NPs did not cause a significant increase in lung oxidative DNA damage in both groups. As expected, DNA damage in the lung of MMS-injected animals showed a significant increase in the % tail intensity (74.50 ± 4.28) compared with the control group.

Our data suggest that inhalation exposure to the tested doses of TiO_2 NPs and SiO_2 NPs do not affect DNA integrity in the rat lung. Nevertheless, further research should be conducted, namely the evaluation of other genotoxicity endpoints to support these findings.

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P11-007

Co-delivery of pemetrexed and quercetin with multi-walled carbon nanotubes displayed synergic effects in pancreatic cancer cells

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Combination of conventional and natural chemotherapeutics was proved to offer synergistic anticancer efficacy, sometimes minimizing adverse effects. In this study, we aimed to investigate *in vitro*, the cytotoxic activity of pemetrexed (PMX) and quercetin (QCN) delivered separately or simultaneously in multi-walled carbon nanotubes

(MWCNTs) and to compare their anticancer potential.

Carboxyl-modified MWCNTs were mixed with QCN, PMX, and respectively QCN-PMX solution. The samples were characterized by FT-IR spectroscopy, DLS, zeta potential and drug release profile. Different doses (between 3.125–50 μg/mL MWCNTs, 2.5–40 μg/mL PMX and respectively 0.25–4 μg/mL QCN) of the obtained nanoformulations (MWCNT-PMX, MWCNT-QCN and MWCNT-PMX-QCN) were tested on two human cell lines: MDA-MB-231 (breast cancer cells) and PANC-1 (pancreatic tumor cells). Biological effects were assessed after 24 h exposure analyzing cell viability, cell morphology, cellular uptake and internalization into lysosomes as well as reactive oxygen species (ROS) production.

The results indicated higher stability of MWCNT-PMX-QCN nanoformulation compared with the other samples and smaller size of MWCNTs when conjugated with drugs (369–460 nm) compared with free MWCNTs (approx. 700 nm) as a result of electrostatic charges and lower aggregation. *In vitro* results showed higher cytotoxic effects of MWCNT-PMX-QCN nanoformulation compared with the single-drug ones in pancreatic cells. In breast cancer cells the cytotoxic effects were less pronounced and quite similar between the three nanoformulations. The MWCNT-PMX-QCN nanoformulation induced significant alterations of pancreatic cell morphology and a decrease of cell viability under 60% at a dose of 25 μg/mL MWCNTs – 20 μg/mL PMX – 2 μg/mL QCN. The evaluation of drug release revealed also the highest percent (approx. 42%) for MWCNT-PMX-QCN sample at pH 7.4 after 24 h. In accordance with these observations, a significant increase of lysosome number (by max. 2.2 fold compared to single-drug samples) and an elevation of the intracellular ROS level (by max. 1.6 fold) at doses over 25 μg/mL MWCNTs – 20 μg/mL PMX – 2 μg/mL QCN were also noticed after 24 h for the same sample confirming the cellular internalization and co-oxidant drug activity in pancreatic cancer cells.

We concluded that the combination of PMX and QCN exhibits synergic effects on pancreatic cancer cells but not in breast cancer cells, showing a superior therapeutic efficacy compared with single-drug nanoformulations by expressing higher stability, internalization rate, and oxidant activity.

P11-008
Therapeutic effects of oxidized single-walled carbon nanotubes loaded with cisplatin on breast cancer multicellular tumor spheroids

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The aim of this study was to evaluate the anti-tumoral efficiency of oxidized single-walled carbon nanotubes (SWCNT-COOH) loaded with cisplatin (CDDP) on breast cancer multicellular tumor spheroids (MCTSs).

SWCNTs were functionalized by acid treatment, resulting SWCNT-COOH. Further, SWCNT-COOH were mixed with dimethylformamide and CDDP to obtain the nanocomposite SWCNT-COOH-CDDP. The efficiency of drug encapsulation was checked by ICP-MS and it was found that the encapsulated CDDP is 192.82 μg/mL.

Breast cancer MCTSs were generated from MDA-MB-231 cells in a medium with 2.5% Matrigel. The toxicity of SWCNT-COOH-CDDP and free components was tested at doses of 1 μg/mL SWCNT-COOH/0.6 μg/mL CDDP and 4 μg/mL SWCNT-COOH/2.52 μg/mL CDDP after 24 and 48 h of incubation. Optical microscopy was used to analyze the morphology of treated and untreated MCTSs, while the presence of lysosomal vesicles was observed by fluorescence microscopy. The proliferative capacity of breast cancer MCTSs was assessed by evaluating the protein expression of proliferating cell nuclear antigen (PCNA) by immunoblotting. The protein expression of cathepsin B and phosphatidylinositol 3-kinase (PI3K) was also analyzed. The evolution of invasive potential of MCTSs was monitored after the embedding of MCTSs in a matrix composed of basement membrane proteins.

The results revealed that after 48 h of incubation with 4 μg/mL SWCNT-COOH-CDDP the dimensions of MCTSs decreased and their spherical morphology was altered probably due to the detachment of cells from the proliferative layer. However, the expression of PCNA remained constant after treatment, suggesting that nanocomposite SWCNT-COOH-CDDP did not affect the proliferative capacity of MCTSs. An increase in the lysosomes number in presence of SWCNT-COOH-CDDP was observed in correlation with the rise of cathepsin B expression, after 24 h of exposure, indicating that their uptake occurred by an endocytic pathway. An up-regulation followed by a down-regulation of PI3K expression after 24h respectively 48h was noticed. Moreover, after 48 h of incubation, the invasive potential of breast cancer MCTSs was significantly inhibited in the presence of 4 μg/mL SWCNT-COOH-CDDP in comparison with 2.52 μg/mL free CDDP.

We concluded that nanocomposite SWCNT-COOH-CDDP showed high efficiency in the transport of CDDP in the breast cancer MCTSs and anti-tumoral activity by inhibiting their invasive potential and initiating probably cell death.

P11-009
Silica nanoparticles induce inflammatory response by interfering with cell autophagy

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Objective: To investigate the mechanisms of inflammation caused by Silica nanoparticles.

Methods: RAW264.7 mouse macrophage cells were cultured and randomly divided into 5 groups: negative control group, positive control group (LPS10ng/ml exposure 24 hours), silica nanoparticle exposure groups (5μg/ml, 10μg/ml, 20μg/ml , 40ug/ml), the exposure time was 24h, the morphological changes of the cells were observed under microscope and the activity of lactate dehydrogenase (LDH) in macrophages, intracellular activity (ROS) concentration was used to detect the oxidative damage of the cells. The cytotoxicity of silica nanoparticles was determined by CCK-8 cell proliferation-toxicity method. The inflammatory responses were tested by ELISA and qPCR methods. The relationship between cell autophagy and cell inflammatory response was detected by Western Blot.

Results: Compared with the negative control group, the cell density was decreased and the irregularly morphological changes were observed. The data of the oxidative damage test showed that in the the exposed groups ROS and LDH activities were higher than negative control group, even in the 40ug/ml exposure group, the activities were higher than the positive control group ($P < 0.05$). Compared with the negative control group, the levels of TNFa in the exposed groups were higher than those in the negative control ($P < 0.05$). The expression at transcription level of the NLRP3, Caspase-1, TNF-α, IL-1β, IL-6 and IL-18 showed an increased trend in the exposed groups. Meanwhile the protein levels of NLRP3, IL-1β, NF-κB, P-NF-κB, Caspase-1, LC3, P62 in the exposed groups were higher than the negative control group. Dose-dependent relationships existed for all tests.

Conclusion: Silica nanoparticles induced oxidative stress and cytotoxicity to the RAW264.7 macrophages, then caused the inflammatory response which may associate with the interference of cell autophagy.

P11-010

In vitro* toxicity of model ZnO-Ag nanoparticles in human lymphocytes and hemocytes of mussel *Mytilus galloprovincialis

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The present study investigates the effects of ZnO-Ag nanoparticles (ZnO-Ag NPs) on two *in vitro* biological models, i.e. human lymphocytes and mussel hemocytes, using a battery of bioassays, commonly linked to cytotoxic and genotoxic/mutagenic, as well as cytotoxic and oxidative effects, respectively. In this regard, different concentrations of ZnO-Ag NPs manufactured through Flame Spray Pyrolysis were tested in cultured human lymphocytes (0.5, 5, 10 and 20 μg mL^{-1}) via the Cytokinesis-Block micronucleus (CBMN) assay and in primary cultures of *Mytilus galloprovincialis* hemocytes (0.1, 0.5 and 1 μg mL^{-1}), using cytotoxic (i.e. neutral red retention time/NRRT assay) and oxidative (determination of superoxide anions, nitric oxide and lipid peroxides) stress indices for determining NPs cytotoxic, oxidative and genotoxic potential in any case. The obtained results were also compared with relevant data derived from bulk metal ions Zn^{+2} and Ag^{+}. According to the latter, none of the ZnO-Ag NPs concentrations exhibited genotoxicity in human lymphocytes, while the Cytokinesis block proliferation index (CBPI), used for the assessment of cytotoxicity, showed ZnO-Ag NPs cytotoxic potential, similar to the results in the case of bulk metal ions Zn^{+2} and Ag^{+}. As far as mussel hemocytes are concerned, the results demonstrated a significant increase of cell death after treatment with ZnO-Ag NPs, with maximum values of cell death at concentration 1 μg mL^{-1}. A significant increase of $O_2^{\bullet-}$ and MDA was shown, compared to those values observed in control cells in each case, whereas a statistically significant decrease of NO was demonstrated. The comparative study of cytotoxic and oxidative effects of ZnO-Ag NPs, $ZnCl_2$ and $AgNO_3$ demonstrates the cytotoxic nature of NPs compared with the bulk metal ions Zn^{+2} and Ag^{+} (ZnO-Ag NPs > $AgNO_3$ > $ZnCl_2$), as well as NPs oxidative potential in the mussel hemocytes.

P11-011

Biological effects of molybdenum(IV) sulfide in the form of nano- and microparticles after intratracheal instillation in rat

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Considering application of molybdenum(IV) sulfide (MoS_2) in the nanosize form as a lubricant and scarcity of data on its biological effect *in vivo* with contradictory results *in vitro*, the study was undertaken to characterize its activity after short- and long-term exposure by intratracheal instillation.

Prepared nano- and microparticles (bulk MoS_2 from US Research Nanomaterials, Inc.) were disk-shaped (97x8.5 nm by TEM) and plates (1.92x0.273 μm by TEM), respectively. Sprague Dawley rats were treated via intratracheal instillation with micro- and nanosized MoS_2 at 1.5 or 5 mg/kg, using single exposure (analysis after 1 and 7 days) or multiple exposures (7 exposures every 2 weeks with analysis after 90 days). The following parameters were assessed: blood hematology, biochemistry (albumin and total protein concentration, triglycerides, urea, total cholesterol and HDL, uric acid, ASPAT, ALAT, GSH-Px activity), cytotoxic effects in bronchoalveolar lavage (BAL), comet assay on blood leukocytes, histopathological evaluation.

No acute effect was observed 1 or 7 days after single exposure of the animals to MoS_2 in both forms. No clinical signs of systemic toxicity were noticed after multiple exposures. Some hematological and biochemical changes were observed, however no uniform pattern of toxic effects was evident.

After 90 days histopathological analysis revealed inflammatory changes in lungs in all animals treated with nano- and microform. Index of histopathological changes (range: 0–4 points) reached on average 1.33 and 1.67 for nanoform and 1.33 and 2.83 for microform (dose 1.5 and 5 mg/kg bw, respectively). In control group small inflammatory lesions in lungs were observed in 5/12 animals (index 0.42). Comet assay showed no significant DNA damage in blood lymphocytes in the exposed groups.

In conclusion, repeated intratracheal exposure to micro- and nanosized MoS_2 can lead to inflammatory changes in the rat respiratory system, slightly stronger for the microform.

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P11-012

Assessment of reactive oxygen species in tobacco (*Nicotiana tabacum* L.) seedlings exposed to silver nanoparticles

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Silver nanoparticles (AgNPs) have wide application in many consumer products due to their unique chemical and physical features enhancing well known antibacterial and antifungal properties of silver. Increase in AgNPs production has raised many concerns about their possible toxicity, and many studies have already indicated that they induce oxidative stress through enhanced production of reactive oxygen species (ROS). Rapid accumulation of ROS and imbalance in their generation and scavenging could result in severe damage of proteins, lipids and DNA. Since many factors control the ROS metabolism, detecting ROS accurately is challenging. The aim of this study was to develop fast and reliable method for quantitative determination of ROS in the plant tissue and to test the method in AgNP-treated tobacco (*Nicotiana tabacum* L.) seedlings. Three weeks old seedlings were treated with 25, 50, 75, 100 and 150 μM polyvinylpyrrolidone (PVP)-coated AgNPs for 7 days. Cytosolic ROS level in the plant extracts was assessed with fluorescent probe dihydroethidium (DHE) that specifically detects superoxide radical, and for quantification of fluorescence microplate reader wavelengths were set at 520 nm for excitation and 600 nm for emission. To optimize the method, optimal incubation time of the reaction was tested by monitoring fluorescence immediately after addition of DHE, and after 5 and 15 minutes of incubation. The linearity of the method was tested by measuring fluorescence of several dilutions of the extracts. Testing the incubation time for the samples showed that incubation could lead to false

results and should be omitted. Moreover, measuring several dilutions of the samples confirmed linearity of the method thus proving that this method could be used for ROS quantification in the plant extracts. Finally, obtained results showed dose-dependent increase of ROS in AgNP-PVP treated tobacco seedlings indicating that oxidative stress is involved in toxicity of AgNPs towards plants.

P11-013

Mechanism of toxicity of amorphous silica nanoparticles in lung epithelial cells and macrophages

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Synthetic amorphous silica nanoparticles (SAS NPs) are the most abundant nanomaterials and widely used in industry and in consumer products. As mechanisms of toxicity are still insufficiently understood for silica NPs, we studied the effects of two different types of nanosilica (colloidal and pyrogenic) in human lung epithelial cells (A549) and in murine RAW264.7 macrophages. Both silica NPs dose-dependently induced membrane leakage and cell death in the absence of serum without obvious involvement of reactive oxygen species. Interestingly, at low concentrations nanosilica triggered autophagy, evidenced by morphological and biochemical hallmarks such as autophagolysosomes or increased levels of LC3-II, which serves to protect cells from cytotoxicity.

According to the oxidative stress model, also nanosilica elicits an, albeit modest, anti-oxidative response as well as pronounced pro-inflammatory reactions and cytotoxicity in macrophages. Interestingly however, these three tiers of toxicity seem to operate separately of each other for nanosilica. Specifically, impeding the anti-oxidative response by scavenging of reactive oxygen species does not prevent the pro-inflammatory and cytotoxic response. Furthermore, blocking the pro-inflammatory response does not impair cell death.

We further investigated the impact of the protein corona on the biological activity of colloidal and pyrogenic nanosilica. Adsorption of serum proteins to the nanosilica surface suppressed cytotoxicity as well as inflammation in both cell lines. Cytotoxicity precedes the onset of pro-inflammatory gene expression and cytokine release as exemplified for IL-8 in A549 cells and TNF-alpha in RAW264.7 macrophages. Formation of a protein corona not only inhibited cellular toxicity, but also the pro-inflammatory response.

As hazard assessment has been guided by the prevailing assumption of a dose-dependent coupling of sequential tiers of toxicity, identification of critical physico-chemical parameters to assist the safe-by-design concept should be enabled by simply monitoring one of the toxicity read-outs. Our results indicate a more complex scenario in the case of nanosilica, which triggers independent pleiotropic effects possibly also related to different material properties and primary cellular targets.

P11-014

Effects of silver nanoparticles and silver nitrate on photosynthesis and photosynthesis-related proteins in tobacco (*Nicotiana tabacum*) – a comparative study

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The small size of nanoparticles (NPs), with dimensions between 1 and 100 nm, results in unique chemical and physical characteristics, which is why they are being implemented in various consumer products. Therefore, one of the important concerns is the potential detrimental impact of NPs on environment. Among different types of available nanomaterials, the most frequently applied are silver nanoparticles (AgNPs) due to antimicrobial properties of silver. As plants are the vital part of ecosystem and the first component of the food chain, the investigation of NPs phytotoxic effects is of particular interest. In this study, we examined the effects of citrate-coated AgNPs and its bulk form ($AgNO_3$) on photosynthesis and leaf proteome of tobacco plants exposed to 100 μM AgNPs and $AgNO_3$ for 7 days. Silver accumulation in leaf tissue was determined by ICP-MS. Changes in photosynthesis were evaluated by measuring the chlorophyll fluorescence parameters, while content of photosynthetic pigments was analysed by HPLC. Two-dimensional gel electrophoresis (2-DE) and MALDI mass spectrometry were employed to reveal the changes in protein expression. Both types of treatments resulted with the similarly increased Ag uptake. Significantly decreased photochemical quenching and increased concentration of violaxantin was recorded after exposure to AgNPs. On the contrary, treatments with $AgNO_3$ did not significantly influence fluorescence parameters, although they induced a negative effect on majority of photosynthetic pigments. Identified proteins with differential expression were found to be mostly photosynthesis-related and down-regulated, although almost half of the proteins exhibited different expression level between AgNPs and $AgNO_3$ exposure. Obtained results indicate that the AgNPs effects observed in tobacco leaves are not simply due to the release of silver ions and can be correlated with distinct impact of silver nanoparticle form.

P11-015

Surface modification of halloysite nanotubes increases surface area and airway toxicity in mice

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Halloysite [$Al_2Si_2O_5(OH)_2$] is an abundant natural clay mineral that can occur as nanotubes of rolled-up aluminum silica sheets (HNTs). HNT size and structure depend on the formation conditions and therefore vary between locations of origin. Diameters are typically < 100 nm, with length to diameter ratios up to 200. More than 50 000 metric tons of HNTs are mined annually. Due to the hollow nanostructure that allows for loading and subsequent release of compounds applications are wide, e.g. HNTs may be loaded with antimicrobial agents (natural essential oils) and subsequently incorporated into polymers to form packaging materials that may increase food shelf life. Considering their high production volume and physico-chemical characteristics, where HNTs have the characteristics of poorly soluble high aspect ratio nanomaterials (HARN), knowledge about their toxicity is highly warranted [Koivisto *et al*. 2018].

We assessed the toxicological response to HNTs following airway exposure, an evident occupational exposure route of concern during mining, processing and handling of HNTs. Both a pristine (NaturalNano, NN) and a HNT modified by surface chemical etching by sul-

furic acid (NNEtched) were assessed. BET surface area was 4–5 times higher for the NNEtched compared to the pristine HNT. First, the potential cytotoxicity of the two HNTs was screened *in vitro* in MutaTM-Mouse lung epithelial cells. Then adult female C57BL/6J BomTac mice were intratracheally instilled once with 6, 18 or 54 µg HNTs in isoflurane anesthesia and compared to vehicle controls and 162 µg Carbon black Printex 90 (positive inflammation control) on day 1, 3, and 28 after instillation. Lung inflammation was determined by the cellular composition of bronchoalveolar lavage (BAL) fluid, acute phase response by *Saa* mRNA levels (real-time quantitative PCR) in lung and liver tissue, and genotoxicity was analyzed by the alkaline comet assay as DNA strand breaks in BAL cells, lung and liver tissue. None of the HNTs were cytotoxic, affected mouse body weight or induced genotoxicity. At the highest dose level, NNEtched increased neutrophil influx and *Saa3* mRNA levels in lung at all assessed time points. At day 1 post-exposure, the induced inflammation correlated well with the deposited particle surface area, indicating that the increase in BET surface area due to etching could explain the difference in the inflammatory potential of the studied HNTs. This is consistent with observations for other nanosized particles.

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P11-016
Surface chemistry can drive the safer applications of cadmium-based quantum dots related to sex-specific neurodevelopmental adverse outcomes

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Engineered nanomaterials (ENMs) are widely applied to a large number of market sectors that impact the lives of billions of people. However, the ability to fully characterize the risks associated with human exposures to ENMs has been limited. Cadmium-selenium containing quantum dots (QDs) have unique optical properties that favor clinical applications. Therefore, with the growing use of QDs in medical applications, there is an increased probability QDs interact with sensitive human receptors. Consequently, there is a need to investigate associated human health risks, specifically those related to neurodevelopmental disorders. *In vitro* human neural progenitor cell (hNPC) lines that originate from different sexes are promising *in vitro* models to evaluate potential sex-specific chemical effects on neurodevelopment. Cytotoxicity of two CdSe/ZnS QDs of differing surface chemistries (ITK™: carboxyl functional group; Qtracker®: polyethylene glycol with no reactive functional group) was quantified by LDH assay on proliferating and differentiating hNPCs from male (NSC-H14) and female (hNP1™) donors. Cadmium chloride was used as control for cytotoxic effects related to Cd^{2+} release. Exposures (days *in vitro* 1, 24 h) to ITK at five different doses (2.5–40 nM) demonstrated a significant dose-dependent decrease in viability of both cell lines during the proliferation stage. Significant response difference to ITK between cells from male (NSC-H14) versus female (hNP1) origin was also observed, and hNPCs of male origin were more sensitive to ITK than cells of female origin. Qtracker, at the same dose range as ITK, did not induce cytotoxicity in the proliferation or differentiation stages of either hNPC line. Cd (2.5–40 nM) induced a significant dose-response increase in viability of differentiating hNP1. Our results indicate that the main contributing factor of the QDs cytotoxicity is not from Cd ion release. In addition, we find the surface coating of QDs affects the cytotoxicity of these ENMs on hNPCs, suggesting that PEGylation can be a strategy for the safer application of QDs in clinical settings. Our findings also demonstrate the need to further evaluate sex-specific neurodevelopmental effects of ENMs.

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P11-017
Cobalt-impregnated tungsten nanoparticles and cobalt ions trigger toxicity in differentiating neuronal cells: potential link to parkinsonian neurodegeneration

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Tire studs, with pins made of cobalt-doped tungsten carbide (WC-Co), are used in many countries during winter to improve the gripping power on icy roads. During their use, cobalt and tungsten based nanoparticles (NPs) may be released. Recent material flow analysis studies performed in the MISTRA Environmental Nanosafety program have shown that there is a high dissipation rate of tungsten in tire studs and that there is presently no functional recycling of the tungsten [Furberg *et al.*, 2019]. In the present study, tungsten and cobalt based NPs were studied to understand their potential neurotoxicity using a retinoic acid (RA) differentiated human neuroblastoma cell line. $CoCl_2$ was also included as ionic control. Differentiated SH-SY5Y cells displayed characteristics of dopaminergic as well as cholinergic neurons. The hydrodynamic size of NPs was between 400–600 nm in MilliQ water. It was also observed that these NPs sediment within 1 h of dispersion in cell culture medium. The zeta potential values were negative and were reduced in cell culture medium. ICP-MS analysis revealed that WC-Co and CO NPs released approximately 77% and 96% Co ions, respectively, in cell culture medium after 24 h. TEM showed that the NPs were internalized in SH-SY5Y cells and triggered ultrastructural changes. Co NPs were specifically internalized in cells through endosome formation and the NPs caused mitochondrial damage. Co NPs were found the most toxic when compared to WC and WC-Co NPs as determined by the Alamar Blue assay and the toxicity was paralleled by the toxicity of the cobalt salt. We also observed that differentiating cells were more sensitive to Co NPs than undifferentiated and differentiated cells. Furthermore, calcium overload and oxidative stress were shown to play a role cell death. Real-time PCR analysis of cholinergic and dopaminergic markers in RA differentiated SH-SY5Y cells revealed overexpression of markers of cholinergic neurons after exposure to Co NPs and Co ions indicative of a relative decline in dopaminergic neurons. Finally, *in silico* analysis of publically available transcriptomics data [Serra *et al.*, 2019] predicted a significant connection between WC-Co NPs, the parkinsonogenic chemical, MPTP and L-dopa, which ameliorates symptoms in patients with Parkinson's disease. Overall, this study provides ini-

tial evidence of the neurodegenerative potential of Co-based NPs in human systems.

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P11-018

This abstract has been withdrawn.

P11-019

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P11-020
Neuro- & biochemical- toxicity of silver nanoparticles and silver nitrate in soil to *Aporrectodea calginosa* earthworms

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Silver nanoparticles (AgNPs) now widely used in many industry applications discharged via the land application of sewage sludge [Meier *et al*. 2016] interact with soil biota, including earthworms. Because there are no regulations on discharge limits, improper discharge of waste from these industries can lead to environmental contamination and damage to ecosystem organisms (Kühnel and Nickei 2014). In this study *Aporrectodea caliginosa* earthworms were exposed to 0 (control), 0.3, 3, 30, 300 and 0 (control), 0.03, 0.3, 3, 10 mg/kg of AgNPs and $AgNO_3$ in soil respectively for 4 weeks and select biochemical and neurotoxicity studies were conducted. The lipid peroxidation (a measure of thiobarbituric acid reactive substances; TBARS) and activities of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase, glutathione S transferase, lipid peroxidation), and nerve conduction velocity (NCV) of the medial giant fibers (MGF) using a novel non-invasive electrophysiological technique were measured in earthworms at 1, 2, 3, and 4 weeks. The TBARS and antioxidant enzyme activities were elevated by both $AgNO_3$ and AgNPs and this was most evident in earthworms at 4 weeks > 3 > 2 > 1. In neurotoxicity studies, MGF NCV progressively decreased in *A. caliginosa* exposed to both AgNPs and $AgNO_3$. Biochemical toxicity was > neurotoxicity. The findings highlight oxidative stress and neurotoxic effects of Ag compounds on earthworms and the importance of government authorities to have legislations in place to prevent excessive soil contamination by AgNPs produced by the expanding nanoparticle industry.

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P11-021
TiO_2 NM 105 response obtained on three different rat models, *vitro*, ALI, and *vivo*

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Due to the growing use of nanomaterials in various industrial processes, the number of workers potentially exposed is increasing even though the toxicological properties of these compounds are not completely known. Since nanoparticles (NP) may become aerosolized, inhalation represents the main route of occupational exposure, and the first tissues to be exposed are therefore those of the respiratory system.

Titanium dioxide (TiO2) is among the most widely produced nanomaterials worldwide. TiO2 NP are used in coatings, paints, self-cleaning windows, food products, toothpaste, pharmaceuticals, and cosmetics including sunscreens.

Tremendous amount of scientific work has been done regarding the assessment of nanomaterials pulmonary toxicity with different models et techniques. *In vitro* models are the most widely used, but the relevance of the observations obtained could be questionable. *In vivo* models are considered more relevant for hazard and risk assessment, but ethical issues exist. Recently, the development of new systems, such as Air Lung Interface (ALI) systems developed by Vitrocell® offer the opportunity for toxicologists to better mimic the *in vivo* conditions, and may help to reduce the use of animals. In order to better caracterize these experimental models and assess their predictivity, we wanted to gain insight about the cellular and molecular mechanisms involved in NP toxicity in *in vitro* and *in vivo* models.

The present work was performed with the objective to compare transcriptomic profiles obtained from three different models exposed to identical amount of TiO_2 nanoparticles.

The three different models were:

1. Rat NR 8383 monocytes/macrophages exposed to TiO_2 NM 105 under submerged conditions
2. Rat NR 8383 monocytes/macrophages exposed to TiO_2 NM 105 *via* the Vitrocell® Cloud system
3. Fischer 344 rats were exposed to a TiO2 nanostructured aerosol by nose-only inhalation for 6 h/day, 5 days/week for 4 weeks Common and divergent toxicity pathways between models were identified. The thorough analysis of the data will help us to increase the predictivity of the *in vitro* models. This work is presented in the H2020 SmartNanoTox project framework.

P11-022
High-throughput hazard-based scoring, ranking and grouping of engineered nanomaterials

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Traditional safety testing is costly and labor-intensive, and does not suffice for keeping pace with steadily increasing innovations that involve engineered nanomaterials (ENMs). High throughput screening (HTS) technology offers opportunity to partly tackle the problem, as it allows for rapid and cost-effective *in vitro* model-based definition of inherent toxicological properties and priority ranking of tested agents for further study. We report a NANOSOLUTIONS FP7 EU-funded HTS-driven case study of 31 ENMs covering nine core structures functionalized by carboxylation, amination/ammoniation and pegylation. Cellular ATP content, cell number changes, apoptosis frequency, DNA damage and nucleic acid oxidative stress markers were assessed for up to 72h in the human lung epithelial cell line BEAS-2B in conditions with or without 10% fetal bovine serum, in-

volving the generation of almost 10^5 data points. The results were fused into a comprehensive, multi-time point and multi-endpoint inclusive toxicity score for rank ordering of the ENMs, including versus reference agents with known toxicity profiles. The ENMs were finally grouped related to score or individual assays applying the US-EPA ToxPi Toxicological Prioritization Index tool. The integrated analysis demonstrated unique toxicity profiles of the respective ENMs, including variable activity relative the end points and/or by showing different dose-dependencies early or later with time. Serum influenced the results by slightly enhancing, having no effect or markedly decreasing the toxicity. Ammoniation coupled to higher toxicity than the other functionalized groups. The scoring analysis clustered the ENMs into several distinguishable groups likely reflecting inherent physiochemical reactivity among other properties. We believe our study to effectively advocate for the general usefulness of HTS technology for safety assessment studies. This technology overall promises to aid the integration of safety testing measures during innovation of ENMs.

P11-023
Orally administered SiO_2 nanoparticles differing in their specific surface area did not induce local or systemic toxicity

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Understanding how the physicochemical properties of nanomaterials influence toxicity is critical to support their grouping and avoid case by case testing. Little attention has been placed on the role of specific surface area on oral toxicity. In this study, four amorphous methyl-coated SiO_2 NPs consisting of two target sizes (100 and 300 nm), and two types of porosity (non-porous and highly porous particles) were used. As a result, their specific surface areas ranged from 10 to 844 m^2/g. Female Swiss mice were administered by oral gavage for 5 consecutive days. Two SiO_2 NP dose levels (100 and 1000 mg/kg b.w.) were tested for each of the four materials. All dispersions were characterized by TEM and Nanoparticle tracking analysis. Porous material dispersions tended to form agglomerates and were rather unstable. Animals were sacrificed one day after the last administration or after a three-week recovery period. No relevant toxicological effects were induced by any of the SiO_2 NPs, as evaluated by body weight, gross pathology, relative organ weights (liver, spleen, kidneys), hematology, blood biochemistry (AST, ALT, creatinine and total protein concentration), genotoxicity (Comet assay in jejunum cells and micronucleus test in peripheral blood erythrocytes), liver and small intestine histopathology, and intestinal inflammation (cytokine determinations in jejunum mucosa and submucosa). The presence of SiO_2 NP in the intestine was evaluated by a hyperspectral imaging microscopy system (CytoViva) using histological samples of jejunum tissue. A high intra-group variability in the percentage of pixels showing a SiO_2 NP spectral signature was found. The highest percentages of positive pixels per sample were recorded in NP-treated animals, but no statistically significant differences in the mean percentages were observed either among the four NPs treatments, or with the control group. (Funded by EU H2020 caLIBRAte project, Grant Agreement No. 686239).

P11-024
Impact of silver nanoparticles on physiological parameters of tobacco seedlings

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Silver nanoparticles (AgNPs) are among the most commonly applied nanomaterials due to their powerful antibacterial and antimicrobial properties that are being exploited in a number of consumer products. As a consequence, AgNPs are expected to enter natural ecosystems where they can undergo biodegradation or bio-accumulate in the food chain and act as a potential environmental hazard. Plants, as primary producers, are very likely to be influenced. Bioaccumulation of AgNPs is dependent on the characteristics of the particles, with mainly their size and surface coating determining their mobility and transport in the environment. In this study, we compared the effects of two differently coated AgNPs [polyvinylpyrrolidone (AgNP-PVP) and cetyltrimethylammonium bromide (AgNP-CTAB)], applied in three concentrations (25, 50 and 100 µM), on photosynthesis and oxidative stress parameters of tobacco (*Nicotiana tabacum*) seedlings. Silver uptake in the plant tissue was determined with inductively coupled plasma mass spectrometry (ICP-MS). Chlorophyll fluorescence parameters were measured by fluorimeter using a saturation pulse method. Dihydroethidium test was used to determine the content of reactive oxygen species (ROS). Ascorbate peroxidase (APX), pyrogallol peroxidase (PPX), superoxide dismutase (SOD) and catalase (CAT) activities were spectrophotometrically measured. Although both types of AgNPs significantly increased the silver content in the plant tissue, the highest amount of Ag was detected in 100 µM AgNP-CTAB treatment. Both AgNPs induced ROS formation that was again much more pronounced with AgNP-CTAB. Changes in antioxidant enzymes activities were also observed. AgNP-PVP significantly decreased PPX and APX activity in all tested concentrations, but no changes were detected in CAT and SOD activity. AgNP-CTAB decreased PPX and SOD activity, and increased CAT activity at the highest concentration. Both AgNP-PVP and AgNP-CTAB lowered most of the chlorophyll fluorescence parameters, indicating a negative influence on the photosynthetic apparatus. Even though both types of AgNPs caused phytotoxic effects on tobacco seedlings, these results indicate that the degree of damage correlates with the surface coating used for AgNPs stabilization.

P11-025
A metabolomic study of the effect of gold nanostars vs gold nanospheres in Wistar rats after a single-dose intravenous administration

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The physical and chemical properties of gold nanoparticles (AuNPs) such as shape, influence their optical and biological effects. Regarding their biological effects, the existing studies fail in consistency and

one of the causes is the low toxicological profile of AuNPs as well as the lack of sensitive methods to detect differences among different types of AuNPs. For this reason, innovative and sensitive approaches such as metabolomics are much needed.

The current work aimed at using a metabolomic approach to compare the effect of gold nanospheres *vs.*gold nanostars (of similar dimeter ~40–48 and coated with 11-mercaptoundecanoic acid) 24h after i.v. administration to Wistar rats ($1.33*10^{11}$AuNPs/Kg). A gas chromatography-mass spectrometry (GC-MS)-based metabolomic study was performed to investigate the metabolomic changes in the liver and spleen of the AuNPs-exposed animals.

Multivariate analysis showed that the metabolic pattern of the liver from animals exposed to gold nanospheres discriminate from the liver exposed to gold nanostars. The spheres produced a significant increase in intracellular metabolites such as palmitic, oleic, and 5,8,11- eicosatrienoic acids while the nanostars increased dimethylglycine, uracil and reduced inosine, uridine, L-lysine, and phosphoric acid. The discriminated metabolites are associated to the biosynthesis of fatty acids, the metabolism of arachidonic acid, pyrimidine and purine, biotin and glycine as well as to the synthesis of aminoacids. Regarding the spleen, the multivariate analysis did not discriminate significantly between the effect of spheres and stars, but some of the discriminated metabolites are involved in the same pathways as in the case of the liver (biosynthesis of fatty acids, pyrimidine and purine metabolism).

Using a metabolomic approach we were able to obtain different metabolic profiles for gold nanospheres vs gold nanostars in the liver. This proves that metabolomics is a very useful tool for the study of the effect of gold nanoparticles and should be taken into consideration as a highly sensitive alternative for comparison studies between different types of AuNPs.

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P11-026
Effects of titanium dioxide nanoparticles on T98G human glioblastoma cells

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The effects of a set of TiO_2 nanoparticles (NPs) (Z potential = +22.8 ± 0.8 mV; mean size determined by transmission electronic microscopy (TEM) = 18 ± 5 nm and mean size after 72 hours in cell culture media determined by dynamic light scattering = 12 nm) on T98G human glioblastoma cells were characterised.

TiO_2 NPs were incorporated into the cells after 72 hours of exposure, as was verified by optic microscopy and by light scattering flow cytometry. TEM confirmed these results showing that NPs remain in the cytoplasm grouped in clusters and causing, apparently, autophagy.

RNAseq experiment showed that the exposure of T98G cells to TiO_2 NPs in non-cytotoxic conditions (20 µg/ml during 72 hours) caused dysregulation in the expression of 1025 genes. The results of the RNAseq were further partly validated in independent experiments using a set of 5 genes.

The analysis of the ontology of the genes with altered expression determined that the more overrepresented biological processes among the differentially expressed genes were blood vessel endothelial cell proliferation involved in sprouting angiogenesis and negative regulation of fibroblast grow factor receptor signalling pathway together with other biological processes related to angiogenesis and vasculogénesis as positive regulation of vascular endothelial growth factor production, positive regulation of angiogenesis and blood vessel development.

Up to 6 different biological processes associated to mechanisms of inflammation were altered among the differentially expressed genes. The overexpression of the pro-inflammatory interleukins 6 and 8 were also verified by immunological methodologies.

In conclusion, TiO_2 NPs are able to cause alterations in glia cells that seriously affect their role in maintenance of the nervous system and can eventually conduct to neurotoxicity.

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P11-027
Precision-cut liver slices as a promising *ex vivo* model for nanosafety studies

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Significant efforts within the nanosafety community are currently focused in the implementation of novel advanced models for *in vitro* testing. Ideal models should mimic as much as possible the complexity of the *in-vivo* environments in which nanomaterials have been found to accumulate. Within this context, Precision-Cut Tissue Slices (PCTS) represent an interesting *ex-vivo* model already validated and extensively used for toxicological studies and drug metabolism [1]: the maintained cellular complexity of the tissue and the possibility of preparing tissue slices from diverse species, including from human tissue, and different organs, are only few of the many advantages of using this model. The use of PCTS can also significantly contribute to the reduction of *in-vivo* studies in accordance with the 3Rs – replacement, reduction and refinement.

Within this context we aimed to test whether PCTS can be used as an *ex vivo* model for nanosafety assessment. We have focused as a first step on Precision Cut Liver Slices (PCLS): most *in vivo* studies show in fact that once NPs reach the blood stream, they mainly end up in the liver and – within this organ – often accumulate in Kupffer macrophages. Then we have used fluorescently labeled carboxylate polystyrene (PS-COOH) NPs and amino-modified polystyrene (PS-NH_2) as model nanoparticles whose behavior and effects at cellular level have already been extensively characterized. [2] In this way, we could determine how *in vivo* accumulation within the liver and *in vitro* effects at cellular level of these well characterized nanoparticles translate in the *ex vivo* model.

Thus, NPs have been added to PCLS in relevant biological media containing serum where a protein corona forms on their surface [3]. NP uptake and distribution in the PCLS have been explored by using confocal fluorescence imaging, in order to determine whether the NPs enter cells and in which cell types they accumulate over time. Eventual effects on the tissue slices have been assessed by histological analysis and by measuring the ATP content, caspase 3/7 activity and TUNEL assay.

Upon exposure to increasing doses of PS-NH_2, toxic effects have been observed in the tissue, including activation of apoptosis, resembling overall what has been shown in *in-vitro* studies with the same NPs. Single cell analysis has been used to determine NP impact in the cells in which they accumulate within the tissue. Despite the large adsorption of NPs on the outer cell layer, uptake of NPs in the tissue was clearly visible inside the section and immune-staining has been used to determine the cell types in which NPs accumulated over time.

Overall, the results suggest that NP behavior in the PCLS can resemble several aspects of what observed *in vivo* and *in vitro*. Additional results obtained in lung and in human liver are also presented.

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P11-028
Effect of carbon nanotubes on pulmonary surfactant

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Purpose: Carbon nanotubes (CNTs) are an important group of nanomaterials (NMs) and due to their outstanding physical and chemical properties have a wide range of applications. It is estimated that CNTs constitute almost 30% value of the total NM market. By 2023, the global CNT market is expected to reach almost 4,000 tonnes, an annual increase of 12.8%. The potential CNT applications indicate the need for a detailed analysis of the impact of carbon nanomaterials on biological membranes. The aim of this study was to evaluate the influence of carbon nanotubes on the rheological properties of the air/liquid interface with the pulmonary surfactant (PS), which is the first barrier separating the inhaled air in pulmonary alveoli from the lung tissue.

Materials and methods: The experiments were carried out with dynamic pendant drop (DPD) method using PAT-1M tensiometer (Sinterface Technologies, Germany). The animal-derived preparation Survanta (Abbott Laboratories, France), intended for treatment of Respiratory Distress Syndrome (RDS) in newborn premature infants, was used as a model PS. The tests were conducted at 36.6 ± 0.2 °C for different CNT concentrations (ranging up to 1 mg/ml) with constant concentration of PS solution (2.5 mg phospholipids/ml). The droplet was oscillated at 10% surface area changes with various frequencies (0.1–0.5 Hz) what corresponded to a range of breathing patterns(2 s –10 s per inspiration-expiration cycle).

Results: It was found that carbon nanotubes studied influence the dynamics of changes in surface tension, loss angle, surface elasticity and surface viscosity of oscillated air-liquid interface. An increase in the concentration of carbon nanotubes causes an increase in surface elasticity and a decrease in loss angle. An increase in the oscillation frequency of the air/liquid interface with PS results in an increase in surface elasticity and a decrease in loss angle and surface viscosity of the air/liquid interface contacted with the tested CNTs.

Conclusion: The change in rheological parameters of the interface indicates a disturbance of viscoelastic properties of the pulmonary surfactant in the presence of the carbon nanotubes.

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P11-029
Effect of surface charge on the genotoxic potential of nanomaterials: hazard classification

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A main challenge in nanotoxicology is to understand, how the physicochemical properties of an engineered nanomaterial (ENM) determine its hazard potential. Particle functionalization may influence the nature and extent of the ENM-biological structures interaction, thereby modulating the potential consequences of ENM exposure. Here, we studied the cytotoxic and genotoxic potential of nine sets of ENMs: silver nanoparticles (NPs), copper NPs, gold NPs (5-nm and 20-nm core sizes), titanium dioxide NPs, titanium dioxide nanorods, multi-walled carbon nanotubes, nanodiamonds, and quantum dots. Each ENM set comprised of a core particle with different surface functionalizations: -COOH (negative charge), -NH_2 (positive charge) and –PEG (polyethylene glycol; neutral charge). Pristine core particles were also included for ENMs with adequate dispersibility. Human bronchial epithelial BEAS-2B cells were exposed to the ENMs at doses chosen based on cytotoxicity (cell counts, dead cells excluded by trypan blue). For a protype of a nanosafety classifier, we devised a point-based classification system for the hazard potential of the ENMs. For cytotoxicity (24- and 48-h exposure), points were given according to the dose when IC50 was reached. For genotoxicity (comet assay, 24 h; micronucleus assay, 48 h), points were given for a positive result, efficiency (lowest dose giving a statistically significant increase) and effectivity (maximum fold-effect in comparison with control). Four categories were established depending on the amount of points: non-(cyto/geno)toxic, weak equivocal, equivocal and (cyto/geno) toxic. Our results showed that the cytotoxicity of all ENMs (except the carbon nanomaterials) was enhanced by the NH_2-functionalization, while the effect of functionalization on genotoxicity depended on the ENM and genotoxicity endpoint. Treatment time affected the cytotoxicity categorization. Differences were observed in genotoxicity categorization based on DNA damage and micronuclei. In conclusion, functionalization affected both the cytotoxicity and genotoxicity of the ENMs. ENM hazard categorization based on a single cell system is restricted to that system and depends on the experimental details.

P11-030
Bioavailability improvement of a monoamine oxidase-B inhibitor using PEGylated PCL-based nanoparticles

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Parkinson's disease is a neurodegenerative disorder, which has as current pharmacological treatment the administration of levodopa

in conjugation with catechol-O-methyltransferase or monoamine oxidase B inhibitors (IMAO-B). Despite new chemical entities have been discovered, most of drug candidates fail in pre- and clinical trials due largely to bioavailability pitfalls, such as cytotoxicity or brain targeting.

In this context, the use of PEGylated nanoparticles (NPs) as drug delivery systems has been reported as an interesting tool to avoid drug toxicity and to increase the stealth capacity of drug candidates to surpass biological barriers. Thus, in the present work, a novel potent, selective and reversible IMAO-B (chromone C27, IC_{50} = 670 ± 130 *p*M) was encapsulated in PEGylated poly(caprolactone) (PCL) NPs by a nanoprecipitation process with an encapsulation efficacy higher than 50%. PEGylated PCL NPs containing C27 (PCL@C27 NPs) present hydrodynamic size lower than 213 nm and high stability in physiological medium. Both free C27 and PCL@C27 NPs did not cause cytotoxic effects in Caco-2 cells for all concentrations tested. However, in both SH-SY5Y and *h*CMEC/D3 cells, C27 (10 µM) caused a significant decrease in metabolic activity, which was not observed after encapsulation. Fluorescent probe-loaded PEGylated PCL NPs (50 µg/mL) were capable to cross plasmatic SH-SY5Y and *h*CMEC/D3 cell membranes and accumulate in the cytoplasm. In addition, PEGylated PCL NPs (100 µg/mL) were found to permeate Caco-2 and *h*CMEC/D3 cell monolayers that have been used as *in vitro* models of the human intestine and BBB barriers, respectively. PEGylated PCL NPs were capable to deliver C27 chromone at concentrations higher than the MAO-B IC_{50} value. Overall, our results provide evidence of the effectiveness of PEGylated PCL NPs to increase neuroactive compounds bioavailability, highlighting their relevance to solve drug discovery pitfalls.

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P11-031
Effects of double-walled carbon nanotubes on the early phase of respiratory syncytial virus infection in mice.

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Double-walled carbon nanotubes (DWCNTs) are important materials in the fields of nanotechnology. Effects of DWCNTs on the pneumonia in respiratory syncytial virus (RSV)-infected mice were already reported by our research group at EuroTox 2018. The aim of this study was to evaluate effects of DWCNTs on primary immunity responding to RSV infection in mice.

DWNT-1 (1 µm in length) and DWNT-15 (15 µm in length) were used in this study. Female (6 weeks old) BALB/c mice were intranasally exposed to DWCNTs (0–0.125 mg/kg) on days 1, 3 and 5 before RSV infection under anesthesia. These mice were intranasally infected with 3.5 x10^5 PFU of RSV under anesthesia.

On day 1 post-infection, the levels of TNF-α, a proinflammatory cytokine, in the bronchoalveolar lavage fluids (BALF) of RSV-infected mice were significantly suppressed due to DWNT-1-exposure (0.125 mg/kg) compared with the control, but not DWNT-15-exposure. Histopathological analysis for lung tissues showed that increase of infiltration of the inflammatory cells around artery and hypertrophy of the epithelial cells due to DWCNTs treatment compared with the control. Especially, ingestion of the carbon tubes in alveolar macrophages was confirmed in DWNT-1-treated mice.

Thus, exposure to DWNT-1 might affect the function of alveolar macrophages/monocytes in an early phase of infection, resulting in the exacerbation of the pneumonia in RSV-infected mice.

P11-032
Tissue distribution of silver chalcogenide quantum dots in mouse model

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Silver chalcogenide quantum dots (QDs), including Ag_2S, Ag_2Se and Ag_2Te, hold great promise in fields of bioimaging, biosensor, photocatalysis, optoelectronics and thermoelectric materials, because of their bright near-infrared (NIR) emission, Cd-free composition and high stability. Especially, the emission peak of silver chalcogenide QDs is within the second NIR window, and thus these QDs exhibit great potentials in high resolution bioimaging with deep penetration. But, their safety issues are still not well addressed, which may impede their development and applications. In this study, we synthesized polyethylene glycol coated silver chalcogenide QDs, and measured and compared their biodistribution in mice after a single intravenous injection. Three kinds of QDs had the same size (5–6 nm) and surface properties (polyethylene glycol), and good dispersibility and stability in physiological solutions. After mice were intravenously injected with QDs at a dose of 8 µmol/kg body weight (silver equivalent), their blood kinetics and tissue distribution profiles were obtained by measuring the Ag contents in tissues at different time points. All three kinds of QDs were quickly removed from blood. Among them, Ag_2Te displayed the longest half-life in blood, while Ag_2Se displayed the lowest half-life. All three QDs mainly accumulated in liver and spleen, and then eliminated along with time. Compared to gradual decrease of Ag_2S and Ag_2Te, Ag_2Se decreased markedly in first 7 days and reached to background levels at day 14. The stability of silver chalcogenide and the different properties of chalcogenide ions in biosystems may contribute to the different biological behaviour of three QDs.

P11-033
Discovery of an inhibitor of multiwall carbon nanotubes-stimulated IL-1β secretion via inflammasome activation

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The NLRP3 inflammasome-mediated IL-1β production is implicated in the pathogenesis of various chronic inflammatory diseases. We have previously shown that multiwall carbon nanotubes (MWCNT) of certain length and needle-like shape can elicit robust NLRP3-inflammasome activation and IL-1β secretion in macrophages. In this study, we explored inhibitors of inflammasome-mediated IL-1β production. PMA-differentiated THP-1 macrophages pretreated with/without chemicals were stimulated with either MWCNT or ATP. Among chemicals tested, K-NK104 caused marked reduction in

MWCNT-induced IL-1β production. This reduction was accompanied by decreased release of mature IL-1β and active caspase-1 fragment, while cellular levels of IL-1β precursor, procaspase-1, and NLRP3 were unaffected. Flow cytometry analysis showed that MWCNT uptake by cells was repressed by this compound. In contrast, extracellular ATP-elicited IL-1β production was unimpaired, suggesting that the NLRP3 inflammasome and its downstream pathways were unaffected by this compound. We conclude that K-NK104 inhibits MWCNT-stimulated inflammasome activation and the consequent IL-1β production by repressing internalization of MWCNT into cells. The target of K-NK104 during this process is now under investigation.

P11-034
Toxicity assessment of engineered and airborne ceramic nanoparticles on a human 3D bronchial epithelium

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Nanotechnology has been contributing to major advances in the ceramic industry. Many nanoscale powders are currently used for production of ceramic materials. At the same time, some processes used in the manufacture of ceramic products have a high potential for nanoparticle (NP) release into the workplace environment that raise occupational health concerns. Therefore, there is an urgent need for assessing the toxicity of these intentionally used or unintentionally generated NPs. This study aimed to investigate the *in vitro* toxicity of engineered (ENPs) and airborne ceramic NPs in a 3D human bronchial epithelial model (*MucilAir™*) under air-liquid interface conditions.

Two commonly used ENPs, zirconium (ZrO_2) and antimony-tin oxide (Sb_2O_3•SnO_2) and the particulate matter (PM) < 2.5 μm and ultrafine (UFP) < 0.2 μm fractions of airborne NPs released during High Velocity Oxy-Fuel (HVOF) spraying were tested. *MucilAir™* cultures were exposed three consecutive days (E1, E2, E3) to different doses of NPs aerosols in a Vitrocell® Cloud 12 system. Cytotoxicity was evaluated by the lactate dehydrogenase (LDH) release (24h after each exposure) and WST-1 metabolization (24 h after the last exposure) assays. Primary and oxidative DNA damage were evaluated in cultures collected 24h after the last exposure by the alkaline and FPG-modified comet assay versions, respectively.

A significant increase in LDH leakage was observed in bronchial cultures exposed to aerosolised ZrO_2 NPs (5.6 μg/cm^2 per exposure) 24h after E1 and E2, while for antimony-tin oxide NP-exposed cultures (11.0 μg/cm^2) this effect was only detected 24h after E1. No significant changes upon cell viability were detected 24h after E3 in both ENP-exposed cultures. Moreover, no significant alterations of primary and oxidative DNA damage levels were detected following exposure to the ENPs aerosols comparing with control cultures. The chemical analysis revealed that airborne HVOF-generated NPs were mainly constituted by WC, CrC and Ni. Exposure to the aerosolised PM 2.5 fraction failed to affect plasma membrane integrity of the *MucilAir™* cultures as evaluated by the LDH release. However, 24 h after the last exposure a significant decrease in cellular metabolic activity in human bronchial epithelium exposed cultures compared to the control cultures was observed, as assessed by the WST-1 assay. On the other hand, cultures exposed to the lowest tested dose of the aerosolised UFP fraction exhibited a significant increase in LDH release only visible at 24 h after the first exposure, whereas no changes in the metabolic activity were detected. On the other hand, only human bronchial cultures exposed to the highest tested dose of the UFP fraction aerosol exhibited a significant increase of oxidative DNA damage comparing with control cultures. Thus, our findings highlight the potential health risks associated with exposure to engineered and unintentional NP emissions derived from ceramic industry processes.

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P11-035
Agglomeration state of titanium-di-oxide (TiO_2) nanomaterials influences the toxicity/biological responses in human bronchial epithelial cells at the air-liquid interface

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Agglomeration of nanomaterials (NMs) is a ubiquitous phenomenon and its dynamic behaviour throughout their life cycle poses a great challenge in assessing its impact on human health. While agglomerates are of prime importance in occupational exposure scenarios, their toxicological relevance remain poorly understood [1,2]. Therefore, the aim of this study was to compare the toxicity/biological responses induced by either agglomerates or individual/unbound particles. Two different sized titania particles, nano-TiO_2 (primary size 17 nm) and a sub-micron TiO_2 (117 nm) were selected for this study. Stable stock dispersions of non-agglomerated particles (median feret min size, 34 and 120 nm) and their respective agglomerates (137 and 309 nm) were prepared using a modified protocol published previously [3]. These dispersions were aerosolized and subsequently administered to human bronchial epithelial cell cultures (16HBE14o-) at the air-liquid interface [4,5], a procedure which is more realistic in terms of inhalation exposure. The cells were exposed to different doses of TiO_2 aerosols using electrostatic deposition. At the end of 4-hour exposure, the effects on cell membrane integrity (LDH release), metabolic activity (WST-1 reduction) and oxidative stress (glutathione depletion) were evaluated. Significant effects were observed only for nano-TiO_2. Non-agglomerated particles (34 nm) induced a dose dependent increase of LDH. Further, they decreased metabolic activity and glutathione levels at the highest dose tested. In contrast to unbound particles, agglomerates of nano-TiO_2 did not induce adverse effects although the deposited mass was similar. Similarly, exposure of cells to comparable doses of sub-micron TiO_2, either in the form of primary or agglomerated particles, also did not provoke toxicity. These results suggest that the agglomeration state of TiO_2 nanomaterials influences the toxicity/biological responses at the air-liquid interface, depending on the primary particle size. In addition to acute cellular toxicity, other end points such as genotoxicity and altered gene expression are currently investigated.

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P11-036
Clearance of multi-walled carbon nanotubes in rat lungs after intratracheal instillation: a comparison of different instillation devices

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Background: An intratracheal instillation test has been used as an alternative exposure method to the inhalation. Quantifying the clearance of test materials is essential, especially for the evaluation of long-biopersistent fibers. We here examined the time course of histological changes and the lung burden after a single administration of multi-walled carbon nanotube (MWCNT).

Materials and Methods: Ten-week-old F344 male rats were intratracheally administered MWNT-7 (Mitsui) dispersed in saline containing 0.5% of Pluronic F-68 at the dose of 125 μg/rat (0.5mg/kg body weight) using two types of devices, an ordinary feeding cannula or a sprayer. MWCNT pulmonary burden was determined at days 1, 28, 54, 84 and 112 after the instillation, using a method involving the adsorption of a marker, benzo[*ghi*]perylene to nanotubes, which can be measured by HPLC.

Results and Discussion: There were no significant differences between the cannula- and sprayer-groups in the histopathological findings, except for grossly visible depositions of MWCNTs in the tracheal epithelium of animals in the sprayer-group. On day 1 after the instillation, isolated fibers or aggregates phagocytosed by macrophages were widely distributed through the parenchyma as well as the visceral pleura, accompanying marked infiltration of neutrophils and eosinophils. Acute inflammation was decreased within 28 days, and alternatively, large MWCNT-laden macrophages and focal alveolar hyperplasias were prominent. On day 112, microgranulomas associated with macrophages engulfing fibers were frequently observed. Clearance of MWCNTs also did not significantly differ between two groups. On day 1, 28, 54, 84 and 112 after instillation by the cannula, averages of the lung burden were 73.8, 68.6, 35.5, 35.6 and 47.3 μg/lung respectively (N = 3). As for those instilled by the sprayer, corresponding lung burdens were 60.7, 58.4, 64.6, 37.5 and 79.7 μg/lung (N = 3). These data suggested that approximately 40 to 50% of administered MWCNTs may be removed by the mucociliary "elevator" within 24 hours, while fibers deposited in the lung were retained for a long time and can induce chronic inflammation through the activation of macrophages.

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P11-037
Prospects for the use of alternative methods for testing the safety of nanomaterials in the Republic of Belarus

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The number of names of nanomaterials and the volume of their application in various fields of science, medicine, energy, industry is growing rapidly not only throughout the world, but in the Republic of Belarus too. The speed of nanotechnology development is ahead of the development of methods for assessing their safety and regulatory documents. Currently in the republic there is no register of nanomaterials. The need to study the potential toxic properties of nanomaterials in products and in the air of the working area is not fixed by law, technical normative legal acts regulating circulation in the market for products containing nanomaterials are not developed.

The aim of this work was to develop methodological approaches for screening safety assessment of nanomaterials on cell cultures.

The objects of research were nanoscale particles of different chemical nature (carbon nanotubes, nanoparticles based on metals and metal salts, etc.), both in suspension and fixed on the carrier.

The main damaging properties of nanomaterials are based on their ability to penetrate and accumulate inside the cell, disrupting the functioning of the internal systems, causing oxidative stress, as well as, when penetrating the cell nucleus, the ability to induce mutations. Thus, for preliminary screening testing of the safety of nanomaterials, it is most appropriate to use a sequential testing scheme based on alternative methods using cell cultures of different origin. On the basis of the main routes of entry of nano-sized particles into the body, cell cultures of similar specifications (A549, CaCo2, skin-muscle embryonic fibroblasts) were selected for research. The developed testing scheme includes a number of methods for determining the general toxic effect, mutagenicity, the ability to assess cell membrane damage and the method of assessing cell membrane damage. method for studying the induction of reactive oxygen species using fluorescein diacetate staining, cytogenetic analysis under a microscope and cytofluorimetric methods).

The study allowed us to develop a specific algorithm for screening assessment of nanomaterials. But the issues of proper sample preparation, as well as a comparative assessment of the number of tested nanoparticles, remained unresolved. We cannot adequately compare the concentrations of large molecular particles (for example, carbon nanotubes, which practically do not penetrate cell membranes, including due to the formation of agglomerates) and low molecular size (for example, silver nanoparticles). Thus, the question of the quantitative determination of nanoparticles for hygienic rationing nanoparticles in products and in the air of the working zone is still open.

P11-038
Skin irritation potential of graphene based materials

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Graphene based materials (GBMs) are innovative 2D nanomaterials obtained by graphite exfoliation. Their unique physicochemical properties stay at the basis of the multiple potential GBMs applications, ranging from electronics to biomedicine. However, little is known about their negative impact on human health, especially after skin contact, which represents one of the major exposure routes to GBMs for humans, especially in occupational settings.

Hence, this study was aimed at investigating skin irritation properties of a panel of GBMs: a few layer graphene (FLG), exfoliated by ball milling of graphite, a FLG exfoliated using sodium dodecyl sulfate (FLG-SDS) or sodium dodecylbenzenesulfonate (FLG-SDBS), a CVD-graphene monolayer disk, obtained by chemical vapor deposition, a graphene oxide (GO) and a reduced GO (rGO). In compliance with the 3Rs principle, skin irritation was assessed using the SkinEthic™ Reconstructed human Epidermis (RhE) model, following the skin Irritation Test-^{42bis} (42 min exposure to GBMs followed by 42 h post-exposure without the materials), a test compliant with the Organization for Economic Co-operation and Development (OECD) Test Guideline (TG) 439.

In general, none of the materials reduced RhE viability at levels lower than those predicting skin irritation (≤ 50%), suggesting no skin irritant properties for the tested GBMs. This result, obtained following the OECD TG 439, was further confirmed by the absence of cytokines (IL-1α, IL-6 and IL-8) release by GBMs-treated RhE and by no observed histological alterations.

On the whole, these results demonstrate, for the first time, that GBMs do not seem to induce skin irritation after a single acute exposure.

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P11-039
Assessment of potential toxicity of new synthesized nanofibers in pulmonary cells *in vitro*

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Introduction: The development and production of new nanomaterials have been increasing in recent years. Various technologies enable the synthesis of unique nanomaterials, including nanofibers. The highest risk of toxicity effect of nanomaterials is emerging in workplaces and environment. Respiratory tract is the primary and the most frequent possibility of entering nanomaterials into the human body, therefore it is important to test the pulmonary toxicity.

Aim: Nanofibers are among the nanomaterials whose cytotoxicity is generally not so much tested comparing with other nanomaterials. Our aim was to determine and to characterize the potential pulmonary toxicity of several different inorganic nanofibers, produced by electrospinning and centrifugal spinning, compared to commercially available nanoparticles using human lung cell line *in vitro*.

Methods: We tested newly synthesized nanofibers to compare their biological effects with commercial TiO_2 nanoparticles. The human lung carcinoma epithelial cells (= A549 cell line) were seeded into well plates. After seeding, the cells were exposed to solutions of nanofibers or nanoparticles at concentrations of 0–100 $\mu g.ml^{-1}$ for up to 24 h. Cell damage after treatment was assessed by cell viability tests and microscopic analysis.

Results and conclusion: The results showed that TiO_2 nanoparticles did not induce any decrease in cell viability after 1 and 4 h. After 24 h, 100 $\mu g.ml^{-1}$ TiO_2 nanoparticles showed significant reduction of cell viability by 10% in comparison with control cells. On the other hand, after 1, 4 and 24 h, any changes in cell viability were not found in A549 cells treated with nanofibers in comparison with control cells. Our results were also supported by microscopic analysis showing no visible cell damage after incubation with nanofibers. In general, commercial TiO_2 nanoparticles are more toxic than tested nanofibers of similar composition.

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P11-040
Evaluation of the impact of TiO_2 and SiO_2 nanofibers on the neuronal cells.

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Introduction: Nanomaterials, such as nanoparticles or nanofibers, have a large potential for use in a number of industry sectors. A number of nanomaterials have been used and new types of structures with improved properties are being produced. In particular, inorganic nanofibers produced by electrospinning possess interesting prospects (porous structure, high surface area and breathability, tunable surface reactivity, dimensions). However, their impact on biological systems has not been well characterized yet. Generally, nanomaterials can have potential hepatotoxic and nephrotoxic, but also neurotoxic effects. The consequences of the presence of nanomaterials in the body are not fully understood. Therefore it is necessary to conduct neurotoxicity studies of these materials.

Aim: *In vitro* testing of inorganic electrospun nanofibers, comparing their effect with nanoparticles in neuronal cells.

Methods: The effect of inorganic nanofibers (TiO_2 and SiO_2) was tested comparing with TiO_2 P25 nanoparticles in human neuroblastoma SH-SY5Y cell line. The SH-SY5Y cells were seeded into 96-well plates at density of 2.5×10^4 cells/well and incubated in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 containing tested nanomaterials at concentrations up to 100 µg/ml. The cells were incubated up to 24 hours. The cell viability was tested and evaluated by the WST-1 test spectrophotometrically.

Results and Conclusion: We found that none of the tested nanofibers caused a decrease in cell viability after 1 to 6 hours incubation period. After 24 hours of incubation, we found that TiO_2 P25 nanoparticles caused a decrease in cell viability below 80% at concentration of 100 µg/ml. Nanofibers, on the other hand, did not induce changes in cell viability. The results on no significant cell damage were also supported by fluorescence microscopic analysis.

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P11-041
Multiparametric platform for safety testing of nanoparticles based on a 3D liver tissue model

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Synthetic nanoparticles (NPs) offer numerous applications in technical fields and biomedicine. Evaluating their potential hazards at an

early stage will provide standards for production processes and preclinical development of NPs. To assess the hazards emanating from NPs we developed a multiparametric platform based on a 3D liver tissue model. The liver plays an important role in the safety assessment of nanoparticles, as it decomposes many drugs and metabolites as well as is a relevant target organ of NPs after entering in the blood circulation. The 3D liver tissue model features two key advantages; 1) It allows the combined measurement of classical, functional, and morphology markers. 2) In contrast to commonly used 2D cell culture, nanoparticle penetration into the tissue can be studied. Knowledge of NP distribution in liver tissue is of relevance for understanding NP induced mechanisms in nanosafety as well as for their delivery efficiency in biomedical applications.

The test platform was established addressing different aspects: 1) characterization of spheroids, 2) NP induced cytotoxicity, 3) hepatocyte function, and 4) NP penetration. Liver microtissues were prepared by 3D cell culture of human hepatocarcinoma HepG2 cells using the hanging-drop method. Microscopy analysis revealed 400 µm sized spheroids with uniform cell arrangement and without holes. The cells expressed a liver specific bile canaliculi related protein (MRP-2). NPs with relevance for biomedical and cosmetic applications owning different element composition (SiO_2, TiO_2, Ag) were tested. Cytotoxicity of the NPs was studied addressing cell viability and oxidative stress. Only Ag NPs caused decrease in cell viability. As a functional marker the gene expression of an important liver-specific CYP P450 enzyme for metabolism of xenobiotics (Cyp1A2) was analyzed. In presence of all tested NPs the basal Cyp1A2 gene expression was not affected. In contrast menadione-induced Cyp1A2 gene expression was altered in presence of TiO_2 and Ag NPs. A combination of modern imaging techniques allowed us to locate the NPs inside the spheroids and to study NP penetration into tissue. NP penetration was shown to be limited to about 20µm from the spheroid border.

The combination of various markers with 3D cell culture technology, microscopy and PCR-based analysis of the gene expression pattern of cells in an integrated test platform will provide an important contribution to NP risk assessment.

P11-042
The ecotoxicity of zinc oxide nanoparticles in sunscreen formulations

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As zinc oxide nanoparticles (ZnO NPs) are effective sun blocking agents and increasingly being used in sunscreen products, however, they will ultimately be released into the environment, and therefore it is very important to investigate the biological effects on aquatic organism. In order to study the toxicity of ZnO nanoparticles in sunscreen to zebrafish embryos, five different groups were set up: (1) sunscreen containing ZnO nanoparticles in (SN-groups); (2) pure ZnO nanoparticles (PN-groups); (3) sunscreen containing ZnCl2 (SI-groups); (4) sunscreen containing all accessories but no ZnO (SA-group); and (5) Zebrafish culture medium (Control). The Zn concentrations in SN-, PN-, SI-groups were 0.1, 1, 10, 20, 50 mg/L. We assessed the acute toxicity and oxidative stress to zebrafish embryos. For the acute toxicity, the suspensions and solutions in each group did not significantly affect the toxicological endpoints in low concentrations groups (0.1, 1 mg/L). In the 10 mg/L concentration groups, SI-10 group had the strongest interference to zebrafish embryos, but in the 20 and 50 mg/L concentrations groups, PN-groups had the strongest interference with zebrafish embryos, followed by SN-groups, and finally SI-groups. Since the sedimentation of ZnO NPs has been increased in a time- and concentration-dependent manner, and the sedimentation of PN-groups was higher than that of SN-groups after exposure for 12 h in the 20 and 50 mg/L concentrations groups, sedimentation is a very important factor for the acute toxicity of ZnO NPs to zebrafish embryos. On the one hand, the LC50 of SN-, PN-, SI-group at 120 hours post fertilization (hpf) were 22.9, 14.898 and 34.73 mg/L, respectively, the zinc ions released by the SN- and PN-groups were less than 10 mg/L. On the other hand, the activity of CAT was significantly reduced in PN-10 group, which should be caused by oxidative stress. So zinc ions released did not play a major role in the ecological toxicity of ZnO nanoparticles to zebrafish embryos. The sunscreen accessories (SA) could alleviate the ZnO nanoparticles toxicity to zebrafish embryos by inhibiting the dissolution of zinc ions and reducing the deposition of ZnO nanoparticles, which provides a good idea to relieve the side effects of ZnO nanomaterials to the aquatic environment. This work was supported by the National Natural Science Foundation of China (21371118, 41573116, 21611130174).

P11-043

This abstract has been withdrawn.

P11-044
In vitro cytotoxicity and genotoxicity evaluation of five different nanoforms of manganese iron oxide

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Manganese iron oxide nanoparticles ($MnFe_2O_4$ NPs) are extensively used nanomaterials due to their unique magnetic and electric characteristics, such as superparamagnetism. However, their potential benefits may be accompanied by human health hazards and risks during exposure. In this study, five nanoforms of $MnFe_2O_4$ (primary particle diameter 5 nm) synthesized by two different methods, coprecipitation (Cp) or thermal decomposition (Td) and coated with citrate (Cit) or meso-2,3-dimercaptossuccinic acid (Dmsa) were toxicologically assessed in human bronchial epithelial BEAS 2B cells. Bare Cp-synthesized NPs were also analysed. Cytotoxicity was measured by luminometric detection of ATP at 6, 24 and 48 hours. DNA damage was assessed by single cell gel electrophoresis (comet) assay, and chromosome damage was assessed by the cytokinesis-block micronucleus (MN) assay. Based on the cytotoxicity results, BEAS 2B cells were exposed to 25.6- 256 µg.mL of $MnFe_2O_4$ NPs for 6 and 24 h in the comet assay, and to 25.6- 256 µg.mL for 48 h in the MN assay. All NPs were uptaken by BEAS 2B cells within 6 hours of treatment, as verified by transmission eletronic microscopy. Dynamic light scattering (DLS)-based characterization of NPs in the ultrapure water showed slightly bigger hydrodynamic size (Hs) for NPs synthesised by thermal decomposition (110 ± 4nm for $MnFe_2O_4$-TdCit, 170 ± 5nm for $MnFe_2O_4$-TdDmsa) than by coprecipitation (101 ± 7nm for $MnFe_2O_4$-Cp, 74 ± 3 nm for $MnFe_2O_4$-CpCit, and 102 ± 4 nm for $MnFe_2O_4$-CpDmsa). None of the $MnFe_2O_4$ NPs did cause any statistically or toxicologically significant cytotoxic or genotoxic effect ($p > 0.05$) in the concentration range up to 153.6 µg.mL^{-} and up to 48 hours of exposure, when analyzed by ANOVA - 1 Way. This is the first *in vitro* comparative research to address the genotoxicity of manganese iron oxide nanoparticles, with the merit of a comparative factorial analy-

sis, which pointed out that cytotoxicity is dictated by concentration, time and coating; in the other hand, regarding the genotoxicity end points, the time of exposure was a statistically and biologically significant factor for the damage to the deoxyribonucleic acid caused by both citrate-coated nanoparticles, independently of the route synthesis, when analyzed by ANOVA – 2 and 4 Way.

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P11-045

Toxicological evaluation of textiles coated with antibacterial metal oxide nanoparticles by 2D and 3D *in vitro* skin model

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Metal oxide (MeO) nanoparticles (NPs), such as copper (CuO) and zinc (ZnO) oxides, thanks to their antibacterial properties, are among the most eligible nanomaterials for the coating of textiles. Nevertheless, there is a general concern about their safety for human health. Within the EU funded H2020 project "PROTECT", the skin toxicity of textiles coated with antibacterial CuO and ZnO nanoparticles was assessed by different *in vitro* approaches.

CuO and ZnO NPs were produced by sonochemical process and characterized by TEM and DLS. Textiles, coated with ZnO and CuO NPs, were subjected to extraction procedure (ISO 10993-12), including 72 hours incubation in artificial sweat solution (AS, pH 4.7 and 6.3) at 37°C. The stability of the NPs at the different AS pHs, so as the release of NPs and/or ions from the textiles after the extraction procedure in AS, was evaluated through CPS and ICP-OES techniques. For the Skin Corrosion test, Epiderm™ 3D *in vitro* skin models (Mattek) were exposed to different concentration of CuO and ZnO in water, according to the OECD TG.431 protocol, while for the Skin Irritation test, the 3D models were exposed to the textile extracts, according to ISO/TC 194/WG 8 for Medical Devices. Balb/3T3 fibroblasts were also used to understand the mechanisms of action of the cytotoxicity trigged by CuO and ZnO NPs. Cell viability, inflammatory mediators release, morphological changes and wound healing process (scratch assay) were investigated.

Data from the Corrosion test showed that CuO and ZnO NPs resulted non-corrosive up to 1000 ppm in water, according to the Globally Harmonized System (GHS) adopted by the OECD. For the Skin Irritation test, a significant reduction of tissue cells viability was induced by both metal oxide NPs extracted from textiles in AS pH 4.7, likely due to the high content of free Cu and Zn ions released in these conditions and detected by ICP-OES. At higher pH, the effects were observed at lower extent, due to the less solubility of NPs at these experimental conditions. Experiments on fibroblasts showed that ZnO NPs strongly affected cell viability and IL-8 release starting from the dose of 20 ppm. Moreover, the release of IL-8 resulted dose-dependent after the exposure to CuO NPs. Microscopy analyses showed that MeO NPs changed fibroblasts morphology and their ability to migrate during the wound healing process. All together, these data highlight that coated textiles seem to be safe on intact skin models, unless NPs dissolution in acid AS occurs. Nevertheless, further experiments are needed in order to understand the MeO NPs toxicity in case of wounded skin, as suggested by data on fibroblasts. In conclusion, NPs physical and chemical properties and appropriate *in vitro* tools are determinant parameters in order to assess NPs safety.

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P11-046

Neuro-inflammation after inhalation exposure to aerosol mixtures of alumina nanoparticles/hydrogen chloride gas in rats

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Alumina nanoparticles (Al_2O_3NPs) have a wide range of applications in several industrial fields such as electronics or energy. These NPs can also be found as pollutants of interest in mixtures containing high amount of hydrogen chloride (HCl) gas after burning of solid composite propellants used for missile or rocket in defence or aerospace fields. The main route of exposure to these mixtures is inhalation but neurotoxicity could occur in addition to pulmonary effects. These co-exposures may represent a health risk for workers. Due to a lack of data related to potential neurotoxic effects, the aim of this study was to investigate brain toxicity.

In order to study cytotoxic effects using several pollutants concentrations, we performed first series of experiments using mouse microvascular endothelial cells (bEnd.3). Cells were exposed to Al_2O_3particles (primary sizes 13 and 500 nm), HCl or Al_2O_3particles/HCl mixtures during 24h. Treatment with HCl induced concentration-dependant decrease of cell viability, cell index and depletion of reduced glutathione. Incubations with Al_2O_3NPs (13 and 500 nm) mixed or not with HCl markedly decreased cell index, but only 500 nm Al_2O_3NPs ± HCl induced depletion of reduced glutathione.

To assess neurotoxicity, Wistar rats were exposed during 4 hours by nose-only inhalation to filtered air (controls), Al_2O_3NPs (primary sizes 13 and 500 nm), HCl gas (5 ppm) or Al_2O_3NPs/HCl gas mixtures. 24 hours post-exposure, aluminum biodistribution was quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and different analytes were quantified in brain homogenates using ELISA (VEGF, IL-1β, IP-10, TNF-α, Fractalkine, IFN-γ, RANTES). While aluminum was undetectable in brains, significant increases of IL-1βand IP-10 were quantified after exposure to 500 nm Al_2O_3particles ± HCl.

These first results show that only exposure to 500 nm Al_2O_3particles ± HCl, but not to 13 nm Al_2O_3NPs, could induce indirect neuroinflammation after inhalation. Additional research is needed to identify mediators inducing this inflammation, but *in vitro* results suggest that a mechanism of oxidative stress generated on microvascular endothelial cells of the blood-brain barrier is probably involved.

P11-047

***In vitro* effects of ZnO and CuO NPs in mixture with DEP: different nano-bio-interactions affect viability and colony forming efficiency of A549 cells**

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Most of the atmospheric ultrafine particles (UFPs) in urban areas derive from combustion sources, especially diesel exhaust particles (DEP), but also from non-exhaust sources or from the unintentional release of engineered nanoparticles (NPs) during production and use. Since the environmental exposure to DEP and NPs occur simultaneously, it is necessary to consider their possible interactive effects in biological system. Commercially available (cZnO, cCuO < 50 nm) from Sigma-Aldrich) and sonochemically synthesized ZnO and CuO NPs (sZnO, sCuO) from Bar-Ilan University, were used alone or in combination with standard DEP (NIST 2975) to expose A549 cells.

After 24–72h exposure to increasing metal oxide NPs concentrations (10–20 µg/ml), with and without DEP at 100 µg/ml, MTT test and Colony Forming Efficiency Assay (CFE) were performed to assess the cytotoxicity. The NP mixtures were characterized by DLS and TEM, while the NP dissolution in cell medium was measured by ICP-OES. In parallel to the cytotoxicity studies, morphological analyses on NP-cell interactions were performed by light and transmission electron microscopy.

The results suggest that the presence of DEP introduced new physico-chemical interactions able to increase the cytotoxicity of cZnO, but to decrease that of sZnO. For CuO NPs, the presence of DEP significantly reduced the cytotoxicity of cCuO and only slightly that of sCuO. This is probably due to different interferences with the metal oxide NP surface and/or to the modulation of ions release.

The results from CFE were coherent with those from MTT. On the basis of the morphology and cell density, four well distinguishable colony types were identified. Cytostatic effects and changes in colony morphology were observed especially after exposure to CuO and DEP+CuO NPs.

TEM analyses revealed that both ZnO and CuO NPs, as well as their mixture with DEP, were abundantly internalized in A549 cells, especially in the endo-lysosomal compartments and multilamellar bodies. We are performing additional investigations to discriminate the modality of nano-bio-interactions of CuO and ZnO in presence of DEP and to analyse cell-cell adhesion molecules and epithelial-to-mesenchymal transition mechanisms.

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P11-048
Oxidative stress in microbes after exposure to iron nanoparticles: analysis of aldehydes as oxidative damage products of lipids and proteins

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Nanoremediation represents a new branch of remediation that employs various nanomaterials to decontaminate polluted matrices of the environment. Despite the large spectrum nanomaterials used, nanoscale zero-valent iron (nZVI) and its related materials represent the most commonly used agents in environmental remediation practice. Application of nZVI and full-scale cleanup operations have been successfully demonstrated in the decontamination of various organic and inorganic pollutants. However, there is still a lack of suitable and standardized tests enabling estimation of ecotoxicity of these new nanomaterials and especially during the remediation attempt. It is of noteworthy, that classical ecotoxicity tests are not useable due to special features of the nanomaterials or interaction with the assays. One of the most important mechanisms of nanoparticle toxicity is oxidative stress. Oxidative stress is established when an imbalance of reactive oxygen species (ROS) occurs. The presence of elevated ROS concentrations or their insufficient catabolism can cause interaction with biomolecules including proteins, carbohydrates, lipids, and nucleic acids, leading to the formation of toxic and mutagenic products.

Therefore, the aim of this contribution was to develop and to test new approaches for evaluation of potentially negative effects caused by nZVI and related nanomaterials We have developed two different protocols, both based on analysis of lipid peroxidation products employing 1) analysis of a typical OS marker malondialdehyde (MDA) using HPLC in microbial cultures and 2) analysis of volatile aldehydes originating from lipids and proteins based on headspace-SPME-GC-MS, analysis that enables the direct determination of the volatile oxidative damage products of lipids and proteins in microbial cultures after exposure to commercial types of nZVI. The MDA assay was tested using nZVI and several novel oxide shell nZVI materials with different oxide thicknesses that proved the reliability of the test. The second approach revealed that the volatile analyses are suitable even for testing of samples during treatment with nZVI based materials.

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P11-049
Antimicrobial properties and cytotoxicity of polymeric nanocomposites in TK6 lymphoblastoid cells

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Metallic nanocomposites have potential in a variety of applications such as, antibacterial formulations to improve the efficacy of antimicrobial agents, specific cell targeted drug delivery and for use as an anti-cancer agent. Although, the potential benefits of metallic nanocomposites are considerable, there is a distinct need to identify any potential cellular damage associated with these nanocomposites. The aim of this study was to characterize metallic nanocomposites, determine the release of drug from synthesised formulations, and assess antimicrobial effects and their potential *in vitro* cytotoxic effects.

TK6 lymphoblastoid-B cells were exposed for 24 hours to five different metallic composites made up of a polymer Poly (D,L-lactide-co-glycolide) used at 5% w/v, a drug amoxicillin 5% w/w, metallic nanoparticles used at 5% w/w including, silver of differing shapes (hexagonal, spherical and nanowires) gold and copper nanoparticles. The fully synthesised formulations were used at various concentrations (0µg/ml–10µg/ml). Physico-chemical characterization on all composites was performed. Drug release of the antibiotic amoxicillin was performed to assess the release of drug based on differences in shape and type of metal used. Antimicrobial efficacy was determined using the disk diffusion method on two bacteria, E.coli and S.aureus. MTT viability assay was used to determine cytotoxicity.

The physiochemical characterisation showed the metallic nanoparticles to be within 1–250nm and the metallic nanocomposites to be between 600nm–1.5µm. Drug release studies showed differential results based on the shape of particles, with the silver hexagonal formulation having the maximum % age release. Antimicrobial studies showed that the hexagonal formulation exhibited the strongest antimicrobial effect. The cytotoxicity showed that the copper composites were the most cytotoxic followed by spherical and hexagonal polymeric nanocomposites with gold being the least toxic.

Interestingly, there was a correlation between release of drug from the nanocomposites and the antimicrobial responses. As metal based composites find their use in as antibacterial agents in humans, these findings are important in guiding the fabrication and biocompatibility of metallic composites.

P11-050
Improved aerosol generation method and newly designed whole body rodent inhalation apparatus for the testing of nanomaterials

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Inhalation study is a gold standard for the assessment of respiratory toxicity of the nanomaterials (NM). To generate well-dispersed aerosol, we developed the "Taquann" dispersion method and a "direct injection" whole body inhalation system for the dispersed sample (designated as "Taquann System" J Toxicol Sci. 2013). The Mitsui MWNT-7 was used to evaluate the performance. Taquann method removes the aggregate/agglomerates effectively and enrich the well-dispersed single fibers without changing the length and width distribution. The method is based on two concepts; liquid-phase fine filtration and critical point drying to avoid re-aggregation by surface tension. The bulk sample of MWNT-7 was suspended and dispersed in tert-butyl alcohol (TBA), filtered by 25 micrometer mesh to remove aggregates/agglomerates, snap-frozen by liquid nitrogen, and vacuumed, mimicking the process of critical point drying (a method used for scanning electron microscope sample preparation). Aliquots of dry dispersed MWCNT were loaded in tube-shaped original cartridges, and the dispersed sample in the cartridges are periodically (every few minutes in most cases) injected to a newly designed inhalation chamber system by the compressed air to maintain a certain range of aerosol concentration. The length distribution of the MWNT recovered from the lungs of the exposed mice was similar to the fibers in original sample and aerosol in the chamber. Now the system is shown to work for Nikkiso MWCNT, three different makes of nano TiOs, potassium titanate whiskers, nano-sized agglomerates of double wall CNT and carbohydrate polymer. The advantages of the Taquann System include relatively cheap small scale equipment compared to traditional whole body inhalation chamber systems, low running cost, high flexibility for various types of NM samples, low chance of scattering of sample due to closed procedures after making TBA suspension, and low loss rate of sample. To date, we have developed four models of inhalation exposure systems (ver.1.0, 2.0, 2.5 and 3.0). The latest model of the Taquann system is now equipped with computer-controlled automatic cartridge loader/injector that can keep the aerosol concentration for up to 6 hours. The capacity of the chamber is for 25 mice or 12 rats per chamber. With respect to MWNT-7, 80% of samples loaded into a cartridge were aerosolized and mass concentration was reached up to 6 mg per cubic meters. These improvements made long-term studies possible for testing chronic effects of NMs. (Supported by Grants from MHLW, Japan.)

P12 – Neurotoxicology

P12-001

This abstract has been withdrawn.

P12-002
Identifying cobalt neurotoxicity targets *in vivo* through RNA-Sequencing

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Background: Implantation of Metal-on-Metal (MoM) hip implants is a common orthopaedic procedure, with an estimated one million patients worldwide. These prostheses have come under recent scrutiny with reported long-term systemic toxicities in multiple organ systems. Cobalt (Co) is suspected of causing this systemic poisoning as a consequence of the progressive wear of Co alloy MoM implants eluting metallic particles and ions into the bloodstream. However, the pathological consequences of Co accumulation, particularly in the brain, are difficult to interpret and its mechanisms of toxicity in neural cells remain obscure. Here we investigate Co neurotoxicity using an *in vivo* rat model.

Methods: Cobalt content in neural tissue was measured by inductively coupled plasma mass spectrometry (ICP-MS) and RNA-Seq data were obtained from the prefrontal cortex and cerebellum of Sprague Dawley rats dosed for 28 days with daily i.p. injections of 1 mg/kg Body Weight of $CoCl_2$ (treatment groups) or dH_2O (controls).

Results: After treatment, Co content was significantly increased in the prefrontal cortex, cerebellum, and hippocampus ($p < 0.01$), and Co levels in blood ($27.14 \pm 2.70 \mu g/l$) were within those of MoM patients. The number of Differentially Expressed Genes (DEGs) ($p < 0.05$) in the prefrontal cortex (3564 up-regulated, 2694 down) and cerebellum (2037 up, 1568 down) indicates a transcriptional response to Co accumulation from the clinically relevant doses of Co used. Transcript molecular classification showed a common metal homeostasis dysregulation in prefrontal cortex and cerebellum, since DEGs from the most populated functional group transcribe for metal ion binding proteins for zinc, calcium, and magnesium. Ion channels and transporters that handle calcium were also dysregulated. Finally, the gene expression of zinc binding phosphodiesterase and carbonic anhydrase families was significantly altered, suggesting that Co may modulate cyclic nucleotide signalling and pH balance, respectively.

Conclusions: Our research reveals that cobalt has neurotoxic effects via accumulation in neural tissue and interference with the transcriptional regulation of different metal ion binding systems. These findings may point towards novel therapeutic targets for patients with cobalt poisoning.

P12-003
Exposure to flame retardant tris (2-butoxyethyl) phosphate induces memory deficit and neuroinflammatory responses in a mouse model of allergic asthma

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Tris (2-butoxyethyl) phosphate (TBEP) is a phosphate ester and used as a flame retardant in various household appliances. However, its potential health effects including neurotoxicity and immunotoxicity are not known clearly. In this study, we aimed to examine the effects of dietary exposure of TBEP on novel object recognition ability and inflammatory markers in the brain of a mouse model of allergic asthma. Tolerable daily intake (TDI) of TBEP 2 μg/kg/day was set as high dose and 1/10, 1/100 from high dose were set as medium and low doses. Five-week-old male C3H/HeJ mice were fed a chow diet containing three doses of TBEP (1.67, 16.7 or 167 μg/kg/day) and ovalbumin (OVA) was given intratracheally every other week from 5 to 11-week-old. At 11 weeks of age, a novel object recognition test was conducted 2 hours after final instillation of OVA. Then the hippocampi were collected to detect neurological and immunological biomarkers using real-time RT-PC method. Mast cell was examined by toluidine blue staining and immunohistochemical staining methods. In addition, microglia activation was investigated by ionized calcium-binding adapter molecule (Iba)-1 immunoreactivity using immunohistochemical analysis. Regarding novel object recognition test, no significant changes of discrimination ability between novel and familiar objects were observed in the control and low or medium-dose TBEP-exposed allergic asthmatic mice. However, impaired discrimination ability was observed in high-dose TBEP-exposed allergic asthmatic mice compared with the control. The mRNA expression levels of memory function-related genes such as the N-methyl-D aspartate (NMDA) receptor subunits NR1 and NR2B, and inflammatory markers interleukin (IL)-1 β, tumor necrosis factor (TNF)-α, oxidative stress marker heme oxygenase (HO)1 were significantly increased in the hippocampus of high-dose TBEP-exposed allergic asthmatic mice. Moreover, mast cells and microglia activation were remarkable in high-dose TBEP-exposed allergic asthmatic mice. Our results indicate the possibility that childhood to young adulthood exposure to a phosphate flame retardant TBEP impaired memory function accompanied with alteration of memory function-related genes and inflammatory markers expression in the hippocampus of allergic asthmatic mice.

P12-004
Brain-derived neurotrophic factor protects against acrylamide-induced neuronal and synaptic injury via the TrkB-MAPK-Erk1/2 pathway

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In this study, we explored the neuroprotective effects of brain-derived neurotrophic factor (BDNF) in the human neuroblastoma cell line (NB-1 cells) after exposure to a potent neurotoxin, acrylamide (ACR). NB-1 cells, NB-1 cells co-cultured with Schwann cells (SCs), and a negative control group were exposed to increasing concentrations of ACR. Cytotoxicity and cell viability were determined by a -(4, 5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide assay. Protein and partial mRNA expression of BDNF, tropomyosin receptor kinase B (TrkB), mitogen-activated protein kinase-extracellular signal-regulated kinases (MAPK-Erk), and Synapsin I were tested by western blotting and reverse transcription polymerase chain reaction. Expression changes after the application of exogenous BDNF to NB-1 cells were also examined. To determine the mechanisms underlying neuronal damage repair, TrkB was blocked with the K252a inhibitor. ACR decreased cell viability in a dose- and time-dependent manner and damaged synapses, as evidenced by a decrease in Synapsin I expression. After ACR exposure, neurons initiated a self-protection mechanism, in which the levels of Synapsin I and BDNF were increased. This mechanism could be strengthened by downstream activation of the TrkB/MAPK/Erk1/2 pathway in the co-culture condition. The application of exogenous BDNF led to increased TrkB, MAPK-Erk, and Synapsin I levels. Thus, we have demonstrated that ACR is a neurotoxin, SCs may play a protective role via the BDNF-TrkB-MAPK-Erk1/2 signaling pathway, and exogenous BDNF can exert neuroprotective effects that can surpass those of SCs. Therefore, BDNF could potentially reverse ACR-induced neuronal damage and could be useful in the prevention and treatment of other neurodegenerative diseases.

P12-005
Gene expression changes induced by Type II piretroids exposure in human neuroblastoma SH-SY5Y cells

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Recent studies, using human neuroblastoma SH-SY5Y cells, indicate that the Type II pyrethroid insecticides alpha-cypermethrin and cyfluthrin affect the expression of genes involved in proinflammatory, apoptosis and oxidative stress pathways. In this study we have initiated a test of the hypothesis that the pyrethroid-induced alterations that could occur during neural development may be responsible for the behavior and cognitive impairment observed for these pyrethroid in adult life. To study the molecular changes taking place during embryogenesis and neurodevelopment as a consequence of pyrethroid exposure we used a neuronal cell line, SH-SY5Y cells. This *in vitro* model allows us to determine whether changes that take place *in vivo* during gestational pyrethroid-exposure occur also in pyrethroid-treated neurons *in vitro*. In the present study, mRNA levels of the following general neuronal markers were examined: (i) tubulin beta 3 (TUBB3) and growth-associated protein-43 (GAP43) both implicated in neurite and axon growth; (ii) neurofilament triplet H protein (NEFH) involved in cytoarchitecture organization; (iii) growth-associated protein 43 (GAP43), which is expressed at high levels during development and stressed by nerve injury adult motor-neurons; (iv) calcium/calmodulin-dependent protein kinase II (CAMK2), alpha (CAMK2A), and beta (CAMK2B) isoforms, both essential for learning and memory formation. We used quantitative real-time RT-PCR to measure the expression of these neural markers. We found that TUBB3, GAP43, NEFH, CAMK2A and CAMK2B genes were upregulated by both pyrethroids. We assume that this counter-regulation may serve as an endogenous protective function. These data suggest that pyrethroid exposure during early life may produce irreversible neuronal dysfunction and reorganization that last into adult life. These data may contribute to clarify the role of early exposure to industrial chemicals such as pyrethroids in neurodegenerative diseases. This work was supported by Project (ALIBIRD-CM Program) Ref. S2013/ABI-2728 from Comunidad de Madrid, and Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

P12-006
Neurotoxicity assessment of silver nanoparticle using human iPS cells

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Developmental neurotoxicity (DNT) has been assessed using experimental animals. Due to complexity of human brain development and

species differences, alternative *in vitro* testing using human cells, such as human iPS cells (iPSCs), has been expected in terms of cost, time-consuming and high throughput. Here we examine the effects of a well-known nanomaterial silver nanoparticles (AgNPs) using iPSCs. Despite their extensive use as anti-bacterial and anti-viral agents in broad consumer products, such as cosmetics and textiles, AgNPs have been concerned various types of cytotoxicity, including DNT. We have focused on neural differentiation process in iPSCs as a possible endpoint of DNT *in vitro*. Exposure to AgNPs reduced the expression of several marker genes, such as a neurogenesis marker OTX2 in the neural induction from iPSCs. Since neural differentiation requires ATP as a source of energy, we examined the intracellular ATP content. AgNPs decreased intracellular ATP levels in iPSCs. To understand the mechanisms by which AgNPs suppressed ATP production, we further examined the effects of AgNPs on mitochondrial dynamics. AgNPs induced mitochondrial fragmentation and reduced the level of mitochondrial fusion protein mitofusin 1 (Mfn1) in iPSCs. Moreover, knockdown of Mfn1 in iPSCs inhibited neural induction via OTX2 downregulation. Taken together, these data suggest that AgNPs induce cytotoxicity via Mfn1-mediated mitochondrial dysfunction in iPSCs. Thus, neural differentiation capability using iPSCs can be used for evaluation of compounds with developmental neurotoxicity.

P12-007
Pin1 is inactivated by environmental pollutant cobalt and contributes to neurodegenerative damage

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There is emerging evidence that the aberrant expression of prolyl *cis-trans* isomerase (Pin1) may underlie the pathogenesis of neurodegenerative diseases. Despite its clinical significance, the molecular mechanisms of Pin1 and age-related effects remain unclear in the environmental toxin-induced neurodegeneration.

Upon the various environmental toxicants tested, only cobalt caused a significant and dose-dependent decrease in Pin1 protein levels and increase in the inactive form of Pin1 levels in human brain glioma (H4) cells, demonstrated by Western blot and immunofluorescent microscopy. Accompanied with Pin1 inactivation, $CoCl_2$ induced neural cell damages including cell-cycle arrest and upregulation of apoptosis rates dose-dependently examined by flow cytometry, Hoechst and Annexin V/PI staining and Western blot of HIF1α and CASPASE 9 protein levels. In addition, $CoCl_2$ resulted in the upregulation of phosphorylated Tau (P-Tau) and disturbance of *cis* and *trans* P-Tau. Thus, we constructed Pin1 knockdown cell lines by lentivirus transfection, followed by exposure to $CoCl_2$ for 24 and 36 h. $CoCl_2$ exaggerated the loss of cell viability, hypoxia and neurodegenerative damages in Pin1 knockdown cells. The similar pattern of $CoCl_2$ was found in Pin1 overexpression cell lines. *In vitro* study indicates that $CoCl_2$ functions alongside with Pin1. To verify the above findings and include age-effect *in vivo*, 2-month-old and 12-month-old of C57BL/6J mice and the corresponding Pin1 knockout (KO) C57BL/6J mice were used. In accordance with *in vitro* studies, in wildtype mice, Pin1 was significantly downregulated by $CoCl_2$ in the hippocampus and cortex. The content of Co^{2+} in blood, cerebral cortex and hippocampus of $CoCl_2$-treated groups were significantly higher than that of control group, accompanied by metal ion disturbance. Neural damage was also found by immunohistochemical examinations, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling (TUNEL) assay and Western blots. Additionally, neurodegenerative indicators such as Tau, P-Tau, *cis-/trans*-P-Tau, APP and $A\beta_{1\text{-}42}$ levels were significantly upregulated. As predicted, the loss of learning, memory and spatial exploration abilities were found in response to $CoCl_2$. Those damages were more predominant in the 12-month-old mice and Pin1 KO mice, compared with 2-month-old mice and wildtype mice, individually. By comparing WT mice with Pin1 KO mice, $CoCl_2$ exposure strengthened the severity of neurodegenerative damage related to Pin1 downregulation, which became even more severe when aging.

In conclusion, $CoCl_2$ triggers neurodegenerative damages associated with Pin1 downregulation, age-dependently both *in vivo* and *in vitro*. Our findings indicate that cobalt could be one of the environmental toxicants that trigger neurodegenerative diseases associated with Pin1.

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P12-008
Novel mitochondrial targets of PM exposure in human olfactory mucosa cells

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Over the last years, a growing body of evidence has reported the association of ambient air pollution exposure with neurodegenerative diseases. Animal studies have shown that pollutant particles can translocate to the brain via the olfactory system, inducing inflammation and neurodegeneration. The olfactory system is known to be impaired in patients with neurodegenerative disease, and individuals living in highly polluted environments are impaired in odor identification. Therefore, human olfactory mucosa (hOM) cells present a great avenue for discovering early markers of air pollutant particle effects along with its link to pathogenesis of neurodegenerative diseases. To date, how air pollutants affect cellular function in live olfactory cells remains poorly addressed. In this study, we are the first to validate hOM cells as an *in vitro* model for interrogating cellular and molecular mechanisms induced by acute exposure to size-segregated particulate matter (PM). Initial characterization of human olfactory biopsy-derived hOM cells revealed the expression of key epithelial stem and neural cell markers. The effects of fine to coarse PM (< 1, < 2.5, < 10μm) on toxicity, inflammation, oxidative stress and mitochondrial function in hOM cells derived from healthy individuals were next assessed by a battery of functional and fluorescence-based methodologies. Results from mechanistic assays and gene expression profiling showed 1) reduced metabolic activity, 2) increased oxidative stress, 3) disruption of mitochondrial function and 4) a mild inflam-

matory response. RNA sequencing revealed a novel mitochondrial target of PM effects in the hOM cultures. Collectively, our findings strongly suggest that the mitochondria are an early target of PM exposure. We propose that the hOM *in vitro* model enables the study of air pollution in the context of discovering early indicators of neurodegeneration.

P12-009
Glutathione depletion and p38 activation trigger production of pro-inflammatory citokines in microglia exposed to mercury (II)

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Mercury (Hg) is widely known by its neurotoxicity albeit immunotoxicity occurs at lower exposure levels. Since microglia cells are the major representatives of the immune system in the CNS, we hypothesize that Hg compounds disrupt microglia homeostasis by interfering with redox regulation of signalling pathways. Thus, this work aims to study in microglia cells the effect of exposure to Hg^{2+}on p50, p65 and p38 nuclear translocation and activation considering the interaction of Hg with the glutathione system.

N9 mouse microglia cells were used as the experimental model and, following exposure to Hg^{2+}, were analysed for viability (MTT assay), GSH activity (DTNB assay), ROS production (DHCF assay), nuclear translocation of p38, p50, p65 (Western blot). Activation of p38 was assessed by measuring its phosphorylation by Western blot. Transcription of genes associated with pro-inflammatory activation of microglia (e.g.IL1-ß; TNF-α) was evaluated by qRT-PCR. LPS exposure (300 ng/mL; 24 h) was used as a positive control for microglia activation. Pre-exposure to N-Acetylcysteine (NAC; 2.5 mM 24 h) and a specific p38 inhibitor (SB 239063; 10 µM 4 h) were used to modulate relevant pathways. All experiments were independently replicated at least 3 times.

Following 24 h of exposure to different concentrations of Hg^{2+} the $EC_{50}$for a reduction in viability was 42.1 ± 3.7 µM. However, subsequent experiments showed that after exposure for 24 h to just 5 µM of Hg^{2+} there was a general increase in ROS levels (»40%) which was accompanied by a very significant depletion (»90%) of GSH.

Upon 6 h of exposure to Hg^{2+}(5 µM), nuclear translocation of p50 was decreased whereas p65's was increased. Most importantly, Hg^{2+} induced a very significant accumulation of p38 in the nucleus (50% higher than in control), which was accompanied by an increase in its phosphorylation.

Likewise, after exposure to Hg^{2+}, transcript levels of both IL1-ß and TNF-α were increased by 50% relatively to control. However, pre-exposure to NAC – which caused a 60% increase in basal GSH levels – reduced transcription of both cytokines by Hg^{2+}back to control levels. Similarly, pre-exposure of N9 cells to the p38 inhibitor SB 239063 hindered activation of cytokine transcription by Hg^{2+}.

These results show that disruption of redox signalling by Hg^{2+}causes activation of inflammatory pathways leading to production of IL1-ß and TNF-α at exposure levels much below cytotoxic concentrations. GSH depletion and p38 activation are major events contributing to enhance cytokine production. This is of significance since it shows that activation of microglia at sub-cytotoxic exposure levels may result in the production of pro-inflammatory factors which may enhance cytotoxicity for neighbouring cells (e.g. neurons).

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P12-010

This abstract has been withdrawn.

P12-011
Analysis of brain transcriptome in MPTP-lesioned adult zebrafish: insights into innate immune-related genes

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Background and Objective: Parkinson's disease (PD) is a common age-related neurodegenerative disease. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, has been used to model PD in multiple model organism. Zebrafish is an alternative vertebrate model for the study of neurotoxicity. The objective of this study is to understand the biomechanism of MPTP induced neurotoxicity in zebrafish.

Methods and Results: Adult zebrafish received an intraperitoneal injection of MPTP. The phenotype and role of genes and proteins related to neurotoxicity were tested by using zebrafish swimming behavior, Realtime-PCR, immunofluorescence and transcriptome analysis approaches. MPTP-lesioned adult zebrafish showed the significant decreases in average speed (75%), average acceleration (71%), mobility rate (22%), distance of activity (64%) and exploration rate (54%). The levels of tyrosine hydroxylase gene and protein were significantly decreased in MPTP group, which represented dopaminergic neuron loss. We performed comparative transcriptomics of the brain from wildtype and MPTP-lesioned zebrafish to identify transcriptional signatures involved in the mechanism of MPTP induced neurotoxicity. MPTP treatment caused alteration of gene expression patten, a total of 863 differently expressed genes (fold change ≥ 2, $P < 0.05$) were identified by RNA-seq. Application of KEGG enrichment algorithms revealed a number of key biological processes perturbed by MPTP. Particularly, MPTP differentially activated biological processes associated with innate immune-related pathways including Toll-like receptor signaling pathway, NOD-like receptor signaling pathway and Cytokine-cytokine receptor interaction, with significant upregulation of *tlr1, tlr3, irf3, irf7, il21r, il7r* genes compared to the wildtype brain.

Conclusions: Our work provides a convenient tool for neurotoxicity study and uses an innovative approach to indicate the potential roles of innate immune in the development of MPTP-induced zebrafish PD model.

P12-012
Phenyl valerate esterase activity of human acetylcholinesterase

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The toxicity of organophosphorus compounds (OPs) cannot be explained only by action on acetylcholinesterase or neuropathy target esterase (NTE). A fraction of the membrane bound phenylvalerate esterase activity (PVase) is associated to NTE, the key initiating molecular event in the OP-induced delayed neuropathy (OPIDN). An enzymatic fraction in chicken brain soluble PVase has been reported to be due to a butyrylcholinesterase protein. We showed that human butyrylcholinesterase (hBuChE) shows PVase activity and that the substrates acethylthiocholine and phenyl valerate showed competition in their activities but with a interaction different to the competitive model of substrates.

In addition, we have observed that human acetylcholinesterase has also phenyl valerate esterase activity, but with lower activity than human butyrylcholinesterase. The level of phenylvalerate esterase activity in cholinesterases depends on the species and the type of cholinesterase.

This work shows that the kinetic interactions between phenyl valerate and acetylthiocholine in human acetylcholinesterase are different to the competitive model of substrates according to the Michaelis-Menten reaction. In addition, PVase activity is enhanced in presence of low acetylthiocholine concentration. The approach introduced in this work suggests that other site could be involved in the interaction with phenyl valerate.

P12-013
Comparison between the cytotoxic effects of pure cylindrospermopsin and containing and non-containing cylindrospermopsin-extracts in the neuronal SH-SY5Y cell line

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Cylindrospermopsin (CYN) is one of the main common cyanotoxins produced by several genera of cyanobacteria. Due to its capacity of causing damage at different levels in the organism is considered a cytotoxin. Among its effects, neurotoxicity is one of the less clear due to the lack of studies. However, some studies demonstrate its neurotoxic potential *in vitro* and *in vivo*. The aim of the present study was to compare the cytotoxic effects between pure CYN and extracts from a producer and a non-producer cyanobacterial cultures in the SH-SY5Y human neuroblastoma cell line. For this purpose, cells were exposed to 0–10 µg/mL CYN, 0–10 µg/mL CYN from an *Chrysosporum ovalisporum* culture extract (CYN+), and the equivalent volume of extract of a non-producer *Cylindrospermopsis raciborskii* (CYN-) culture. The cytotoxicity assays performed were the MTS tetrazolium salt reduction (MTS), the neutral red uptake (NR), and the total protein content (PC) assays. The results provided a more sensitive response in the MTS assay in all cases, obtaining EC_{50} values after 24 hours of exposure of 0.866 ± 0.131, 1.111 ± 0.325, and 5.658 ± 1.180 µg/mL after exposure to CYN, CYN+ and CYN-, respectively, and 0.322 ± 0.081, 0.691 ± 0.165 and 5.164 ± 1.620 µg/mL, respectively, after 48 hours. Thus, it can be concluded that pure CYN exerts higher cytotoxicity in this cell line compared to the CYN+ extract from *C. ovalisporum*. Furthermore, it is important to highlight that some compounds different to CYN present in the extracts from non-producer (CYN-) could promote cytotoxic damage by themselves, playing a role in the cell-damaging potential of cyanobacterial cultures.

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P12-014

This abstract has been withdrawn.

P12-015
Behavioral effects of cypermethrin, lambdacyhalothrin, and betacyfluthrin

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Pyrethroids are among the most commonly used insecticides. Their main mode of action is blocking voltage dependent sodium channels in neuron membranes.

The aim of the study was to compare behavioral effects after exposure to cypermethrin (CYP), lambdacyhalothrin (LCH), and betacyfluthrin (BCF) in a model of subacute poisoning in mice.

A total of 64 mice were divided into 8 groups of 8 animals:
1. control females
2. control males
3. females receiving 12mg/kg CYP
4. males receiving 12mg/kg CYP
5. females receiving 2mg/kg LCH
6. males receiving 2mg/kg LCH
7. females receiving 20mg/kg BCF
8. males receiving 20mg/kg BCF

Animas were tested in a Y maze on day 1 and 7 in order to measure their spontaneous locomotor activity and fresh spatial memory. They were also trained in passive avoidance (PA) on day1 and examined on day 2 and day 7. The PA task is regarded as a measure of long-term memory retention.

The mean time (±SD) of memory retention in PA on day 2 was: for CYP females 165.7 ± 40.3s, CYP males 180 ± 0.0s, for LCH females 100.3 ± 85.2s ($p<0.05$ *vs* controls), for LCH males 153.5 ± 54.4s, for BCF females and males 180 ± 0.0s. On day 7 the mean time of memory retention in PA was: for CYP females 98.38 ± 68.8s, CYP males 152.2 ± 31.8s, for LCH females 95.13 ± 90.7s, for males 95.75 ± 74.9s, for BCF females and males 180 ± 0.0s.

Measuring locomotor activity in a Y-maze on day 1 CYP females had 45.38 ± 12.74 arm entries, CYP males 36.75 ± 6.96, LCH females 53.88 ± 13.59, LCH males 34.75 ± 7.29, BCF females 27.25 ± 10 ($p < 0.05$ vs controls) males 29.38 ± 9.88. On day 7: CYP females 34.5 ± 19.73, CYP males 32.75 ± 10.54, LCH females 46.63 ± 23.22, LCH males 35.13 ± 3.6, BCF females 29.5 ± 10.66 ($p<0.05$ *vs* controls), BCF males 21.12 ± 10.58 ($p<0,05$ *vs* controls).

The number of logical alternation in the Y maze on day 1 was: for CYP females 62.29 ± 8.48, CYP males 69.43 ± 9.85, LCH females 60.81 ± 15.06, LCH males 60.98 ± 16.72, BCF females 64.84 ± 16.72, BCF males 62.14 ± 15.28. On day 7 the numbers of logical alternation was for CYP females 59,54 ± 10,95, CYP males 62.75 ± 10.96, LCH females 54.16 ± 9.33, LCH males 68.34 ± 11.52, BCF females 65.48 ± 18.07, BCF males 65.14 ± 11.84.

To sum up: subacute poisoning with LCH significantly decreases memory retention in females on day 2. BCF reduces locomotor activity in females on day 1 and in males and females on day 7. LCH is the least neurotoxic of the three pyrethroids.

P12-016
Integration of PBPK with ROS SB model for PFOS induced neurotoxicity

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PFOS is one of the most abundant perfluoroalkyl substances (PFASs) in environment with wide exposure to the human population through contaminated food, water, consumer products and occupa-

tional exposure. Various epidemiological, *in vitro* and *in vivo* studies have found the causal link between the exposure of PFOS and developmental neurotoxicity. Several studies have indicated that PFOS cause oxidative damage in neurons by generating ROS like peroxide and free radicals. Consequently, ROS alters antioxidant response elements (ARE), disturb redox signalling and activation of apoptotic pathway which may increase the neuronal cell death. In-silico modelling along with high throughput *in vitro* assays is gaining attention to assess the environmental chemicals related human health effects. The objective of this study is to develop an integrated tool that describes both the kinetic and dynamic effects of PFOS via coupling physiologically based pharmacokinetics (PBPK) to a systems biology model of ROS. This integrative approach will be applied for the cohort of pregnant women, where neuronal damage in fetuses will be investigated. A PBPK model describing pharmacokinetics of PFOS in pregnant women and a systems biology (SB) model describing the ROS generation was made. PBPK along with the systems biology model will be used to understand the mechanistic pathway regarding PFOS induced neurotoxicity. Developed PBPK coupled ROS SB model is able to demonstrate the effects of PFOS on ROS, thus predicting effects of ROS on ARE and consequently mitochondrial damage and alteration in ATP production as a proxy for neuronal survivability.

P12-017
Fusarium mycotoxins alter neuronal network activity in surviving rat brain slices

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Mycotoxins are toxic secondary metabolites produced by microscopic fungi often infecting agricultural crops. The most common *Fusarium* toxins – fumonisin B1 (FB1), deoxynivalenol (DON) and zearalenone (ZEA) – are structurally stable and able to enter into food chain. Consumption of grain containing toxin, whose amount exceeds tolerable daily intake (TDI) may cause adverse health effects. FB1 is known as a *de novo* sphingolipid biosynthesis depressor, DON is a powerful protein synthesis inhibitor, while ZEA can interact with estrogen receptors. Recent data suggest that these toxins may also have acute effects on neural cell cultures as well. Thus, we decided to examine with electrophysiological techniques whether they alter neuronal network activities after acute treatment.

In order to carry out *ex vivo* experiments, rat brain slices were incubated for 30 minutes in artificial cerebro-spinal fluid (ACSF), which contained each *Fusarium* toxin (FB1, DON or ZEA) in different concentrations (10, 50 and 100 µM). After the pre-treatment, electrically evoked field potentials in the hippocampus and seizure-like events in the neocortex were investigated. In addition, neuronal activation pattern was studied after intraperitoneal injection of FB1 (7.5 mg/kg bw), DON (1 mg/kg bw) or ZEA (5 mg/kg bw), by counting c-Fos positive cells.

Basic excitability in the hippocampus was increased by FB1, but long-term potentiation (LTP) was not altered, while DON and ZEA inhibited both excitability and LTP. Seizure-like events in the neocortex were not altered by FB1, but DON delayed the appearance of bursts, and ZEA increased the frequency of events. The number of activated neurons increased mostly in the nucleus accumbens, but we can see similar tendencies in other studied brain areas as well.

Based on our results, each *Fusarium* toxin has different acute effects on neuronal networks following direct exposure of rat brain slices to toxin-containing ACSF. However, after intraperitoneal injection, the toxins may not penetrate sufficiently through the blood-brain barrier to yield effective concentration required for significantly increased neuronal activation. Therefore, activation was detected only in few brain regions.

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P12-018
Effect of *Fusarium* mycotoxins on behavior and neuronal network activity after subchronic exposure in rat

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Mycotoxins are toxic secondary metabolites produced by microscopic fungi; the most common *Fusarium* toxins – fumonisin B1 (FB1), deoxynivalenol (DON) and zearalenone (ZEA) – may contaminate the food chain. FB1 is known for inhibiting *de novo* sphingolipid biosynthesis, DON effectively decreases protein synthesis inhibitor while ZEA can interact with estrogen receptors. There is increasing evidence that these substances may affect nervous system function as well.

In the present study, neuronal effects of *Fusarium* toxins were studied in subacute toxicological experiments. Rats of both sexes were treated for 28 days via gavage with FB1 (50 and 500 µg/kg), DON (20 and 200 µg/kg) or ZEA (20 and 200 µg/kg).

Previous experiments indicated that the toxins cause changes activity of neurons in brain areas implicated in anxiety- and depression-related behaviors. For this reason, specific behavioral tests were performed on the treated rats. DON decreased open arm entry number in the elevated plus maze test, indicating increased anxiety level. ZEA increased the time spent with immobility in the forced swim test, which is characteristic of depression-like behavior.

Sleep-wake activity pattern was studied with chronic EEG recording. None of the mycotoxins caused any significant change in the length and circadian distribution of wakefulness, but ZEA slightly decreased the time spent with light sleep. Neuronal network activity on the microcircuit level was studied on rat brain slices of hippocampus and neocortex with the means of field potential recording; excitability, plasticity and seizure susceptibility were examined. FB1 and DON slightly increased excitability in both areas, while ZEA modified seizure-like event pattern in male rats. The higher dose of ZEA also inhibited the development of long-term potentiation in hippocampal slices.

To summarize, the three *Fusarium* toxins exert different effects on the nervous system, from the microcircuit to the behavioral level; severity of alterations may depend on the dose, sex and exact experimental endpoint.

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P12-019
Deoxynivalenol affects neuronal activity and impairs motivational behavior in mothers

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Deoxynivalenol (DON) or vomitoxin, is a trichothecene mycotoxin produced by Fusarium graminearum and culmorum. Mycotoxins or secondary metabolic products of mould fungi, are micro-pollutants, which may affect human and animal health. The neuronal and behavioural actions of DON were analyzed in the present study. To address which neurons can be affected by DON, the neuronal activation pattern following intraperitoneal injection of DON (1 mg/kg) was investigated in adult male rats while control animals received physiological saline solution. Neuronal activity was assessed by c-Fos immunohistochemistry. DON induced significant c-Fos activation in only a few brain regions, including the accumbens nucleus, the medial prefrontal cortex and the ventral tegmental area. Further double labeling studies suggested that in the accumbens nucleus, a subpopulation of medium spiny neurons may be activated by DON treatment. The activation pattern suggested that DON influenced the reward system of the brain. To study the behavioural relevance of this activation, we examined the effect of DON on a special goal-directed behaviour, the pup-carrying behaviour in mother rats. Pup retrieval latencies were increased by DON administration, and DON-treated mother rats spent less time with nursing, suggesting reduced maternal motivation. Consistently with the behavioural inhibition, electrophysiological recording on rat brain slices indicated that *in vitro* field responses evoked by electrical stimulation also decreased in the nucleus accumbens as a result of DON pretreatment.

The data imply that acute uptake of the mycotoxin DON can influence the reward circuit of the brain and exert negative behavioural actions.

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P12-020
Screening of various neural induced hiPSCs (hiNPCs) for the use in (developmental) neurotoxicity assays

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For most chemicals in use today, the (developmental) neurotoxic ((D)NT) potential is unknown. This data gap needs to be filled, as the exposure to these chemicals might contribute to neurological diseases in children and adults. Animal experiments are currently used for the testing of (D)NT endpoints, but they are very time-consuming, expensive and the results are often difficult to interpret due to high variation of endpoints. In addition, there are known species differences between rodents and man. Therefore, there is a need for alternative testing methods to assess the (D)NT potential of chemicals. One approach is to use human neural cell-based *in vitro*-systems to analyze effects on developmental neurotoxicity endpoints like proliferation, differentiation and migration of neural progenitor cells (NPC) as well as for acute neurotoxicity by assessing the influence of substances on a functional neuronal network. To avoid ethical concerns and to ensure enough cell material for medium to high throughput testing, we use human induced pluripotent stem cells (hiPSCs) instead of primary human cells and differentiate them into the neural lineage. Thereby we generate 3D cell aggregates called neurospheres (hiNPCs).

For standardized substance testing it is essential that the neural induction (NI) is highly reproducible and quality criteria need to be defined and established. Therefore we neurally induce different iPS-lines and compare them by monitoring their differentiation status via FACS and qPCR analysis. In addition we plate them on an extracellular matrix to differentiate them into neurons and astrocytes and analyze their radial migration by immunocytochemistry and high content image analysis. To test their ability to form functional neuronal networks, we measure their electrophysiological activity on microelectrode arrays (MEAs). With these results, we will be able to define quality criteria which have to be met by the initial NI culture as well as the time frame in which the cells can be used for (developmental) neurotoxicity assessment.

P12-021
Biocompatibility evaluation of medical devices coming into contact with brain tissue

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The evaluation of local effects in brain tissue is a requirement according to ISO 10993-6: 2016 "Biological evaluation of medical devices – Part 6: Tests for local effects after implantation" for assessing the biological response of the neural tissue to an implanted material. Depending on its clinical use, the material can either be implanted in the brain parenchyma or be placed directly on the parenchymal surface in rats or rabbits. The evaluation must consist in the comparison of the biological response of the test item with the response of a control sample (medical devices with well-known clinical acceptability and biocompatibility characteristics). The local effects of the material to be tested are evaluated at the macroscopic and microscopic level, after the animals have been evaluated clinically.

Intracerebral implantation was performed in rats under general anaesthesia and strict aseptic conditions. In one group, a solid test item (rod shape) was inserted intracerebrally in one hemisphere caudally from the bregma while in one further group, other animals received a liquid test item, which was applied topically on the parenchymal surface of brain. For periods of 1 or 4 weeks, the animals were observed for general clinical findings and detailed behaviour. At the end of the implantation period the animals were necropsied and the brain was stored in 4% neutral-buffered formaldehyde.

Implantation sites were processed to microscopic slides and stained with haematoxylin and eosin, Fluoro-Jade® C (degenerating neurons) and Glial Fibrillary Acidic Protein (GFAP, astrocyte biomarker). Histopathological findings with the solid test item consisted of reactive macrophages, swollen astrocytes, reactive microglia cells, neuronal necrosis, neuronophagia and satellitosis, degenerating neurons, hemorrhage, hemosiderin, and ependymal reaction. The glial reactions

formed a gliosis and were present in all affected sections. Implantation sites with fluid item caused focal meningeal fibrosis or lymphocytic infiltration, and in one case, a focal submeningeal gliosis with reactive astrocytes was noted. Overall, the intracerebral implantation of the solid material showed a higher irritative potential than the implantation of the liquid material on parenchymal surface due to the traumatic impact of implantation.

References

ISO 10993-6: 2016 "Biological evaluation of medical devices – Part 6: Tests for local effects after implantation"

P12-022
Quantitative evaluation of the Key Events Relationships (KERs) resulting in impairment of learning and memory abilities (OECD AOP13) to support regulatory decision-making

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Exposures to environmental chemicals during early life are suspected to contribute to the increasing incidence of neurodevelopmental disorders in children, such as lowered IQ, learning disabilities, attention deficit hyperactivity disorder (ADHD) and autism. This rise has major societal and financial consequences. There is undoubtedly a substantial genetic component to these disorders [1]. However, findings from neuropathology, brain gene expression, twin and sibling concordance/recurrence risk analyses, all suggest that environmental influences (e.g. chemicals and drugs) in the prenatal period also impact the risk of developing these disorders. The adverse outcome pathway (AOP) concept is a revolutionary advance in toxicological science, based on the application of mechanistic information. An AOP represents the existing knowledge concerning the causal links between a molecular initiating event (MIE) and the cascade of key events (KEs) that lead to a specific adverse outcome of regulatory concern [2]. Well-developed AOPs are expected to guide the identification of experimental testing and non-testing approaches to support regulatory decision-making [3]. However, AOPs are currently a theoretical concept, and activities within regulatory, industry and academic institutions, are still trying to determine how the use of AOPs can support regulatory decision-making. Quantitative metrics obtained in human physiologically relevant vitro models coupled to *in silico* modelling to construct quantitative AOP (qAOP) and followed by *in vitro – in vivo* extrapolations (IVIVE) are key to set exposure thresholds and allow the use of AOPs in risk assessment. Therefore, we are currently using the human 3D iPSC-derived brain system (BrainSpheres) developed by the authors [4] to study already endorsed developmental brain AOPs. Several KEs of AOP13 "*Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities*" are quantitatively evaluated after exposure to MIE triggers, and then a qAOP will be constructed to model the effects of xenobiotics within the BrainSpheres. Calcium signalling and multielectrode array have confirmed the presence of functional NMDR in BrainSpheres, and have also allowed to establish a dose-response of NMDAR inhibition after Lead exposure, finding the IC50 around 50 µM. The ultimate goal of this work is to increase the potential of the application of AOPs for regulatory decision-making and improve our capability to predict human toxic exposure thresholds without the need for animal testing.

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P12-023
Could Ochratoxin A be a possible etiological factor of Parkinson's disease?

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Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies which contain aggregates of alpha-synuclein (α-syn). There is increasing evidence that the transmission of the pathology between neurons plays a central role in disease progression. The neurodegenerative process might start in the enteric nervous system and spread via the vagus nerve to the lower brainstem, a process that precedes degeneration of the dopaminergic neurons.

People living in a rural area might be at significantly increased risk of getting PD due to exposure to potential neurotoxins present in the environment. Mycotoxins are a group of natural-occurring fungal secondary metabolites contaminating a huge variety of crops. Very few studies have been specifically designed to evaluate the neurotoxic effects of mycotoxins. However, one of the most relevant mycotoxins in terms of genotoxicity/carcinogenicity, ochratoxin A (OTA), has shown some neurotoxic effects *in vivo*.

The aim of the present project was to determine the effect and mechanisms of OTA on α-syn *in vitro* in both intestinal and neuroblastoma cell lines.

For that purpose, i) Caco-2 cells were transfected with a plasmid to express wild-type (WT) and α-syn and ii) a stable SH-SY5Y cell line overexpressing WT α-syn was selected. Cytotoxicity assay (Celltiter Blue assay) was carried out to select a range of subtoxic concentrations. A range of 25 to 500 nM OTA was selected for the subsequent evaluation of α-syn (Western blot) after 72 h of exposure. The lower doses of OTA (25, 50 nM) had no effect upon α-syn however doses between 75 to 200 nM increased significantly the intracellular α-syn levels. Moreover, proteins (hsc70 and LAMP-2A) involved in chaperone-mediated autophagy, a pathway known to degrade α-syn were evaluated. Although no significant changes were observed in hsc70 a clear downregulation of LAMP-2A was observed at concentration 100 nM. Decreased LAMP-2A levels in cell lines reduced chaperone-mediated autophagy activity and increased the half-life of α-syn.

Our results provide preliminary data to understand the potential neurodegenerative effect of OTA as well as contribute with an experimental system able to detect other long-term effect neurotoxins.

P12-024

This abstract has been withdrawn.

P12-025

Establishment of *in vitro* assays for regulatory developmental neurotoxicity testing

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The activation of key neurodevelopmental processes (KNDP) is an essential requirement for brain development. It is assumed that at least one of these processes is impaired during chemical exposure, leading to developmental neurotoxicity (DNT). To assess this DNT hazard, animal-free new approach methods (NAM) have been developed, which model certain KNDP *in vitro*. To allow conclusion on the DNT hazard of hits the information from a screen assay has to be combined with additional data and with information on the test method.

Based on a set of selected pesticides we are performing primary hit testing with different KNDP methods. Primary fetal human neural progenitor cells (NPC), growing in 3D cell aggregates called neurospheres, cover the KNDP NPC proliferation, migration and differentiation into neural effector cells (astrocytes, neurons and oligodendrocytes) as well as neuronal morphology. A further test method models neurite outgrowth of immature human dorsal root ganglia cells. To improve the readiness and highlight robustness and reliability of the test methods we are performing an exchange of these methods between two laboratories. Additionally, as a hit confirmation, human induced pluripotent stem cell (hiPSC)-derived iNPC are being differentiated into neurons and astroglia cells. A second hit confirmation for the neurite outgrowth assay of dorsal root ganglia cells is being performed with immortalized primary central neurons from an 8-week old mesencephalon to observe effects on the neurite outgrowth. Beside data from tests for structural impairment, we aim to establish the neuronal network formation (NNF) assay based on human iPSCs to test neuronal network activity on 24-well microelectrode arrays (MEA). Previous data show that some pesticides as rotenone affect more than one endpoint while others are likely to be specific for only one of the tests (e.g. disruption of oligodendrocyte maturation or the neural network function).

We will present first results of this study according to the experimental progress.

P12-026

Synthetic cannabinoids 5F-PB22 and THJ-2201 promote *in vitro* CB1 receptor-dependent neuronal differentiation at *in vivo*-relevant concentrations

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The widespread recreational use of Synthetic Cannabinoids (SCs), a vast group of new psychoactive substances that activate cannabinoid receptors (CB1R, CB2R) with stronger potency than THC (the main psychoactive substance of cannabis), represents a major public health issue. SC use during and before pregnancy is especially alarming due to the possible onset of neurodevelopment disorders in the offspring. In the present study, the role of 2 commonly abused SCs, 5F-PB22 and THJ-2201, on *in vitro* neuronal differentiation and proliferation was evaluated in NG108-15 neuroblastoma cells.

Cells were differentiated for 3 days in serum-starved (1% FBS) culture medium supplemented with forskolin and retinoic acid, and exposed to SCs at non-toxic, *in vivo*-relevant concentrations (≤ 1 nM), either in acute (single addition at day 0) or repeated (one addition every 24 h) exposure settings. Differentiation ratios (number of neurites per total cell number), total neurite length and neuronal marker expression (e.g. β3-tubulin, p73) were assessed. Cell proliferation was followed up to 72 h (Sulforhodamine B assay).

5F-PB22 and THJ-2201 only impaired metabolic activity, measured by the MTT assay, at high concentrations (EC_{50} of 779 µM and 299 µM, respectively). Exposure of NG108-15 cells to 1 pM – 1 nM of either SC raised differentiation ratios (near 2-fold) and total neurite length, compared to vehicle-treated cells. Regulation of such processes depended on CB1R activation, as its inhibition with the selective antagonist SR141716A abrogated SC-induced effects. Interestingly, repeated 5F-PB22 exposure was required to reach effects similar to a single THJ-2201 dose. Different neuronal marker expression levels (higher 5F-PB22-induced β3-tubulin and p73 expression, compared to THJ-2201) suggest that neuronal maturation state varied between the tested SCs. Of note, none of the 2 SCs affected cell proliferation.

Overall, we report first-hand the CB1R-mediated enhancement of neurodifferentiation by 5F-PB22 and THJ-2201 at concentrations below 1 nM. Still, further research is required to identify the underlying action mechanisms and the consequences for neurodevelopment *in vivo*.

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P12-027

Neurotoxicity evaluation of acute pesticide exposure on Human progenitor neural stem cells

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Neurodevelopment in humans is susceptible to damage through exposure to toxic chemicals including pesticides. Pesticide-induced neurotoxicity was investigated using the human neural progenitor cells. Both undifferentiated and differentiated cells were challenged with either of two commonly employed organophosphorus pesticides, Azamethiphos-oxon or Chlorpyrifos-oxon, or the carbamate pesticide, Aldicarb, over a concentration range of 0.3–300 µM for 24 hrs. Cellular metabolic activities and cell viability were assessed using MTT, LDH, and ATP assays. Reactive oxygen species (ROS) generated in response to pesticide exposures were quantified using a dichlorofluorescein diacetate assay. Cellular protein modification and damage was also investigated using gel electrophoresis, and Western blotting. Cell metabolic activity and viability decreased in a pesticide concentration-dependent fashion. The inhibitor concentration producing 50% cell death (IC_{50}) in undifferentiated cells was 12.01 ± 1.128 µM, 12.16 ± 1.982 µM and 14.3 ± 2.393 µM for Azamethiphos-oxon, Chlorphyrifos-oxon and Aldicarb, respectively, whereas differentiated cells were more sensitive to pesticides with IC_{50}s of 11.883 ± 2.043 µM, 8.2475 ± 0.8896 µM, and 13.88 ± 2.844 µM, respectively. Differentiated cells were also more susceptible to ROS damage than undifferentiated cells, with ROS damaged proteins increased in a concentration-dependent fashion. Collectively, our results demonstrate the vulnerability of neural stem cells to a toxic insult from a range of commonly encountered pesticides.

P12-028
The increase in lipid peroxidation in the rat brain after acute exposure to Pb and/or Cd

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Among metals lead (Pb) and cadmium (Cd) are leading industrial and environmental pollutants. It is well known that the nervous system is the main target organ of Pb toxicity. However, there is growing evidence that Cd as well can produce toxic effects associated with nervous system. Having in mind that one of the main mechanisms of metal toxicity is induction of prooxidants and antioxidants imbalance, the aim of the study was to investigate whether the combined acute treatment with Pb and Cd has more pronounced effects than intoxication with single toxic agent on the parameters of oxidative stress: malondialdehyde (MDA), total antioxidative status (TAS) and total oxidative status (TOS) in the rats brain.

Three experimental groups (6-8 Wistar rats) received single oral treatment of 150 mg Pb/kg b.w. (Pb group), 15 mg Cd/kg b.w. (Cd group), or mixture of these two doses (Pb+Cd group). Control group was untreated. All animals were sacrificed 24 h after treatment, and brains were removed and homogenized for further analysis of MDA, TAS and TOS.

Co-treatment with Pb and Cd induced significant increase of MDA in rat brain compared to the controls, and both groups treated with only one metal. Interestingly, single treatment of Pb or Cd did not change MDA levels when compared to the control group. On the other hand, TAS levels were significantly increased in all treated groups, while no significant changes in TOS levels were observed

The results of the present study indicated that Pb and Cd mixture exhibited more pronounced toxic effect on lipid peroxidation status in rats brain when compared to Pb or Cd single treatment. Further studies are needed to determine whether this observed exacerbation of oxidative stress in brain following mixture administration is the result of toxicokinetic or toxicodynamic interactions between these two toxic metals.

P12-029
Feasibility studies for prediction models analysing concentration response data from high content image analyses

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Testing of chemicals for their potential to induce neurodevelopmental toxicity (DNT) *in vitro* is a stepwise process that starts with the *in vitro* experiments followed by data generation, data analyses and finally decision-making based on the *in vitro* findings. Each of these steps is crucial, highly dependent on the preceding one and has a lot of criteria to regard. Here we propose a data evaluation tool for effective, versatile and robust data evaluation, analyses and interpretation of *in vitro* concentration-response toxicity data. For DNT testing, we use primary human neural progenitor cells (NPC) that grow as neurospheres in suspension culture. After plating cells on the extracellular matrix protein laminin, they radially migrate out of the neurosphere and thereby differentiate into neurons and glia cells. For simultaneous analyses of the multiple endpoints in this *in vitro* model, i.e. neuronal and glia migration and differentiation, as well as neuronal morphology analyses, we engage the software 'Omnisphero' that was specifically designed in our lab for analysing data generated with High Content Image Analyses (HCA) of spheroid based neural models. The output of Omnisphero are concentration-response curves over multiple endpoints for each compound, making an automated evaluation of data necessary. Here, we present our efforts of creating an R based data evaluation workflow, where several fitting models are generated and compared, thus applying the optimal fit for each set of data. Based on the optimal fit, different prediction models are developed and compared to find the one that best represents the *in vitro* data and thus enables data interpretation.

The here proposed statistical workflow takes several statistical models for concentration-response fitting and analysis into account and thus enables more robust, informative and versatile concentration-response analysis.

P12-030
Repeated intravenous administrations of macrocyclic Gadolinium Based Contrast Agents in rats: evaluation of gadolinium retention in different organs

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Gadolinium Based Contrast Agents (GBCAs) are paramagnetic agents used in magnetic resonance imaging (MRI) as they add relevant diagnostic information to the anatomical resolution of the MR images. Recent studies have found that tiny amounts of gadolinium can be retained in the brain and other tissues after cumulative administration of GBCAs. Using a healthy rat animal model, we compared the residual gadolinium levels in cerebrum, cerebellum, liver, kidney (right), skin, femur and blood after repeated exposure to four commercially-available macrocyclic GBCAs.

Sixty-five male Sprague-Dawley rats were divided into four treated groups (ProHance®, Dotarem®, Clariscan™, Gadovist®, n=15) and one control group (saline, n=5). Exposed animals received 20 GBCA administrations (four per week for five weeks) at 0.6 mmol/kg, a dose corresponding to a clinical dose of 0.1 mmol/kg based on the extrapolation factor set for rats in FDA Human Equivalent Dose (HED) guidance. After a wash-out period of 28-days, animals were sacrificed and tissues harvested for gadolinium (Gd) determination. Gd content was determined by Inductively Coupled Plasma Mass-Spectrometry. Since GBCAs are mainly excreted through the kidneys, we also dissected and processed to slides the left kidney for histopathologic evaluation.

Significantly ($p \leq 0.005$; all evaluations) lower levels of Gd were noted with ProHance than with Dotarem, Clariscan or Gadovist in all soft tissues. Significantly ($p \leq 0.01$) higher Gd levels were noted with Gadovist in the femur compared to all other GBCAs. No GBCA-induced macroscopic or microscopic findings were noted in the left kidney.

In conclusion, our findings reveal considerably lower retained levels of Gd in brain and soft body tissues of rats at 28 days after the cumulative administration of a total ProHance dose of 12 mmol/kg, than after equivalent cumulative doses of Dotarem, Clariscan and Gadovist administered under identical conditions. Moreover, no evidence of GBCA-induced renal changes was observed histopathologically at the tested cumulative dose.

P12-031
Effect of uranium on multipotency of neural stem cells in a primary neurosphere culture model

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Uranium exposure situations are diverse and originate from its natural presence in the environment, and from its use in specific professional activities in relation with the nuclear industry (extraction, nuclear fuel cycles, and dismantling operations). Uranium internal contamination can occur *via* ingestion of contaminated food and drinking water or *via* inhalation of particulate aerosols containing uranium dust. This latest situation is the main cause of contamination in nuclear occupational activities. These contaminations raise concern in terms of potential consequences on human health. They appear to have negative impact on the brain as experimental studies have shown that uranium exposure *via* ingestion or inhalation can lead to cognitive impairments in rats. Neurogenesis disruption has been proposed to underlie these effects. To address this question, we used *in vitro* neurosphere primary cultures from rat embryo's telencephalon at embryonic day 13. We studied uranium impact on multipotency of neural stem cell within a range of concentrations (10, 50, 100 µM) *versus* control over 7 days of contamination. Our results show a significant effect on cell survival *via* a decrease of the absolute number of all cell types: neurons, astrocytes and mature oligodendrocytes at 50 and 100 µM. Among cells surviving after 7 days of contamination, analysis of apoptotic gene expression tend to suggest an adaptive response *via* Bax/Bcl2 balance in favour of cell survival at 100 µM condition, that will need further investigations. In this condition (100 µM), neurons exhibit an aborted morphology with a reduction of the axon and dendrite length correlated with a significant decrease of gene expression GAP43 known to be involved in dendritic arborization development. Regarding gliogenesis, uranium seems to have a direct action on the maintenance of a population of glial progenitors Olig2 positive, linked with a significant increase of NeuroG3 gene expression at 100 µM. All together, these results suggest that uranium exerts a specific action on late cell maturation phases rather than on early determination stages.

P12-032
Doxorubicin and mitoxantrone effects on the brain of differently aged mice: an *in vivo* chemobrain study

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Chemobrain is the designation given to chemotherapy-induced cognitive dysfunction. Doxorubicin (DOX) and mitoxantrone (MTX) are two widely use chemotherapeutic agents with a broad spectrum of activity against cancer; however, their toxicity has been shown to occur in several organs. Still, their neurological effects are largely unknown. This work aimed to evaluate the redox and energetic status in the brain of differently aged CD-1 mice [juvenile (4 weeks), adults (3 months) and old (18–20 months)] after exposure to clinically relevant doses of DOX and MTX. Animals received biweekly intraperitoneal administrations, for 3 weeks. All age groups received a total cumulative dose of 6 mg/kg MTX or a total cumulative dose of 18 mg/kg DOX, except the old group that received a total cumulative dose of 9 mg/kg DOX. The control groups received same number of saline injections. Throughout the protocol, animal well-being, as well as body weight and food and water consumption were determined. Mice were euthanized one week (adults and old animals) or seventeen days (juvenile) after the last drug injection. To evaluate the brain's oxidative stress, total glutathione (GSHt), reduced glutathione (GSH) and oxidized glutathione (GSSG) levels were determined, as well as the GSH/GSSG ratio. To evaluate the brain's energetic status, ATP levels were measured. In adult and juvenile mice, DOX (18 mg/kg) caused weight decrease after the last injection. In fact, as early as day 10, these DOX groups revealed lower food intake than their respective controls. Brain levels of GSHt, GSH and GSH/GSSG ratio were decreased in DOX adults, but DOX infant brains had no changes in these parameters. Nonetheless, DOX (18 mg/kg) increased brain ATP levels in juvenile mice. MTX did not cause significant changes in the brain glutathione levels or ATP levels in any of the groups tested. This data suggest that DOX significantly impairs the redox status of the adult brain while increasing ATP in juvenile mice, and DOX neurotoxicity requires further research.

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P12-033
The unfinished symphony: neurotoxicity potential and mitochondrial-mediated mechanisms of synthetic cathinones in dopaminergic human neuronal SH-SY5Y cells

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Synthetic cathinones (SCs) emerged as a popular "legal" alternatives to the "traditional" psychostimulant drugs in recent years. Increasing reported hospital admissions leading to fatalities has raised concerns. However, the precise mechanism of drug toxicity is unclear. This paucity of understanding raises an interest to investigate *in-vitro* neurotoxicity and mechanistic pathways. SCs-induced mitochondrial dysfunction has been hypothesized to be a crucial factor in the onset of neurotoxicity. For this reason, butylone and pentylone were supplemented in the human neuronal cell line, SH-SY5Y to evaluate the neurotoxicity potential and potency using trypan blue and lactate dehydrogenase assays over the dose range 1 to 10 mM. Cells were cultured in commercial DMEM/F12 media and plated at a optimal density when cell confluency reached 70–80%. Cells were then differentiated to a neuronal phenotype using 10 µM retinoic acid (RA) in the media for 3 days, followed by a mixture of 10 µM RA and 81 nM 12-*O*-tetradecanoylphorbol-13-acetate in the media for another 3 days. To define the mechanisms underlying neurotoxicity, measurements included: markers of oxidative stress, mitochondrial bioenergetics impairment, intracellular calcium (Ca^{2+}) and cell death pathways

were evaluated at two doses for each drug tested, EC_{15} (butylone 4.67 and pentylone 3.05 mM) and EC_{40} (butylone 5.91 and pentylone 4.06 mM), together with EC_{15} (2.60 mM) and EC_{40} (3.43 mM) of popular synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV). After 24 h of exposure, both butylone and pentylone exhibited a dose-dependent cytotoxicity, characterized by significant ($p<0.0001$ vs control) production of reactive oxygen species, decreased mitochondrial respiration, depletion of adenosine triphosphate contents and increased intracellular Ca^{2+} concentrations. Activation of caspases 3, and 7 indicated that these synthetic cathinones induced neurotoxicity primarily via the intrinsic apoptotic pathway. These data provide important insight into the calcium involvement in mitochondrial pathophysiology mechanism, ultimately identifying potentially novel therapeutic targets in treating acute-neurological complications or at least ameliorate the deleterious consequences arising from the illicit use of butylone and pentylone.

P13 – Occupational toxicology

P13-001
Are PON and GST polymorphisms associated with advanced oxidation protein products in pesticide-exposed subjects?

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Purpose: Recent studies have suggested oxidative stress as one of the mechanisms for the adverse health effects of pesticides [1]. Oxidation generates several molecules, such as advanced oxidation protein products (AOPP), which could represent useful biomarkers of oxidative stress.

Glutathione S-tranferases (GSTs) and PON family genes are enzymes involved in the detoxification of xenobiotics, sharing antioxidant effect; genetic differences in expression and activity of these enzymes are often due to polymorphic alleles. These polymorphisms alter enzyme activity and consequently susceptibility towards many toxic compounds. The present study was aimed to assess the contribution of genetic polymorphisms of pesticide-metabolizing enzymes on AOPP production as a biomarker of oxidative stress.

Methods: 45 healthy Caucasian males (age 42.08 ± 12.78) working as greenhouse farmers were enrolled. Genomic DNA was isolated from peripheral blood lymphocytes. Genotyping of the PON2 S331C and GSTM1 polymorphisms was performed by PCR.

The serum concentrations of AOPP were determined by a microplate absorbance reader, as previously described [2].

Data were analyzed using Kruskaal-Wallis test followed by Dunn's post hoc test using Prism version 6.01 (GraphPad software, La Jolla, USA).

Results: Subjects were exposed to a mixture of pesticides with prevalent use of chlorpyrifos. No infectious or inflammatory diseases and no drug use was reported in the subjects in the three months preceding the survey. The majority of subjects had an adequate intake of food rich in antioxidants, did not smoke and did not abuse alcohol. All subjects used personal protective equipment.

Increased AOPP levels were observed in the subjects with S331CG and S331GG (2.25 ± 1.54 and 2.21 ± 1.22 nmol/ml respectively, mean ± SD) mutated genotype, compared with WT subjects (1.32 ± 0.814 nmol/ml). A similar trend was observed in subjects with deleted GSDTM1 genotype, compared with WT (2.01 ± 1.40 and 1.48 ± 0.99 nmol/ml respectively).

The results of the present study indicate that measurement of AOPP levels may provide a useful biomarker for the oxidative effect of chronic pesticide exposure, and polymorphic genes encoding PON2 and GSTM1 can be genetic determinants of pesticide toxicity.

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P13-002
Low-dose exposure to lead and neurobehavioral effects

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Purpose: Exposure to inorganic lead (Pb) in the environmental and occupational settings continues to be a serious public health problem. At high exposure levels, lead is known to cause encephalopathy, kidney damage, anaemia and toxicity to the reproductive system. This survey was conducted to evaluate the association between occupational exposure to low-dose Pb and mood states using biological markers and a validated and standardized test [1,2].

Methods: Thirtysix male workers, employed in a battery recycling plant and participating to an health surveillance program, were enrolled for the present study.

Biomarkers of exposure and effect (PbB, blood lead; ZPP, Zn protoporphirin) and a neuropsychological test (POMS, Profile of Mood States) were evaluated in the exposed workers compared to 36 age-matched control subjects.

Results: Mean PbB level resulted 56.7 ± 13.9 µg/dL in exposed workers and 15.5 ± 1.6 µg/dL in control subjects; ZPP was 53.9 ± 23.6 and 23.5 ± 1.4 µg/dL in workers and controls respectively.

Environmental assessment of workplace lead levels was over the threshold limit value of 0.05 mg/m^3 set by the American Conference of Governmental Industrial Hygienists (ACGIH).

The values of tension, depression, aggressiveness, tiredness and confusion resulted higher in the exposed workers than in controls. An inverse trend was found for the vigour, that resulted higher for the controls. In addition, Poisson regression test performed on single psychoemotional factors, has allowed to evidence a significant influence of Pb e ZPP levels on tension, anxiety and depression.

Authors found that blood lead levels considered borderline for occupational exposure in workers currently exposed to low-dose lead were associated with tension, anxiety, hostility and depression.

Therefore, neurobehavioral effects may occur also at concentrations several orders of magnitude below the clinical threshold for acute lead poisoning.

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P13-003
Glucorticoids: different approaches in PDE and OEL evaluation, but similar values

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The approach followed for setting PDE limits is the same outlined in ICH Q3C consensus guideline on residual solvents, in ICH Q3D consensus guideline on elemental impurities and in the EMA guideline EMA/CHMP/CVMP/SWP/169430/2012. The Permitted Daily Exposure (PDE) value for APIs is based on scientific evaluation of all available toxicological and pharmacological data, taking into account several different adjustment factors (Safety Factors or Uncertainty Factors) and the specific absorption/bioavailability of the compound under evaluation according to the selected Point of Departure (PoD). The same approach has been used for the calculation of the OEL values.

Glucocorticoids are a group of drugs with various anti-inflammatory and immunosuppressant as well as metabolic and endocrine effects. Systemic glucocorticoids are used for hormone replacement therapy (e.g., in Addison disease), for acute or chronic inflammatory diseases (e.g., rheumatoid arthritis), and for immunosuppression (e.g., after organ transplants). Local glucocorticoids are used to treat conditions like dermatoses, asthma, and anterior uveitis.

An extensive literature search has been carried out on fourteen glucocorticoids in order to find data useful to their PDE and OEL derivations.

Different criteria have been used for the selection of the Point of Departure (POD) in each calculation: NOAEL (the highest tested dose at which no "critical" effect is observed) or LOAEL (the lowest-observed-adverse-effect level) if no NOAEL is obtained. For some compounds, toxicological profile is poorly characterized and there were no useful quali-quantitative data to be used to allow a NOAEL or LOAEL determination, so the PDE and the OEL values for these four compounds were based on the lowest recommended daily dose (MED).

Although different approaches have been used, the results obtained are very similar. Indeed, the resulting calculated PDE and OEL values for each glucocorticoids range from 0.4 to 2 µg/day and from 0.1 to 0.8 µg/m^3, respectively. After the OEL determination, all compounds need the same containment strategy.

P13-004
The effects of umbilical cord Mesenchymal stem cells on the pulmonary fibrosis *in silico*sis rats

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Objective: To explore the effects of the umbilical cord mesenchymal stem cells(UC-MSCs) on the pulmonary fibrosis *in silico*sis rats.

Methods: SPF male Sprague Dawley rats were randomly divided into control group, silica model group and UC-MSCs treatment group with 12 rats each group · SiO_2 intra-tracheal injection (0.5 ml of 50 mg/mL/rat) were applied to silica model group and UC-MSCs treatment groups. After that UC-MSCs treatment group received 1 mL UC-MSCs suspension (3×10^6 cells/mL) by tail vein injection on the 29th, 36th, 43rd and 50th day after exposure to the first silica suspension. On the 60th and 75th day after exposure to silica suspension, all animals were examed for pulmonary CT. Then the rats were euthanized on 75th day after the first exposure to silica · Lung's histopathological examination of the rats from all the groups were carried out. The content of hydroxyproline in lungs,TGF-β1and IL-6 in serum were examined. The microRNA expression profiles were identified by Illumia sequencing.

Results: The lung's histopathological examination showed a lot of inflammatory cell aggregation and collagen fiber deposition in silica model group, while in the UC-MSCs treatment group, there were less inflammatory cells and collagen fiber. The rats from silica model groups had higher HYP, TGF-β1and IL-6 than the rats from UC-MSCs treatment group and control group. For CT examination, different-sized granular high-density shadows or reticular fibrous shadows were found diffusely distributed in the lungs of the rats in silica model group. Lung field of rats in UC-MSCs treatment group were less high density shadows, and more clear. Lung fields of rats in the control group were clear and no obvious high-density shadow. 7 miRNAs were found to be up-regulated, whereas 19 miRNAs were found to be down-regulated in UC-MSCs treated group compared with the silicosis group. In the UC-MSCs treated group,the expression of miR-449a/c , miR-133a/b, miR-34c, miR-384, miR-135a increased, and were related to changes in mRNA for regulating the fibrosis process and macrophage function. The changes of microRNAs were verified by real time QPCR.

Conclusion: UC-MSCs can alleviate the pulmonary fibrosis in silica model rats through microRNAs to regulating fiblosis preoces and macrophage function,and UC-MSCs can also down-regulate the level of TGF-β1 and IL-6 to decrease fibrosis.

P13-005
Use of the local lymph node assay: 5-bromo-2-deoxyuridine flow cytometry method to predict the skin sensitization potential of PHMG, PGH, TRICLOSAN and mixtures of these compounds with the excipient propylene glycol

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In commercial biocidal products such as polyhexamethylene guanidine (PHMG), oligo (2-(2-ethoxy) ethoxyethyl guanidine chloride (PGH), 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan) often serves as an antimicrobial agent, and the excipient propylene glycol (PG) is used to dissolve the active ingredients. The skin sensitization (SS) potentials of each of these substances are still being debated. Moreover, mixtures of PHMG, PGH or triclosan with PG have not been evaluated for SS potency. The Local lymph node assay: 5-bromo-2-deoxyuridine-Flow Cytometry Method (LLNA: BrdU-FCM), which was developed and validated by Korean Scientists, and adopted as OECD TG 442B at 2018, served to address these issues. All the experimental procedures were undertaken following the OECD TG. Test concentrations without systemic toxicities were determined as followings through 2-stage pre-screening tests: PHMG: 5, 10, 25%; PGH and triclosan: 2.5, 5, 10%; PG: 25, 50, 100%. The stimulation index (SI) versus AOO vehicle (acetone:olive oil = 4:1) was dose-dependently increased to 0.99 ± 0.13 for 5%, 1.62 ± 0.36 for 10%, 4.43 ± 0.76 for 25% for PHMG, 1.62 ± 0.23 for 2.5%, 2.43 ± 0.16 for 5%, 15.00 ± 1.91 for 10% for PGH, and 1.10 ± 0.09 for 2.5%, 2.40 ± 0.50 for 5%, 6.19 ± 0.57 for 10% for triclosan. Since the SI ≥ 2.7 is considered skin sensitization positive, these three test substances were predicted as skin sensitizer, but PG was predicted as a non-sensitizer (0.92 ± 0.19 for 25%, 1.48 ± 0.37 for 50%, 1.16 ± 0.18 for 100%). Concerning a broad mixture ratio in manufacturing companies, the mixture ratios were decided as 1:4, 4:1, 9:1 weight/volume for PHMG, PGH, triclosan versus PG. Mixtures of PHMG, PGH, triclosan with PG were all positive in terms of SS potential but SS potency was mitigated as the proportion of PG increased. Since humans can be occupationally or environmentally exposed to mixtures of excipients with active ingredients such as biocides, the

present study may give insights into further investigations of the SS potentials of various chemical mixtures. [supported by Korea National Research Foundation, Project no. 2017R1D1A3B03032723].

P13-006
Occupational lung cancer risk caused by CrVI assessed using human biomonitoring data

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Hexavalent chromium (CrVI) compounds are known lung carcinogens, and their use is subject to authorisation under REACH. However, occupational exposure to CrVI remains a relevant concern, as CrVI compounds are still widely used in authorised industrial applications, mostly due to their superiority in producing hard and corrosion tolerant coatings. CrVI fumes are also formed, e.g., in manufacturing and welding of stainless steel. Such process-generated fumes are not covered by REACH.

We calculated lung cancer risks due to occupational CrVI exposure based on human biomonitoring (HBM) data. As cumulative exposure is essential in CrVI-related cancer incidence increase, data covering a ~40-year period (1980–2016) were used, originating from a database of the Finnish Institute of Occupational Health (FIOH). It consists of the urinary Cr (U-Cr) samples sent to FIOH for exposure monitoring by occupational health care units. Published equations were used to convert the data (p95) into corresponding air levels and to calculate lung cancer risks.

The measured U-Cr levels decreased substantially over the 40-y period. One of the highest measured U-Cr levels was in welders: 0.77 µmol/l in the 1980s (n = 3232), down to 0.13 µmol/l in the 2010s (n = 5348). The estimated corresponding CrVI air levels were 10–19 and 2–3 µg/m^3 in the 1980s and 2010s, respectively. The 40-y cumulative exposure was estimated as 216–384 µg/m^3, and the resulting attributive risk (AR; lung cancer) 27–40%. However, the estimates for welders include uncertainties: 1) the correlation equations are based on plating activities, 2) U-Cr reflects total Cr exposure and welders are also exposed to CrIII, 3) HBM reflects exposure also via other than the inhalatory route, which are not particularly relevant to lung cancer. For platers, the measured U-Cr levels in the 1980s and 2010s were 0.46 µmol/l (n = 771) and 0.12 µmol/l (n = 3631), respectively, corresponding to air levels of 6–11 and 2–3 µg/m^3. The cumulative 40-y exposure level was calculated as 162–282 µg/m^3, resulting to an AR of 22–33%.

Even though the use of HBM data may in some cases result in overestimation of risk, it provides a useful tool for assessing risks of adverse health effects, as it reflects actual and total internal dose via all exposure routes.

P13-007
Safety assessment of copper nanoparticles developed for printable electronics

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Unique electrical properties of copper nanoparticles (Cu NPs) are being utilized in the development of conductive inks for printable electronics. Here, the safety of workers developing Cu NPs coated with polyvinyl pyrrolidone (PVP) in laboratory scale was assessed based on published toxicological data on Cu NPs and exposure measurements by on-line measurement of particle number concentration and size distribution (size range 6 nm–10 µm).

The toxicity data available on Cu NPs considers mainly the oxidized form of Cu (CuO). CuO NPs possess stronger toxic potential *in vitro* as compared to micron-sized CuO or soluble copper compounds, most likely explained by the efficient uptake of NPs, followed by intracellular release of copper ions. The few inhalation studies on CuO NPs in mice and rats suggest that inhaled CuO NPs can induce inflammatory responses and histological changes in rodent lungs. Intratracheal instillation of CuO NPs has been shown to induce oxidative stress, inflammation and even neoplastic lesions in rats. Based on sparse *in vitro* data on PVP-coated CuO NPs, the coating is not expected to increase the toxicity of Cu NPs.

Exposure measurements were carried out in four different work tasks involving Cu NPs: synthesis of Cu NPs, handling of Cu NP powder, precursor mixing and cleaning procedure of the sample. A significant increase in the particle number concentration was detected only inside the fume hood when Cu NPs were handled as dry powder. However, no simultaneous increase was detected in the breathing zone of the workers, indicating an effective capture of the released Cu NPs by the fume hood.

Although the current knowledge about the health hazards of Cu NPs is limited, the available data indicate that inhalation exposure to Cu NPs may cause pulmonary toxicity. In this case, workers' exposure was considered negligible due to small scale process combined with effective use of control measures. Thus, the health risk was assessed to be low. The recommendations given included ensuring flawless and efficient operation of the fume hoods, changing the form of the material from powder to liquid or paste, when applicable, wearing a fit tested respirator (FFP3) in reactor cleaning and maintenance, and compiling a procedure for accidental situations.

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P13-008
Two years of DNA damage monitoring in males and females occupationally exposed to nanoparticles

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Due to the increase of nanomaterials (NM) application in many areas of human life during the last decades, assessment of genotoxicity of NM and nanoparticles (NP) is one of the main objectives of genetic toxicology. Despite this fact, human cytogenetic studies focused on micronuclei (MN) formation following the exposure to NP are still rare. Moreover, no relevant information on possible differences in

sensitivity of males and females to NP exposure is available.

In this study we analyzed 4x (in September 2016 and 2017; pre-shift and post-shift each year) samples in a group of workers (both genders), working long time in nanocomposites research, and matched controls. Detail aerosol exposure monitoring of particulate matter (PM) including nano-sized fractions was completed during working shift in sampling days. The micronucleus assay using Pan-Centromeric Chromosome Paint was applied to recognize, beside the frequency of total MN in binucleated cells (BNC), also other types of chromosomal damage (losses and breaks), including the centromere positive (CEN+) and centromere negative (CEN-) micronuclei. Moreover, whole-chromosome painting for autosome #1 and both gonosomes (X and Y) were applied with the aim to identify the particular structural and numerical chromosomal aberrations.

Obtained results showed consistently: (i) differences in the risk of exposure to NP related to individual working processes; (ii) differences in chemical composition of nano-fraction; (iii) possible adaptation to chronic exposure of NP (total MN); (iv) acute exposure (2.5 h) could be a reason for the CEN+ MN increase; (v) females seem to be more sensitive to chromosomal losses. Additional data suggested increased frequency of numerical aberrations in gonosomes.

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P13-009
Influence of genetic variance on biomarker levels after occupational exposure to 1,6-hexamethylene diisocyanate (HDI) monomer and HDI isocyanurate

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Isocyanates are a leading cause of occupational asthma globally, in which 5–15% of exposed workers develop isocyanate-induced asthma. However, very little is known about the mechanism of isocyanate-caused skin and respiratory sensitization. In order to close part of the knowledge gap, we investigated the influence of genetics in conjunction with skin and inhalation exposures to 1,6-hexamethylene diisocyanate (HDI) monomer its trimer HDI isocyanurate on plasma and urine biomarker levels of 1,6-diaminohexane (HDA) and trisaminohexyl isocyanurate (TAHI) in a population of 33 workers who spray automobiles with isocyanate-containing polyurethane paints. Linear mixed model analyses indicated that HDI monomer and isocyanurate skin and inhalation exposures are both important modifiers of HDA and TAHI biomarker levels, respectively. Therefore, in order to assess how genetics impacts biomarker levels, we used genome-wide single nucleotide polymorphism (SNP) microarray data (Affymetrix 6.0), a false discovery rate < 0.10, and both skin and inhalation exposure levels as covariates in this model. Seven SNPs were significantly associated with HDA levels in plasma, five SNPs were associated with HDA in urine, eight SNPs were associated with TAHI levels in urine, while no SNPs reached significance for TAHI in plasma. Furthermore, the heterozygous genotype and homozygous minor allele genotype for these 20 SNPs were associated with an average of 10–16-fold higher biomarker levels compared to the homozygous major allele. To evaluate the potential biological pathways impacted by these SNPs, NCBI gene database was used to determine the genes proximal to each of the significant SNPs and then those genes were input into GeneMANIA and DAVID bioinformatics databases to infer gene-ontology based predicted network associations. The predicted molecular functions included transcription regulation, calcium ion transport, and TGF-β signaling. Our results demonstrate that genetics is an important modifier of biomarker levels following occupational exposure to HDI. In future studies, these SNPs can be used to study isocyanate toxicokinetics and to identify individuals who are susceptible to developing isocyanate-induced asthma.

P13-010
Case report of the rapid successful treatment of methemoglobinemia caused by occupational exposure to aniline

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Purpose: Aniline is a colorless aromatic liquid that is widely used in the manufacturing of synthetic dyes. Occupational non-oral exposure this chemical has led to fast absorbed by all other routes and induces methemoglobinemia.

Case Report: A 53-years-old man who worked with aniline in a chemical plant was admitted to a hospital 1 hour later with typical signs of methemoglobinemia – dizziness, cyanosis, nausea, unconsciousness *et al*. The exam was remarkable for coma. Initial vital signs were as follows: temperature 37.5°C, blood pressure 100/60 mm Hg, pulse 120 beats per minute, with a respiratory rate 36/min., and pulseoximetry of 72.0% (serum methemoglobin level was moderately high 54.0%). He was administered oxygen supplementation through a high concentration mask, infusion of 5% dextrose and slow IV injection of 10 ml methylene blue and 30 ml of sodium thiosulfate. The patient's state rapidly improved after 12 hours of hospitalization without any complaints in the subsequent 8 days. His blood and urine analysis was normal. Neither further biological sign of haemolysis, nor organ dysfunction was observed, so that the patient was discharged on the ninth day.

Conclusion: This observation shows that rapid recovery is possible in serious acute aniline poisoning provided tissular oxygenation is promptly restored by generous oxygen supplements and proper antidotal treatment with methylene blue and sodium thiosulfate.

P13-011
Risk assessment for an aniline derivative ortho-toluidine by using human biomonitoring data and bioequivalent method in the HBM4EU project

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Ortho-toluidine (CAS 95-53-4) is an aniline derivative which is considered to be an animal and human carcinogen, and may cause methemoglobinemia. o-Toluidine is used as a curing agent in epoxy resins and as intermediate in producing herbicides, dyes, and rubber chemicals. It is listed in the candidate list of substances of very high concern for authorization under REACH. A risk assessment (RA) was performed in the HBM4EU project for o-toluidine by utilizing human biomonitoring (HBM) data since the possible health risks should be monitored, especially for workers.

After hazard characterization and exposure assessment, a literature search was conducted on studies concerning o-toluidine HBM data. The biomonitoring equivalent (BE) methodology was used for

the comparison to estimate the urinary levels corresponding to the external intake levels. For the RA, the results of the BE method were compared to the available HBM studies and occupational exposure levels.

The existing cancer RA resulted in a Benchmark Dose causing 10% urinary bladder tumour incidence above background level (BMD_{10}) of 42.2 mg/kg bw/day in rats, corresponding to an inhaled dose scaled to humans of 210 mg/m^3 at occupational exposure. Converted to mg/bw/working day this BMD_{10} corresponds to 30 mg/kg/day. Using the BE method, this level corresponds to a urinary level of 1000 mg/L by assuming a 70-kg bw, a 1.5 L/day urinary volume and 75% excretion.

In conclusion, by applying the BE methodology and based on HBM studies, the workers exposed to o-toluidine have a cancer risk of 1:20 000 in the worst-case scenario (0.5 mg/L in urine). The exposure levels calculated based on HBM data were below the binding occupational exposure level (BOELV, 0.44 mg/m^3) set under the EU Carcinogens and Mutagens Directive. However, results should be considered carefully due to uncertainties and the limited number of HBM data. There is clearly a need for further HBM studies and data on the biokinetics of o-toluidine exposure.

P13-012
Occupational exposure to monoclonal antibodies in Portuguese health units: are there reasons for concern?

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Background: The use of monoclonal antibodies (MABs) has been increasing in healthcare, namely, in the treatment of malignant and non-malignant diseases. These molecules are widely used in monotherapy and in chemotherapy cycles along with cytotoxic drugs. Currently the use is also increasing in veterinary practice. Thus, a considerable number of health professionals (e.g. pharmacists and pharmacy technicians, nurses, veterinarians, physicians and other) can be exposed to this new therapeutic class.

Aim: To give an overview of the main guidelines to safe handling of MABs worldwide; To estimate the occupational risk of handling MABs in three Portuguese health institutions.

Methods: A detailed literature search on *B-on* "*all for all*", *PubMed* (includes *Medline*) and *Web of Science* was carried out using various combinations of corresponding descriptors and free text terms such as "antibodies, monoclonal", "occupational exposure", "safe handling", "management" and "risk assessment". A direct observation of the workplaces and tasks implicating the handle of the MABs in a general hospital, an ophthalmic clinic and in a veterinary hospital was also performed. This allow understanding the medication pathway at each health institution, to describe the most used monoclonal antibodies and the location (ward/pharmacy) where they are prepared or administered, critical moments where exposure can occur and recommend new procedures if needed.

Results: Healthcare workers involved in the preparation or administration of MABs should be aware of the potential occupational exposure risks. Recommendations to allocate the preparation and administration of MABs according to their toxicity profile should be performed taking into account the literature evidence.

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P13-013
Evaluation of inflammatory biomarkers in agate grinding workers in Iran

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Objective: Silicosis is a chronic progressive and life threatening occupational disease which is usually recognized at later stages of the disease. The aim of this study was to evaluate the levels of LDH (Lactate Dehydrogenase), CA125, HS-CRP (high sensitive C-reactive protein), MDA, SOD, MMP9 (Matrix Metalloproteinase, MMP2 and Copper as early biomarkers for silicosis.

Methods: Three groups were recruited in this study: 1) 12 agate grinding workers with silicosis, 2) 26 agate grinding workers exposed to silica dust but without silicosis and 3) 17 Healthy individuals (control group). Required data were collected in annual medical survey through face to face interview, general health questionnaire, spirometry and chest radiography. Serum samples of the participants were analyzed for LDH, CA125, Copper, HS-CRP, MDA, SOD, MMP2 and MMP9. Diagnosis of silicosis was based on history of occupational exposure to silica dust and chest x-ray findings by an occupational medicine specialist. Data were analyzed using SPSS 20 and statistical tests including ANOVA, levene's and chi-square tests.

Results: A total of 55 male workers were included in this study. The mean age of participants was 40.12 ± 9.56 years and the mean of employment duration was18.27 ± 12.54years which were statistically different between the groups. 145 (8%) of all the individuals were current smokers. According to the analysis, there were significant differences according to spirometric parameters and plasma levels of MMP2, MMP9, HS-CRP and CA125 between 3 groups ($P \leq 0.05$). In patients with silicosis all the spirometric parameters were lower compared to the other 2 groups. MMP2, MMP9, serum hs-CRP and CA125 concentrations were significantly higher in cases compared with controls. Significant correlations were also observed between values of HS-CRP and CA125 and spirometric parameters.

Conclusion: These findings indicate that HS-CRP and CA125 are increased *in silico*sis patients, suggesting that these biomarkers are involved in the onset of disease and correlate with severity of silicosis.

P13-014
Experimental study of toxicity and derive occupational exposure limit to 6-chlorohexan-1-ol

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In chemical and petrochemical production (for example, the manufacture of polyurethane foams, sealants, adhesives), 6-chlorohexan-1-ol is used, which can be released into the air during the production process and affect workers. According to the ECHA (European Chemicals Agency) 6-chlorohexan-1-ol database (CAS No. 2009-83-8) is a substance that can be harmful if swallowed and inhaled, can cause eye irritation, can cause mutations in the Ames test, does not cause sensitization and skin irritation, there is no information about the toxicity of the substance with repeated exposure. The purpose of the research was the study of the toxicity and hazard of 6-chlorohexan-

1-ol in subacute and subchronic experiments on rats to substantiate the hygienic standard in the air of the working area for professional use of the substance.

In the subacute experiment (intragastric administration to rats for 14 days), the threshold of acute action (Lim_{ac} – 420 mg/kg) was set to change behavioral parameters ($p < 0.05$ against the background of control). In a subchronic experiment (60 days), rats were intragastrically administered with 0,1 DL_{50} of 6-chlorohexan-1-ol – 375 mg/kg. The animals were found to have changes in morpho-functional parameters ($p < 0,05$ against control): body weight, neutrophil and lymphocyte content in the blood, glutathione system (SH-groups, reduced glutathione and glutatintransferase), cellular and humoral immunity (blood granulocytes, lysozyme and antimicrobial blood activity). The revealed changes were functional (reversible) and disappeared after 30 days of the recovery period. The experiment performed allows us to conclude that the subchronic dose-monotonic administration of 6-chlorohexan-1-ol to rats leads to activation of the mechanisms of antioxidant and nonspecific immune defense, which are of an adaptive nature. Using the logarithmic equations that take into account the experimental data and the physicochemical properties of the substance (molecular weight and volatility), an estimated occupational exposure limit of 6-chlorohexan-1-ol in the working area air is calculated – 8 mg/m^3.

P13-015

This abstract has been withdrawn.

P13-016

The genetics of occupational asthma development among workers exposed to diisocyanates: a systematic review with meta-analysis

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Diisocyanates are a widely used class of chemicals that pose an occupational safety concern for workers in the spray-paint and spray-foam insulation industries. Epidemiologic studies indicate that 5–15% of workers exposed to diisocyanates develop diisocyanate-induced occupational asthma (diisocyanate asthma, DA). Because only a subset of workers develops this disease, genetic susceptibility may play a role in the development of DA. As such, many researchers have studied genetic markers that may increase workers' susceptibility for DA. The purpose of this systematic review was to compile the results on genetic susceptibility markers for DA and to meta-analyze the results for the most commonly studied genes. Three databases (Embase, Pubmed, and Scopus) were searched and 166 non-duplicate publications were identified, of which 24 relevant occupational studies were included in this review. The genome-wide association studies and candidate-gene studies on DA susceptibility identified single nucleotide polymorphisms (SNPs) within 71 different genes. Multiple studies reported on SNPs within 17 genes and, thus, those genes were included in meta-analysis: CDH17, CTNNA3, GSTM1, GSTM3, GSTP1, GSTT1, HLA-A, HLA-B, HLA-C, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRB1, NAT1, NAT2, TNF-α, and ZBTB16. These 17 genes code for proteins that are involved in many processes including xenobiotic presentation, lymphocyte and T-cell activation, response to oxidative stress, cell-cell adhesions, and interaction with histone deacetylase. Knowledge about the genetic markers that impact susceptibility to developing asthma after exposure to diisocyanates could help to determine the etiology of the disease and to identify more effective ways to protect workers' health.

P13-017

Health risk associated with delta-aminolevulinic acid dehydratase (ALAD) gene polymorphism (rs1800435C/G) in Bulgarian workers from battery recycling industry

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According to data from the Association of Bulgarian Association of Metallurgical Industry in Bulgaria for the period 2012–2016 the production of primary and secondary lead including the production of batteries in Bulgaria has increased almost double.

A number of recent studies (WHO, IARC Monographs, 2010) found that chronic exposure to lead leads to lead poisoning at a higher frequency than expected. This implies reviewing the data and conducting new studies to assess the health effects of chronic low-exposure exposure.

Questions arise – what indicators are being explored; are they suitable? Are new strategies and biomarkers needed? Is there a traceability of results in dynamics; what are the risk reduction measures that are sustainable?

The aim of the present study is to determine the allelic frequency and distribution of individual haplotypes among Bulgarian population based on ALAD gene polymorphism (rs1800435C/G) and possibility to use as a prediction biomarker for prevention of lead intoxication.

The subject of this study is 80 workers from Bulgarian battery recycling industry, professionally exposed to lead. The following biomarkers was measured: Hematological parameters; blood lead content, delta aminolevulinic acid (DALA) levels in urine and rs1800435C/G polymorphism distribution.

All persons have signed informed consent for conducting the research.

The results of the molecular-genetic analysis demonstrates that 23% of the study population is heterozygous for ALAD-2. According to the literature, there is an increased risk for the health of these persons. Our results show trends in higher blood lead concentrations and DALA levels and decreased hemoglobin levels and white blood cells count in ALAD-2 heterozygous subjects.

The present study is first for the Bulgarian population. Tendencies of dependence between the lead of the excretion of lead and the genotype distribution are established. It is difficult to decide which genotype is genotype "at risk" because the results show that each genotype is susceptible to one or more adverse effects than others. Genetic polymorphism seems to have a strong impact on lead absorption and bioaccumulation, but its role in affecting the neurotoxicity of lead is still unclear.

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P13-018
Serum metabolomics of occupational noise exposure workers in China

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Occupational noise exposure has become a major public health problem in China. Previous studies have suggested that noise exposure is associated with hearing loss, psychiatric disorder and cardiovascular disease. In the present study, we sought to explore the hazards of occupational noise exposure and screen tentative metabolic markers from the perspective of serum metabolomics. The 1:1:1 matched case-control design was used in this study, 100 cases of occupational noise exposure workers (50 cases with noise-induced hearing loss while 50 cases without) were compared with 50 healthy workers by physical examination data like gender, age, smoking and drinking history. The average length of occupational noise exposure was 8.37 ± 7.58 years in NIHL group, and 8.54 ± 7.37 years in non-NIHL group. The noise intensity in the workplace was 80dB(A)-85dB(A). A nontargeted metabolomics approach based on UPLC-QTOF-MS was performed on serum samples to identify differentially expressed metabolites. The results showed significant differences in serum metabolic profile between exposure workers and healthy workers. The metabolite that was upregulated in both exposure groups is Oleamide. Eight metabolites including arachidonic acid, L-kynurenine, docosahexaenoic acid, dihomolinoleic acid, L-methionine, 13-HOTE, 6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one and sphinganine 1-phosphate were downregulated in both exposure groups. Researches have proved that arachidonic acid metabolites metabolized by CYP450 in kidney are altered in diabetes, hepatorenal syndrome, and in various models of hypertension. The L-kynurenine is associated with nervous system disorders. Oleamide is being studied as a potential medical treatment for mood and sleep disorders. Our findings suggest that occupational noise exposure can cause disturbances in tryptophan metabolism, glycine and serine metabolism, and lipid metabolism pathway which are related to the autonomic nervous system and endocrine system. In conclusion, the present study provides new insights into the health effect caused by occupational noise exposure, and the hygienic significance of the abnormal metabolites will be investigated in further studies.

P13-019
2,4-Dimethylaniline may contribute to the occurrence of bladder cancer among workers in a chemical factory

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Ten cases of bladder cancer was reported recently among workers in a chemical factory in Fukui of Japan, and several aromatic amines have been used for nearly thirty years there. All of the ten cases of cancer had the records of exposure to *o*-toluidine, a known human carcinogen classified into group I chemical by the International Agency for Research on Cancer, and nine cases were also exposed to 2,4-dimethylaniline (2,4-DMA). Information about the genotoxicity and carcinogenesis of 2,4-DMA is limited and inconsistent. The International Agency for Research on Cancer classifies 2,4-DMA as a Group 3 chemical, indicating no evidence of carcinogenicity to humans. Our study aimed to investigate whether and how 2,4-DMA is of genotoxic effects and its possible contribution to the occurrence of occupational bladder cancer. Methods: human urothelial (1T1) and hepatocyte (WRL-68) cells were treated with 2,4-DMA at different concentrations for 1-24 hr, and phosphorylated histone H2AX (γ-H2AX), a marker of DNA double strand breaks, was detected by western blot and immunofluorescence staining. To explore the mechanism underlying the genotoxic effects, reactive oxygen species (ROS) production following 2,4-DMA exposure and the mediation of CYP2E1 were evaluated. Results: it was showed that 2,4-DMA induced γ-H2AX in a dose-dependent way in both cell lines, and this effect was even comparable to *o*-toluidine. The double-strand breaks formed in 1T1 cells after 2,4-DMA treatment was confirmed by the biased sinusoidal field gel electrophoresis. In the mechanistic investigations, we found that 2,4-DMA induced intracellular ROS, an effect clearly attenuated by disulfiram, a strong inhibitor of CYP2E1. Furthermore, CYP2E1 inhibitors and the antioxidant, NAC, also attenuated γ-H2AX generation following exposure to 2,4-DMA. Conclusions: our results suggest that 2,4-DMA can strongly induce γ-H2AX via ROS produced by CYP2E1-mediated metabolism. Exposure to 2,4-DMA over a long period of time may have contributed to the development of bladder cancer. Our results suggested the necessity of re-assessment on the carcinogenicity of 2,4-DMA.

P13-020
Frequency of GSTP1 and GSTM1 null genotype in batik textile worker in Yogyakarta, Indonesia

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Background: Glutathione S-transferases (GSTs) is composed of multiple isoenzymes. Some of the isoenzymes of GSTs are GSTP1 and GSTM1. Both GSTP1 and GSTM1 is known to have an important role in detoxifying various xenobiotics, including textile production related toxicant, some of which are suspected to be hazardous towards human health. Yogyakarta is one of the most productive batik textile business. Therefore many people, especially women, are work in batik textile factory in which they are exposed to potentially hazardous substance related to batik textile production.

Aim of work: To find the susceptibility of batik textile worker towards hazardous substance in batik textile production related to GSTM1 and GSTP1 polymorphism.

Patients and methods: A total of 40 batik textile workers were genotyped for GSTP 1 and GSTM 1 using a specific primer to detect null genotype of GSTP1 and GSTM1 using conventional PCR.

Results: In our study, we found that the frequency of GSTP1 null genotype is 5% and the frequency of GSTM1 null genotype is 17,5%.

Conclusion: We demonstrated that GSTM1 null genotype or GSTT1 null genotypes are low in batik textile worker in Yogyakarta, Indonesia.

P13-021
CamkII Beta might protect the toxicity induced by benzene in G6PD deficient cells

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic disease, which affects nearly 400 million people worldwide.

Calcium/calmodulin-dependent protein kinase type II beta chain (CAMK2B) is a member of the serine/threonine protein kinase family and belongs to the Ca2+/calmodulin-dependent protein kinase subfamily. Here, the mRNA expression of CAMKIIB of G6PD deficient mice (G6pdxa-m1Neu mice) was found 41.8 times that of normal C3H mice in bone marrow cells. The mRNA expression of CAMK2B in G6PD low-expression mice and normal mice decreased by half after exposure to 150 mg/ (kg · d) benzene by subcutaneous exposure, but the mRNA expression level of CAMK2B in G6PD low-expression mouse was still 38 times that of normal mice. In order to investigate the role of camk2B in high expression of g6pd deficiency, K562 cells were treated with KN93, a chemical inhibitor of CAMK2B, to construct low expression CAMK2B cells. The proliferation rate of low-expression CAMK2B cells decreased and the apoptosis rate increased significantly compared with control cells. The generation of the mitochondrial ATP and the membrane potential was reduced in CAMK2B low expression cells. Then, K562 cells with low expression of CAMK2B and normal K562 cells were treated with 1,4-Benzoquinone. The toxicity of cell proliferation, apoptosis and the mitochondrial damage was increased in CAMK2B low expression cells with 1,4-BQ treatment. Therefore,a high expression of Camk2B may be a defect in balancing the low expression of G6PD and protect the toxicity induced by benzene in G6PD deficient mice. For further study, we hope to identify the mechanism of how G6PD deficient regulate a high expression in CAMK2B, that might be contribute a new insight for G6PD deficient genetic disorder. (This work was supported by the National Natural Science Foundation of China (Grants no. 81573120, 81730087)).

P13-022
Risk assessment of occupational exposure to DINP, DIDP and DPHP in plastics sector

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Background: The use of DiNP, DiDP and DPHP has increased in plastic product manufacturing to substitute older phthalates like DEHP. DiNP has shown anti-androgenic effects but with much lower potency than e.g. DEHP, whereas DiDP and DPHP have not clearly shown these effects. No occupational exposure limits (OELs) or biological limit values (BLVs) have been set for DiNP, DiDP or DPHP. Only few human biomonitoring (HBM) data exists on the exposure of workers to these phthalates, including the data from our own biomonitoring study in plastics workers.

Methods: For the risk assessment (RA) of DiNP, DiDP and DPHP, we used our own HBM data from Finnish plastics workers complemented with published data. DNELs calculated by ECHA were used for DiNP and DiDP RA after the adjustment for occupational exposure. For DPHP, a DNEL based on the published BMDL10 level for thyroid effects was calculated. One compartment model based methodology was applied to calculate biomonitoring equivalents (BEs) for these DNELs, and measured biomarker levels were compared to the BEs to calculate risk characterization ratios (RCRs).

Results and conlusion: Using the BE approach based on the DNEL values, the calculated BE for occupational population was 1.0 mg/L for both cx-MiNP (metabolite of DiNP) and cx-MiDP (metabolite of DIDP). The BE estimated for OH-MPHP (metabolite of DPHP) was 0.6 mg/L. RCRs were all well below 1, being the highest for DiNP (RCR = 0.3 in worst case scenario). For DiDP and DPHP, RCRs were below 0.1, indicating a very low risk. Even though the BE approach used here is quite rough and gives only an estimate on the level corresponding the external DNEL value, in many cases it can be considered sufficient for RA. Current data on occupational exposure to these three phthalates is, however, very limited, and therefore exposure assessment should be viewed with caution. As these phthalates are being widely used to replace the already restricted phthalates, more occupational exposure data is needed.

P15 – Pulmonary toxicology

P15-001
Comparative assessment of reconstituted human airway epithelium 3D models derived from large and small airway epithelial cells exposed to whole cigarette smoke

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Purpose: Based on the field of injury concept, cigarette smoke (CS) is thought to elicit common molecular changes throughout the respiratory tract. The recapitulation and investigation of this concept with *in vitro* observations might help understand the mechanism of action further. In this study, we used two different reconstituted human airway epithelium 3D models derived from large and small airway epithelial cells in a CS exposure test to investigate their potential and limitations for use in a field of injury concept *in vitro* study.

Methods: MucilAir and SmallAir from the same donor were individually exposed to 1R6F reference CS using the Vitrocell exposure system. Cultures were exposed to 1R6F smoke at three different concentrations prepared by controlling the dilution flow (1, 2, and 4 L/min). Cultures exposed to air were used as controls. Four repeated exposures were performed per day with a 5-min exposure and 60-min interval between exposures. Cultures at 4, 24, 48, and 72 h post-exposure timepoints were subjected to the following analyses: cytotoxicity, tissue integrity, histology, cytokine secretion, and microarray.

Results: Although there was a concentration-dependent increase in cytotoxicity, destruction of pseudostratified morphology and decreased tissue integrity were observed in MucilAir and SmallAir exposed to 1R6F smoke. SmallAir was more vulnerable to tissue damage than MucilAir. Cytokine release was increased in both models exposed to 1R6F smoke at all timepoints, and cytokine levels were higher in MucilAir than in SmallAir. The highest number of differentially expressed genes (DEGs) was observed 24 h post-exposure to 1R6F smoke at all concentrations in MucilAir, while the increased number of DEGs lasted for 72 h post-exposure at 1 and 2 L/min concentrations in SmallAir. These DEGs were similar in both models, and were predicted to be related to oxidative stress and inflammatory response.

Conclusion: CS-inducible biological effects on large and small airways were similar, but vulnerability and time-dependent reactivity were different. These findings provide informative insights into the CS effect aligned with the airway field of injury concept.

P15-002
Effect of cigarette smoke extract on the functional expression of P-glycoprotein in human lung-derived A549/P-gp cells

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Purpose: The lung is the organ directly exposed to cigarette smoke in smokers, and cigarette smoking is known to affect various functional proteins in the lung. The alveolar epithelium is comprised of two morphologically and functionally different cell types, squamous

type I cells and cuboidal type II cells. Using rat primary cultured alveolar epithelial cells, we previously reported that P-glycoprotein (P-gp; ABCB1) was expressed in type I-like cells, but not in type II cells, and that cigarette smoke extract (CSE) directly inhibited P-gp activity in alveolar epithelial cells. However, in order to understand the change in alveolar P-gp activity in smokers, the effect of long-term treatment with CSE should be examined. For this purpose, we have established A549/P-gp cell line naturally and stably expressing P-gp, because P-gp expression in native A549, an alveolar epithelial cell line derived from human lung, was negligible. In this study, we examined the effect of long-term treatment with CSE on P-gp expression and function using A549/P-gp cells.

Methods: Expression of MDR1 mRNA and P-gp protein was measured by real-time PCR analysis and western blotting, respectively. P-gp activity in A549/P-gp cells was measured by uptake experiments using rhodamine 123 as a substrate. Intracellular reactive oxygen species (ROS) level was estimated by flow cytometry using dihydroethidium as a fluorescence probe.

Results: A549/P-gp cells were pretreated with various concentrations of CSE for 96 hours, and P-gp activity was measured in the absence of CSE. CSE treatment suppressed P-gp activity in a concentration-dependent manner. MDR1 mRNA expression and P-gp protein level were also suppressed by the long-term treatment with CSE. Intracellular ROS level was increased by CSE treatment, which was suppressed by α-tocopherol. In addition, CSE-induced suppression of P-gp activity was also attenuated by co-treatment with α-tocopherol. In conclusion, long-term treatment of A549/P-gp cells with CSE suppressed P-gp activity as well as its expression in alveolar epithelial cells, and ROS may be involved in the suppression of P-gp by CSE. The role of intracellular signaling pathways including MAPK pathways in CSE-induced suppression of P-gp will also be discussed.

P15-003
Dose-dependent cytotoxicity assessment of nitrogen dioxide following pure or compounded exposures through the air liquid interface: *in vitro*

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As a major air pollutant from vehicle emission, nitrogen dioxide (NO_2) is associated with various respiratory diseases. However, few studies investigate the dose-dependent effect of NO_2 following pure or compounded exposure using the newly developed air-liquid interface (ALI) exposure system with airway epithelial cells to simulate the real life airway inhalation exposure route. This study aimed to investigate the dose-dependent cytotoxicity in human A549 cells that exposed to pure NO_2 or gasoline engine exhausts (marked for compounded exposure of NO_2) using the ALI exposure system. Following exposure to pure or compounded NO_2 through ALI at a flow rate of 15 ml/min/well for 1 h, the cell relative viability (CRV) of A549 cells was analyzed using MTT assay. The benchmark dose (BMD) and limit of benchmark dose (BMDL) were calculated to evaluate the cytotoxicity of NO_2 according the benchmark dose software developed by the U.S Environmental Protection Agency. Our results revealed that the CRV of A549 cells was significant decreased along with the increased concentration of NO_2 in both pure and compounded exposure circumstances ($p<0.05$). The BMD and BMDL of NO_2-induced cytotoxicity estimated by the best fitting model were 4.40 mg/m^3 and 2.74 mg/m^3 for pure exposure, 2.83 mg/m^3 and 1.96 mg/m^3 for compounded exposure, respectively. Taken together, our findings clearly show an increased dose-dependent cytotoxicity in A549 cells following the ALI exposure to NO_2 from pure exposure to compounded exposure, which provides basic data for evaluating the toxic effect of NO_2 through inhalation. (Funding Support: National Natural Science Foundation of China (No. 81472955))

P15-004
Expression of receptors for adhesion molecules in monocytes exposed to urban particulate matter is independent of size and composition of the particles.

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Exposure to urban particulate matter has been related to increases in mortality and visits to emergency rooms. Among the many described effects, local and systemic outcomes have been reported. Endothelial dysfunction and activation of monocytes are among the systemic effects of inhaled particles. In this study, we did evaluate urban particulate matter with aerodynamic sizes of 10 (PM_{10}) and 2.5 ($PM_{2.5}$) μm collected from November 2012 to May 2013 in the central region of Mexico City, a place with high population density and heavy traffic. The particles were collected using a high volume sampler on cellulose nitrate membranes and mechanically dislodged from the membranes [1]. The recovered particles were characterized for their content of metals, total carbon, organic carbon, elemental carbon, polycyclic aromatic hydrocarbons (PAHs), phthalates, endotoxins, and size distribution. Human monocytes were exposed to 0.001, 0.003, 0.01, 0.03, 0.3, 3.0 and 30 μg/mL of the different particles and we did evaluate the expression of early (sLex, PSGL-1) and late (LFA-1, VLA-4, αV-β3) receptors for adhesion molecules by means of flow cytometry. Unexposed cultures were used as negative controls and cultures exposed to 10 ng/mL of TNFα were used as positive controls.

In the following table we show the ranges of concentrations for metals, total carbon, PAHs and endotoxins for PM_{10} and $PM_{2.5}$:

	PM_{10}	$PM_{2.5}$
Metals (μg/mg)	142–231	80–227
Total Carbon (μg/mg	179–218	280–333
Organic Carbon (μg/mg)	125–151	80–227
Elemental Carbon (μg/mg)	42–78	50–93
HAPs (μg/mg)	32–107	37–213
Phthalates (ng/mg)	33–176	22–230
Endotoxin (EU/mg)	24–182	19–226
Size distribution (μm)	1.96±2.56	1.77±2.70

Range of concentrations for different components present in PM_{10} and $PM_{2.5}$ from Mexico City

Despite large variations in the content of different components present in PM_{10} and $PM_{2.5}$ and the variations among the different months, the pattern of expression for the early and late receptors for adhesion molecules was similar, showing no statistical difference when size or month of sampling was considered. At concentrations

of 0.001 and 0.003 μg/mL, the expression of the receptors was no different to unexposed monocytes, but from 0.01 μg/mL the expression was similar to that induced by the positive control (TNFα), which was about 5 times the basal level. Only at the highest concentration of 30 μg/mL, the intensity of the expression of all the receptors was stronger than that induced by TNFα, reaching more than 20 times the basal level for sLex, PSGL-1 and VLA-4.

These results indicate that the activation of monocytes in relation to the expression of receptors for adhesion molecules is related to the mass of particles and not to the composition or size distribution.

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P15-005
E-cigarettes induce lower biological responses than conventional cigarettes: a comparison of *in vitro* toxicity following repeated whole aerosol exposure to human bronchial tissue for 4 weeks

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Numerous public health bodies and governments worldwide have indicated that e-cigarettes have a central role to play in combustible tobacco harm reduction. With the increasing popularity of the Next Generation Nicotine Delivery Products, it is important to assess their potential biological impact in a robust, advanced, human-relevant biological systems, more closely modelling human exposure scenario.

This study compared the *in vitro* toxicological responses of a 3D organotypic model of the human airway epithelia (MucilAir™, Epithelix) following repeated exposures to either *my*blu™ aerosol (Tobacco flavour e-liquid 1.6% [w/w] nicotine) or Kentucky Reference Cigarette (3R4F) smoke. MucilAir™ tissues were repeatedly exposed at the air liquid interface (ALI) for 4 weeks to either 30, 60 or 90 puffs of aerosol/smoke using Imperial Brands' Smoke Aerosol Exposure *In Vitro* System (SAEIVS). The smoke/aerosol was generated using the Health Canada Intense smoking regime for 3R4F (55mL/2s/30s) and the CORESTA Recommended Method N°81 (55mL/3s/30s) for *my*blu™. Cigarette smoke was diluted with filtered air (1:17) whilst *my*blu™ aerosol was applied undiluted. Endpoints measured included Cilia Beat Frequency (CBF) and Cilia Active Area (CAA), cytotoxicity (LDH), and Transepithelial Electrical Resistance (TEER). Inflammatory markers (IL-1β; IL-6; IL-8; TNF-α; MMP-1, 3, 9) secreted into the culture media were measured using MESO Scale QuickPlex™. Tissue histology was assessed using H&E/Alcian Blue immunostaining. Fox-J1 and MUC-5-AC were used to stain for ciliated cells and mucin, respectively. The nicotine dosimetry was performed to assess smoke/vapour delivery to the *in vitro* model.

Data demonstrates a dose response to diluted cigarette smoke in various endpoints assessed, including changes to tissue morphology, significant increase in inflammatory markers, CBF & CAA at all doses tested. *my*blu™ aerosol did not induce such changes, even at highest dose, when tested undiluted for 4 weeks.

The results from various functional and mechanistic endpoints assessed, adds to the weight-of-evidence approach to substantiate the harm reduction potential of e-cigarettes for adult smokers. The study also shows that the *in vitro* 3D organotypic lung model is a sensitive and robust tool for the assessment of lung toxicity.

P15-006
Toxicity of combustion-derived particles emitted from different biomass sources in human bronchial epithelial cells

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Biomass burning is recognized as a main source of air pollutants. Combustion-derived particles (CDPs) have been linked to several respiratory diseases, including lung cancer. In the present study we investigated effects of CDPs originating from different sources on epithelial-to-mesenchymal transition (EMT), a crucial step in the carcinogenic process. The aim of the study was to characterize and compare the relative role of the particle core versus extractable organic compounds.

CDPs (PM10) were collected from a stove fueled with pellet, charcoal or wood, respectively, and chemically characterized. Human bronchial epithelial cells (HBEC3-KT) were exposed to 2.5 μg/cm^2 of whole PM, organic extracts and washed particles. The endpoints measured included cell viability, inflammatory responses, and cell migration.

CDPs showed different chemical compositions: pellet PM was enriched in metals, while charcoal and wood ones have higher PAHs content.

The results showed that CDPs differentially modulated cell viability and proliferation, and induced alterations in cell migration. Interestingly, our data revealed that the effects induced by the particles and by the adsorbed chemicals depended on the PM source; whereas exposure to washed pellet and wood PMs in general gave less response than whole particles and organic extracts, responses induced by washed charcoal were higher than from pristine particles. Additional studies on the expression of genes involved in these processes will provide additional information on the toxicological mechanisms.

In conclusion, the present study suggests that specific components attached to the particles could be responsible for the diverse effects observed following exposure to pellet and wood PMs; whereas with regard to charcoal, the PM as such appeared more toxic. The study highlights the importance of studying CDPs from different biomass sources and that more targeted strategies should be implemented to reduce the biological impact caused by the emission of biomass-propelled heating systems and to prevent hazardous health effects.

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P15-007
The pulmonary damages induced by Polyhexamethyleneguanidine phosphate (PHMG-p) are irreversible

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Polyhexamethyleneguanidine phosphate (PHMG-p) was used as a disinfectant to prevent contamination or growth of bacteria. The causal relationship was revealed between PHMG-p inhalation exposure and induction of pulmonary fibrosis. However, little is known about recovery of PHMG-p induced damage, especially lung damage.

To determine recovery possibility of PHMG-p-induced damage, we evaluated change of damage in recovery period. Male specific-pathogen-free Sprague Dawley rats aged 7-8 weeks were nose-only exposed of aerosolized PHMG-p. PHMG-p aerosol was generated by constant atomizer and clean air was added to adjust the target concentration. The rats were exposed for 6 hours/day, 5 day/week for 4 weeks. Hematological change, organ weight and histopathologic change were examined. PHMG-p exposed rat showed decrease in body weight and food intake, hematological alteration, organ weight change, and histopathologic change were observed. The change of food intake, hematological alteration and organ weight except lung weight were returned to normal range in recovery period. However, PHMG-p-induced pulmonary damage included increase of lung weight and severity of microscopic findings in lung were irreversible during recovery period.

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P15-008
Increased throughput and cryopreservation of precision-cut lung slices extend the utility of human-relevant, 3-dimensional pulmonary test systems

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Human-relevant, *in vitro/ex vivo* assays are considered an ethical and economically viable manner by which to screen the thousands of chemicals requiring hazard assessment. Of the 3-dimensional models, human precision-cut lung slices (PCLS) are often considered the most physiologically relevant pulmonary test system, but lower throughput and difficulties in cryopreservation have hampered PCLS use. We have modified a tissue slicer to accommodate 3 tissue cores for simultaneous slicing. Increased slice production was quantified using agarose and tissue cores in the slicer. To evaluate cryopreservation of PCLS, we have tested 5 cryopreservation formulations using PCLS (frozen on the day of slicing, or after overnight culture). Thawed slice viability in each of the groups was assessed with the WST-8 viability assay, prior to fixation and histological evaluation. The slicer modification resulted in 2.8-fold and 2.4-fold more slices from agarose cores, and lung cores, respectively. Cryopreservation efforts indicated freezing after slicing yields better average viability (48–73% of fresh, non-frozen control) than culturing overnight and freezing (13–54% of control) when assessing health over 4 days, post-thaw. Cryopreservation buffers containing University of Wisconsin preservation solution preserved viability the best (54% – 90% of non-frozen control). Histological findings concurred with WST-8 viability results and indicated the retention of healthy lung tissue features (> 75% of normal), post-thaw. The increased PCLS production indicates larger (or multiple) studies can be initiated from one donor lung. The promising cryopreservation results suggest slices can be banked and utilized at a later date, potentially even allowing the same donor's tissue to be used repeatedly.

P15-009
Excipients for orally inhaled drug products – How can cell culture models facilitate drug development and replace animal experiments?

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Cellular-based *in vitro* models of the respiratory tract support data for safety and efficacy testing of orally inhaled drug products [1]. Recent studies consider more complex co-culture systems, physiological relevant air-liquid conditions and aerosol deposition. However, there are still no guidelines (f. e. OECD, FDA) considering cellular based *in vitro* tools of the lungs. A possible explanation is a missing *in vitro - in vivo* correlation, which proves relevance for replacing animal tests or even predicting human based data. In this study we compared *in vitro* cytotoxicity data of pharmaceutical excipients (IC50) with their related FDA approved concentration and their GHS classification. Calu-3, A549 and hAELVi cells were used as simple monolayers addressing conducting airways and the alveolar space. A standardized MTT assay was applied to determine a dose-response curve for IC50 calculation. In order to predict the GHS classification, excipients were tested up to 10 mg/mL (1%) and ranked according to the *in vitro* hazard classification from *Sauer et al* [2]. Some FDA parenteral and pulmonal approved excipients were above 10 mg/mL and further studies were performed to determine the IC50 when possible. For some compounds, the solubility of a compound was reached before an IC50 was measured. Furthermore, we investigated inflammatory responses of selected compounds on differentiated THP-1 macrophages (dTHP-1) focussing on the release of TNF-α and Interleukin-8. The cellular models were able to predict some *in vivo* aspects, but the immune response data generated by dTHP-1 show – so far – high variability between experiments. Data on cytotoxicity outlined a suitable working range for formulation development which is in accordance with FDA and GHS data. Our future work will focus on combining different *in vitro* assays in order to establish an *in vitro* test strategy reducing animal experiments.

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P15-010
Study on the test of the inhalation exposure of sodium dichloroisocyanurate (NaDCC) aerosols for the inhalation toxicity testing

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Sodium dichloroisocyanurate (NaDCC) is one form of chlorine used for disinfection or biocide [1]. The NaDCC is used to disinfect water and is now widely available for household water treatment [2,3]. The inhalation safety data (i.e., inhalation toxicity data) of NaDCC are needed because NaDCC can be inhaled depending on its use conditions and approaches. To test the inhalation toxicity, the inhalation exposure methods to expose the target materials to experimental animals are preferentially established considering the reactivity, stability, and concentration levels of target materials [4,5]. In this study, the test for development of inhalation exposure method for inhalation toxicity testing of NaDCC aerosols were conducted: (1) stability test of NaDCC solution concentration, (2) confirmation of maximum exposure concentration of NaDCC aerosols, (3) comparison of chlorine concentrations between NaDCC solution and NaDCC aerosols, and (4) validation of sampling method for collection of NaDCC aerosols. The concentrations of free available chlorine in 15% NaDCC solutions were maintained for six hours after preparation of the NaDCC solution: (1) mean concentrations and of free chlorine (0, 0.5, 1, 3, and 6 hours after preparation of NaDCC solution, n = 5) = 9.87 ± 0.33% (distilled water (DW) solvent) and 9.69 ± 0.29% (tap water (TW) solvent) and (2) mean mass fraction of free chlorine in NaDCC solutions (0, 0.5, 1, 3, and 6 hours after preparation of NaDCC solution, n = 5) = 65.8 ± 2.17% (DW) and 64.6 1.95% (TW). The maximum exposure concentration of NaDCC aerosols were 0.069 ± 0.011 mg/L of nose-only inhalation chamber and 1.61 ± 0.05 mg/L of whole-body chamber, respectively. The maximum exposure concentrations are higher than the LC_{50} value of NaDCC reported by the US EPA (Sprague Dawley Rat; LC_{50} < 1.17 mg/L, > 0.27mg/L; four hours exposure). The mass fraction of free chlorine in NaDCC aerosols collected by glass fiber filter averaged 51.1 ± 2.81% and the mass fraction values were similar, regardless of solvent types (DW vs. TW) and chamber types (whole-body chamber vs. nose-only inhalation chamber). The combined free chlorine were detected from the NaDCC aerosols at mean 4.53 ± 0.67%. The combined chlorine can be made by the steps of NaDCC aerosolization and filter sampling. It is expected that the reliable inhalation toxicity data for disinfectants are obtained by conducting the test for the establishment of the optimal inhalation exposure method presented in this study.

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P15-011
Functionalization of carbon nanotubes change their toxicity mechanisms induced in alveolar macrophages

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Functionalized multiwall carbon nanotubes (MWCNTs) have become the focus of increased research interest, particularly in their application as tools in areas such as the biomedical field. Despite the benefits associated with functionalization of the MWCNTs, particularly in overcoming issues relating to solubility, several studies have demonstrated that these functionalized nanoparticles display toxicity. For this study, three well-characterized MWCNTs of similar diameter and length but varying function groups were investigated: a pristine non-functionalized MWCNT (NM403), an anionic MWCNT with a carboxyl group (NRCWE-042) and finally a cationic MWCNT with an amino group (NRCWE-049). This study proposes that the MWCNT toxicity mechanisms are charge dependent and employed cytotoxicity assays, transcriptomics and proteomics to assess the toxicity of the three different MWCNTs using the NR8383 rat alveolar macrophage cell line as an *in vitro* model. The study findings indicated that all three MWCNTs altered ribosomal protein translation, cytoskeleton arrangement and induced inflammation. Additionally, functionalization of the MWCNTs was also shown to alter normal signaling pathways, providing evidence of dysregulation of the mTOR signaling pathway in conjunction with increased *Lamtor* gene expression. Furthermore, the type of functionalization was also shown to be important, with the cationic MWCNT activating the transcription factor EB and inducing cell death *via* autophagy while the anionic MWCNT altering eukaryotic translation initiation factor 4 (EIF4) and phosphoprotein 70 ribosomal protein S6 kinase (p70S6K) signaling pathway as well as upregulation *Tlr2* gene expression. This study also provides evidence that lysosomal stress could be considered as a biomarker of effect to cationic carbon nanotubes. Considering all of the study findings, it can be concluded that functionalization of CNTs is directly linked to their observed toxicity in macrophages.

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P15-012
Comparison of toxicity of Oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride and Polyhexamethylene-guanidine phosphate in mice

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Oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH) and Polyhexamethylene-guanidine phosphate (PHMG) are polymeric biocides with a guanidine group. They are causative agents of the tragic humidifier disinfectant incident, which had caused death of over 179 people in Korea. The aim of this study was to assess and compare the toxic effects of PGH and PHMG when they are directly exposed to the lungs. To assess the toxicity of PGH and PHMG, 0.0125%, 0.0325%, and 0.0625% PGH or PHMG were instilled into the lungs of mice and the mice were necropsied at day 7 and day 14. Body weights, cytokine production, and histopathological examination were performed and T cell subset distribution was evaluated by flow cytometry assay. The body weights of the intermediate- and high-dose PGH or PHMG groups reduced right after instillation. The mice of the PGH group gained weight from day 4 onward, whereas the body weight of the PHMG-P groups did not recovered. Lung weights were significantly increased in the intermediate- and high-dose PGH or PHMG groups. Thymic atrophy was detected in the intermediate- and high-dose PHMG groups. Both PGH and PHMG-P induced immune cells infiltration, atrophy/necrosis of bronchial epithelium in the lungs. Lung fibrosis was observed only in the PHMG groups. Interestingly, the inflammatory changes were weakened in the PGH group at day 14, but exacerbated in the PHMG group, though the inflammation grades of the low-and intermediate-dose PGH group were higher than those of the PHMG group at day 7. Production of proinflammatory cytokines were increased at day 7 and then restored to baseline levels except IL-1β

in the PGH group, whereas the PHMG group showed increasing cytokine levels at days 7 and 14. Because thymic atropy was detected in PHMG group, we studied the subset distribution of $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^+CD8^+$ in the thymus of both groups. The percentage and total numbers of $CD4^+/CD8^+$ cells were markedly suppressed in the PHMG group, whereas there were no changes in the PGH group. T cells are known to play an important role in limiting the innate immune responses. Aberrant T cells development might leads to an inappropriate resolution of inflammation with fibrotic changes in the PHMG group. PGH and PHMG leads to pulmonary inflammation when they were exposed to the lung. And Exposure of PHMG to the lungs showed more severe adverse effect than that of PGH.

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P15-013
Optimization and validation of VITROCELL® 24/48 *in vitro* inhalation exposure system ready for testing petroleum-derived substances

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There is an increasing demand to implement *in vitro* alternatives to *in vivo* experimentation in different research areas to comply with the 3R concept. As the inhalation route is one of the most relevant routes of exposure, alternatives approaches would need realistic lung cell models (*e.g.* 3D), realistic inhalation exposure systems (*i.e.* air-liquid interface (ALI)), and proper dosimetry techniques to increase the predictive ability of *in vitro* cell models and therefore accelerate the shift from *in vivo* towards *in vitro* testing. The ultimate goal of the 'PETRALI' project is to develop an alternative method for *in vivo* inhalation testing of petroleum-derived substances. This is preceded by (i) development and validation of a generation facility to obtain vapors from oil derivatives; (ii) optimization and validation of VITROCELL® 24/48 exposure system for negative control (clean air), positive control (nitrogen dioxide), and ethylbenzene (EB) testing. VITROCELL® 24/48 is designed to perform a dose-response profile in one run. Up to 6 dilutions with 6 inserts can be used for exposure to compounds and 6 inserts in the same system are used for negative and positive control exposure, respectively. A generation facility was successfully developed at VITO to volatilize a single compound (EB) and complex substances (gasoline). Experiments with VITROCELL® 24/48 were performed to test different humidification systems, trumpet heights, flows, and cell models (A549, Calu-3, MucilAir™) to find the most optimal settings. Quality control charts for cell viability of negative and positive control exposures were established [1,2] according to expectations. Generation up to 33000 mg/m^3 EB gave no effect on A549 cell viability. Chemical analysis showed very low deposition (< 1%) of EB. Generation of up to 50000 mg/m^3 resulted in 53% cell viability compared to clean air. Additional endpoints, such as inflammation and oxidative stress, will be evaluated in A549 as well. An overview of the optimization and validation of VITROCELL® 24/48 using EB will be shown and further evaluation will define when the system is ready for testing petroleum-derived substances, *e.g.* gasoline.

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The VITROCELL® 24/48 *in vitro* inhalation exposure system was awarded to VITO by the PETA International Science Consortium.

P15-014
AhR knockout alters formation of prostaglandins in a human model of alveolar epithelial type II cells

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Type II alveolar epithelial (AEII) cells play a major role in the maintenance of lung homeostasis, as they produce surfactant, serve as stem/progenitor cell population, and, in conjunction with immune cells, contribute to regulation of immune response and inflammation within lung tissue. Formation of prostaglandins, which are known as important inflammatory regulators, has been shown to have a major impact on lung anti-microbial defense and tissue damage. Several studies support the hypothesis that the aryl hydrocarbon receptor (AhR), transcription factor that mediates cellular responses to airborne pollutants, such as polycyclic aromatic hydrocarbons (PAHs), could play an indirect role in regulation of expression/activity of major inducible enzyme(s) involved prostaglandin production, such as cyclooxygenase-2. However, the role of AhR in prostaglandin production has not been largely explored in the context of human AEII cells. Therefore, in the present study, we employed both wild-type and AhR knockout human adenocarcinoma A549 cells as a surrogate model for human AEII cells, in order to comprehensively evaluate the functional role of AhR in regulation of prostaglandin synthesis (and other eicosanoids). The LC/MS/MS analysis of eicosanoids in cell culture media, derived from A549 cells, revealed that loss of AhR was associated with a minor increase of arachidonic acid levels and some of its lipoxygenase-derived metabolites. In contrast, the AhR knockout led to a striking increase in the levels of some prostaglandins, such as PGE2 (and its metabolite, 13, 14 dihydro-15-keto-PGE2) and PGF2alpha. Levels of several other prostaglandins, such as PGD2, PGA2 and PGF2beta were also increased in A549 AhR knockout cell medium, albeit to a lower extent. Importantly, when A549 cells were treated with a model inflammatory cytokine, tumor necrosis factor-alpha, this led to a massive up-regulation of PGE2, PGF2alpha, PGD2 and PGA2 production in AhR knockout cells, which was up to several orders of magnitude higher than in wild type A549 cells. The production

of PGE2 (as well additional prostaglandins identified in the present study), known to play primarily protective roles in the context of lung injury, could be thus significantly altered by deregulation of AhR activity in human AEII cells. Therefore, we are currently exploring the impact of AhR knockout (and model environmental AhR ligands, such as PAHs and their mixtures) on principal enzymes involved in eicosanoid synthesis, or other inflammatory mediators in A549 cells, as well as in additional AEII cell models. [This study was supported by the Czech Science Foundation, grant No. 18-00145S.]

P15-015

Safety assessment of compounds – an in chemico/*in vitro* test strategy for inhalable substances from chemical, consumer goods and pharmaceutical industry

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The goal of our joint research project (BMBF 031L0128A-D) is to reduce the number of animal experiments needed for safety assessment by establishing a standardized in chemico/*in vitro* test strategy. Different and complex respiratory cell culture based models with different endpoints of often unknown relevance for human safety assessments have been described. However, a set of predictive markers to identify the human respiratory toxicity potential of a broad variety of inhalable substances still needs to be determined. Based on available *in vivo* data of chosen test materials and mechanistic knowledge, our project focuses on inflammatory effects, modulation of epithelial barrier function and activation of the innate immune system. Our objective is to establish the simplest but appropriate test strategy. Starting from the first line of defense in the alveolar region, we are studying human macrophages (differentiated THP-1, blood-derived macrophages and alveolar macrophages) followed by coculture of macrophages (differentiated THP-1) with alveolar epithelial cells hAELVi (human Alveolar Epithelial Lentivirus immortalized), and consequently further target cells. We include validated approaches for analyzing protein reactivity. Established protocols will be tested with representative compounds consisting of chemicals, pharmaceuticals and nanomaterials. A significant part of the work is dedicated to the optimization and standardization of research protocols and their performance in different laboratories in order to come to a robust protocol for routine assessment of the compound-induced effects. The standardized protocol and data gained here should benefit investigators and considerably contribute to reduction of the currently high number of so far indispensable animal experiments.

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P15-016

Cadmium and lead mixture and lung cancer development: toxicogenomic data mining approach

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Lung cancer is one of the biggest problems in pulmology and the leading cause of cancer mortality worldwide. Recently, there has been an increasing attention for the influence of environmental pollutants in the etiopathogenesis of this multifactorial disease. Among them, metals, such as cadmium (Cd) and lead (Pb), are considered of great significance, both because of their toxicity and increasing human exposure.

The aim of this *in silico* study was to analyze the individual and combined effects of cadmium and lead on the expression and activity of genes associated with the development of lung cancer by using the toxicogenomic data mining approach.

The Comparative Toxicogenomic Database (CTD) and its tools (Batch Query, MyVenn, VennViewer and Set Analyzer) were used to obtain the information about the interactions of investigated metals with genes/proteins associated with lung cancer development. Data on the function of genes were obtained from the GeneCards database, while GeneMania prediction server revealed detailed gene interactions.

Cadmium interacted with a total of 2645 and lead with a total of 3058 genes, of which 109 (Cd), and 70 (Pb) were associated with the development of lung cancer (Batch Query). MyVenn CTD tool revealed that lead and cadmium interacted with 48 common genes (additively/synergistically with 21 and antagonistically with 7 genes). The most important are the following genes: AKT1, CRP, FAS, GPX1, GSTP1, GSTT1, HMOX1, IL10, IL1B, JUN, KRAS, MAPK1, MAPK3, NOS2, TNF and TP53. They participate in 189 different metabolic pathways associated with the development of lung cancer (Set Analyzer), including MAPK signaling pathway and TNF signal pathway that affect a wide range of biological processes, such as cell growth, adhesion, transcription, translation, cytoskeletal redistribution, cell proliferation, differentiation, apoptosis. GeneMania server revealed that most of these genes were in co-expression (54.28%) and physical interaction (22.70%).

These results, confirming both individual and combined effect of Cd and Pb on genes important for lung cancer development, could be considered the basis for further *in vitro* and *in vivo* investigation in order to clarify the mechanisms of the development of this disease. Additionally, identified genes/proteins could serve as potential biomarkers and could be included for assessment of mixture toxicity of investigated metals in future (project: III 46009).

References

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P15-017

This abstract has been withdrawn.

P15-018

Development of immunocompetent human airway epithelial models with macrophages for inhalation toxicity evaluation of airborne substances

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In addition to its barrier function, airway epithelia plays also a key role in immune responses in respiratory system. Among the immune cells, airway macrophages are particularly important and active for eliminating inhaled airborne particles such as allergens as well as microbes. Their activation must be tightly regulated by both soluble factors in the lumen of the airways and through cell-cell interactions. To a better understanding of the complex crosstalk between the epithelial cells and macrophages, *in vitro* immunocompetent models

are developed based on three dimensional (3D) fully differentiated human airway epithelia cultured at air-liquid interface, co-cultured with human fibroblasts and surrogates of airway macrophages.

We report herein a simple and transposable procedure for the long term co-culture of MucilAir™ or SmallAir™ with fibroblasts and THP-1 derived M0-like macrophages. Using serum free ImmunAir™ culture medium, co-cultures were successfully maintained and functional for two weeks incorporating 5 cells types (basal, ciliated, goblet or club cells, fibroblasts and M0 macrophages).

The phenotypic identification of successively differentiated macrophages were performed by quantitative PCR of ten cellular markers. The co-culture was exposed to different stimuli and both cell types responded to these challenges. Repeated apical exposure to TNF-a and LPS increased the release of interleukin 8, in a dose dependent manner. Effect of respiratory irritants and pollens applied topically will be presented.

These data suggest that this new generation of immunocompetent airway human models may be useful for inhalation toxicity evaluation of airborne substances.

P15-019
Cytotoxic effect of real-time gasoline engine emissions exposure on BEAS-2B cells and MucilAir™

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Road traffic is a major cause of air pollution-related adverse health effects despite technological improvements and a considerable decrease in type approval limits. *In vitro* toxicity tests of emissions are usually done by the treatment of model cell lines with extractable organic matter (EOM) under submerged conditions. The aim of our study was to focus on more realistic exposure conditions such as air-liquid interface (ALI) and physical interaction of solid particles and gaseous pollutants with cells. This study reports on an in-house developed air-liquid interface exposure system for direct exposure of lung cell cultures to conditioned exhaust fumes. This approach involved a proportional sampling of exhaust using a partial flow dilution tunnel, conditioning of the sample to a stable temperature, humidity and CO_2 content. Parallel exposure to exhaust fumes and control air was achieved using 4 separate exposure boxes (2 control and 2 exposed). For this study, we used human lung cell line BEAS-2B grown at the air-liquid interface and 3D lung tissue model MucilAir™ (Epithelix Sarl, Geneva). To investigate the adverse effects, a typical direct injection spark ignition petrol engine was mounted on an engine dynamometer and operated according to the World Harmonized Light Duty Vehicle Test Cycle (WLTC). BEAS-2B cells were maintained in our exposure device with 0.2 l/min of filtered humidified air supplemented with approximately 5% of CO_2 for >10 h with no visible changes. Based on the preliminary data, two exposure periods were selected: one-day and repeated 5-days exposure. The cytotoxicity measured as lactate dehydrogenase (LDH) release into media showed a time-dependent increase in BEAS-2B cells but not in MucilAir™. Changes in sample morphology we observed after 5-days exposure in MucilAir™ as slower cilia beating frequency. In conclusion, our exposure device is able to maintain cell culture under ALI conditions and it is possible to use it for a wide variety of exposure schemes. This work was supported by the grant of the Czech Science Foundation (18-04719S).

P16 – Regulatory toxicology

P16-001
Efficient creation of electronic SEND datasets between CRO – establishment of the global SEND alliance (G-SEND) –

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The Standard for Exchange of Nonclinical Data (SEND), adopted by the US Food and Drug Administration (FDA), is a set of regulations for digitalization and standardization of nonclinical study data; thus, related organizations have begun implementing processes in support of SEND. SEND provides electronic data standards created by the Clinical Data Interchange Standards Consortium (CDISC), and CDISC also collaborates in the implementation of SEND. Furthermore, the Pharmaceutical Users Software Exchange (PhUSE), which includes members of the US FDA, has conducted various activities to promote realistic and effective methods to implement SEND. As we surveyed in 2018, there is a significant variation in the efficiency and quality of SEND data implementation across pharmaceutical companies and contractors (CROs) globally. To address this problem, the Global SEND Alliance (G-SEND) was established in August 2018 to facilitate the coordination and standardization of SEND datasets across CROs in Asia. This presentaion reports the first method for organizationally and jointly creating consistent SEND datasets between CROs using G-SEND.

P16-002
Promoting the uptake of alternatives to animal testing through the development of eLearning tools

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In order to further promote the implementation of Directive 2010/63/EU, the European Commission issued calls for a number of related projects last year. One of these projects is aimed at facilitating the uptake of non-animal alternatives by developing two e-learning modules. The contract for this project was awarded to a consortium consisting of SYRCLE, the Swiss 3R Competence Centre, Institute for In Vitro Sciences, Pharma Launcher and Ecorys UK. This consortium will develop two modules, i.e., one e-learning module focused on searching for existing non-animal alternatives (including systematic reviews) and one module targeted at researchers who want to develop reliable and relevant non-animal alternatives for regulatory use taking into account Good *In Vitro* Method Practices (GIVIMP). The quality of the developed modules will be assessed by external review groups. The learning outcomes will be presented as well as the design of the assignments through which these outcomes will be realised.

P16-003
Toxicological assessment of flavored e-liquids in Sprague-Dawley rats in an OECD sub-chronic inhalation study complemented by systems toxicology endpoints

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Flavor substances are an important element that is commonly added to e-liquids for sensory pleasure, but relatively few studies have been performed to evaluate their toxicity via inhalation exposure. The toxicity of a mixture of flavor substances in an e-liquid was characterized in a 90-day inhalation study according to the OECD 413 Testing Guideline, using 28 flavor group representatives (FGR) selected by grouping 179 flavors into 28 distinct groups based on chemical structure, where substances predicted to show the highest potential toxicological effect from each group were chosen as FGR. Sprague-Dawley rats were exposed for six hours/day, five days/week for at least 13 weeks to aerosols of vehicle control, e-liquid (propylene glycol [PG], vegetable glycerin [VG], and nicotine), e-liquid with three concentrations of FGR mixture, or PG/VG with medium concentration of FGR mixture. The target test atmosphere concentrations of nicotine, PG, and VG were 23 µg/L, 1520 µg/L and 1890 µg/L, respectively. The concentrations of the 28 flavors were derived from current maximum levels used in products. The results indicated that inhalation of the flavored e-liquid caused very minimal local and systemic toxic effects. No significant changes were detected in the number of inflammatory cells and inflammatory markers in the bronchoalveolar lavage fluid of rats in all groups, indicating limited pulmonary inflammation. The systemic effects related to exposure to the FGR mixture were limited and mainly nicotine-mediated, including changes in hematology, blood chemistry, and organ weights. There were minimal histopathological findings noted, but some findings, such as laryngeal squamous metaplasia, were seen in some rats of all groups, including vehicle control. Macro- and microscopic findings in spleen, adrenal, and thymus were considered due to procedure-related stress. The FGR mixture added to the e-liquid did not induce a measurable biological response on the transcriptome level, as seen from nose, lung, and liver samples in the current study, except a nicotine effect on metabolic processes. In summary, the results revealed findings mainly associated with nicotine exposure and limited synergistic effects caused by flavors.

P16-004
Aluminium salts in vaccines: from ancient concepts to current knowledge

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Aluminum (Al) salts Al oxy-hydroxide (AlOOH, Alhydrogel®) and Al hydroxyphosphate (AlOHPO4, Adju-Phos®) are particulate compounds widely used as immunologic adjuvants in about 60% of human vaccines. Today the exact degree of safety of Al-containing vaccines is still the subject of persistent disagreement and the WHO even notes that "adjuvant safety is an important and neglected field". Concerns linked to the use of Al particles emerged 20 years ago following recognition of their causative role in the so-called macrophagic myofasciitis (MMF) lesion detected in patients with myalgic encephalomyelitis/chronic fatigue syndrome, revealing an unexpectedly long-lasting biopersistence of Al within immune cells. Currently growing worries concern the potential role played by Al-adjuvant exposure in a large scale of diseases, among them chronic fatigue syndrome, Gulf war syndrome or autism spectrum disorders. In this field, the Autoimmune (Autoinflammatory) Syndrome Induced by Adjuvants" (ASIA) has been delineated.

Our poster presents i) key points about the use of Al salts in vaccines; ii) recent experimental data from both human and animal studies showing persistence, systemic translocation and adverse effects following Al-adjuvant exposure; iii) the three old dogma commonly cited to suggest that Al-based adjuvants are innocuous that are currently put on trial according to recent knowledge.

Although the benefits of vaccination are not questioned, we strongly suggest that novel experimental studies of Al-adjuvants toxicokinetics should be performed on the long-term, including both neonatal and adult exposures, to ensure their safety and restore population confidence in Al-containing vaccines.

P16-005
180-day toxicological research of GM soybean line MON87701×MON89788: the results of morphological examination

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As a part of the state registration of GM soybean line MON87701 × MON89788 in the Russian Federation, a comprehensive safety assessment was conducted, which included chronic toxicological research on rats *in vivo*. This article presents the results of morphological examination of internal organs of rats that consumed GM soybean for 180 days.

During this study organs of 32 Wistar rats were examined (16 in the control and test groups each). The rats were subjected to euthanasia by decapitation. Microscopic examination of internal organs was conducted at the time of autopsy: both groups showed no pathological changes and anatomically correct organ structure of typical sizes and forms, chest cavity and abdominal cavity position of organs was in the norm, the capsules, mucous membranes and serous membranes were moist, smooth and shiny, without focal changes, of a tightly elastic consistency, lymph nodes were not enlarged, lungs were airy to the touch, freely lying in the pleural cavity, pleurodiaphragmatic adhesions were absent.

Thymus, heart, lungs, liver, kidneys, adrenals, spleen, small intestine, testicles, prostate gland were histologically investigated. Organs were fixed in 10% formalin solution, preparations stained with hematoxiline-eosin and van Gieson's stain. Histological preparations were assessed in light microscope AxioImager Zl. Morphometry was performed with AxioVision 4.8.

Histological structure of the investigated organs showed no deviations. No pathological changes in tissue structure or endemic hemorrhage have been detected. Morphometric analysis of the liver, kidneys, spleen structure showed no differences between the groups: for control and test groups the average values of diameters of the renal glomeruli, the renal proximal tubules, the lumen of renal proximal tubules were 96,32 ± 1,07 and 95,25 ± 1,14 µm; 29,01 ± 0,39 and 28,16 ± 0,44 µm, 17,23 ± 0,56 and 16,95 ± 0,57 µm, respectively; the diameters of the white pulp of the spleen were 288,11 ± 4,1 and 284,46 ± 3,95 µm; the diameters of the hepatocytes were 13,85 ± 0,43 and 13,04 ± 0,34 µm.

Results of the morphological examination, together with hematological and biochemical examination, diagnosis of antioxidant status and monooxygenase system enzymes' activity in the liver, did not

reveal any toxic effect of GM soybean line MON87701 × MON89788 in comparison with its traditional counterpart.

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P16-006

This abstract has been withdrawn.

P16-007
Results of preclinical and clinical safety studies of the novel adenoviral gene therapy

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Mobilan is a recombinant bicistronic non-replicating adenoviral immunotherapeutic drug that directs expression of human toll-like receptor 5 (hTLR5) and its specific agonistic ligand, 502s, which is a recombinant form of the natural TLR5 ligand, flagellin. Mechanism of action involves transduction of tumor cells with Mobilan, which leads to constitutive autocrine stimulation of TLR5 pathway. This results in strong induction of the innate immune system with subsequent development of adaptive anti-tumor responses. Mobilan has been engineered for immunotherapy of prostate cancer and designed to be intratumorally injected into both lobes of the prostate gland.

A standard regulatory set of preclinical safety studies of this drug included toxicity studies with a single (acute toxicity; outbred white mice and rats; i.v. and i.m. injections) and multiple (chronic toxicity; outbred white rats and Chinchilla rabbits; i.m. injections; 10^9, 10^{10} and 10^{11} virus particles (v.p.) per dose), mutagenic activity (mouse bone marrow chromosomal aberration test; DNA-comet assay), immunotoxicity, allergenicity, and reproductive toxicity. Standard *in vivo* test systems were used and Mobilan was injected intramuscularly in two doses (10^9 and 10^{10} v.p. per dose) except for the cases specified above.

The results of toxicological studies did not reveal any factors that impede the clinical trials of Mobilan, however, it is necessary to consider its potential risks for patients with bleeding disorders or suffering from chronic inflammatory diseases. The maximum dose (10^{10} v.p./dose) without the observed adverse effect (NOAEL) was determined. Since standard approaches to interspecific dose recalculation are not applicable to such drugs, the NOAEL defined for animals with a safety factor of at least 10 (10^9 v.p./dose) was used in clinical trials as a starting dose.

Key safety results of Phase I clinical trials in patients with local non-metastatic prostate cancer demonstrated that favorable tolerability was observed in patients administered with Mobilan in several dose levels (10^9–10^{11} v.p. per ml per intraprostatic injection). Two reversible SAEs possibly related to Mobilan were documented: severe pollakiuria with leukocytosis and elevated C-reactive protein level in patient administered with 10^9 v.p. and acute prostatitis in patient administered with $3{\cdot}10^9$ v.p.

P16-008
R-ODAF: an omics data analysis framework for regulatory application

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High throughput technologies to analyze biological molecules (genes, protein, metabolites …) are collectively known as "omics". The use of omics technologies for toxicology research and scientific publications is expanding. However, till date no omics data has been used to support a chemical regulatory application.

Regulatory agencies report that the "truth" of toxicity is difficult to assess when using omics data, mainly because of 2 issues:

1.) Multiple platforms are available for detection and analysis of a single type of biological molecule. Due to high technical variability between the platforms, the data is sometimes difficult to correlate within and between different platforms;
2.) Conclusions obtained from omics analysis are prone to pipeline-dependent differences because the choice of bioinformatics pipeline (pre-processing and statistical analysis) impacts the obtained lists of biological systems significantly affected by the compounds of interest.

In order to address these issues, a consensus on an omics analysis framework (ODAF) for regulatory application needs to be achieved. The purpose of the current research is to test and develop a regulatory ODAF (R-ODAF) proposal for the toxicogenomics community with the ambition to enable the regulatory bodies to consider omics as a relevant data type to support compound submissions.

Because transcriptomics data is by far the most abundantly available in toxicogenomics, the CEFIC LRI-C4 project focusses on generating a standardized procedure for the analysis of transcriptomics data, obtained using the three major platforms: microarrays, RNA sequencing and the new TempO-Seq® technology.

P16-009
A systematic review of the monocyte activation test: How much proof is good enough?

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Pyrogenic contamination of parenteral pharmaceuticals is considered a serious public health risk and can result in symptoms ranging from mild reactions (e.g. fever) to septic shock and death. Therefore, for injectable formulations, pyrogen testing is mandatory during the routine quality control of injectable products by regulatory agencies, as well as during the manufacturing process.

Over the last 70 years, various pyrogen testing methods have been introduced, namely: in the 1940s, the Rabbit Pyrogen Test (RPT), which is an *in vivo* test that measures the fever reaction as an endpoint; in the 1970s, the Limulus Amoebocyte Lysate (LAL) test (also referred to as the bacterial endotoxin test), which is an animal-based *in vitro* test that uses the haemolymph of the horseshoe crab and only represents a partial replacement of the rabbit test, as it solely detects endotoxin; and in 1995, the Monocyte Activation Test (MAT), which is a non-animal based *in vitro* pyrogen test that represents a full replacement of the rabbit test.

Article 12 of the Directive 2010/63/EU specifies that an alternative to animal testing should be used whenever such method prevails. The

MAT was validated as a replacement for the RPT and the LAL by the EU Reference Laboratory for alternatives to animal testing (ECVAM) back in 2000 and adopted by the European Pharmacopoeia in 2013. We conducted a systematic review comparing the performance of the MAT against that of the two widely used animal-based pyrogen testing methods. From a scientific perspective, the results clearly demonstrate that the MAT does not have the limitations of the animal-based tests, thus outperforming the latter. The RPT fails to detect human-specific pyrogens and the LAL does not detect non-endotoxin pyrogens.

We are certain that the MAT represents an extraordinary opportunity to safeguard public health and simultaneously end the suffering of 400,000 rabbits worldwide per year used in the RPT and that it could contribute to the conservation of the critically endangered 450 million-year-old horseshoe crab used in the LAL and the birds up the food chain that depend on them. We strongly urge the biomedical and pharmaceutical industries to adhere to article 12 of the Directive 2010/63/EU and make it a priority to replace the animal-based methods with the *in vitro* alternative.

P16-010
Cannabinoid toxicity: computational assessment of (eco)toxic effects

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The significant increase of cannabinoid application for the therapeutic and recreational purposes raises question, how safe is their uncritical use by majority of consumers. Especially, the new synthetic cannabinoids are continuously produced as designer drugs, but their toxic effects are even not evaluated. The time and cost consuming chemical risk assessment of new designer drugs of course is not concern of illegal market. Fortunately, at least fast and costless *in silico* safety profiling by publically or commercially available models can be performed. We made it for 120 natural and synthetic cannabinoids. When quantitative structure-activity relationship (QSAR) models are used for regulation purposes it is recommended to run as much as possible of available QSAR models for the endpoint of interest, since agreement among predictions generated from several independent QSAR models increases the confidence on the predictions (*ECHA, Practical Guide – How to use and report (Q)SARs 3.1*). For prediction of various toxic endpoints of health and environment many validated QSAR models are publically available. By using several of them from VEGA, TEST and QSAR toolbox we evaluated cannabinoid toxicity. Independent and consensus predictions were performed including toxic health effect like mutagenicity, carcinogenicity, developmental toxicity, skin sensitization, endocrine disruption and hepatotoxicity. Cannabinoids were detected also in wastewater, thus the evaluation of their ecotoxic effects is of interest too. Therefore, properties of cannabinoid bioaccumulation, degradability and toxicity towards fish, water flea and algae were determined. In general all cannabinoids were estimated as developmental toxicants. Majority of them are also endocrine disruptors and potential carcinogens. In environment they are ready degradable. For natural cannabinoids much more reliable predictions can be done, while majority of synthetic cannabinoids unfortunately failed out of applicability domain of models. The compendium of predictive QSAR models used in this study can be implemented for preliminary chemical safety profiling of practically any other substance of interest.

P16-011
Assessment of endocrine disruption potential of ozone using the ECHA/EFSA guidance document on identifying endocrine-disrupting chemicals: experiences gained and challenges faced

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Biocidal and plant protection products to be used in the European Union are required to undergo an assessment for endocrine disruption (ED) potential pursuant to the Biocidal Products Regulation (EU 528/2012) and the Plant Protection Products Regulation (EC 1107/2009), respectively. A guidance document (GD) on the identification of endocrine-disrupting chemicals (EDCs) was published by the European Chemicals Agency (ECHA) and European Food Safety Authority (EFSA) and serves as a tool for applicants to identify EDCs using a defined set of ED criteria.

Ozone is currently regulated as a biocidal active substance; therefore, an assessment of ozone for ED potential is required. This task was undertaken by following the key steps laid out in the ECHA/EFSA GD: gather all relevant information, evaluate relevance/reliability, assemble and assess the lines of evidence using an Excel template prepared by ECHA/EFSA and draw conclusion with the extended option of conducting a mode of action (MoA) analysis.

There are indications of ozone triggering endocrine activity, such as altered hormone levels, that are mediated by the oestrogen, androgen, thyroid or steroidogenic (EATS) modalities, but there is limited evidence of EATS-mediated endocrine adverse effects. On the other hand, there are indications of ozone exposure leading to non-EATS-related activities and effects such as altered stress hormone responses and impaired metabolism. To gain a better understanding of these non-EATS activities and effects, a MoA analysis of ozone was performed. The effects of ozone are primarily mediated by local effects in the respiratory tract (e.g. local inflammation) due to its strong oxidising properties, which subsequently leads to oxidative stress that can subsequently trigger altered hormonal or metabolic responses. Overall, for both mammalian and environmental species, there is no biologically plausible link between endocrine activity and adverse effects observed from ozone. Therefore, ozone does not meet the ED criteria with respect to human health or environment relevance.

The ECHA/EFSA GD provides a clear approach of performing the ED assessment of chemicals; however, challenges were faced during the data compilation and assembling the lines of evidence. Recommendations are made on how to deal with these challenges.

P16-012
Food derived from genetically modified animals: formation of safety assessment system and new approaches to toxicological research

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The intensive development of animal genetic engineering and entering of genetically modified (GM) animal-derived food at the food market has led to necessity of safety assessment system development, as well as to harmonization of Russian and international regulatory and methodical documents. According to the requirements of the European Union a full-scale veterinary examination of GM animals is considered to be the start point of safety assessment. The next stage of assessment involves the comprehensive studies aimed at the establishing of substantial equivalence of animal-derived GM food. In general, the set of studies is very similar to the approach used for GM

plants, with the exception of only the first stage of the veterinary examination.

The Russian system of GM plants safety assessment includes the execution of scale investigations, such as general toxicological research and the study of specific types of toxicity. Such approach provides with the most complete and reliable information on potential reprotoxic, genotoxic, immunotoxic etc. effects of GM organism, as well as enables to reveal possible unintended effects of modification. At the same time the necessity of creation of animal-derived GM food safety assessment system inspired us to update of toxicological methods set. One of the strategic points of further development we believe the involvement of new knowledge in the toxicological studies (for example, advances in the oncology which allow not only to detect tumors at an early stage, but also to predict the risk of developing tumors, can be used in the study of mutagenesis and carcinogenesis). The search of sensitive biomarkers that respond to adverse effects is a constant and important aspect of scientific work that would not lose its relevance in the coming decades. Also an important research direction is the search for new models that will increase the research informativity: first, the development of models with traditionally used laboratory animals (e.g. models of adaptive potential reducing which allow to decompensate the adaptation processes of healthy organism and to identify the effects of negative impact); second, the use of new biological objects that facilitate extrapolation to humans (here can be possible the range from cell cultures and individual organs to GM organisms and synthetic biology-derived organisms, which are similar in their biochemical, physiological, pathological reactions with the humans); third, the possibility of use computer simulation within toxicological studies.

Thus, the further improvement of methodical approaches in the safety assessment of novel food determines the necessity of efforts integration not only medical and biological scientists, but also specialists in the field of mathematical analysis, computer science, analytical chemistry and other areas.

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P16-013
Risk for human health from five phthalates used in plastic food contact materials (FCM): a cumulative risk assessment by the European Food Safety Authority (EFSA)

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EFSA has updated its 2005 risk assessments for five phthalates which are authorised for use in plastic FCM and may possibly migrate into food: di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP). EFSA reconfirmed the same critical effects and individual Tolerable Daily Intakes (TDIs; mg/kg bw per day) that it had established in 2005 for such phthalates, i.e. reproductive effects for DBP (0.01), BBP (0.5), DEHP (0.05), and liver effects for DINP and DIDP (0.15 each). The possibility of a cumulative risk for humans deriving from co-exposure to phthalates causing similar effects was investigated, since consumers may simultaneously be exposed to several phthalates from the diet and other sources. A common mode of action, i.e. reduction in fetal testosterone levels, was considered plausible for DBP, BBP and DEHP. DINP also affected fetal testosterone levels even though liver is recognised as its main toxicity target. DIDP did not affect fetal testosterone levels. Thus, a group-TDI was proposed for DBP, BBP, DEHP and DINP by using the Relative Potency Factor (RPF) approach. DEHP was chosen as the index compound due to its most robust dataset and the group-TDI was set to 0.05 mg/kg bw per day, expressed as DEHP equivalents. The RPFs were calculated as the ratio between the TDI of the index compound and the individual TDIs for DBP (5.0) and BBP (0.1). For DINP, an additional assessment factor was introduced to cover for the 3-fold lower NOAEL for liver effects compared to that for reproductive effects and the resulting RPF for DINP was 0.3. For the four grouped-phthalates, an aggregated potency-adjusted dietary exposure (expressed as DEHP equivalents by applying the RPFs) was estimated to contribute to up to 23% of the group-TDI in the worst-case scenario. For DIDP, dietary exposure was estimated to be 1,500-fold below its individual TDI. This draft assessment is under public consultation until 14 April 2019, and will be revised afterwards based on the comments received.

P16-014
Benchmark dose uncertainty as a possible indicator of the biological relevance of toxicological endpoint

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Benchmark dose (BMD) analysis sometimes results in high uncertainty of BMD interval (upper/lower limit ratio, BMDU/L) associated with predefined effect size. Data sets resulted in high BMD uncertainty are qualified as low quality ones. We suppose that the level of BMD uncertainty may reflect also toxicological relevance of endpoint. Previously toxicity/safety profile assessment of novel central acetylcholinesterase reactivator S-XX was performed. S-XX was administered (i.m.) at doses 0-10-50-100-200 mg/kg to rats females (n = 6). Red blood endpoints found to be affected. Different NOAELs were established for HCT (10 mg/kg), RBC (50 mg/kg), HGB (100mg/kg), MCH (100mg/kg). Statistical deviations unrelated to dose were found for MCHC.

Purpose: To analyze uncertainty levels of BMD determined for the number of hematological endpoints within one study.

Methods: BMD-analysis performed using PROAST66.24, critical effect size 10%.

Results: In contrast to NOAELs, BMDLs for HGB, RBC, and HCT were found to be similar (from 12 to 16mg/kg) and lowest between other red blood parameters. BMDU/L were similar (5.5–6.5) as well, and low (< 10), reflecting acceptable data quality. BMDLs for other hematological endpoints – MCH, and MCHC were 81, and 224 mg/kg, as well BMDU/Ls were substantially higher – 89, and 49, indicating lower relevance of these endpoints.

Conclusion: Despite different NOAELs established for hematological endpoints, BMD methodology allowed distinguishing set of endpoints with similar BMD with low uncertainty. These endpoints might be considered as the most relevant hematological endpoints of the particular study.

P16-015
Sources of uncertainty in the threshold of toxicological concern approach

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The probabilistic approach using the genotoxicity and non-cancer (Cramer class) Thresholds of Toxicological Concern (TTC) is often perceived as accepting a higher risk than traditional, substance-specific risk assessments. However, robust scientific activities to describe the sources of uncertainty within the TTC approach have not yet been conducted or published. An ILSI Europe Expert Group was formed to examine how much uncertainty may be associated with the application of the TTC approach as compared to a substance-specific risk assessment, thus developing scientific knowledge about the sources of uncertainty being specific to the TTC. The initial phase of the project focuses on qualitative description and ranking of the identified sources of uncertainty, with a subsequent quantitative assessment.

Uncertainties addressing the development of the TTC approach include, but are not limited to, the variability of animal studies, the accurate use of uncertainty ("safety") factors, choice of the point of departure (NO(A)EL, BMDL, TD50), overall database quality and data distribution, and the choice of the 5th percentile for threshold selection.

Potential uncertainties that stem from the practical application of the TTC approach are: chemical space covered by the reference database, excluded substance groups, uncertainties associated with the use of *in silico* prediction vs. experimental data, the applicability of one TTC value to cover different toxicological endpoints (repeated dose toxicity, DART, endocrine disruption, immunotoxicity, etc.), and the influence of Cramer class misclassification.

The level of uncertainty was found to be similar for some factors, irrespective if the risk assessment is based on TTC or substance-specific data. Examples include the inherent uncertainty and variability of animal studies or the accuracy of assays employed to assess the mutagenicity of the substance.

P16-016
Similarity assessment of peroxisome proliferators based on intracellular metabolomics in HepG2 cells

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BASF and metanomics established the database MetaMap®Tox containing the plasma metabolome of more than 800 compounds derived 28-day studies in rats. In 2016, we have published a case study on the value of such *in vivo* metabolomics data for read-across within a group of phenoxy carboxylic acid herbicides [van Ravenzwaay *et al.*, 2016]. In this case study, we identified 2,4-DP as the best out of two potential source compounds to predict the 90 day-toxicity of the target compound MCPP. Over the last few years, a highly stable and reproducible liver *in vitro* model was established, in which the intracellular metabolome of HepG2 cells can be specifically altered through treatment with different hepatotoxicants. Within the EU-funded Horizon 2020 project EU-ToxRisk, we have now analysed the intracellular metabolome of HepG2 cells treated with different classes of peroxisome proliferators (phenoxy carboxylic acid herbicides, pharmacologically active peroxisome proliferators, DEHP and MEHP). The metabolome consisted of 236 unique metabolites, thereof 35 amino acids and derivatives, 11 carbohydrates and related compounds, 54 lipids, 14 energy metabolites, 6 nucleobases, 14 vitamins and cofactors as well as other miscellaneous or unknown metabolites. Most of the treatments resulted in clear changes of the intracellular metabolome in HepG2 cells at at least the highest sub-cytotoxic concentration used. In a multivariate statistical approach (PCA) clear separations from the control treatments along the first principal component were seen for the herbicides, pharmacologically active PPARalpha agonists as well as for MEHP. Furthermore, within the group of herbicides, the results show that for MCPP, the most similar treatment is 2,4-DP, whereas MCPA and 2,4-D are less similar. This result is in line with the outcome of the abovementioned *in vivo* case study.

P16-017
Impurities in cosmetic products: which are the most common, and how to assess them in a cosmetic safety report ?

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While assessing the safety of cosmetic product, an important part of the approach is to evaluate whether the cosmetic product contains or not substances that have not been intentionally added to the formulation. These substances, also commonly called impurities, are unintended substances which can appear as traces in the finished product. To guarantee the consumer safety, a safe level should be established for each of impurity. When no safe level has been established by the cosmetic Regulation, it has to be determined on a case-by-case analysis. This safe level is then compared to the exposure of the consumer from the finished product, to determine if there is a risk for health. However, information concerning the exact content of impurities in cosmetic products is often very poor. The aim of this study was to perform a wide review of different categories of cosmetic products on the market, in order to determine the major impurities and their occurrence. Then, an approach to determine their safe level was proposed.

P16-018
Read-across approach using molecular descriptors for the prediction of rat repeated-dose toxicity

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Introduction: Read-across is an approach to predict the toxicity of untested substances, based on the similarity in the chemical structures and/or other characteristics of substances with existing toxicity information. However, since current read-across approaches are subjective, expert-driven methods in terms of the similarity judgment, there are concerns on objectivity and/or reproducibility. In this study, we tested a possible use of molecular descriptors to judge chemical similarity for read-across approach of repeated-dose toxicity (RDT) prediction. [Methods] The results of rat 28-day RDT and 42-day combined RDT and reproductive/developmental toxicity tests (458 substances and 432 endpoints (EPs)) were obtained from the toxicity database HESS (NITE, Japan). Liver function/injury-related EPs were divided into 6 groups, and anemia- and kidney injury-associated EPs were grouped, respectively, and a total of 8 groups were used. Molecular descriptors were calculated using Dragon 7 (Talete) and Euclidean distances between substances were calculated with the normalized descriptors. As verification substances, 20 substances

were randomly selected and their EPs (8 groups) were compared with those of top 10 neighboring substances

Results and Discussion: We tested 4 descriptor sets: A) calculable 2385 descriptors, excluding constant values, B) 101 functional group-related descriptors, C) molecular weight-, hydrophobicity- and polar surface area-related 5 descriptors, D) extended-connectivity fingerprints (ECFP1024, maximum diameter of 4). Neighboring substances of each verification substance were different depending on the descriptor set used, although similar results were obtained with sets A, C and D for certain substances. Their relative distances between verification and neighboring substances varied for each descriptor set. The toxicity similarity with neighbors was also different depending on the descriptor set and verification substances. The overall accordance was higher for substances with low toxicity (e.g. negative for all 8 EP groups) than those with multiple EPs. These results suggest that the molecular descriptors can be used for read-across of RDT prediction although the selection of appropriate descriptors and objective/statistical determination of neighboring substances are needed.

P16-019
Investigating human cytochrome P450-related variability using PBK models for chemical risk assessment

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A 100-fold default uncertainty factor (UF) has been applied for over 60 years to account for interspecies differences and human variability in safe levels of threshold toxicants in non-cancer risk assessment. Pathway-related UFs quantifying human variability in a range of metabolic pathways have been proposed as intermediate options between default UFs and chemical-specific adjustment factors. Physiologically based kinetic (PBK) models are well suited to model pathway-related UFs as they simulate human variability in chemical disposition.

Here, we propose a methodology for human risk assessment based on *in vitro* systems and PBK models that includes variability. Techniques are now available to determine isoform specific metabolism and kinetics parameters for chemicals using *in vitro* human cell systems. Human variability distributions for metabolic pathways estimated from previous work can be used to estimate lognormal distribution for clearance of chemicals. These distributions are used in a PBK model with Markov-Chain Monte Carlo and physiological parameters taken from the online software PopGen. Other parameters specific to the studied compound such as partition coefficients are estimated using quantitative structure-activity relationship (QSAR).

Three different case studies have been realized: 1) the application of the methodology to a data rich chemical, chlorpyrifos, in order to validate the methodology by comparing the results with data from literature; 2) an illustration of the utility of using variability distributions for metabolism in the human risk assessment of a data poor compound, phosmet 3) and triflumuron.

The proposed methodology avoids the use of default UFs which are both overly and not conservative enough.

The views in this publication do not necessarily represent those of EFSA, Anses, ISS and are the authors only.

P16-020
Assessment of the specificity of tyrosine kinase inhibitors in relation to their cardiovascular toxicity, cutaneous toxicity and hepatotoxicity in cancer treatment

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Cancers remain the leading cause of death in France. The availability of new and more effective treatments with acceptable tolerance is still essential to improve patient survival. In drug development, anticancer drugs represent 60% of the drugs on the market in Europe. Chemotherapy was the first treatment in development and was largely used these last decades. However new therapeutics including immunotherapy, antibody drug-conjugate, tyrosine kinase inhibitors (TKI) have emerged because of chemotherapy's side effects and its low remission rate. Targeted therapies such as TKI are less harmful and more effective than chemotherapy treatments because their action is specific on the tumor process.

Tyrosine kinase enzymes activate proteins involved in cell proliferation, survival, migration, differentiation, angiogenesis... Their inhibitors block these enzymes and in doing so, the tumor growth. They can be divided into multi-kinase or single-kinase inhibitors and are related to potential toxicity, resistance mechanisms, pharmacokinetics, selectivity and tumor environment.

On-targets and off-targets effects related to cardiotoxicity, cutaneous toxicity, and hepatotoxicity are the most commonly emerging toxicities seen with the TKI. In the well-known marketed TKI, sumatinib has been associated to cardiotoxicity and both erlotinib and gefitinib have been associated to cutaneous toxicity and hepatotoxicity.

We present here a review of the different TKI families on the market and in clinical development in France. We will discuss their specificities of action in light of their on-target and off-target effects. We will focus particularly on cardiotoxicity, cutaneous toxicity and hepatotoxicity. Finally, we will compare the toxic effects observed in both non-clinical and clinical development in order to predict the side effects observed in Human. Data from French clinical trial authorization as well marketing Authorization application will be analyzed. We will suggest new approaches optimization of non-clinical models to improve side effects detection in Human.

P16-021
"Hypoallergenic" cosmetic products: regulatory review and scientific approach – a practical case

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Cosmetic products are regulated by two main pieces of legislation, applicable throughout the entire European Community: Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products and Commission Regulation (EU) No 655/2013 laying down common criteria for the justification of claims used in relation to cosmetic products. Implementation rules for cosmetic claim Regulation have not been clear from the beginning, so discussions followed and other documents were drafted. On 3rd July 2017 the subworking group on claims released the updated Technical document on cosmetic claims, which should become applicable to all Member States as of 1st July 2019.

Concerning the specific type of claim "hypoallergenic", Annex IV of the technical document provides a better definition of the term, and offers additional recommendations for supporting this claim. Evidence that a cosmetic product has a very low allergenic potential should be based on scientifically robust and statistically reliable data, coming both from the substances and the finished product. In this study, a practical approach was tested and implemented for the safety assessment of "hypoallergenic" products, in compliance with the latest revision of the Technical document on cosmetic claims.

P16-022
EFSA safety assessment of food additives: data and methodology used for the assessment of dietary exposure for different European countries and population groups

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Purpose: To assess chronic dietary exposure to food additives in different European countries and population groups.

Methods: The European Food Safety Authority's (EFSA) Panel on Food Additives and Flavourings (FAF) estimates chronic dietary exposure to food additives with the purpose of re-evaluating food additives that were previously authorized in Europe. For this, EFSA uses concentration values (usage and/or analytical occurrence data) reported by food industry and European countries. These are combined, at individual level, with national food consumption data from the EFSA Comprehensive European Food Consumption Database including data from 33 dietary surveys from 19 European countries and considering six different population groups (infants, toddlers, children, adolescents, adults and the elderly). Dietary exposure is assessed based on two different sets of data: (a) Maximum permitted levels (MPLs) of use set down in the EU legislation (defined as regulatory maximum level exposure assessment scenario) and (b) usage levels and/or analytical occurrence data (defined as refined exposure assessment scenario). The refined exposure assessment scenario is sub-divided into the brand-loyal consumer scenario and the non-brand-loyal consumer scenario. Additional exposure scenarios considering consumers of specific food (e.g. food supplements) are also estimated, as appropriate.

Results: Since 2014, this methodology has been applied in more than 60 food additive exposure assessments conducted as part of scientific opinions of the EFSA FAF Panel (previously Panel on Food Additives and Nutrient Sources added to Food (ANS)). For example, under the non-brand-loyal scenario, the highest 95th percentile of exposure to silicates (E 552–553) and the second highest 95th percentile of exposure to quillaia (E 999) was estimated in toddlers up to 27.3 and 0.8 mg/kg body weight/day, respectively. The estimates under the brand-loyal scenario in toddlers resulted in exposures of 65.0 and 1.0 mg/kg body weight/day, respectively. For the regulatory maximum level exposure assessment scenario, the 95th percentile of exposure to silicates (E 552–553) was estimated in toddlers up to 72.9 and 9.6 mg/kg body weight/day, respectively.

Conclusions: Detailed and up-to-date information on food additive concentration values (usage and/or analytical occurrence data) and food consumption data enables the assessment of chronic dietary exposure to food additives to more realistic levels.

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EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2019. Scientific Opinion on the re-evaluation of Quillaia extract (E 999) as a food additive and safety of the proposed extension of use. EFSA Journal 2019;17(3):5622, 50 pp. https://doi.org/10.2903/j.efsa.2019.5622

P16-023
Hierarchical Bayesian meta-analysis of human variability in PON1 metabolism for the refinement of uncertainty factors in chemical risk assessment

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Human paraoxonase (PON) exhibits a broad substrate specificity and a range of important activities, including drug metabolism, hydrolyzation of a number of organophosphorus compounds as well as the oxon metabolites of organophosphorothionates, more toxic than the parent, including insecticides and nerve agents. PON1 activity was polymorphically distributed in human populations and the frequency of the low activity phenotype varied among populations of different ethnic origins.

Here, inter-individual differences in PON1 activity have been investigated through a systematic review. All data were extracted in a structured database and meta-analyses were performed using a hierarchical Bayesian model in the R freeware to derive parameter, route and ethnic-specific variability distributions for PON1 activity. Two different approaches were applied. 1) First, non-genotyped data were meta-analysed in order to provide a distribution of PON1 activity. 2) Derivation of genotype-specific variability distributions using fast metabolizer as reference group to compare with other polymorphism. Reference group was respectively PON1*192 RR, PON1*55 RR and PON1-108 CC.

Overall, subgroup-specific distributions for PON1-variability provided the basis to derive PON1-related uncertainty factors (UF) to cover 95th or 99th percentiles of the population and were compared with the human default toxicokinetic UF (3.16). The results indicated that differences in activity related to PON1*192 are much higher than differences related to PON1*55 and PON1-108. The PON1-related UFs in healthy adults were within the default toxicokinetic UF except for the slow metabolizers PON1*192 QQ and PON1*55MM**.** From these results, an uncertainty factor of eight would be needed to protect 95% of the slow metabolizers and 10 to cover 99%.

These distributions allow to: 1) apply PON1-related UFs in the risk assessment process for compounds for which *in vitro* PON1metabolism evidence are available without the need for animal data; 2) integrate PON1-related variability distributions with *in vitro* metabolism data into physiologically based kinetic (PBK) models for quantitative *in vitro in vivo* extrapolation (QIVIVE); 3) estimate UFs

in the risk assessment process using variability distributions on metabolism.

The views in this publication do not necessarily represent those of EFSA, Anses, ISS and are the authors only.

P16-024
Non-dietary risk assessment of secondary metabolites of micro-organisms in plant protection products

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Plant protection products containing micro-organisms (MPCP) like bacteria or fungi as active substances (MPCA) are regarded as safe for both the environment and human health. Recently, secondary metabolites (SM) formed by these micro-organisms have come into focus and it is required that a formal non-dietary risk assessment is conducted. The use of the relevant EFSA model (EFSA, 2014) in conjunction with the threshold of toxicological concern (TTC) concept (EFSA, 2016) has been proposed (OECD, 2018). For SM of unknown toxicity, a QSAR evaluation for genotoxicity is required.

Here we describe generic preliminary first tier estimations which demonstrate a safe use in high or low crops considering a reasonable application rate of 1 kg MPCP per ha/day. The application rate of the secondary metabolite is calculated with its concentration and considered in the EFSA model together with appropriate default values.

Operator exposure depends on the formulation type (liquid, granular or powder) and crop type (high or low crops). MPCA containing SM at concentrations of < 0.2 to 1000 ppm or < 2 to 2000 ppm are predicted to be safe for operators in high and low crops, respectively. When additional personal protective equipment such as protective gloves (and face mask) during mixing/loading are considered safe concentration range from < 100 ppm to < 10000 ppm.

For workers estimated exposure depends on crop type and tasks and are predicted to be safe at SM concentrations of < 1000 ppm (< 5000 ppm with gloves) for re-entry in vineyards or orchards and < 5000 ppm (< 10000 ppm with gloves) in arable crops or vegetables.

First tier estimations predict a safe exposure level for child residents up to 100 ppm upon application in high crops and < 1000 ppm in low crops. However, the model overpredicts resident inhalation exposure for low application rates and a higher tier refinement is possible.

These safe concentrations of secondary metabolites in the MPCA apply to non-genotoxic metabolites of low volatility and give only a generic orientation. A formal and detailed risk assessment considering the level of SM in the MPCP and the intended uses is required.

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P16-025
Assessment of bisphenol AF as an endocrine disruptor

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Identification of Endocrine Disruptors (ED) is the first step to minimize human and environmental exposure. Scientific criteria and guidance for ED assessment have recently been established for pesticides in the EU [1]. Bisphenol A (BPA) is a widely used chemical classified as toxic for reproduction and identified as ED under REACH. Its potential adverse effects have resulted in restrictions for certain uses and increased use of BPA-analogs as safer alternatives. However, the potential toxicity of most of them are still unknown. Bisphenol AF (BPAF) is a structural BPA-analog with greater estrogen and anti-androgen activity in several *in vitro* studies [2].

The aim of this work was to assess the ED properties of BPAF for human health by applying the recently established EU criteria and guidance.

A systematic literature review was performed by a non-targeted search (CAS, chemical name and synonyms) in WOS, Pubmed and Scopus databases. Title and abstract screening using RAYYAN (rayyan.qcri.org) and full text selection was performed. All relevant information was extracted and systematically reported. Reliability and relevance of data was assessed using SciRAP (www.scirap.org). Data was synthesized into lines of evidence for endocrine activity and adversity, respectively, and weight of evidence evaluation was performed.

Ninety-six of 456 identified studies were selected based on title and abstract and 72 were finally included in the dossier after full text analysis. Relevant extracted information included 461 parameters evaluated in mammals, fish and several cell lines. Lines of evidence for endocrine activity showed predominance of estrogenic mechanisms *in vitro* (activation of estrogen receptors, cell proliferation) and *in vivo* (estradiol and testosterone levels). Adverse effects included gonads histopathology, alterations of prostate, testes, seminal vesicle, mammary gland, and disturbance of the estrus cycle, indicating estrogenic and anti-androgenic effects.

There is strong evidence that BPAF has endocrine activity and causes endocrine-related adverse effects based on the EU criteria. A mode of action analysis is required to demonstrate the biological link between the endocrine activity and adversity. This study illustrates the application of the EU criteria and guidance for ED assessment for a non-pesticide.

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P16-026
The use of *in silico* models for the prediction of mutagenicity

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In silico methods have been gaining recognition and relevance across different industries. They are used to predict the potential toxicity of chemicals and an important advantage of these tools is their capability to provide an immediate and accurate estimate of potential toxicity hazards. This feeds into chemical prioritisation and early-stage risk assessments, giving a reliable indication of any further biological testing requirements. The hazard identification step within toxicological risk assessment often begins with using (quantitative) structure-activity relationship (Q)SAR models and in the case of mutagenicity, Ames activity is investigated. The International Council for Harmonisation (ICH) M7 guidance for the assessment and control of mutagenic impurities in pharmaceutical products recommends the application of *in silico* prediction techniques as part of the hazard identification and risk assessment strategy. The guideline advises the use of two complementary *in silico* models. We have explored the use of an expert rule-based system (Derek Nexus) and a statistical-based system (Leadscope Model Applier) for the prediction of mutagenic

potential. Twenty-five compounds were investigated covering ECHA harmonised and self-notified mutagens, and mutagens and non-mutagens identified from a literature search. These were analysed using the (Q)SAR models to evaluate their sensitivity and specificity for the endpoint of mutagenicity against public data. The sensitivity of both programmes individually was 100%. The specificity when using one programme alone was 73% and this was increased to 91% when two *in silico* models were combined. This demonstrates that by combining complementary models, the number of false positive predictions can be reduced and increases the confidence in predictions when used in combination.

P16-027
Six-month repeated dose toxicity of subcutaneously administered BM41, a novel allergen immunotherapy candidate, in Wistar rats

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Birch pollen allergy is one of the most common respiratory disease in Europe. "BM4SIT – Innovations for Allergy" (www.bm4sit.eu) is an EU-funded project evaluating the efficacy of a novel Allergen Immunotherapy (AIT) candidate for the treatment of birch pollen allergy. A vaccine based on a hypoallergenic variant of Bet v 1, the major birch pollen allergen, called BM41 is designed to reduce allergic side effects and be more effective in the modulation of the allergic towards an anti-inflammatory immune response.

This repeated dose toxicity study was designed 1) to provide information on the major toxic effects of BM41 and 2) to indicate possible target organs and 3) to provide an estimate of the no-observed-adverse-effect level (NOAEL) of exposure after bi-weekly subcutaneous administration over a period of six months in Wistar rats. The study was performed according to OECD principles of Good Laboratory Practice (GLP) using 90 adult Wistar rats. The animals were allocated into three treatment groups to receive either Placebo only, Low dose of BM41 (20 µg) or High dose of BM41 (40 µg). For the adjuvant alhydrogel was used. Clinical signs, morbidity and mortality, body weights, water and food consumption were monitored during the experimental period. Blood, urine and tissue samples were collected at the end of the study for hematology, clinical chemistry, immunological and coagulation tests, urinalysis and for histopathological evaluation. Animals from the Main groups were sacrificed one week after the last dose (study week 24) while Recovery groups were kept for six more weeks (up to study week 30) after the treatment period for observation of reversibility or persistence of any toxic effects.

No animals in moribund state or having significant toxic symptoms were found and no mortality was recorded. Observed microscopic findings were either considered similar in Placebo and test item treated animals, and thus not considered related to treatment with BM41, incidental or within background changes seen microscopically in rats of this age.

This study demonstrates that 20 µg and 40 µg of BM41 did not cause significant effects on vital signs and did not produce toxicologically significant adverse effects. The dose 40 µg BM41/0,5 mg Alum/0.9% NaCl in 250 µl reflects a no-observed-adverse-effect level (NOAEL) of exposure.

P16-028
Nonclinical development of products intended for treatment of damaged skin

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For pharmaceutical products intended for dermal application, the minipig is an ideal animal model due to the close similarity of the skin of this species to human skin. Under normal conditions, the skin constitutes a relatively profound barrier to the external surroundings. However, in some human skin diseases, superficial abrasions and sores develop, potentially increasing systemic absorption of substances intended for topical skin use. This issue is not covered when using healthy animals with intact skin in regulatory toxicity testing of products intended for dermal application. For such studies, development of a model for repeated administration on damaged skin is becoming relevant and we have used the Göttingen minipig in regulatory toxicity testing of dermal products, to address this issue. The model includes an initial wound healing phase. Depending on the duration of the study, on completion of the healing phase of the wounds, a different route of administration, for example subcutaneous dosing to give good systemic exposure to the Test Item, can be considered. This model has been accepted by the regulatory authorities. Surgically applied wounds are used in the study design as these can be inflicted in a very precise and reproducible way. It is not considered ethical to wound the animals repeatedly, therefore the initial wound healing phase being combined with a second dose route if necessary. This meets requirements for longer duration non-clinical studies and maximises systemic exposure of the test compound. All routine guideline requirements for evaluation of systemic toxicity in non-rodent species are integrated into the study design. We conclude that the combination of a wound healing study with a second dosing route constitutes a valid method for testing test compounds intended for use on damaged skin in humans. This poster presents different study design options that have been used in regulatory studies and based on the study outcomes, evaluates their appropriateness.

P16-029
Critical review of the human database used for performance evaluation of defined approaches to skin sensitisation testing

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An estimated 15–20% of the general population suffer from contact allergy [Peiser *et al*., 2012]. Human predictive patch tests (HPPT) have been employed to explore the sensitising properties of chemicals for decades, e.g. the Human Maximisation Test [Kligman 1966] or the Human Repeated Insult Patch Test [Politano and Api, 2008]. HPPT data were used in the validation of the Local Lymph Node Assay [ICCVAM 1999] and have been integrated with other information to compare the relative sensitising potencies of different chemicals [Api *et al*., 2017; Basketter *et al*., 2014]. In 2018, an OECD expert group began characterising the performance of published Defined Approaches (DAs) to skin sensitisation testing and assessment [OECD, 2016]. Under this activity, the authors of this presentation curated a large HPPT dataset and analysed its suitability to serve as a reference point for

DA performance in terms of classifying sensitisers using the potency categories provided by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). HPPT data reliability and inherent variability were critically reviewed to determine the potential role of these data in binary hazard classification and potency subcategorisation, using HPPT study reports previously collated at NICEATM and later reviewed for quality and extended with additional data by BfR. Results from this activity are presented, with a more comprehensive review forthcoming. The final curated HPPT database will be available to the public to facilitate additional activities such as screening or modelling.

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P16-030
The use of dosimetric modeling in the derivation of acute inhalable DNELs for nickel metal and nickel compounds

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Under REACH legislation in the European Union, acute inhalation Derived No Effect Levels (DNELs) for local and systemic effects are required for substances that are classified for acute toxicity by inhalation or that cause local adverse effects. These DNELs are compared to worker exposure levels for the characterization of risk and to guide the selection of risk management measures. For nickel compounds, acute systemic DNELs can be based on the results of single-exposure mortality studies in rats. However, for local effects, there are no acute studies where lung toxicity was examined in any detail. Instead, acute local DNELs are based on lung inflammation associated with 16 or 28 day repeated exposure studies to nickel metal or nickel compounds. These rodent inhalation studies utilize exposure to nickel particulates in the respirable size range (MMAD ≤ 4 µm). By contrast, workers are usually exposed to more complex aerosols made up of larger inhalable particles (MMAD ≤ 100 µm), with fewer workers exposed to respirable-sized particles exclusively. Therefore, both inhalable and respirable DNELs were derived for systemic and local effects for comparison to workplace exposures. Dosimetric modeling estimates of interspecies differences in deposition (MPPD v2.1) were used to calculate human equivalent concentrations (HECs) based on pulmonary particle deposition (for systemic effects) or retention (for local effects) in rats. This modeling also allowed the incorporation of respirable or inhalable workplace particle size ranges in the calculations. Once HECs for nickel that were equivalent to points of departure from the animal studies were calculated, assessment factors were applied for remaining toxicokinetic and toxicodynamic differences. In addition, nickel-specific duration of exposure adjustments were incorporated into the local-effects DNEL calculations. The dosimetrically derived inhalable and respirable DNELs are more appropriate for worker risk characterization under REACH than those based solely on animal exposure concentration and particle size distributions.

P16-031
Proposal for a selection of priority biocide mixtures in consumer products: screening the potential synergistic toxicity on pulmonary fibrosis

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Different biocidal products have been broadly developed for industrial, professional, and consumer uses to restrain any unwanted harmful organism. Biocides can be also added as additives into other substances for keeping chemical products and articles from biological influx and contamination. In European Union (EU), a new biocidal product regulation (BPR) applied from 2013 for enhancing the authorization process of biocides. In a similar way, a new Korean biocides regulation, entitled "Consumer Chemical Products and Biocides Safety Act (also known as K-BPR)", recently entered into force in South Korea in January 2019. Korea Ministry of Environment very recently initiated R&D programs, "The Environmental Health Action Program" and "Technology Program for Establishing Biocide Safety Management", for supporting the implementation of the K-BPR.

These regulations make chemical risk assessors consider the mixture toxicity between active substances, or between active substances and other additives in the authorization process of biocidal products, if any. This is due to the fact that biocides may trigger the mixture toxicity effects on human health, and the environment if they interact with other biocides or substances at the same time and place due to their mixture toxicity (also known as cocktail effect or combined toxicity). Under those regulations, an additive toxicity approach based on concentration addition model has been frequently recommended as a default method to evaluate the toxicity of mixtures when there is no evidence on toxicological interactions among mixture components. However, available data on such interactions in mixtures still lack. Combined inhalation exposures to biocide mixtures by consumer products can occur mainly using sprays and powders. Combined inhalation exposures to airborne toxicants may also cause pulmonary or even systemic inflammation.

Therefore, the objectives of this study were i) to conduct a meta-study for investigating possible combinations of biocides in consumer products based on the EU and Korea chemical databases on consumer products, and ii) to screen biocide mixtures which may cause potential synergistic toxicity on pulmonary fibrosis using a scoring and ranking system. This study highlights a priority list of biocide mixtures that need to be assessed as a priority by toxicity testing to identify their synergistic toxicity on pulmonary fibrosis.

P16-032
Computational Toxicology @ German Toxicology Society

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The working group „Computational Toxicology" was founded in 2018 as a specialty section of the German toxicological society (GT). Its main purpose as an expert platform in this emerging field of toxicology is the advancement of computational methods and their application in regulatory risk assessment. We aim to facilitate research and training by enhancing the communication of its members, contribut-

ing to symposia, meetings, and hosting or supporting educational events. Relevant tools and methods include QSAR, read-across, building and analysing toxicological databases, PBTK and metabolism predictions.

General assemblies are held during the annual meetings of the German Society for Experimental and Clinical Pharmacology and Toxicology (DGPT). At the annual meeting in February 2018, the specialty section held its first symposium on computational toxicology. Contributing to continuous education, an "Advanced Course in Computational Pharmacology and Toxicology" was offered jointly with the Clinical Pharmacology (DGKliPha) subdivision of the DGPT.

Members have been actively engaged in international consortia sharing toxicity data, such as the IMI eTOX/eTRANSAFE, and contribute to the "*in silico* toxicology protocols" consortium which aims to standardise *in silico* tool use and their interpretation. Furthermore, members are engaged as lecturers for Computational Toxicology at universities and in education courses offered by the German Toxicology Society ("Fachtoxikologe GT") for certification as European Registered Toxicologist.

The specialty section currently has 27 members from 15 institutions in Germany, Finland, Switzerland, and the USA. At Eurotox 2019 we ask you and other interested members of academia, industry and authorities to get in touch and discuss future collaboration. E-mail us at: mail@comptox.de

P16-033
Assessment of priority tobacco additives per the requirements of the EU Tobacco Products Directive (2014/40/EU)

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Non-clinical and clinical assessments were performed to fulfill the regulatory requirement per Art. 6 (2) of the EU Tobacco Products Directive 2014/40/EU, under which Member States shall require manufacturers and importers of cigarettes and roll-your-own tobacco containing an additive that is included in the priority list established by Commission Implementing Decision (EU) 2016/787 to carry out comprehensive studies. The results of smoke chemistry (39 World Health Organizations smoke emissions), *in vitro* toxicology, and clinical studies performed by independent Contract Research Organizations are presented. Minor changes in smoke chemistry parameters were observed when comparing emissions from test cigarettes containing priority additives with emissions from additive-free reference cigarettes, and only two of the additives (sorbitol and guar gum) tested led to significant increases in a limited number of smoke constituents. These changes were not observed when sorbitol or guar gum were tested in a mixture with other priority additives. None of the priority additives resulted in increases in *in vitro* toxicity in the Ames, micronucleus, and neutral red uptake assays. In the clinical study, two distinct endpoints were investigated, namely measuring plasma nicotine pharmacokinetics as a measure of nicotine uptake and analyses of changes in smoker puffing behavior as a measure of cigarette smoke inhalation. This clinical study indicated that the inclusion of none of the priority additives, either as single additive or as part of a chemical mixture, facilitated nicotine uptake. Furthermore, the data did not suggest that differences in the inhalation pattern of cigarette smoke of any of the priority additives tested occurred when compared with the additive-free reference cigarette.

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European Union, Priority list of additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations In: Commision, E., (Ed.), Decision (EU) 2016/787, 2016.

P16-034
Read-across approach, based on a combined use of five *in silico* tools, predicts practically identical true compound toxicity

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The cosmetic industry has faced challenges in recent years regarding insufficient data for the safety assessment of new raw materials due to the animal testing ban. A commonly used method for filling data gaps is read-across. However, uncertainty remains, which has an impact on the reliability and acceptance of this approach. To improve this, we investigated five *in silico* tools, ToxRead, AMBIT, COSMOS, ChemTunes.ToxGPS and OECD Toolbox, to establish a relevant next generation safety assessment (NGSA) strategy based on a combined use of these tools. We hypothesize this strategy can raise confidence in predictions and lower the uncertainty. With this objective, we conducted case studies of 12 cosmetic ingredients with full toxicological profiles to determine if the read-across outcome, based on our novel NGSA, predicts the toxicity correctly. The combination of these five tools identified sufficient, relevant analogues with toxicological data, and allowed a successful read-across and prediction of the toxicity of the target compounds. For example, 14 relevant analogues were found for cinnamyl alcohol, a known skin sensitizer. 71% of these showed at least one positive experimental or QSAR-based result for sensitization. This correctly predicted the sensitizing potential of the target. Regarding systemic toxicity, the lowest point of departure, a NOAEL of 53 mg/kg bw/d, was covered by the lowest NOAEL found among the identified analogues (53.4 mg/kg bw/d). For genotoxicity, 35.7% of the analogues were negative and 35.7% had positive experimental results; 28.6% had no data. However, the hazard prediction feature of ChemTunes.ToxGPS predicted a genotoxic potential for 57% of all analogues. As a worst-case scenario, we predicted the target to be genotoxic. This is in line with the experimental results of cinnamyl alcohol, which shows mainly negative results but also an alert for genotoxicity.

Based on our results, we began developing a decision tree to provide guidance on when to use all five tools or when a limited number of tools could be sufficient (e.g. new material with no prevalence vs. new material with high prevalence).

In conclusion, our investigations indicate that a combined use of the *in silico* tools presented leads to a reliable and sufficiently conservative safety assessment approach for cosmetic compounds.

P16-035
A QSAR and read-across methodology for genotoxicity endpoints to support registration of agrochemicals in Europe

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As part of the registration of agrochemicals in Europe QSAR and read-across may be used to assess the genotoxicity potential of metabolites and impurities reducing the reliance on *in vitro* and *in vivo* data generation.

QSAR and read-across usage have been embedded in the pharmaceutical industry (e.g. ICH M7 guidelines) and as part of REACH requirements for a number of years. In the agrochemical industry, the acceptance of the use of these methods has been limited in a regulatory context. However, with the EFSA publication of Definition of Residue (not yet adopted) and Impurities guidance there has been a rapid expansion into QSAR/read-across processes to address these endpoints.

A transparent QSAR and read-across methodology based on the current guidance has been developed to support metabolites and impurities of agrochemical active ingredients. Three QSAR systems (DEREK Nexus, CAESAR and the OECD QSAR Toolbox) are used in concert and chemical grouping based on QSAR alerts and structural similarity/chemical reactivity with respect to genotoxic endpoints proposed. Additionally, metabolites with existing genotoxicity data are identified based on similarity and included in the assessment as representative compounds in the grouping approach.

Based on the output, additional genotoxicity testing may be proposed to support the grouping approach.

A degree of regulatory conservatism is taken into account when proposing a chemical grouping strategy based on the QSAR and read-across assessment.

An example of the QSAR and read-across approach will be demonstrated.

This approach can be transferred to support various endpoints where an understanding of the genotoxic potential is required for multiple chemical entities, e.g metabolites or impurities, while still maintaining a robust scientific methodology.

The aim is to use the methodology to support active ingredient submissions in Europe and beyond where a QSAR and read-across assessment is required.

P16-036
Incorporation of rabbit suitability as a test species in a framework to evaluate an adequate adaption for PNDT 2nd species information requirement under REACH

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Testing for pre-natal developmental toxicity (PNDT) in two species is an Annex X requirement under REACH. The preferred species are rat and rabbit. Due to the known sensitivity of rabbits to gastro intestinal (GI) imbalances ECHA initiated a project to investigate the impact of rabbit GI toxicity in PNDT studies. In this investigation ECHA found rabbits were more sensitive than rats for REACH substances with respect to lower maternal mLOAEL, more frequent GI toxicity, abortions, and mortality. Of interest this differs from investigations of PNDT studies conducted with pharmaceuticals where no species sensitivities were identified [Theunissen *et al.*], which suggests there could be a fundamental difference in the utility of rabbits for PNDT testing of REACH substances perhaps due to specific physical chemical properties not shared by pharmaceuticals. In particular ECHA identified that substances classified for skin irritation or corrosion, or substances with low water solubility (< 1 mg/l) led to GI effects more frequently in rabbits. In their discussion ECHA recommended conducting a dose range finding (DRF) study on substances with these properties to conclude on the suitability of rabbits as test species. In accordance with ECHA's recommendation a PNDT DRF study was conducted on a REACH registered UVCB substance where greater than 90% of the constituents had water solubility lower than 1 mg/l. Consistent with the ECHA evaluation our study suggests that the rabbit is not a suitable test species for the tested substance. The water solubility criteria has been included into a framework we developed to assess objectively our substances for the 2nd species information requirement under REACH. The framework has established a decision logic for considerations on whether the general adaptation possibilities of Annex XI of the REACH Regulation are adequate to generate the necessary information as required by ECHA. For this substance it has been determined that, in accordance with Annex XI section 3, an exposure based waiving adaptation is also adequate. The framework, results of the DRF study, and exposure based waiving adaption are presented here.

P16-037
Applying pathway-oriented thinking to problem formulation for planning a systematic review: a case study with aluminium-containing antiperspirants and female breast cancer risk

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The use of evidence-based methods for evaluating human health risks from environmental chemical exposures is still in its infancy. Case-studies showing how Systematic Review principles and methods can be translated to the chemical risk assessment context are needed for advancing best practices in evidence-based toxicology. We have developed a stepwise approach to Problem Formulation, using aluminium-containing antiperspirants (Al-AP) and female breast cancer risk as a case-study. Regulatory bodies have concluded, albeit with high uncertainty, that Al-AP are not a risk factor for female breast cancer, however the existing evidence has not been systematically reviewed and critically evaluated. Since this is a broad consumer health topic with direct relevance to regulatory decision-making, our aim was to explore how evidence mapping and pathway-oriented thinking can be applied to Problem Formulation to support *planning*, *scoping*, and *framing* primary and secondary PECO (*Population, Exposure, Control, Outcome*) questions in the broader context of health risk assessment. We mapped the grey (regulatory toxicology) literature to identify the conceptual boundaries, breadth and depth of analysis, research and regulatory activities, and major knowledge gaps and research needs; as well as to evaluate the feasibility and value to conduct a Systematic Review on the topic. A conceptual model (analytical framework) was developed that maps and causally links key research questions, working hypotheses, routes of exposure, pathways of toxicity, and primary and secondary health outcomes, based on a three-level hierarchy integrating the various dimensions of a health risk assessment: risk (first level), hazard and exposure (second

level), and mechanistic and biokinetic (third level) related information. The model can be used in a transparent, objective and iterative manner, as a dynamic and central tool to lay out the methodological foundation of a Systematic Review on the topic.

P16-038
How to develop the best strategy to meet the reproductive toxicity information requirements within the EU REACH regulation

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Prenatal developmental toxicity studies in one or two species and/or an extended one-generation reproductive toxicity study (EOGRTS, since March 2015) are imposed on EU REACH registrants at the higher tonnage levels (> 100 tonnes per year). Selecting the most appropriate strategy to meet these information requirements is critical for the registrant as it may significantly impact the budget needed as well as the timeline for dossier completion. The uncertainties related to the outcome of these higher tiered tests may be challenging in terms of data interpretation and decisions on appropriate risk management measures. The elaboration of the testing strategy starts with the evaluation of all relevant existing information and especially repeated dose toxicity and reproductive toxicity data. When further *in vivo* testing is required, refinement of complex study designs is required for animal welfare reasons. More specifically the design of the modular EOGRTS needs to be well-defined, referring to the premating exposure duration, dose selection, and potential additional cohorts for assessment of F2 generation, neurotoxicity or immunotoxicity. The refined study design elaborated for the test to be performed should be described in a testing proposal submitted by the Lead Registrant, together with considerations for alternative testing methods. Authorities can request reproductive toxicity testing combined with a subchronic toxicity study, either in parallel or in a tiered approach. Stepwise testing would facilitate optimized study designs by intermittent data interpretation. A comprehensive analysis of publicly available information on ongoing dossier evaluations and decision-making processes will be presented. Generating additional information of this magnitude also depends on collaboration with experienced testing facilities offering sufficient capacities. Extension of imposed timelines might need to be considered in light of limited capacity at the testing facilities.

Strategies for endpoint coverage will be presented and challenges for impacted registrants with substances in the Annex IX and/or Annex X tonnage band are highlighted.

P16-039
Assessment strategy for the identification of endocrine disruptors under the biocidal products and plant protection products regulations

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In 2018, the new scientific criteria for the determination of endocrine disrupting (ED) properties became applicable to the Biocidal Products Regulation (BPR) (EU) No. 528/2012 and the Plant Protection Products Regulation (PPPR) (EC) No. 1107/2009. In the same year, ECHA and EFSA have jointly published the Guidance document [1] on how to identify endocrine disruptors in accordance with these criteria.

According to the ED criteria, which are limited to hazard identification, a substance shall be considered as having ED properties: if it shows an adverse effect, if it shows endocrine activity, and if there is a biologically plausible link between the adverse effect and the endocrine activity (i.e. it has an endocrine mode of action). The criteria require a weight of evidence approach, taking into account all available information: this includes a systematic review of the scientific literature. Separate conclusions are required on whether the criteria are met with respect to humans and non-target organisms.

The current data requirements under the BPR and PPPR contain more mammalian studies that may be informative on ED properties than studies on other taxonomic groups. Thus, in line with the general principle to avoid unnecessary animal testing, the assessment strategy in the guidance recommends to strive for a conclusion on the ED properties with regard to humans first, followed by a conclusion on mammals as non-target organisms based on the same data set. Only when the ED criteria are not met for mammals as non-target organisms, there will be a need to proceed to other taxonomic groups. Depending on the available data, additional data might need to be generated.

The guidance document addresses the necessary steps to establish whether the ED criteria are met. It describes the gathering and evaluation of all relevant information for the ED assessment, how to conduct a mode of action analysis (including assessing essentiality, consistency and specificity), and when and how to apply a weight of evidence approach. To facilitate the assessment, parameters have been assigned to different groups, depending on whether they provide information on endocrine activity, on adversity or both. First experiences with the practical application of the ED Guidance, including the template for data collection, are discussed.

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P16-040
Influence of acidity and chlorinated compounds on the formation of 3-MCPD, 2-MCPD E glycidyl esters during the deodorization of bleached palm oil

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Esters of 3-MCPD (3-MCPDE), 2-MCPD (2-MCPDE) and glycidol (GE) are contaminants formed during heat treatment of foods, especially refined palm oil and derived products, which have raised attention in recent years. Literature indicates that in the case of 3-MCPDE and 2-MCPDE, the occurrence of a chlorine 'donor' in crude oils is a crucial issue, while for GE the main precursors have been identified as diacylglycerols and monoacylglycerols. During deodorization, the formation of HCl from thermal-catalyzed decomposition of organic chlorinated compounds has been reported and the high medium acidity may play an important role in the formation of these substances, which is still under study. The focus of this work is to evaluate the influence of inorganic acids and chlorinated compounds, in combination or not, on the formation of 3-MCPDE, 2-MCPDE and GE. For that, solutions of sodium chloride (NaCl), hydrochloric acid (HCl), hydrobromic acid (HBr) and sulfuric acid (H_2SO_4) at a concentration of 10 mg/kg were added to bleached palm oil during the steam

injection of the deodorization process. The following treatments were investigated: T1 = NaCl; T2 = HCl; T3 = HBr; T4 = H_2SO_4; and T5 = H_2SO_4+NaCl. Demineralized water was used in a further treatment as control. The deodorization process was conducted in laboratory scale batch deodorizer at 250 °C during 120 minutes, using 1% stripping steam. In the control treatment, concentrations (mg/kg) of 3-MCPDE, 2-MPCDE and GE were 2.4, 1.2 and 1.4, respectively. In comparison to the control, all treatments showed an increase in the formation of MCPDE, with levels of 3.2 (T1), 4.5 (T2), 2.8 (T3), 2.8 (T4) and 3.7 (T5) mg/kg for 3-MCPDE, and 1.6 (T1), 2.3 (T2), 1.5 (T3), 1.4 (T4) and 1.9 (T5) mg/kg for 2-MCPDE. HCl presented the highest impact on the formation of chlorinated contaminants while HBr and H_2SO_4 showed the lowest contribution, indicating that acidity is an important condition to the reaction, but chlorinated ions are the limiting factor. The small increase observed in T3 and T4 could be a result of chlorines already present in the oil. Regarding GE, the concentration increased only with HBr probably due to their conversion into 3-monobromopropane-1,2-diol. These results support the fact that inorganic acids and chlorides are effective to promote the formation of chlorinated contaminants.

P16-041
A review of the toxicological information available for Dicyclopentadiene (DCPD) pertinent to its assessment to potentially cause endocrine disruption

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Dicyclopentadiene (CAS No. 77-73-6), abbreviated as DCPD, is an olefinic hydrocarbon which is manufactured and imported into the European Union in quantities greater than 1,000 tons per year. Concerns related to foetotoxic effects observed in reproductive toxicity studies at high doses led the REACH registrants to self-classify DCPD as a Category 2 reproductive toxicant under the EU CLP Regulation. These also led to a review of DCPD by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) under the European Chemical Agency (ECHA)'s Community Rolling Action Plan (CoRAP).

To elucidate whether the observed developmental effects may be triggered by an endocrine mode of action, CEFIC's Lower Olefins Sector Group (LOSG), composed of the main DCPD interested petrochemical companies, formed an ad-hoc toxicology expert working group to review the scientific evidence for this hypothesis. The LOSG group followed OECD (2018) and EFSA/ECHA (2018) principles for the assessment of endocrine disrupting properties by identifying, collating and assessing the existing information pertaining to the potential endocrine activity and adversity of DCPD. Existing *in vitro* and *in vivo* information was complemented with additional structure activity modelling using ECHA-recommended (Q)SAR software tools. Lines of evidence were then assembled and assessed following a weight of evidence approach.

The objective of this poster is to present and discuss the data underlying the outcome of this review. Overall, taking the information from (Q)SAR, mechanistic *in vitro* and OECD conceptual framework level 4 and 5 *in vivo* studies into account, lines of evidence for endocrine-mediated adversity could not be established. Hence, the weight of evidence supports the conclusion that DCPD does not cause developmental toxicity via an endocrine mode of action.

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P16-042
Can diet-induced obesity and food restriction separate body weight-related from drug-related findings in rats following treatment with an anti-obesity compound?

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Preclinical safety evaluation of anti-obesity drug candidates is a challenge, since marked body weight loss in normal weight rodents has detrimental effects on various organ systems. We hypothesize that the observed pathological changes in normal weight, healthy, chow fed, young rats, treated with a potent anti-obesity drug are attributed to secondary effects related to drug-induced weight-loss rather than being a direct effect of drug toxicity *per se*. It was therefore anticipated that 1) obese rats would develop less pathological findings compared to normal weight animals, due to excess energy storage and 2) that food restriction (FR) and drug dosing would result in a similar pattern of findings when inducing a comparable body weight loss.

To test this hypothesis, male and female Sprague-Dawley rats (n =12–16/sex/group) were fed *ad libitum* either a standard control chow diet or a 45 kJ% high fat diet (HFD) for 23 weeks to achieve diet-induced obesity (DIO). Subsequently, the animals underwent either FR or treatment with daily subcutaneous doses of an anti-obesity drug candidate for 4 weeks to induce a 20% body weight loss.

As previously shown, HFD resulted in only a modest additional weight gain in comparison to chow-feeding [Rojas *et al.*] and DIO did not prevent the development of histological changes in drug-treated rats. Notably, some histopathological changes (e.g. in testis and kidneys) were exclusively detected in drug-treated DIO males, suggesting a higher susceptibility of DIO rats to develop findings in certain organ systems. Apart from thymus atrophy and increased macrophage infiltration in females, drug-treatment and FR shared only a few identical findings related to elevated fuel mobilization in response to energy deprivation such as reduced adipocyte size. Specific findings (e.g. in pancreas, salivary and Brunner's glands) were additionally found in chow as well as HFD fed drug-treated rats, indicating that a direct effect of the drug was accountable for these changes.

In conclusion, FR seems successful in differentiating body weight-related from drug-related findings, whereas DIO did not prevent findings induced by the anti-obesity drug. Factors such as age, degree of obesity, difference in nutritional constituents, stress level and mode of weight loss may affect the outcome of the study.

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P16-043
Tobacco and tobacco products test results before and after the implementation of the 2014/40 EU tobacco directive

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In 2016 and 2017 tests were carried out on randomly bought tobacco and tobacco related products for the purpose of screening the market situation before and after the implementation of the 2014/40 EU Tobacco Products Directive. In this research test results for cigarettes, e-cigarettes and e-liquids were compared. The tests were carried out in accordance with EU Directives (2001/37/EC and 2014/40/EU) and requirements of national legislation (Ordinance on Health Safety of Consumer Items (OG 125/2009).

Results: Before and after implementation of Tobacco Products Directive mold was found in cigarette samples. Results also showed that organochlorinated pesticides in tobacco, content of lead and arsenic, as well as product declarations were in compliance with the requirements of the legislations. Carbon monoxide, nicotine and tar content in smoke condensate in samples from 2017 were within limits of compliance. In cigarete samples from 2016 elevated content of carbon monoxide in the smoke condensate were found. Cigarete samples from 2016 also had 5% of the tar content higher than the declared value. In samples from 2016 and 2017 lead and cadmium were found in mouthpieces. Lead concentrations in 30% of tested mouthpieces from 2016 were above MAC values according to the requirements of Commission Regulation (EU) No. 836/2012 amending Annex XVII to Regulation (EC) No 1907/2006 (REACH). It was found that acrylic copolymer mouthpiece measured 10 m/m% of lead. Tests were repeated in 2017, when two other samples with unacceptable lead content were found. Those samples were also acrylate-based materials (polyacrylamide, styrene-acrylate copolymer applied to a stainless steel base). In 2016 there were no legal restrictions regarding the concentration of nicotine of 20mg/mL in e-liquid. That was one of the reason why in 50% of the tested samples nicotine concentration were above this value and highest measured concentration was 28.9 mg/mL. In samples tested in 2017, the nicotine concentration in all samples was compliant with the 2014/40/EU Directive. In addition, the labelling verification according to the Directive was conducted, as well as screening for selected allergens in products and comparison with the declared ingredients (in 2017). Only 25% of the samples complied with all the requirements.

Conclusion: Comparison of the test results proved a positive effect of the implementation of the new Tobacco Products Directive has had on the tobacco industry and tobacco related products. Since there is a wide variety of products available on the market and the variety of manufacturers, an uneven interpretation of labelling requirements has been noted. Hazard identification, monitoring of the market and smoking prevalence has to be part of health prevention, especially for the young population for whom tobacco products are banned but still very attractive.

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Directive 2001/37/EC of the European Parliament and of the Council of 5 June 2001 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco products

Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

P16-044
Systematic evaluation of *in vitro* data for hazard and risk assessment – development of the SciRAP tool

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There is currently a rapid development of *in vitro* methods as alternatives to animal studies for investigating health effects of chemical exposure, driven by stakeholder needs, academic research interests and increased regulatory focus on the 3R concept [1,2]. *In vitro* data also provide valuable mechanistic information to hazard and risk assessment of chemicals, for example in the assessment of endocrine disruptors according to new EU regulations [3]. However, tools for systematic evaluation of the reliability and relevance of *in vitro* data have been lacking. Here we present the development of a tool for evaluating *in vitro* data on the Science in Risk Assessment and Policy (SciRAP) on-line platform. Criteria for evaluating reliability, addressing aspects of both reporting and methodological quality, as well as relevance of *in vitro* studies were developed based primarily on requirements and recommendations in OECD test guidelines and corresponding guidance documents. This first version of the criteria is now available on the SciRAP platform (www.scirap.org) and is currently being tested for completeness and practical use by experts in the field of *in vitro* testing and health risk assessment. The output of a study evaluation using the SciRAP method is a colour profile, which provides a transparent overview of how the evaluator judged each criterion [4]. These colour profiles can be used as basis for evidence integration in hazard and risk assessment. Ongoing studies, using the SciRAP tool for evaluating *in vivo* studies, demonstrate how the SciRAP method can be adjusted for judging risk of bias domains when applying a systematic review approach. Future studies are planned to illustrate how evaluations of *in vitro* studies using the SciRAP tool can be used to integrate mechanistic data in hazard and risk assessment.

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P16-045
EDC-MixRisk: novel whole mixture approach to improve risk assessment of EDC-mixtures

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Endocrine disrupting chemicals (EDCs) are linked to serious health problems such as diabetes, obesity, neurodevelopmental disorders and reproductive problems. We are exposed on a daily basis to a cocktail of EDCs that potentially interact and amplify each other's effects. EDC-MixRisk, a H2020 project, has studied the effects of prenatal exposure to mixtures of potential EDCs on the development and health of children. The objectives of the project were i) Identification of mixtures of EDCs that are associated with multiple adverse health outcomes; ii) Identification of molecular mechanisms and pathways underlying these associations and iii) Development of methods for risk assessment of EDC-mixtures.

EDC-MixRisk has developed a novel, integrated approach which is grounded on interdisciplinary collaboration, including epidemiology, experimental biology and regulatory toxicology. Three health domains were addressed: 1) growth and metabolism, 2) neurodevelopment and 3) sexual development. By using whole mixture approach and epidemiology data from the Swedish pregnancy cohort SELMA, relevant EDC mixtures associated with adverse health outcomes in humans were identified. Then, reference mixtures were created to mimic real-life internal exposures, and these mixtures were tested in various cell and animal models. The experimental data were used to establish new methods and strategies for mixture risk assessment in order to complement current approaches and to better address environmental exposures.

The epidemiological analysis showed that prenatal exposure to mixtures of EDCs was associated with various effects in children's health and development, some effects being sex specific. The mixtures, tested in the variety of experimental models, affected hormone-regulated and disease-relevant outcomes at concentrations found in the pregnant women. By applying this novel whole-mixture approach, we found a higher number of children at risk compared to estimates by current methods based on a single compound assessment. The results call for a comprehensive and harmonized approach across policy and regulatory silos to tackle combined exposures to hazardous chemical mixtures, as current regulations seem to systematically underestimate health risks associated with co-exposures to EDCs or potential EDCs.

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P16-046
Feasibility study for the applicability of the ECHA/EFSA guidance for the identification of endocrine disruptors: the example of α-cypermethrin

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Regulation 528/2012 on biocide products and Regulation 1107/2009 on plant protection products ask that the new approved active substances should not present endocrine disruptor activity. To support applicants ECHA and EFSA together have prepared and published a detailed guidance on how to collect and present data to demonstrate the presence or absence of endocrine disruptor activity. In order to understand the applicability of the ECHA/EFSA guidance, available data for an already approved substance was used to test the procedure proposed by ECHA/EFSA and understand if a conclusion on endocrine disruptor activity was possible. For this aim, α-cypermethrin was selected as it is already approved as both pesticide and biocide. This substance is also in the list of substances requiring additional testing according to the EPA (Environmental Protection Agency) EDSP (Endocrine Disruptor Screening Program) program.

The data that were analysed included the studies that are present in the RAR (Renewal Assessment Report) for the approval as pesticide with the addition of new studies that were performed after the publication of the RAR. All these data were inserted in the Excel table contained in Annex E of the guideline and analysed following the provided instruction.

Even disregarding minor problems for example related to adapt the template to accept *in vitro* studies, some other identified limitations are:

1.) There is still uncertainty in the classification of an effect to indicate an endocrine disruptor activity.
2.) The *in vivo* studies that have been performed in the past are not suitable for the new requirements. The risk is that many new *in vivo* studies will be required in the future.
3.) There is no incentive in the use of *in vitro* studies even though they can be useful in the elucidation of endocrine disruptor mechanism with more relevance to human organism.
4.) Endocrine disruptor activity is generally still limited to the area of reproductive/developmental toxicity studies, with little attention to thyroid mediated effects.

Conclusion is that additional endpoints should be included in the template, with more emphasis to *in vitro* tests, whose development and application should be encouraged to reduce the number of new *in vivo* studies and increase toxicological predictivity.

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P16-047
SweNanoSafe – a national platform promoting safe handling of nanomaterials

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The increased production and use of engineered nanomaterials (ENMs) in consumer and industrial products has raised environmental, health and safety concerns. There is a need for more knowledge on the properties and potential risks of ENMs as well as requirements for protective measures throughout their life cycle. To promote the safe handling of nanomaterials, the Swedish National Platform for Nanosafety was established in 2016. The platform is an assignment from the Swedish Government with the aim to, in cooperation with various stakeholders, ensure knowledge building and exchange on environmental, health and safety issues of nanomaterials. The aim is also to increase knowledge on hindrances to safe handling of nanomaterials and on how these hindrances can be addressed. The platform consists of a Steering Committee, a Project Team, a Cooperation Council, an Expert Panel and a web-based forum to facilitate

knowledge transfer (www.swenanosafe.se). The Cooperation Council gathers representatives from various stakeholders i.e., authorities, industry, NGO's and academia. The Expert Panel and the recently established research network provides expertise from different disciplines within the field of nanosafety. Safety and sustainability aspects of nanomaterials concern their whole life cycle, such as synthesis, development, production, use and management of waste. Currently, through a range of activities, information needs and knowledge gaps, together with other hindrances to the safe handling of nanomaterials, are being identified within the areas of regulations and guidance, research and development, education, as well as knowledge and information exchange. Actions to overcome various hindrances to the safe handling of nanomaterials will also be proposed by the platform, thereby promoting a coordinated approach to issues of nanosafety in Sweden.

P16-048
Impact of the new ERA guidance on the conduction of pharmacokinetic and toxicity studies

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In November 2018 the EMA issued a new guideline draft prescribing the testing course for the Environmental Risk Assessment (ERA) and the involvement of data from the preclinical studies. An analysis of the recent and draft guidances is shown and discussed.

An impact of the guideline update will be to affect the study design of preclinical studies due to the phase-out of the Predicted Environmental Concentration (PEC) refinement on the basis of marketing data.

The ERA is a requirement for the registration of a Human Medicinal Product (HMP) as module 1.6 in the (electronic) Common Technical Document ((e) CTD) format. Although a dossier can be rejected if the ERA lacks, the outcome of it cannot be a reason for denial of the Market Authorisation Application (MAA). A leaflet warning however, may be a consequence of an unfavourable ERA. Therefore the conduct of specific environmental fate and effect studies is often considered rather late, but it must be commenced more than one year before submission in order to present a complete dossier. Authorities often do not consider some unfinished studies as an incomplete dossier and normally grant an extension, provided a letter of commitment had been signed.

Accordingly, the ERA and its requirements are more or less out of mind during the toxicity and pharmacokinetic studies necessary for registration of a HMP. These studies always played a certain role in the ERA in that they had to be considered in the CMR (Carcinogenicity, Mutagenicity and toxicity to Reproduction) assessment and thus the applicability of the environmental action limit. As this is only a question arising for Active Pharmaceutical Ingredients (API) with a maximum daily dose below 2 mg, the impact of the toxicity studies can in all other cases be neglected. Much more important for the avoidance of an environmental leaflet warning (with negative marketing impact) is the recalculation of the PEC, which used to be possible on the basis of marketing data once the base set of data was determined. This option was key to avoid a leaflet waring but is now phased out according to the new draft guidance update.

In consequence the calculation of a Factor of excretion ($F_{excreta}$) is the only remaining option for PEC mitigation, but it depends of API and metabolite quantification in both, the faeces and urine, which is thus of significantly increased importance.

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P16-049
Hazard assessment of hydrazine, a possible migration c ontaminant from drinking water apparatus

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The Drinking Water Quality Standards for lifetime exposure of contaminants have been established for 51 items under the Japanese Water Supply Act (JWSA). In addition, non-legally binding target values are also notified for "Complementary Items" and some of "Items for Further Study" that can be detected in drinking water or water sources. Chemical contaminants can be leached to drinking water from the water supply system, but such chemical contaminants were not well evaluated. Therefore, we searched chemicals that can be migrated from drinking water apparatus to identify and evaluate hazard of migration contaminants in drinking water. Firstly, we made a list of chemical items that used in drinking water apparatus by reference to the Japan Water Works Association (JWWA) publications. Twenty-five items out of ca. 150 items appeared in JWWA publications were found to be used in materials directory contact to drinking water, and 18 out of 25 items were already evaluated under JWSA. However, seven items recognized as "Items for Further Study" were lack of information for their toxicity and detected levels, and the target values were not yet established. Therefore, we subsequently conducted screening assessment of these seven items using publicly available risk assessment reports to identify their hazard. As a result, the lowest health-based value (Tolerable Daily Intake: TDI or Virtually Safe Dose: VSD) was provisionally obtained for hydrazine from seven items we evaluated. Then, we decided to further evaluate hydrazine to derive a health-based target value in drinking water because of high toxicity potential, high water solubility and a wide range of industrial use. The health-based target value of hydrazine in drinking water was calculated to be 0.005 mg/L with body weight (50 kg for adults) and drinking water intake (2L/day) by using the VSD at 10^{-5} risk of 2.1×10^{-4} mg/kg/day, which is based on hepatocellular adenomas and carcinomas in rats in a two-year drinking water study (OECD TG 451). Our health-based target value will be useful to identify a possible risk of hydrazine intake via drinking water. ACKNOWLEGMENT: This study was supported by a Health and Labour Sciences Research Grant (H28-Kenki-Ippan-005) from the Ministry of Health, Labour and Welfare, Japan.

P16-050
What is the risk of drinking water downstream from sites polluted with polycyclic aromatic hydrocarbons (PAHs)? Comparative toxicity of oxygenated polycyclic aromatic compounds (O-PACs) to associated PAHs.

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Introduction: In industrialized countries, a lot of PAH-contaminated sites can be identified. PAH high toxicity has already been

demonstrated. However, other polycyclic aromatic compounds (PACs) can be found at these sites and may therefore contribute to the risk for humans and the environment such as oxygenated PACs (O-PACs). O-PACs are emitted from the same sources as PAHs and can be formed by oxidation of the parent PAHs. They show a higher mobility and persistence in soils than PAH, and thus, a possible risk for human by drinking groundwater. In order to better assess the health risk associated to PAH-contaminated sites (former coking plants, gasworks or wood preservation facilities), 11 O-PACs were selected for their frequency of occurrence in groundwater and structural diversity.

Method: A literature review summarizing existing data was performed on various toxicological endpoints for all 11 O-PACs. All results were gathered and analyzed in order to compare their toxicity to the associated PAH with the most similar structure. Since O-PACs are not extensively described in the databases, results were completed with (Q)SAR predictions and Threshold Toxicological Concern (TTC) safety assessment.

Results: 3 of these compounds were already investigated. Anthraquinone (ANTQ), dibenzofuran (DBF) and 9H-fluorenone (9HF) were compared to their respectively associated PAH: anthracene, acenaphthene and fluorene. In the overall toxicity comparison of ANTQ to its parent compound anthracene, ANTQ seams to represent a greater danger based on a more important carcinogenicity. On the other hand, DBF and 9HF present the same level of toxicity on every studied endpoint compared to acenaphthene and fluorene.

Conclusion: This preliminary work demonstrated that O-PACs present at least the same level of toxicity than their associated PAH, suggesting that a follow-up of those molecules could be implemented for groundwater in order to assess its quality.

P16-051
The role of chemical analysis in supporting the European Union's ban on characterising flavors in tobacco products

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Introduction: In light of the evidence of flavoured tobacco products facilitating initiation of tobacco consumption and affecting consumption patterns, the European Union (EU) Tobacco Products Directive (TPD) requires Members States (MS) to prohibit the placing on the market of tobacco products with a characterising flavour, specifically boxed cigarettes and roll-your-own tobacco.

Methods: The objective of the EUREST-FLAVOURS project is to support the European Commission in the specification of the methodology to support the decision on whether a tobacco product has a characterising flavour.

Results: The approach for specifying the methodology for whether a tobacco product imparts a characterising flavour is based on a comparison of the smelling properties of test products with those of reference products through sensory analysis, complemented by a chemical assessment of the product composition through chemical analyses.

Conclusions: The EUREST-FLAVOURS project is developing clear science-based decision criteria that a tobacco product has a characterising flavour. Chemical analysis will contribute to supporting evidence that a tobacco product contains flavour compounds in order to support the EU TPD ban on characterising flavours.

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Disclaimer: The content of this working document represents the views of the EUREST-FLAVOUR Consortium and is its sole responsibility; it can in no way be taken to reflect the views of the European Commission and/or Chafea or any other body of the European Union.

P16-052
Analysis of level 1 and 2 of the OECD Guidance Document 150 for Evaluating Chemicals for Endocrine Disruption and applicability in the EU

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OECD Guidance Document (GD) 150 for Evaluating Chemicals for Endocrine Disruption describes 5 levels with increasing complexity for the definition of the endocrine disruptor activity of chemicals.

Level 1 regulates Existing data and existing or new non-test information, including modelling programs to collect existing information and perform the preliminary assessment of the substance. Scope of level 2 is *in vitro* assays providing data to elucidate selected endocrine mechanism(s)/pathway(s). This step is very important to demonstrate the endocrine disruptor mechanism at the basis of an adverse effect, with special attention to the species-specific mechanisms.

With the focus on human toxicology and the EU market, α-cypermethrin was taken as case study to test the procedure described for the Level 1 and 2 in order to define opportunities and limits of the approach.

Analysis of α-cypermethrin was performed using the list of databases present in Annex D of the ECHA/EFSA guidance for the identification of endocrine disruptors. More than a hundred studies was retrieved, but in many cases the exact tested isomers was not specified and in general pubic available studies do not report enough details for the definition of endocrine disruptor activity. Appendix D reports also very interesting modelling programs that could provide the useful link between the chemical structure and a possible concern. Use and consultation of the programs is often cumbersome, requesting special expertise.

Regarding level 2, there are already some validated *in vitro* methods and many others are well advanced in the acceptability for the elucidation of specific AOP (Adverse Outcome Pathways) offering a tremendous opportunity for the demonstration of a possible ED activity. The applicability of level 2 requires the availability of CROs (Contract Reasearch Laboratories) to execute the experiments. The authors performed a detailed search of any possible lab that may offer the service. In spite of the efforts only 20 labs were found eligible for *in vitro* testing. An enquiry was sent to all of them, with reply from 16 and only 6 confirmed the possibility to offer the service for *in vitro* testing to assess endocrine disruptor properties. Two of them are also developing new systems for the assessment of thyroid disfunction, which has still no official OECD guidelines. The average cost to perform the whole set of tests is about 25,000€ per substance.

Conclusion is that *in vitro* tests for the assessment of ED properties is a useful opportunity but needs stimulus for wider applicability.

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P16-053
Comparison of single, paired and group housing effects on cardiovascular parameters and body temperature in telemetered cynomolgus monkeys

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Animal research in the European Union (EU) is regulated under Directive 2010/63/EU (protection of animals used for scientific purposes). This directive clearly indicates that animals, except those which are naturally solitary, should be socially housed in stable groups of compatible individuals. In the present investigation, we compared three housing conditions (single, paired and group housing) in four well-acclimated male telemeter-implanted cynomolgus monkeys. Body temperature (BT) and cardiovascular parameters, including heart rate (HR), arterial blood pressure [systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP)] and ECG parameters (PQ interval, QRS complex and QT interval) were continuously recorded by telemetry over a period of 19 hours (from 16:00 to 11:00). The animals were housed in ETS-123 compliant cages and data were recorded first under group housing and then under single and paired housing conditions using a cross-over design. When compared to single housing conditions, paired housing had no significant effect on cardiovascular parameters, but the group housing configuration led to significant decreases in HR from 19:30 to 6:00 [maximum effect (Emax) at 21:00: 106 ±9 *vs.* 139 ±4 beats/min, $p < 0.001$], DAP from 18:00 to 3:00 (Emax at 21:00: 64 ±3 *vs.* 87 ±6 mmHg, $p < 0.001$), MAP from 19:00 to 1:00 (Emax at 21:00: 83 ±3 *vs.* 106 ±7 mmHg, $p < 0.001$) and increases in QT interval (Emax at 00:00: 297 ±15 *vs.* 247 ±3 ms, $p < 0.01$). There were no statistically significant changes between single and group housed animals in SAP or QT corrected for changes in HR according to the Bazett (QTcB) formula or the individual QT correction method (QTca). In group housed animals, there was a statistically significant increase in body temperature from 16:00 to 17:30 and from 7:00 to 8:00, reaching an Emax at 16:30 (38.8 ±0 *vs.* 38.2 ±0 °C, $p < 0.01$). Based on quantitative cardiovascular parameters, the present preliminary findings suggest a benefit of group housing conditions over single or paired housing in cynomolgus monkeys. Paired housing conditions had no benefit over the single housing environment under our experimental conditions. These preliminary findings support the use of group housing in studies of cardiovascular safety assessment.

P16-054
***In silico* acute toxicity protocols and models**

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In silico toxicology is an important alternative approach to animal testing that provides a fast and inexpensive prediction of toxicity. While computational approaches can quickly calculate a prediction, the process of selecting and acquiring models, performing an expert review, integrating experimental data and model results, and documenting conclusions and uncertainties can be time-consuming and difficult to reproduce. It is also challenging to defend the results, primarily due to a lack of published procedures for performing an *in silico* assessment. To support the development of such protocols, a 60-member international cross-industry consortium has been assembled including representatives from international regulatory agencies and government research laboratories in the United States, Canada, Japan and Europe, as well as large companies from various industrial sectors (e.g., pharmaceutical, food, cosmetics, agrochemicals), academic groups and other stakeholders. The protocols ensure that any *in silico* assessments are performed in a consistent, repeatable, well-documented and defendable manner so as to support their broader acceptance. To support the implementation of the acute toxicity protocol, a series of *in silico* models to predict acute toxicity were developed that are based on GHS categories from acute rat oral toxicity studies. A battery of structural fragment-based models and alerts were used to predict these categories. The overall predictive accuracy is 74% and is based on a predicting the correct GHS category or an adjacent more conservative category.

P16-055
New TTC database compilation to support thresholds of toxicological concern in the risk assessment of antimicrobials beyond Cramer Classes

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Threshold of Toxicological Concern (TTC) is an alternative method applied in the risk/safety assessment for substances whose exposure is very low and when appropriate data are not available. The aim of this work was to expand the original Munro TTC dataset through integration of existing public data sources to extend TTC approach to antimicrobials. Global antimicrobial inventory was defined based on records from US EPA (319), EFSA (170), and ECHA (240) spanning the chemical types of disinfectants, antimicrobial, biocides, and preservatives. The expanded database includes over 1600 chemicals and data from several well-established datasets, e.g., COSMOS TTC, MUNRO, EFSA, EPA IRIS and ToxRefDB. Strict study inclusion criteria (e.g., study type/duration, route of exposure, species, number of doses) have been applied. Approximately 85% of the AM inventory is Cramer Class III, which can be considered simplistic to apply 90 mg/day for most of the antimicrobials (AMs). Instead of using Cramer Decision Tree, AM category concept was developed to bin the compounds structurally, which then were further delineated to sub-categories according to their potency. This large database increases the robustness of the chemical domains already covered by the Munro dataset and enables performing chemoinformatics analysis to go beyond the Cramer decision tree. In this study, a set of AM chemotypes based on ToxPrint chemotypes is identified to develop categories, taking into account the physical and biological properties that are related more directly to toxicity. Potency categories of antimicrobial chemotypes are then developed by correlating with NO(A)EL values. The possibility of grouping chemicals into potency categories using the chemotypes is then validated against the full dataset. Using these AM categories, several use cases such as caffeine, organophosphate, iodo-2-propynl butylcarbamate, etidronic acid, and ZnPTO were demonstrated to set up frame work for potential thresholds. This new method intends to reduce the need for chronic animal testing of active antimicrobial ingredients in premarket reviews while reduce animal testing of metabolites or impurities.

P16-056
Tyrosinaemia: factors affecting production & excretion of HPPA during inhibition of HPPD

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Tyrosineaminotranferase (TAT) is the first and rate limiting enzyme of tyrosine catabolism and when 4-hydroxyphenylpyruvate dioxygenase (HPPD) is inhibited the amount of 4-hydroxyphenylpyruvate (HPPA) increases, is then removed to the general circulation and transported to the kidney where it is actively transported to urine with related metabolites, collectively known as phenolic acids.

The extent of the steady-state tyrosinaemia induced upon inhibition of HPPD in mice and rats has been shown to be inversely correlated to the hepatic activity of (TAT) in that species. This correlation extrapolates well to humans where the hepatic activity of TAT and the extent of the tyrosinaemia in healthy humans has been reported to be similar to mice.

The activity of TAT in rabbit and dog have also been reported. The activity of TAT in the rabbit is similar to that of the female rat consistent with the extent of the tyrosinaemia in each species. Unlike female rat, the rabbit does not suffer ocular effects, despite ocular exposure to tyrosine, suggesting a further defence mechanism is active within the eye. The activity of hepatic TAT in the dog is significantly higher than in mice which should indicate a tyrosinaemia less pronounced than that in mice. However, the degree of tyrosinaemia in the dog is greater than 1,000 nmol/ml, the threshold for ocular toxicity to be expressed, the characteristic, tyrosine-mediated ocular lesion of the dog has been reported with different inhibitors of HPPD.

From this information, the dog clearly contradicts the association of TAT activity with the maximal extent of tyrosinaemia once HPPD is inhibited in rats, mice, rabbits and humans. The present study examines the kinetic variables, during the inhibition of HPPD, that may influence the disposition of HPPA following its production from tyrosine. This in turn allows definition of the relative contribution to the development and extent of tyrosinaemia across species of hepatic TAT activity in production of HPPA, versus those factors that control the removal of HPPA from systemic circulation. This work extends our understanding of the mechanism that controls tyrosine-mediated ocular toxicity in laboratory animal species and the consequence for humans.

P16-057
GHS "Serious Eye Damage" mixture classification: predictive capacity of the calculation method versus test data

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Purpose: Under UN GHS (and thus also EU CLP) the eye hazard classification of a chemical mixture is primarily to be based on appropriate data for that mixture or (via bridging) data for similar mixtures. If no such data are available, the calculation method (based on additivity) shall be used. Whereas this is intended as a last resort in a tiered approach, to avoid animal testing and due to limited availability of validated in-vitro tests, it is nevertheless frequently used in some product categories. The current review assesses how well the calculation method can reproduce data-based classification for actual mixtures, as reported in the literature.

Method: Eye hazard classification based on conclusive test data for 430 mixtures (crop protection products and detergents) was sourced from 5 peer reviewed papers. The corresponding UN GHS calculation method results were either also published in the papers, or were reconstructed based on the reported composition information. False positive and false negative rates for the calculation's outcome were determined, for "Cat1" (serious eye damage) versus "not Cat1".

Results & Discussion: 70% of the reviewed mixtures were not classified as Cat1 based on data. A prominent proportion (47%) of these had a false positive additivity result of Cat1. On the other hand, 15% of the true Cat1 mixtures (based on data) resulted in a false negative calculation. The false negatives rate is substantially better than the reproducibility of the standard animal test (Draize), for which 27% of false negatives are reported. The high false positives rate indicates a general tendency of over-prediction. The driving parameter in the calculation method is the cut off/concentration limit of 3%. Above this level, a mixture's constituent that is classified for Cat1 serious eye damage will trigger this same classification for the mixture itself. The findings suggest that this threshold is defined too conservatively to achieve a good concordance of the calculation method with the data-based classification. Such additional conservatism may not be required to ensure an adequately precautionary approach, because the classification based on the standard *in vivo* method is in itself over-predictive of effects in man.

P16-058

This abstract has been withdrawn.

P16-059
Analysis of mycotoxins and toxic elements in laboratory animals feed

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Purpose: Laboratory animals are the most widely models used in experiments in toxicological research. The lack of a standardized diet for laboratory animals can have profound effects on their health and can lead to less reproducible research outcomes [1,2]. The laboratory feeds are commonly used by lab animal breeders and researchers and could be a potential source of toxic compounds and elements. Dietary toxicants such as mycotoxins and toxic elements are important to measure because these are ubiquitous contaminants [3,4,5]. The presence of mycotoxins in European feed has been reported worldwide for decades. Moreover, their co-occurrence in a feed is an important problem affecting animal health due to their multidirectional toxicity [6]. Toxic elements are a group of such compounds, that can accumulate in the body leading to developmental abnormalities, reduced growth, and increased rates of mortality.

Methods: Forty samples of feed for laboratory animals (mouse, rat, hamster, guinea pig, rabbit, zebrafish) were collected from breeders in Poland. The samples came from domestic and foreign manufacturers. The concentrations of mycotoxins (aflatoxins, deoxynivalenol, ochratoxin A, zearalenone and enniatins) were analyzed by liquid chromatography coupled with tandem mass spectrometry

(LC-MS/MS). Toxic elements (arsenic, cadmium, lead, and mercury) were determined using inductively-coupled plasma mass spectrometry (ICP-MS) and atomic absorption (AAS) methods.

Results: Mycotoxins and toxic elements were detected in all study samples of feed for laboratory animals. The most frequently detected (95%) in lab feed were enniatins at the concentration ranging from 1–2025 µg/kg. Zearalenone (4–218 µg/kg) and deoxynivalenol (100–500 µg/kg) were quantified in 76% and 56% samples, respectively. Only in 3 samples, ochratoxin A occurred (0.1–4.7 µg/kg). Arsenic and cadmium in concentrations 50–100 µg/kg were detectable in 44% and 49% samples of feed, respectively. Lead (50–100 µg/kg) and mercury (1–5 µg/kg) were detected in 58% and 59%, respectively.

Conclusion: This results demonstrated that the occurrence of mycotoxins and toxic elements in feed for laboratory animals are significant. Chronic consumption of these diets can be considered as a risk for animals health. Consequently, lead to obtaining false study results, increased the number of animals used in experiments and greater difficulty in extrapolating outcomes to humans. Efforts directed at analytical control of laboratory animal feed will improve the reliability of toxicity tests in biomedical research and regulatory toxicology.

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P16-060
Comparison of two commercially available systems proposed for oral administration of capsules in rats

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Two commercially available systems for the oral administration of capsules to rats were tested to select the most appropriate device for use: a flexible tube (Instech Laboratories) and a stainless steel dosing applicator (Torpac Inc.). Ten female Sprague-Dawley rats (304–338g) were allocated to 2 groups and received empty standard size-9 gelatine capsules once daily for 4 days by the oral route using one of the two administration systems. On Day 5, the rats received a coated placebo size-9 capsule (filled with Avicel PH-102) by the same method as on Days 1-4. The animals were habituated to the administration procedure using a 3mm diameter plastic gavage tube (on 3 successive days). Air or liquid (water or corn oil) was administered to facilitate movement of the capsule into the stomach. With the Torpac rigid tube, it was difficult to insert a sufficient length of tube into the esophagus. It was also difficult to withdraw the tube from the esophagus, and the capsule had to be carefully placed into the tube to ensure that the direction (thick side first) was correct. The animals in this group showed some signs of distress, such as vocalization, and loud breathing was noted in 2/5 animals one hour after administration on Days 4 and 5. With the Instech flexible tube, flushing with oil or water to eject the capsule was at times difficult due to pressure at the junction between the tube and the syringe, which had a tendency to come apart when the syringe plunger was pushed (therefore a screw syringe was used). The capsule had to be carefully placed into the tube with the appropriate orientation (thin side of empty capsules first and thick side of the coated capsules first) for administration to work properly. Insertion of the flexible tube was easy, and similar to our currently used technique for oral gavage administrations. The animals did not show signs of distress at handling or after administration using the Instech tube method. No regurgitation was observed in either group. Body weight change was not impacted by either administration method, and there were no macroscopic lesions in the digestive tract. In conclusion, using the Instech procedure and flushing with water or corn oil via a screw syringe is considered to be an appropriate method for use in local tolerance studies on the rat gastrointestinal tract.

P16-061
Intravitreal drugs: how define safety limits for high concern impurities

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Intravitreal drug administrations have become an efficient approach to deliver drugs at therapeutic levels. The advantage of this route of administration is an immediate and increased therapeutic effect at the intended site. The intravitreal injection, in fact, is used to administer active ingredients directly into the posterior chamber of the eye, to assure a direct pharmacological effect of the drug and to rapidly reach and maintain pharmacological concentrations. On the other hands, the intravitreal injection route presents several unique challenges. The eye is an extremely sensitive organ, there is a limited collection of excipients acceptable for intravitreal injection compared with other delivery routes. As intravitreal injection is an invasive route, therefore there is always a small but significant risk of infection with each new injection. Moreover, considering the very low doses and volume (less than 0.10 mL per eye), in the setting limits of actual or potential high concern impurities, such as genotoxic or sensitizing impurities as well as elemental impurities, a non-standard approach should be followed to assure safety levels of contaminants in the site of administration.

The relevant guidelines are ICH M7 and ICH Q3D for genotoxic and elemental impurities respectively. Moreover, considering the very sensitive route of administration, a safety assessment of potential or actual sensitizing impurities should be performed, even if not mandatory and formally required by international guidance.

The purpose of the present work was to describe the pragmatic approach employed in Angelini to set specific safety limits of such impurities in intravitreal drugs.

Potential genotoxic impurities were evaluated following the principles outlined by the ICH M7 guideline. However specific safety factors were adopted in defining appropriate safety limits.

Since the ICH Q3D guideline does not provide PDEs for elemental impurities for the intravitreal route, a case-by-case approach was followed to define appropriate limits considering the doses/exposure and the expected local effects.

In addition, a safety evaluation was performed to highlight the sensitizing potential of impurities and specific limits have been set.

P16-062
novel methods for estimating noael confidence bounds and optimising similarity measures for read-across workflows

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Read-across of toxicological information to fill data gaps relies on the efficient identification of analogues associated with high quality data. Analogues are evaluated based on chemical similarity to target and reliability of the study data. Molecular fingerprints have proved to be a key means of identifying structurally similar compounds when applied in similarity measures, e.g., the Tanimoto coefficient. However, current molecular fingerprints are somewhat limited in terms of their mechanistic basis. This study evaluated similarity measures based on various common molecular fingerprints (including Morgan, FeatMorgan, RDKit Topological, MACCS Keys, ToxPrint chemotypes). The performance of the various approaches to determine molecular similarity was assessed in a systematic manner by evaluating the quality of the analogues. Criteria were developed to compare types of fingerprints with regard to: coverage and diversity; information density; consistency of local neighbours; differentiating power between similar and dissimilar compounds; and similarity thresholds. In read-across for repeated-dose toxicity endpoints, the estimation of NOAEL ranges of a target molecule is desired based on study results of analogues. The suitability of the read-across depends on analogue quality and reliability of the study data available for the analogue. Overall molecular fingerprints representing more mechanistic basis. e.g. ToxPrint chemotypes, tend to result higher quality analogues. Subsequently higher quality analogues with reliable study data are in general expected to have lower uncertainty in their NOAEL values. To assess these concepts, a dataset of 900 structures with systemic NOAEL values from repeated-dose toxicity studies was curated from various public sources (e.g., COSMOS DB). Distributions of NOAEL differences for each pair in the dataset were established, and lower and upper confidence bounds of NOAEL values for a target were estimated based on analogues within a given range of similarities to the target. This novel method allows estimation of confidence intervals on the NOAEL value of the target based on well-qualified toxicity data and chemical similarity. This rigorous approach expands the applicability of analogue-based read-across estimations for repeated-dose toxicity.

P16-063
Predictive capacity of the iSafeRat EICM: eye irritation/corrosion prediction model (QSAR)

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Currently, there are no *in vitro* nor *in silico* methods to replace the *in vivo* Draize method [1,2,3] to classify eye irritation UN GHS Category 2 (Cat. 2) substances. IENn the chemical regulatory field *in vitro* methods (*e.g.:* BCOP [1], ICE [2]) cover Cat. 1 (serious eye damage) and chemicals not requiring classification for eye irritation/corrosion (NC) with a "no prediction can be made range". RhCE [3] *in vitro* methods are not able to distinguish Cat. 1 and Cat. 2. *iSafeRat EICM* [4,5] aims to predict both irritation and corrosion potency of chemicals aiming to fill the data gap Cat. 2 for the chemicals in its applicability domain (AD), replacing animal testing.

Herein we compare *iSafeRat EICM*'s predictive capacity to that of *in vitro* methods as stated in the OECD guidelines [1,2,3] assuring the same positive criteria was used for comparison.

Compared to *in vivo* [6] fully validated data, classified according to the UN GHS classification system, *iSafeRat EICM* has a prediction accuracy of 90% (including training and external validation data sets and Cat. 1, 2 and NC substances) within its AD. While the accuracy of *in vitro* test methods ranges between 69–84%. The *iSafeRat EICM* has 98% specificity (*in vitro*: 63–100%), 90% sensitivity (*in vitro*: 63–100%), 2% false positives (*in vitro*: 4–69%) and 10% false negatives (*in vitro*: 0–37%).

iSafeRat EICM's predictive capacity is comparable to the highest performing *in vitro* models within its AD. Furthermore, it can accurately predict Cat. 2, which cannot be identified at all using *in vitro* methods.

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P16-064
Identification and quantification of fragrance allergens in aromas for e-cigarettes

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Electronic cigarettes have been gaining popularity in recent years, although they have been available on the market for over a decade. However, to this day it has not been possible to determine a coherent, supported by scientific research statement about the impact on health of the use of these devices [1,2].

An electronic cigarette is a device whose operating principle is based on heating a special solution (so-called e-liquid) and creating an aerosol that is inhaled by the user. The traditional e-liquid consists of three ingredients: a base – a mixture of propylene glycol and glycerol in varying ratios, nicotine and aroma, which is a mixture of fragrances and flavors compositions, giving the e-liquid a pleasant taste during "vaping". In addition to commercially available ready-made e-liquids, individual components can be easily bought, which allow to create a customized mixture by the user himself.

Aromas (fragrances and flavors compositions), consist of organic compounds (most often aldehydes, alcohols, esters and/or terpenes) of synthetic or natural origin, usually in the form of multicomponent mixtures. In contrast to ready-made e-liquids already containing nicotine, aromas alone are not covered by legal regulations and are not subject to any control system in Poland. Therefore, there is no obligation for manufacturer to specify ingredients on the packaging.

Fragrances are one of the most sensitizing groups of compounds added to cosmetics or food products [3]. In aromas used for preparing e-liquids, these substances are present in high concentrations – they can cause respiratory or contact allergic reactions, which the user, due to the lack of specified composition on the packaging, may not be aware of.

The aim of the work was a qualitative and quantitative analysis of fragrance allergens in aromas used to preparation of e-liquids. The analyzes were carried out using gas chromatography with a flame ionization detector (GC-FID) and gas chromatography mass spectrometry (GC-MS). 40 commercially available aromas with different

flavors were analyzed. The results of the research show the presence of fragrance allergens in the majority of the analyzed aromas.

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P16-065
CLARITY-BPA Study: analysis for non-monotonic dose-responses

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A recently published study sponsored by the European Food Safety Authority (EFSA) described a methodology for evaluating non-monotonic dose responses (NMDR) by assessing study findings according to 6 checkpoints. The publication (Varret, 2018, Toxicol. Appl. Pharmacol. 339:10) suggests researchers consider a meta-analysis of available data when a finding fulfills at least 5 of the 6 checkpoints. This methodology was applied to the results of a large U.S. government-sponsored 2-year bisphenol A (BPA) rat study. This BPA study, called the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA) study, was a collaborative effort between the U.S. Food and Drug Administration (FDA), the National Toxicology Program (NTP), the National Institute for Environmental Health Sciences (NIEHS), and 14 academic scientists. It was designed to address some of the lingering toxicological issues associated with BPA, including its possible role in endocrine disruption and the potential to induce NMDR, by combining standard guideline-compliant research practices (the Core study) with innovative studies conducted by academics (Grantee studies). Within the Core study, rats were exposed to BPA at doses of 2.5, 25, 250, 2,500, and 25,000 µg/kg/day by oral gavage. Treatment and clinical endpoints were examined throughout the 2-year study period. The evaluation presented herein provides additional analyses of statistically significant findings beyond those conducted by the researchers. In the Core study, only 2 of the statistically significant findings met at least 5 of the 6 checkpoint requirements for NMDR. These were clinical chemistry changes in serum: an increase in percent basophils and decreased total bile acids. However, further evaluation showed these 2 findings to not be biologically relevant. In conclusion, this analysis found little evidence for NMDR or biologically relevant changes associated with BPA treatment.

P16-066
Prediction of adverse effects in preclinical subchronic studies by analysis of adverse effects from shorter-term studies using e.g. the RepDose database

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Preclinical animal toxicity studies aim to identify the chemicals short- and long-term functional and morphologic adverse effects. In the interest of reduction of de novo animal testing, we here explore the relationship between the occurrence of short-term effects (subacute treatment period) and adverse effects in longer-term studies (subchronic treatment).

For this approach we used the high quality data from the databases (DB) RepDose (www.fraunhofer-repdose.de) enriched with complementary studies from ToxRef DB (US EPA) and Hess DB (NEDO). This results in a dataset of 37.766 adverse effects from ~2000 chemicals in 970 subacute and 2.360 subchronic studies. The analysis was restricted to 277 compounds, which had at least one subacute and subchronic study. For ~70 compounds the adverse events were reported in same species (Rat) and in a same administration route (dietary) in both long- and short-term studies. Reported adverse events in short-term studies in Rat and dietary were applied as a diagnostic criteria for longer term events.

The investigations were carried out by Bayesian analyses based on the calculation of positive and negative likelihood ratio in KNIME Analytics platform. The sensitivity and specificity of each test were used for determining the diagnostic power of the tests and the diagnostic power was used to identify the connection between subacute and subchronic apical findings.

The investigation showed that many adverse effects in short-term studies can predict adverse outcomes in longer exposure. The realtion of toxic effects in most frequently affected organs such as liver and kidney and clinical chemistry parameters are shown.

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P16-067

This abstract has been withdrawn.

P16-068
Towards an automated workflow for adverse outcome pathway hypothesis: the use case of non-genotoxic-induced hepatocellular carcinoma

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The Adverse Outcome Pathway (AOP) concept as a tool for gathering and linking of information at different levels of biological organization has been largely accepted by regulatory bodies and its usability has been recognized by scientists and regulators. The process of AOP generation, however, is still done manually by experts screening through evidences and extracting probable associations. To facilitate this process and increase the reliability of the findings, we have developed an automated workflow for AOP hypothesis generation.

In brief, high-throughput screening, gene expression, *in vivo* and disease data for chemicals was gathered from ToxCast and the Comparative Toxicogenomics Database (CTD), and subjected to frequent itemset mining to look for relationships between genes, pathways and diseases that co-occur across datasets by using the chemicals as the aggregating variable for the analysis. This was supplemented by pathway mapping using Reactome to fill in gaps and identify events occurring at the cellular/tissue levels. Furthermore, *in vivo* data from TG-Gates (using several time-points and dose levels) was integrated to finally derive a gene, pathway, biochemical/hematological, histopathological and disease information network from which specific disease sub-networks can be queried.

To test the workflow, non-genotoxic-induced hepatocellular carcinoma (HCC) was selected. The first module of frequent itemset mining yielded over 200 genes (from ToxCast and CTD) belonging to approximately 20 major pathways. These were further refined by the inclusion of the TG-Gates module which resulted in the identification of several non-genotoxic-specific HCC-connected biomarker genes,

biochemical parameters and histopathological findings repeatedly deregulated among dose levels and time-points.

With this study, we proved that computational predicted constructs could support the process of AOP development by using pre-existing knowledge in a fast and unbiased manner.

P16-069
A new *in silico* method to predict with high probability the absence of potential for endocrine disruption

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Endocrine disruption (ED) potential of substances is of high concern for human health and environment, as reflected in the updated chemical regulations. Since 2018, Biocide and Pesticide Regulations require examination of ED potential of the active substance and co-formulants, while the definition of ED and ECHA/EFSA guidelines to assess whether substances meet the endocrine criteria were published in 2018 [1]. Under pressure to reduce animal testing and given the complication and cost of studies to determine ED properties, *in silico* methods may be advantageously used. However, screening models are inaccurate and insufficient considering the gravity of the subject.

We have designed an *in silico* battery to predict with high probability the absence of ED potential for a substance (Non EDC) meaning that the substance does not meet the criteria of the best understood ED modes of action (MoAs), *i.e.* related to estrogenic, androgenic, thyroidal and steroidogenic (EATS) modalities as described in EFSA/ECHA guidance [1]. Our approach comprises 3 steps:

1.) identify 2D structural alerts in the chemical structure responsible for the ED MoA, *i.e.* toxicophores. This first model is operational to assess ligands of oestrogen and androgen receptors, mainly based on the data included in the EDKB database [2] (more than 1400 substances). Validation statistics show < 1% false positives (EDCs predicted as Non EDCs).
2.) molecular modelling of the interaction between substances and proteins, *i.e.* molecular docking. Molecular mechanics are used to determine the interaction strength between a substance and known limit conformations of receptors and enzymes derived from co-crystallized protein with agonist or antagonist ligands. This method is still under development.
3.) use of available *in silico* screening models for ED properties. Such models are included in tools like OECD QSAR Toolbox or Danish QSAR Database.

Finally, a consensus of the predictions is obtained via the 3 steps. No alerts for ED MoA means high certainty that the substance does not act with the well understood ED MoAs. If alerts occur, literature searches or further testing to assess the ED properties of the substance is advised. The results of our *in silico* assessment will help orient testing by providing clues of which biological target will likely be disrupted.

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P16-070

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P16-071
Can the battery of *in vitro* and *in silico* methods resolve current deadlocks with skin sensitisation?

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In the context of 3R principles to minimise animal testing, several *in vitro* chemistry-based (DPRA, GSH reactivity) and cell-based methods (MUSST, hCLAT, Keratinosens) have been developed and validated to identify potential skin sensitising chemicals according to the OECD guidelines. However, their application to evaluate the skin potency is still not feasible. Besides, these *in vitro* methods are only able to cover specific events of the skin sensitisation AOP and metabolism is not always into account. Moreover, no formal decision tree is yet adopted on how to combine the results from *in chemico* and *in vitro* methods. More recent methods such as SENS-IS and GARD (currently under OECD validation) have encompassed the limitations of monolayer culture model allowing a better assessment of the sensitisation potency of chemicals.

In silico approaches including read-across and (Q)SAR models are also gaining acceptance within various regulatory frameworks provided they are scientifically valid and respect the recommended OECD principles. In practice, no single (Q)SAR model is currently capable to conclude on the final sensitisation potential of chemicals, however a battery of QSAR predictions including models capable to cover metabolism and mechanisms of action can further assist the classical *in vitro* assessment. This is especially true when the battery results from *in vitro* studies are inconclusive.

This work will discuss various scenarios in which *in silico* methods can be complementary to the *in vitro* assessment to reach final conclusions. This will be illustrated by a case study of Trioctanoin for which no clear conclusions were possible from existing toxicological profile about its safe use as a cosmetic ingredient. Existing experimental studies on this compound and its read-across analogues suggests a negative skin sensitisation potential, although metabolism may have not been taken into account. To get further evidence, we performed an *in silico* evaluation on Trioctanoin as well as its potential metabolites generated using metabolism simulators and mechanism of action tools. Neither the parent compound, nor any of its metabolites were predicted as skin sensitisers. Based on the combined results from *in silico* and *in vitro* studies, we concluded that the skin sensitisation potential of Trioctanion was negative.

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P16-072
Benchmark dose modeling for hematologic effects of occupational benzene exposure

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Benzene is a primary constituent of petrochemical feedstocks used to manufacture other products, and can be present in some petroleum streams. It may cause a specific target organ toxicity to the bone marrow, resulting in effects ranging from subclinical (decreased blood cell counts) to severe (acute myeloid leukemia, aplastic anemia). In the context of developing a Derived No Effect Level it was proposed that utilizing subclinical blood effects as a point of departure would protect exposed individuals from both decreased blood cell counts as well as more serious manifestations of toxicity. Benzene has been reported to affect multiple blood count parameters, while most consistently reported are changes to neutrophil (granulocyte) counts. An innovative approach could combine a benchmark dose approach to blood effects for protection from later, more serious effects. Applying both literature- and effect size-based approaches, we identified a 22% decrease in neutrophil count as a conservative benchmark response. Multiple statistical models in PROAST 65.2 appear to suitably fit the results from Qu, *et al.*, (2003) with a mean value of 12 ppm (BMD) and lower and upper confidence interval values of 1.7 and 44.2 from the Hill model (similar to Exponential 3 model). Given that multiple models provided suitable fits a Bayesian Model Averaging approach could be applied using several approaches.

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P16-073
Evaluation of sexual maturity in the RasH2 mouse model

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The RasH2 transgenic mouse is one of the mouse models accepted by regulatory agencies as an alternative model to carcinogenicity studies. The RasH2 mouse contains multiple copies of the human c-Ha-ras proto-oncogene. This mouse model is one of the most sensitive to both genotoxic and non-genotoxic carcinogens.

Age of the animals is one of the most important parameter for designing toxicology studies and sexual maturity process is critical to distinguish juvenile from mature animals. Despite its increasing use in carcinogenicity studies, little is known about the sexual maturity profile of the RasH2 mouse model. The aim of this study was to evaluate the sexual maturity onset for both RasH2 males and females between 6 and 9 weeks old of age, using histopathological examination of the reproductive organs and sperm analysis.

Histopathological evaluation showed that testicular maturity was already present in 6 weeks old male mice. Consistent with this finding, we did not observe any differences in sperm count between 6, 7, 8 and 9 weeks old animals, suggesting that spermatozoid production is fully efficient at 6 weeks of age in RasH2 males. However, analysis of sperm motility and morphology revealed a significantly lower population of progressive spermatozoids and a lower proportion of normal spermatozoids in up to six out of ten 6 weeks old males, when compared with older animals. Altogether these results demonstrate that RasH2 males are considered to be sexually mature from 6 weeks of age, on the basis of histological and sperm count data, with evidence of functional sexual maturity from 7 weeks of age. In females, histopathological evaluation did not show any significant differences in the examined organs across age.

P16-074
EuroMix handbook for mixture risk assessment

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Focus on risks to human health from combined exposure to multiple substances ("chemical mixtures") has increased in the last couple of decades. There has been a rise in awareness and concern in the community, especially concerning unintentional environmental exposure to unknown chemical mixtures. EuroMix Horizon2020 project has developed methodology and tools for mixture risk assessment and provides a handbook for mixture risk assessment. The handbook is consistent with and expands upon the recent documents on mixture risk assessment published by OECD and EFSA.

The handbook contains concise descriptions of the EuroMix methodology and tools with reference to the EuroMix toolbox. The EuroMix toolbox is a web-based platform where toxicity and exposure data can be uploaded and mixture risk assessment can be performed. Annexes in the handbook provide detailed information or useful templates. Illustrative examples are also included as annexes.

The EuroMix methodology is component-based, tiered and very flexible, enabling assessment of both data-rich and data-poor substances. Substances are grouped based on toxicological considerations in assessment groups. Grouping based on other characteristics can also be applied in the EuroMix toolbox. Toxicity and exposure information for each substance in the assessment group is used for estimation of the combined risk using the dose-addition hypothesis and relative potency factors approach. The concept of adverse outcome pathways forms the basis for the toxicological considerations for grouping as well as for the identification of endpoints that can be measured or predicted to derive toxicity data and relative potency factors. The adverse outcome pathway approach supports the use of *in vitro* data in a tiered testing strategy. *In silico* modelling can be used for grouping and for setting test priorities. The dietary exposure assessment of mixtures is based on probabilistic methodology considering the individual consumption and concentration data and allowing estimation of different percentiles of exposure to the mixture. The EuroMix handbook and toolbox provide practical support to apply the OECD and EFSA guidance on mixture risk assessment.

P16-075
Role of kinetically derived maximum dose (KMD) in top-dose selection for chronic repeated dose toxicity studies

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Based on the importance of toxicokinetic data in understanding systemic exposure, OECD Health Testing Guidance includes and emphasizes that toxicokinetic data can be used to improve selection of doses for repeated dose mammalian toxicity studies. The KMD approach selects a dose-range more relevant for risk assessment purposes. Doses based on toxicokinetic data are quantitatively relevant to real-world human exposures as compared to testing at the limit dose. Since use of the Kinetically-Derived Maximum Dose (KMD) approach can result in test doses lower than those associated with the long-standing conventional Maximum Tolerated Dose (MTD) dose selection approach, challenges have been raised that this potentially compromises identification of health hazards used in regulatory classification and labeling of chemicals. Presentation of case studies associated with KMD *vs* MTD dose selection strategies will illustrate the following: 1) Testing at KMD selected dose levels offers appropriate protection of human health, particularly when knowledge of human exposures is rapidly expanding.; 2) KMD is consistent with current knowledge of dose-dependent transitions of toxicity responses.; 3) KMD evaluations can be retroactively applied to previous classification/labeling/risk assessments based on data from MTD testing.; 4) KMD approach testing honors commitments to reducing animal testing and minimizing animal stress.; and 5) The opportunity to remove inter-and intraspecies uncertainty factors exists with knowledge of systemic dose. Some of the chemicals highlighted will be Sulfoxaflor (route selection), 2,4-D (saturated renal clearance and toxicity), Arylex (pharmacodynamic response), ethyl benzene (post-hoc study analysis), acetaminophen (saturation of metabolic conjugation pathways), and ethyl tertiary butyl ether, afidopyropen (mode of action). These examples will illustrate the importance of understanding systemic dose and toxicokinetics of a chemical and its metabolites in top-dose selection, study interpretation and human relevance.

P17 – Renal toxicology

P17-001
The effect of subacute poisoning with fenpropathrin on TNF alpha and interleukin 1 beta in mice kidneys

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Pyrethroids are insecticides of mainly neurotoxic properties. They are divided into 2 types. Fenpropathrin (FEN) has features of Type I and Type II pyrethroids. There are data that pyrethroids apart from neurotoxic properties, can be also nephrotoxic and immunotoxic.

The aim of the study was to assess the influence of fenpropathrin on kidney function and concentration of proinflammatory cytokines: TNF alpha and interleukin 1 beta in mice kidneys.

16 female mice were divided into two groups: control and the group receiving FEN at the dose of 11.9mg/kg *ip* for 28 consecutive days. On day 29 blood samples were obtained to measure serum creatinine concentration. The animals were sacrificed, and kidneys were obtained in order to measure TNF alpha and interleukin 1 beta in mice kidneys with use of ELISA assay.

The concentration of creatinine was (mean ± SD) in controls 0.2 ± 0.0 mg/dl, in the group exposed to FEN 0.225 ± 0.046 mg/dl. TNF alpha concentration in the kidneys of controls was 6.154 ± 1.597 pg/ml and in the group intoxicated with FEN it was 6.318 ± 1.012 pg/ml. Interleukin 1 beta concentration in the kidneys of controls was 4.67 ± 1.154 pg/ml while in the group intoxicated with FEN 27.983 ± 26.382 pg/ml ($p < 0.05$).

In conclusion: FEN affects kidney function and increases the concentration of proinflammatory interleukin 1 beta in mice kidneys, which supports the hypothesis about nephrotoxic and immunotoxic properties of this compound.

P17-002

This abstract has been withdrawn.

P17-003
Protective effects of *Dendropanax Morbifera* against cisplatin-induced nephrotoxicity without blocking chemotherapeutic efficacy in animal models

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Cisplatin is a widely used chemotherapeutic agent for the treatment of a broad-spectrum of solid tumors. However, its clinical use is limited by occurs acute kidney injury (AKI) in many patients. Despite intensive research, there is no successful protective therapy against cisplatin-induced AKI. The aim of the present study was to investigate the renoprotective effects of *dendropanax morbifera* (DM) on cisplatin-induced AKI and which can be effectively targeted during cisplatin chemotherapy. In the experimental design, four groups of male Sprague-Dawley rats; Control (vehicle); cisplatin (6 mg/kg, i.p.); DM (25 mg/kg, oral) for 5 days; and DM (25 mg/kg, oral) 2 h before cisplatin injection were used. In the present study, injection of cisplatin resulted in reduction of body weight, increased blood urea nitrogen (BUN) and creatinine and pro-inflammatory cytokine levels including IL-6 and TNF-α along with alteration in normal histological architecture of kidney. Urinary excretion of protein-based nephrotoxicity biomarkers such as selenium-binding protein 1 (SBP1), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and tissue inhibitor of metalloproteinase-1 (TIMP-1) also increased in the cisplatin-treated group. On the contrary, DM significantly protected cisplatin-induced nephrotoxicity which was evident by significant reduction of renal injury biomarkers (BUN, creatinine, and KIM-1, NGAL, and SBP1). DM treatment markedly reduced cisplatin-induced oxidative stress in the kidney by increasing endogenous antioxidants activities (SOD and catalase). Further, DM treatment also reduced the levels of pro-inflammatory cytokines. In particular, protective effect of DM was clearly observed in histopathological examination wherein, kidneys from DM treatment markedly reduced cisplatin-induced severe kidney damages in the proximal tubules. In tumor xenograft model, DM did not affect cisplatin-mediated anticancer activity in transfected colon cancer cells, but enhanced the chemotherapeutic activity of cisplatin as well as exhibited protective effects on cisplatin-induced AKI. Taken together, these results demonstrate a protective role of DM in cisplatin-induced nephrotoxicity and support as a reliable strategy used for renoprotective agent during cisplatin-based cancer therapy.

P17-004
A mechanistic model incorporating IVIVE to quantify a proposed AOP on the nephrotoxicity of NSAIDs

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Non-steroidal anti-inflammatory drugs (NSAIDs) are recognised as nephrotoxicants that change intraglomerular haemodynamics and produce an excess of reactive oxygen species and inflammatory changes in the kidney. Adverse outcome pathways (AOPs) have been proposed for the nephrotoxicity of NSAIDs. One AOP is initiated by NSAIDs interacting with organic anion transporters located in the basolateral membrane of proximal tubular cells. There, these substances subsequently accumulate and uncouple or inhibit mitochondrial oxidative phosphorylation which may lead to acute tubular necrosis and acute renal failure. Mechanistic models enable the mathematical description of kinetic processes in defined compartments and hence a better understanding of the concentrations reached in the cell. The purpose of this study was to develop a mechanistic model of the kidney and run it using specific parameters for salicylic acid (SA) to investigate whether a quantitative relationship may be established between the therapeutic doses of SA and toxicity events in proximal tubular cells. The model was parameterised with physiologically based and, when available, kinetic data for SA and related compounds. *In vitro* transporter data were scaled to total kidney tissue level using an *in vitro* to *in vivo* extrapolation (IVIVE) approach. At 2.20 mM, the upper bound of therapeutic SA blood concentration reaching the kidney, concentrations predicted for the proximal tubular cell compartments were between 0.755 and 0.775 mM. The results indicated that at a blood concentration of 2.20 mM the molecular initiating event of adversely effecting mitochondrial oxidative phosphorylation of proximal tubular cells is triggered. At SA concentrations as low as 0.4 mM, permeability transition is observed in rat kidney mitochondria which is triggered by the substance's interaction with the respiratory chain and associated with necrotic cell death. Also, the results showed that the mechanistic kidney model adequately predicts concentrations reached in various parts of the kidney. Validation of the model with additional datasets is necessary to assess the specificity of results.

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P17-005
Overexpression of organic anion transporters in HEK293 reveals high affinity for Aristolochic acid 1

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The herbal derived toxin Aristolochic acid I (AAI), used in traditional medicines and found in contaminated grain products, is considered to be the major cause of Balkan endemic and Chinese herb nephropathy, both associated with renal fibrosis and upper urothelial cancer. Although the carcinogenic potential is attributed to AAI DNA adduct formation, the nephrotoxic mechanism is still under debate. Renal fibrosis is presumed to result from continuously sustained proximal tubular epithelial cell (PTEC) cytotoxicity. Organic anion transporters (OAT), specifically OAT1, OAT3 at the basolateral and OAT4 at the luminal side of human PTEC, are assumed to be important for reaching cellular AAI concentrations critical for PTEC viability.

Thus, the aim of this project was to determine the relative affinity of AAI to OAT1 and OAT3 in comparison to known substrates. HEK293 cells lacking endogenous expression of these transporters were stably transfected with OAT1-, OAT3- or control-eGFP constructs. Confocal microscopy verified localization of OAT1- and OAT3-eGFP to the cytoplasmic membrane, whereas control-eGFP cells demonstrated a ubiquitous intracellular eGFP signal. Western Blot analysis additionally confirmed OAT1 and OAT3 expression with a predicted size of about 120 kDa. Functionality of the transporters was confirmed via the concentration- and time-dependent uptake of radioactive labeled estrone sulfate and fluorescent 6-carboxyfluorescein (6-CF). Competitive inhibition of 6-CF transport with other OAT substrates showed variable affinity of the substrates for OAT-1 and OAT-3, i.e. *para*-aminohippuric acid (IC_{50}: 118 µM and 440 µM), probenecid (IC_{50}: 53 µM and 5 µM), and estrone sulfate (IC_{50}: 428 µM and 5 µM). In contrast to the latter, AAI competed with 6-CF uptake the strongest resulting in relative IC_{50}values of 1.9 and 1.2 µM for OAT1 and OAT3, respectively.

The demonstrated high affinity of AAI for OAT1 and OAT3 strongly suggests that observed PTEC-cytotoxicity stems from AAI (presumably plasma albumin bound) import available from the basal vasculature. OAT4 mediated AAI transport is currently under investigation and will elucidate the contribution of OAT4 for AAI loading from the primary urine, or conversely the evasion of AAI from the cells.

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P17-006
Protective effect of SIRT-1 inhibitor, EX527, against high fat diet-induced nephrotoxicity

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Diabetes nephropathy (DN) is the leading cause of chronic kidney diseases in patients starting transplantation or renal replacement therapy. Previous study indicated that a selective SIRT1 inhibitor exhibits multiple biological functions including antidiabetic potentiality. The aim of this study was to investigate the protective mechanisms of EX527 on high fat-diet (HFD)-induced nephrotoxicity in ZDF rats. The development of DN is clearly observed followed by 60% fat diet for 21 weeks. The changes of body and kidney weights were significantly increased in HFD rats. Total cholesterol, triglyceride, LDL, blood urea nitrogen (BUN), creatinine levels were significantly increased in HFD induced diabetic rats. However, these biochemical parameters were significantly reduced in HFD rats followed by the treatment with EX527. In histopathological analysis, EX527 protected HFD-induced severe kidney injury damage. Urinary excretion of micro albumin and 4-hydroxyproline levels were significantly decreased in HFD rats by EX-527 treatment. Furthermore, urinary secretion of protein biomarkers (KIM-1, NGAL, SBP-1, and vimentin) associated with nephrotoxicity were dramatically reduced in HFD rats by EX-527 treatment. In particular, HFD-induced abnormal levels of oxi-

dative stress molecules (MDA, SOD, Catalase, and GSH) and proliflammatory cytokines were significantly restored after treatment with EX527. Kidney fibrosis biomarkers (α-SMA, TGF-β, vimentin, α-tubulin, fibronectin and collagen-1) was restored significantly followed by the treatment of EX527. The down-regulation of SIRT-1, SIRT-3 and SIRT-4 were noticed in HFD-induced rats, whereas SIRT-3 expression was up-regulated followed by the treatment with EX-527. In conclusion, this study strongly suggests EX527 exerts a protective effect against HFD-induced DN via ameliorating oxidative stress and inflammation.

P17-007
Applying immunoaffinity-proteomics to validate and identify drug-induced kidney injury biomarkers in Cynomolgus monkey's urine

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Drug-induced kidney injury (DIKI) is still one of the major reasons for failure in drug development. This two-phase study was conducted in cynomolgus to evaluate the potential usefulness of novel biomarkers of nephrotoxicity.

First, in a 10-day (D) dose-range finding study, groups of 3 Cynomolgus males received the nephrotoxic antibiotic gentamicin, at dose-levels of 10, 25, or 50 mg/kg/day for 10 days. Urine samples were collected on different days. Minimal to mild proximal tubular injury was histologically confirmed at 10 mg/kg/day while moderate to severe injury was observed at 25 and 50 mg/kg/day, respectively. Several kidney safety biomarkers Osteopontin (SPP1), Cystatin-C, Clusterin (CLU), Retinol Binding protein 4 (RBP4), Alpha-1-microglobulin and Neutrophil gelatinase-associated lipocalin (NGAL) were quantified in the urine samples via a peptide-centric mass spectrometry-based immunoassay panel (IP-LC/MS). In the IP-LC/MS assay, targeted peptides representing the targeted biomarkers are enriched by antibodies which recognize a short epitope motif (TXP-antibodies). As results, SPP1, CLU and RBP4 were best to reflect the nephrotoxicity in the monkey's urine.

Based on the aforementioned results, we followed-up the lowest nephrotoxic dose of gentamicin (10 mg/ kg/ day, n = 6 or 4) for 10 days and a 2-week recovery to explore the efficiency and the sensitivity of the urinary biomarkers. Here, urinary RBP4 was mostly affected with 6 to 19 – fold higher in the treated monkeys versus controls depending on the day of treatment.

This indicate the applicability of the IP-LC/MS assay to detect changes in urine-based proximal tubular injury biomarkers in monkeys. By utilizing our short epitope motif enrichment strategy, the developed assay can be applied in dogs, human, mouse and rat.

Still, not many data about proteome changes in DIKI -monkeys is available. Therefore, we will conduct a toxicoproteomics study to identify novel protein biomarker candidates for monitoring and detecting early events in DIKI. For this, 50 different TXP-antibodies will be selected to fractionate digests of kidney tissue samples collected from the above-mentioned gentamicin low dose -study. The immunoprecipitated peptides will be analysed by high-resolution nLC mass spectrometry to quantify regulated proteins. Applying such approach, we would avoid conventional tryptic fragments appear in conventional bottom-up proteomic studies, by this we aim to maximize our knowledge regarding proteome changes in nephrotoxicity.

The experiment has been conducted in compliance with applicable regulations for tests on animal.

P17-008
Intravenous glutamine infusion is not toxic in partially nephrectomized rats

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Rationale: Intravenous glutamine infusion is contraindicated in patients with severe renal insufficiency (creatinine clearance < 25 ml/min). Clinical trials, however, raised the question whether glutamine infusion is also safe in patients with mild or moderate kidney injury. To address this concern we performed a non-clinical trial in partly nephrectomized rats instead of healthy animals to qualify glutamine from a toxicological point of view.

Methods: 5/6 nephrectomized rats received continuous intravenous infusion of either Dipeptiven® (alanyl-glutamine) or saline for 9 consecutive days. Standard toxicological parameters including clinical chemistry were analysed.

Results: Rats infused with Dipeptiven® only showed transiently increased plasma urea and ALT levels on single occasions during the treatment period, while creatinine levels were unchanged.

Conclusions: This study provides evidence that Dipeptiven® infusion was not toxic in rats with moderate kidney injury and supports the safety of Dipeptiven® administration in this subgroup of human patients.

P17-009
Nephrotoxicity of uranium after low-dose chronic exposure of Nrf2 KO mice

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Uranium is a radioelement present in the environment naturally and also due to human activities. Therefore, exposure of the population to uranium mainly occurs at low dose through drinking water. The kidney is the main target organ of uranium. Biomarkers of U toxicity have been identified but the mechanisms involved in kidney response at low dose are still lacking [1,2].

Pro/anti-oxidative equilibrium is a defense mechanism frequently involved in acute uranium toxicity. However, we have previously shown that a strengthening of this system was observed during chronic exposure to low doses of uranium [3]. A study conducted on animals deficient in Nrf2 (KO), a transcription factor involved in the regulation of the antioxidant system was carried out, via an exposure for 4 months of male and female C57Bl/6N Nrf2 WT or KO mice. Drinking water contamination with uranium (between 1 and 160 $mg.L^{-1}$) leads to an increased uranium tissue content in the kidneys, liver and bones of Nrf2 KO animals compared to WT. It is also higher in females for the 3 organs studied. It results in increased urinary levels of several biomarkers of renal tubular damage and inflammation in uranium-exposed animals (NGAL, OPN, β2-microglobulin and Cystatin C) that is also more pronounced in females. Although the protein levels of KIM-1 and Clusterin are not modified in urines, it appears that exposure to uranium could lead to renal tubular damage. Antioxidant enzymes expression are also modified following exposure to uranium, especially for the highest dose (160 mg/L), but without any no-

table difference between WT and KO. Overall, we show that the tissue accumulation of uranium is Nrf2- and sex-dependent; that biological disturbances are greater in Nrf2-KO animals indicating a role for Redox control, and that females would be more sensitive to the nephrotoxicity of uranium.

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P17-010
Generation and characterisation of induced pluripotent stem cells- derived renal proximal tubular-like cells

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The kidney plays a vital role in whole body homeostasis, via blood filtration, reabsorption of required substances and excretion of excess and waste substances. The proximal tubule region is the major workhorse of the nephron and is also one of the most susceptible regions to injury by xenobiotics. Thus the proximal tubule is an important tissue to assess in integrated testing chemical safety assessment approaches.

The main objective of this study was to explore the possibility of differentiating induced human Pluripotent Stem cells (iPSC) into cells representing a proximal tubule phenotype for application to chemical safety assessment and personalised medicine. iPSC cells were differentiated using a 2-step protocol employing specific small molecules and growth factors. Differentiation was characterised by following the expression of pluripotency markers, renal development markers and proximal tubular markers via immunofluorescence, western blot analysis and RNA sequencing. The data demonstrate a temporal transition from pluripotent tissue, to intermediate mesoderm, renal vesicles and finally to a renal phenotype. The last stage could be maintained for up to 10 days. RNA sequencing was cross referenced with the network biology platform CellNet, which confirmed a renal tissue type and absence of similarities to other organs in the database. The cells were positive for megalin, were sensitive to parathyroid hormone and insensitive to vasopressin which are all characteristic traits of the proximal tubule nephron region. This iPSC derived renal proximal tubular-like model will be further characterised with respect to phase I and phase II metabolism and xenobiotic transport capabilities.

P17-011
Investigation on chemical induced mitochondrial toxicity in human proximal tubular epithelial cells

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The proximal tubule performs constitutive reabsorption of water, amino acids, protein, glucose and ions which is driven by energy dependent Na-K-ATPase. The energy required for this process is generated through oxidative phosphorylation and beta oxidation of fatty acids in the mitochondria. Proximal tubule cells have a high content of mitochondria, which make them especially sensitive to compounds which can injure mitochondria or impair their function. Mitochondrial impairment is a frequent mode of toxicity, that is often identified only late in the drug development pipeline. Thus, there is a need to develop a preclinical screen to identify potential renal mitochondrial liabilities.

The human proximal tubular cell line RPTEC/TERT1 was exposed to 22 electron transport chain (ETC) complex inhibitors of complex I, complex II and complex III. Mitochondrial function was investigated by monitoring glycolysis (lactate production, extracellular acidification rates (ECAR)), mitochondrial membrane potential (MMP) and oxygen consumption rates (OCR, Seahorse Bioanalyser). Transcriptomic studies were also performed using TempO-Seq analysis.

Resazurin reduction in combination with lactate production, the JC-1 assay, the seahorse assay and the TempO-seq analysis performed well to detect mitochondrial liabilities and exhibited similar potency rankings. The data will be used to support the development of a renal quantitative Adverse Outcome Pathway for chemical induced mitochondrial renal diseases, such as Fanconi Syndrome.

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P18 – Reproduction

P18-001
GM stack soybean MON87701×MON89788 reproduction toxicity investigation

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The system of genetically modified (GM) organisms safety assessment in the Russian Federation within the framework of new GM lines state registration includes a large-scale toxicological studies. Since 2011, according to established researcher practice, the reproduction toxicity study of GMO (generative function, pre- and postnatal development) is one of the obligatory stages.

This publication presents the results of GM stack soybean MON87701 × MON89788 evaluation in the *in vivo* reproduction toxicity experiment on Wistar rats. The animals were divided into two groups, fed with rodent diet with inclusion of GM soybean ('test' group) and non-GM near-isogenic counterpart ('control' group) soy varieties. The soy was included into the diet at maximum possible level (~44%) not causing nutritional imbalance or metabolic disturbance for the experimental animals. Rats were monitored for body weight, feed consumption, and general health. The assessment of reproductive system was focused on the generative (indices of mating) and endocrine gonads function of parent animals' and on pre-/postnatal offspring's development. Prenatal development was assessed on 14–15 females of each group, that were euthanized on the 20th day of pregnancy (one day prior to the expected day of delivery). Postnatal offspring development was being assessed during the first month of pups' life (29 and 28 litters in test and control group, respectively).

Analyses of reproductive function (mating efficiency level, ranges of serum estradiol, progesterone and testosterone), offspring prenatal development (number of ovarian corpora lutea, resorptions, implantation sites, number of live and dead fetuses, pre- and post-implantation losses), postnatal development (number of live and dead pups, dynamic of body weight and length, physical developmental parameters) revealed no biologically meaningful differences between

test and control groups. All parameters did not exceed physiological range. The results of the reproduction toxicity assessment along with other biomedical research data indicate the safety of the GM MON87701 × MON89788 soybean stack.

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P18-002
Two-generation reproduction toxicity studies of novel food sources: chronobiologic features

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The procedure of new food sources safety assessment includes a two-generation reproduction toxicity study on laboratory animals. A duration of such studies determines the need of exogenous factors background effects standardization (fluctuations of atmospheric pressure, humidity, geomagnetic activity, etc.) during the experiment. Since the development of adaptation to these factors has been formed throughout the whole period of mammals evolution, the seasonal variability of some physiological and biochemical parameters cannot be mitigated even in the controlled laboratory conditions. Thus, when analyzing the results of the reproduction toxicity experiments it is necessary to take into account the chronobiologic features of laboratory animals.

This publication presents the results of research, that was pointed at investigation of seasonal factors influence on the reproductive system function of Wistar rats. The reproductive function in the autumn/winter seasons and spring/summer seasons was evaluated with the indices of mating, postnatal development of the offspring (number of live and dead pups, dynamic of body weight and length, physical developmental parameters).

All parameters did not exceed physiological range and did not form clearly traceable trends. The indices of mating were ~94% regardless of season of the year. The offspring born in the autumn/winter and spring/summer seasons showed the survival rate as 99.4% and 99.7%, and the males/females ratio in litter as 56/44 and 53/47, respectively. Analyses of body weight and length dynamic also revealed no biologically meaningful differences between groups.

Thus, the analysis of the obtained data did not reveal a correlation with seasonal factors. Values of all studied parameters did not fall outside the limits physiological norm and did not form obviously traced tendencies.

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P18-003

This abstract has been withdrawn.

P18-004

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P18-005

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P18-006
Validation of a novel human stem cell-based gene expression assay for *in vitro* DART assessment

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Testing for developmental and reproductive toxicology (DART) is a crucial part of the toxicological risk assessment. Today, DART mostly relies on animal testing, although alternative *in vitro* tests, such as embryonic stem cells based assays, are used. However, these *in vitro* assays often do not provide mechanistic insight and the results are difficult to translate to human risk due to inter-species differences.

To improve *in vitro* identification of developmental toxicants, we identified potential biomarkers in human induced pluripotent stem cells (hiPSC), marking different developmental stages from pluripotent stem cells to terminally differentiated cells. To test whether compounds affect development, first we optimised the differentiation protocols for hiPSC towards cardiomyocytes, hepatocytes and neural rosettes and confirmed the expression of selected biomarkers (OCT4, BMP4, MYH6, FOXA2, SOX17, AFP, ALB, PAX6) by qPCR. During differentiation, expression of the pluripotency marker OCT4 decreased, while expression increased for matured tissue markers MYH6 in cardiomyocytes, ALB and AFP in hepatocytes and Pax6 during neuronal rosette formation.

Next, we exposed differentiating hiPSC cells to 15 teratogenic and non-teratogenic compounds. We observed a marked downregulation of the cardiomyocyte-specific biomarker MYH6, hepatocyte-specific markers ALB and AFP and/or neural specific biomarker PAX6 during teratogenic compound treatment 5-FU, thalidomide, retinoic acid, diphenylhydantoin, bitertanol, triadimenol and methoxyacetic acid. The late differentiation markers were not affected after mono-butylphthalate treatment, but the early mesoderm specific marker BMP4 was down-regulated. Two potential teratogenic azole fungicides, fluconazole and carbendazim, did not reduce the expression of any of the biomarkers. Three out of five non-teratogenic compounds, acrylamide, dimethyl phthalate and saccharin, did not reduce the biomarker expression in either of the three differentiation protocols and were correctly identified as non-teratogenic

Following the differentiation program by using selected biomarkers allows the quantitative analyses of potential teratogen exposure and provides mechanistic insight into the potential teratogenic mode of action of compounds.

P18-007
Copper nanoparticles alter cell viability and steroidogenic activity of gonadal cells

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With the rapid development and widespread use of nanoparticles (NPs) in many industrial and biomedical applications, the environmental and occupational exposure of humans and animals to NPs is dramatically increasing. The results of recent studies have reported that NPs may pose adverse effects on male and female reproductive health by altering normal testis and ovarian structure, spermatogenesis and sperm quality, oogenesis, follicle maturation and sex hormone levels. The present study aimed to investigate dose-dependent and time-course effects of copper (Cu) NPs of different size on viabil-

ity and steroidogenic activity of ovarian granulosa (GCs) and testis Leydig cells *in vitro*.

The immortalized human GC line COV434, primary GCs isolated from porcine ovarian follicles (3–5 mm in diameter) and mouse somatic Leydig TM3 cells were cultured with Cu NPs of different size (1–50 nm; 0.001–1 μg/ml; 0.4–40 μM) under basal conditions or in the presence of gonadotropins (follicle-stimulating hormone, FSH or luteinizing hormone, LH; both 100 ng/ml) and/or androstenedione (100 nM) for different time periods (24, 48, and 72 h). Cell viability was assessed by MTT and CytoTox-ONE Homogenous Membrane Integrity (LDH) assays. Steroid hormone (progesterone, estradiol, and testosterone) levels in culture media were measured by radioimmunoassay commercial kits.

Treatment of human COV434 and porcine GCs, and mouse Leydig TM3 cells with tested Cu NPs induced a significant concentration- and time-dependent inhibition of cell viability. Exposure of human and porcine GCs, and Leydig cells to Cu NPs altered basal as well as stimulated progesterone and estradiol, and testosterone secretion, respectively by cells after 48 and 72 h of culture. The effects of Cu NPs were dependent on their size and the way of their preparation.

The obtained results indicate that disruption of gonadal cell functional state via NPs may affect steroidogenic output and thus perturb mammalian reproductive function. Possible mechanisms of Cu NPs adverse effects should be further elucidated.

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P18-008
Molecular mechanisms behind blood vessel formation in human *in vitro* cellular vasculogenesis and angiogenesis model and their connection with teratogenesis

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Purpose: New blood vessels are formed by two distinct processes, vasculogenesis and angiogenesis. In this work, the molecular mechanisms behind vasculogenesis and angiogenesis was investigated in *in vitro* model based on co-culture of HUVEC and hASC cells. The model has been validated and standardized for routine use to study inhibitors of blood vessel formation [Toimela *et al*. 2016]. Disturbances in blood vessel formation during embryonal development are one of the main routes leading to embryonal malformations and defects, also notified in OECD AOPs (Project 1.6).

Methods: hASC-HUVEC co-cultures were established and the cultures were stimulated for six days to form vascular structures. Total RNA samples were collected on day 0, day 1, day 3 and day 6 during the process vasculature was formed. The assay included positive and negative (test substance solvent i.e. 0.5% DMSO) controls. The molecular mechanism by which valproic acid, a commonly prescribed drug and known teratogen, inhibit formation of vasculature were investigated using RNA-Seq with next-generation sequencing (NGS) analysis on the RNA samples. RNA-sequencing data was aligned to human genome using STAR aligner, gene expression levels were quantified with featureCounts program, and tested for differential expression between sample groups using DESeq2. Resulting lists of differentially expressed genes were utilized to identify pathways with altered expression using Ingenuity Pathway Analysis software and David functional annotation tool.

Results: The biological pathways behind formation of vasculature were identified. Further, genes and pathways associated to mechanism by which Valporic acid inhibit formation of vasculature were identified.

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P18-009
Hazard identification of pesticide reproductive toxicity – different methodological approaches

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Results of own reproductive toxicity studies of five pesticides in gonadotoxic activity identification test-system were compared with manufacturing firm data obtained in test-systems of two and three generation reproduction toxicity studies in the rats.

For the comparative analysis, the compounds that had a toxic effect on the reproductive system in the test-system for identification gonadotoxic activity but not showing signs of systemic toxicity were selected: α-cypermethrin, mancoceb, metribuzin, pyrimifos-methyl, chloromequat chloride.

In the test-system for identification gonadotoxic activity, the ability to destructive effect on the testes and epididymis morphology and function of sex hormones was revealed in all studied pesticides. This ability characterized by a change in the testes and epididymis weight (α-cypermethrin, metribuzin, pyrimifos-methyl), deterioration of the sperm parameters (pyrimifos-methyl, chloromequat chloride), and violation of the periodicity and duration of the estrous cycle stages in females (α-cypermethrin, mancoceb, metribuzin).

When α-cypermethrin exposed to males, such changes as a decrease in conception and fertility index were noted; mancoceb alters sexual behavior in males, leading to an increase in the duration of the precoital interval when mated with untreated females; metribuzin and pyrimiphos-methyl, when exposed to males, induce an increase in intrauterine death of embryos and fetuses in untreated females.

The following LOAELs of test compounds is established: α-cypermethrin – 2,0 mg/kg/b.w., mancoceb – 25 mg/kg/b.w., metribuzin - when exposed to ♂♂ < 0,4 mg/kg/b.w., and to ♀♀ - 7,5 mg/kg/b.w., pyrimifos-methyl - when exposed to ♂♂ 5,0 mg/kg/b.w., chloromequat chloride - 50,0 mg/kg/b.w.

In the test-system of 2- and 3-generations reproduction toxicity study, α-cypermethrin, mancoceb, metribuzin, pyrimifos-methyl, chloromequat chloride did not show reproductive toxicity.

The presence of endocrine-destructive potential in the studied pesticides is confirmed by the numerous results of independent studies.

The results obtained showed a higher sensitivity, informativity and diagnostic significance of the gonadotoxic activity identification methodology in comparison with the methodology of the 2- and 3-generation reproduction toxicity studies.

P18-010
Irreversibility of non-monotonic and monotonic dose-response curves of pesticide Lambda-Cyhalothrin antiandrogenic effect

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Research methods: Lambda-cyhalothrin (LCT) 98.06% of purity was administered by oral gavage to three groups of animals in doses 0,3; 3,0 and 10 mg/kg of body weight for 11 weeks. After the end of

the exposure period, part of the males was selected to study the parameters of sperm and blood serum testosterone levels, while the remaining males were used for a recovery period without exposure for one full cycle of spermatogenesis (70 days). Morpho-functional indicators of the gonad state and the level of total testosterone in the blood serum were studied in all males after exposure and recovery period.

Results: Tested LCT causes antiandrogenic effect which characterized by impaired of spermatogenesis and oligospermia, as well as a change in the testosterone content in the blood serum of experimental animals. Dose dependence of the severity of oligospermia and spermatozoa adynamia is linear in nature both before and after the recovery period, increasing markedly at the end of the recovery period. While the response level of testosterone to increase of the dose is non-monotonic. The most pronounced significant decrease in the level of testosterone is noted at the end of exposure at a dose of 3.0 mg/kg of body weight. When exposed to the minimum and maximum doses, there is a tendency for this parameter to decrease. After the recovery period, the minimum and maximum doses cause the tendency to increase in testosterone, while the middle dose of LCT, significantly induces a decrease in the content of this hormone.

Conclusions: The analysis of the qualitative and quantitative characteristics of the observed effects at the end of the exposure and recovery periods allows presuming that the tested LCT is irreversible xenoagonists of estrogenic receptors with an intermediate degree of activity, causing damage to Sertoli cells and the spermatogonial population of the germinative cells, depending on the dose level of exposure. The parameters characterizing the processes of spermatogenesis, and the testosterone content did not reach the control level during the recovery period; this indicates the irreversibility of the anti-androgenic effect for 10 weeks, and possibly the complete irreversibility of the observed effects.

P18-011
Optimising the design of minipig embryofetal studies

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Rats and rabbits are the routine species of choice for developmental toxicity (embryofetal development (EFD)) studies. If for any reason these species are found unsuitable (e.g. due to issues of metabolism) another species is chosen, and a commonly used non-rodent species is the minipig. In our laboratories, we have long experience with EFD studies in the Gottingen minipig. When working with non-standard species for studies of this nature, a reliable study design producing robust data is imperative, whilst taking into consideration the requirements of the guidelines. The ICH S5 and OECD 414 guidelines make clear the study designs. We have experience from more than 10 studies and the data generated from these studies enables us to continually review and refine the study designs to ensure reliable and consistent results. Consideration is given to factors such as efficient synchronisation of estrus with the use of Regumate® (altrenogest) to maximise mating success, and we have a pregnancy rate of close to 100%. In this way the required number of pregnant sows for the study can be accurately estimated, eliminating excess and contributing to Reduction in animal use. Further, this also allows precise scheduling of the number of sows mated per day according to the facility capacity to perform the caesarean sections on the required day of gestation. Our mating success and the litter sizes in our studies are superior to the published literature (for example, data from the animal breeder). This poster presents background control animal data for all of the fundamental litter-based parameters and demonstrates the robustness of the methods used.

P18-012
The effects of perfluorooctanoic acid (PFOA) on fetal and adult rat testis

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Perfluorooctanoicacid (PFOA) is widely dispersed synthetic chemical and it accumulates in living organisms. Male reproductive disorders have increased and may have their origin in fetal life. This study was designed to investigate the effects of PFOA on fetal and adult rat testis *in vitro*. Fetal testes (ED 17.5) or seminiferous tubule segments (stage VII-VIII) were cultured in 4 different PFOA concentrations: DMSO only, PFOA 10, 50 and 100 µg/ml for 24 h. Afterwards, cAMP, progesterone, testosterone and StAR protein levels were measured from the fetal testes culture. Apoptotic fetal Sertoli (SC) and Leydig cells (LC) were detected by using immunofluorescence and TUNEL. Number of apoptotic adult testicular cells were detected from squash samples by immunohistochemistry using cleaved caspase-3. Flow cytometry analyze was made for adult testicular cells using vimentin and FxCycle. Present study shows that PFOA has effect on steroidogenesis; the levels of cAMP, progesterone and testosterone as well as the expression of StAR decreased significantly in PFOA 100 µg/ml. Apoptotic cells increased and PFOA affected different testicular cell populations significantly by decreasing the amount of diploid, proliferating, meiotic I and G2/M –phase cells in adult rat testis. PFOA did not affect fetal, proliferating or adult rat SCs. In addition, we detected an increased tendency of apoptotic fetal LCs but the difference was not significant.

P18-013
Activation of sigma-1, MT_1 and MT_3 receptors prevents pre- and postnatal disturbances in rat offspring induced by cigarette smoke and ethanol exposure

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Background: Prenatal maternal smoking as well as alcohol exposure can result in a range of physical, neuropatological and behavioral alterations [1,2]. Fabomotizole (afobazole) is an effective drug with the safety profile for treatment generalized anxiety disorder in Russia, it has pronounced cytoprotective, neuroprotective and antioxidative effect via activation sigma-1, MT_1 and MT_3 receptors [3].

Recently it was shown a strong relationship between DNA damage in the embryo cells during fetal development and cognitive dysfunction in postnatal offspring in the streptozotocin-induced diabetes model prevented by fabomotizole [4]. The aim of the present work is assessment of fabomotizole effects on developmental abnormalities of rat offspring after maternal ethanol or cigarette smoke exposure.

Methods: Pregnant outbred rats were administered ethanol (4.3 g/kg/day, 40% v., orally) from gestational gay 10 (GD10) to GD19. Exposure to cigarette smoke from 4 cigarettes with filter, containing 13 mg of tar and 1 mg nicotine, was performed throughout the pregnancy once a day for 20 minutes in the chambers 72 dm^3. Fabomotizole (1 and 10 mg/kg, orally, daily) was administered 15 minutes prior to the ethanol intake or exposure in the "smoking" chambers. DNA damage in placenta and fetus cells was evaluated on GD13. Rates of embryo- and fetal development were measured on GD20. Parameters of postnatal development were assessed by the unconditional reflexes formation ("turning on the plane" and "avoiding the edge" tests) and

muscle strength ("horizontal rope" test) on postnatal day 5 (PD5). The same animals were examined in the tests "T-shaped maze" and "Extrapolation disposal" to assess congnitive function on PD60.

Results: Cigarette smoke or ethanol exposure led to significant increase in DNA damage in placenta and embryo cells, morphological disturbances of fetuses, retardation of sensory-motor reflexes formation and muscle tone in PD5 offspring as well as cognitive disorders revealed in the "T-shaped maze" and "Extrapolation disposal" tests on PD60 ($p < 0.05$). Prenatal fabomotizole diminished DNA damage in embryo and placental tissues to the level of naïve control, decreased the number of fetuses with abnormal internal organs and impaired ossification and prevented the changes in reflexes formation and muscle strength in the PD5 offspring as well as dose-dependently reduced the disturbances, accelerated adaptation in an unfamiliar environment, and reproduction of cognitive tasks in PD60.

Conclusion: Fabomotizole in the range of anxiolytic and neuroprotective doses via multitargeting action corrects developmental disturbances in rat offspring exposed to prenatal cigarette smoke or ethanol. Thus, fabomotizole is promising for further studies as means of warning of a delay physical development, learning and memory in offspring.

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P18-014
Recent findings on reproductive, developmental and systemic toxicity of propyl paraben show no evidence of endocrine activity

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Alkyl esters of p-hydroxybenzoic acid (parabens) like methyl-, ethyl-, propyl- and butyl paraben are widely used as preservatives in cosmetics, food and pharmaceuticals. It is well known that parabens are rapidly hydrolyzed to nontoxic p-hydroxybenzoic acid, conjugated and excreted through urine resulting in a very low concentration of the parent compound in blood and urine. Although parabens explicitly fulfil all OECD and ECHA RAAF criteria for category formation, a proposal for respective grouping and read-across was rejected by ECHA and individual substance evaluations take place by various MSCAs.

Concerns about potential endocrine activity of parabens which are primarily based on *in vitro* findings indicate an increased endocrine activity of parabens with increasing chain length (methyl- < ethyl- < propyl- < butyl paraben). However, the "potency" remained several orders of magnitude below the activity of the natural endogenous estrogen 17ß-estradiol. Despite clear shortcomings and limitations in available *in vivo* data which is questioning a biological relevance of these *in vitro* findings, methyl-, ethyl- and propyl paraben have been selected to CoRAP as suspected endocrine disruptor and extensive sets of identical higher tier animal studies are required for all three parabens by ECHA. The data requirements comprise *inter alia* subchronic toxicity (OECD 408), developmental toxicity (OECD 414) and full EOGRTS (OECD 443).

The data presented here for propylparaben as example, clearly demonstrate that repeated oral exposure of rats did not result in any finding of toxicological relevance. The NOAELs in these studies were uniformly placed at 1000 mg/kg body weight per day. Even more important, the data do not support any biological meaningful endocrine activity of parabens.

P18-015
Safeguarding food safety: rapid screening of phosphodiesterase 5 (PDE5) inhibitors as adulterants in selected food matrices using enzyme assay

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The global incidence of adulterated food products warrants a faster detection method to address the food safety concern. This study developed an enzyme assay procedure to rapidly screen phosphodiesterase 5 (PDE5) inhibitors found as adulterants in selected food matrices promoted to improve male sexual performance.

The assay used a fluorescein amidite (FAM)-labelled cyclic guanosine monophosphate (cGMP) as a substrate for PDE5 enzyme activity, aided by the presence of phosphate binding beads on its fluorescence polarisation. First, the enzyme assay was validated using certified reference materials of sildenafil. A dose-response-inhibition curve was plotted using a non-linear fit of log inhibitor versus response at a concentration ranged from 1×10^{-4} to 1 µM. Next, three blank food matrices free from any analyte of interest were submitted to the developed assay procedure to verify the interference's effect. The resulting values were utilised as thresholds for positive identification. The applicability of the developed procedure was then established using five samples suspected to be adulterated with PDE5 inhibitors obtained from Malaysia and Australia. Finally, the same samples were submitted to a liquid chromatography-quadrupole time of flight (LC-QTOF) analysis to confirm the adulterants' identities.

The validation results showed that sildenafil inhibits the PDE5 enzyme, exhibiting a symmetrical sigmoidal shape curve with an IC_{50} of 4.3×10^{-3} µM, ensuring the robustness of the assay performance. The results also displayed an excellent enzyme-substrate activity which was deemed fit for application to potentially adulterated samples. The blank samples yielded the percentage of PDE5 enzyme inhibition as 18.2% for chewing gum, 6.6% for hard candy, and 13.8% for jelly. The real samples outcome returned a percentage range of inhibition from 77.3% to 100.6%, indicating the presence of PDE5 inhibitors in all products, in agreement with the confirmatory LC-QTOF analysis.

The procedure proposed in this study provides a rapid screening and straightforward data interpretation to make a quick preliminary decision in separating adulterated and non-adulterated food products. It would be gainful in tackling the problems of food safety, such as adulteration with PDE5 inhibitors, to protect public health.

P18-016
Comparative evaluation of bisphenol A analogues *in silico*

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The growing concern about widely used bisphenol A (BPA) as a chemical that destroys the endocrine system and its possible effects on human health have prompted the exclusion of BPA from consumer products, often referred to as "BPA free". Similarly, structured analogues of BPA are widely used, but much less is known about their potential toxicity or estrogenic activity. Therefore, it is necessary to evaluate such chemicals in order to determine safer ones.

The goal is to conduct a comparative assessment of BFA compounds *in silico* and identify safer substances for further testing *in vitro*.

Methods: *in silico* approach based on OECD QSAR Toolbox.

For the detection of bisphenols in the general list of potentially endocrine disruptive properties, an assessment was made based on data models based on the chemical structure of substances (QSAR-forecasts). Based on the mechanisms described for the properties of endocrine disruption of BPA, and as end points, measures were taken of the potential properties of endocrine disruption of each substance: α-agonism of the estrogen receptor and antagonism of the androgen receptor. It was predicted that out of 100 bisphenols, 75% of the substances are capable of activating the α-estrogen receptor and 65% of the substances are capable of inhibiting the signaling of the androgen receptor (antagonism). Overall, 90% had a positive prognosis for estrogen α-receptor activation or an androgen receptor antagonism (or both). Based on the analyzed literature data on the use of analogues of BPA, those chemicals that are more often encountered during production were selected for more detailed analysis and further *in vitro* testing.

P18-017
Embryotoxicity of Sodium Valproate is correlated to the dysregulation of autophagy

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Valproic acid (VPA) has been clinically used as a traditional first-line antiepileptic drug through many anti-epilepsy drugs emerged, such as sodium phenytoin, phenobarbital, gabapentin, lamotrigine, zonisamide, etc. Epilepsy is a serious chronic and devastating neurologic disorder characterized by spontaneous, transient, recurrent and unprovoked seizures. Current drugs for the treatment of epilepsy include sodium phenytoin, phenobarbital, diazepam, valproic acid, etc. Recent studies showed VPA therapeutic roles on cancer and brain-injure diseases. However, VPA has potential teratogenicity, which is a non-ignorable risk for fetal development when a pregnant woman with epilepsy uses VPA. Therefore, it is important to clarify the teratogenic mechanism of anti-epilepsy drugs. Zebrafish is an ideal animal for studying embryonic development, medicinal toxicology and pharmacology *in vivo*. Autophagy is a host mechanism to maintain intracellular homeostasis and a defense mechanism against invasion by pathogenic microorganisms. Many studies have reported VPA could induce autophagy. In this work, we investigate the correlation between the VPA teratogenicity and autophagy mechanism. Zebrafish embryos were treated with VPA (50, 100 and 200 μg/ml) at three different administration periods (6–10 hpf, 10–24 hpf, 6–24 hpf), and observed under a microscope and collected for western blotting at 48 hpf. The results showed that zebrafish embryos deformed, including deficient cardiac development with pericardial cyst edema, invaginated yolk, short and bent body, small head and eyes and weak color or colorless of body surface. The severity of these deformed phenotypes dependent on the time length and embryonic periods exposed in VPA; the organogenesis is the most sensitive stage in zebrafish. We verified that in zebrafish VPA up-regulated the levels of autophagy marker LC3B-II protein and selective adaptor p62 protein and autophagy related ATG3, ATG5, ATG7 and ATG10 which participated in formation of two ubiquitination complexes for autophagy production. Meanwhile, VPA also activated the apoptosis pathway. These results indicate that the embryotoxicity of VPA probably is resulted from its induction of a deficient autophagy and apoptosis.

P18-018
Extended one-generation reproductive toxicity of Thiamethoxam in rats

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Purpose: Thiamethoxam, a second generation neonicotinoid insecticide was assessed for its systemic and reproductive toxicity, developmental neurotoxicity and immunotoxicity through an extended one generation toxicity study (EOGRTS, OECD No. 443).

Methods: Thiomethoxam was administered to groups of Wistar rats at doses of 15, 50 and 150 mg/kg/day orally. Treatment of males was initiated 2 weeks before cohabitation and continued for 70 consecutive days whereas treatment of females began 2 weeks before cohabitation and continued until weaning of the F1 offspring. In view of reducing the number of animals without compromising on parameters to be assessed, cohort 1A was covered in the parental generation. F1 offspring assigned to cohorts 2A, 2B and 3. Groups of cohort 2A animals were dosed for 70 consecutive days post weaning to assess adult developmental neurotoxicity. Brain histopathology was evaluated in weaned animals (Cohort 2B) for developmental neurotoxicity on lactation day 21. Animals of cohort 3 were treated for 56 days and assessed for developmental immunotoxicity.

Results: No systemic/reproductive toxicity or abnormal changes in fertility parameters (sperm parameters, mating index, pre-coital interval, gestation index, litter size, number of live pups and sex ratio) were observed in parental generation. In Cohort 2A, attainment of puberty was delayed by 4 days in males treated at 150 mg/kg. Examination of reproductive parameters indicated that there was no evidence of treatment related effects that would trigger the need of a second generation. Brain histomorphometry analysis revealed decrease in overall width of hippocampus in males treated at 150 mg/kg. A significant decrease in acoustic startle response (65 to 120 db), total and ambulatory counts at 50 and 150 mg/kg was also observed. In weaned animals (Cohort 2B), no treatment related gross or histopathological findings were observed in brain. Evaluation of developmental immunotoxicity (Cohort 3) of thiamethoxam, did not exhibit the ability to mount an antibody (IgM and/or IgG) response up to 150 mg/kg in a T-cell dependent antibody response functional assay.

Based on the findings, NOAEL of thiamethoxam was considered as 15 mg/kg for development & neurobehavior endpoints and 150 mg/kg for its reproductive & immunotoxic potential.

P18-019
Assessment of a framework to identify analogues for read-across: case study

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Read-across is an alternative method for filling data gaps based on an analogue or chemical category approach. OECD and ECHA developed the most important guidance for read-across, but a methodology is not well defined yet. This study intends to explore the performance of a framework for analogues identification in read-across, through a case of study to predict the human aromatase (CYP19A1) binding of 2-aminobenzothiazol. A set of *in vitro* CYP19A1 binding data was collected from Tox21 as candidates for read-across. An automated framework was developed to select most suitable analogues, based on the evaluation of structural, physical-chemical and biological similarities, and was implemented in KNIME. For the structural similarity assessment Tanimoto index was used as similarity measure. To evaluate the physical-chemical similarity relevant properties were determined. Mechanistic structural alerts for CYP19A1 binding and Rat liver S9 metabolism were used to explore biological similarity. The final list of analogues for read-across was defined by: 1) identifying the intersection between structural and physical-chemical similarities, 2) retrieving only compounds with structural alerts in common with the target and, 3) retrieving only compounds with common metabolites. In the end, two analogues were identified: 2-Amino-6-methoxybenzothiazole and 2-Amino-6-ethoxybenzothiazole. The activity for both analogues was in concordance with the experimental activity of the target confirming the real-life validity of the here presented framework. Deeper analysis must be performed to explore new cases. Different integration approaches, parameters, interactive threshold values, and uncertainties must be consider to refine the framework.

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P18-020
Extended One Generation Reproductive Toxicity Study- EORGTS (OECD 443): How to successfully integrate additional parameters to meet specific regulatory and scientific requirements

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Driven by the motivation to reduce the number of animals used for safety evaluation of chemicals, the EORGTS was developed to allow systemic toxicity evaluation in rats covering a wide range of parameters in the fields of reproduction and developmental toxicity, neurotoxicity and immunotoxicity, including also the evaluation of endocrine disrupting properties of test materials. The OECD 443 guideline together with its corresponding guidance document No. 151 offer details on how to conduct such a complex study but at the same time calls upon the registrant to go beyond and include further parameters if deemed scientifically necessary.

We will present a study design which allows the inclusion of additional offspring in some existing or additional cohorts in order to meet specific regulatory requirement such as the assesment of learning and memory behaviour, which is not a standard requirement of the OECD 443 guideline, and discuss what are the key elements to ensure the success of such an evaluation.

In addition, we will present examples of specific stains that can be used on brain tissue in order to address more specifically potential neurotoxicity (Fluoro-Jade® C and Glial Fibrillary Acidic Protein (GFAP)) when the toxicity profile of similar compounds or literature suggest potential risk for neuronal degeneration. In other spare offspring, specific brain tissues (e.g. hippocampus) or other organs can also be sampled on various postnatal days before or at weaning in order to address dedicated anatomical regions and perform histology or molecular analysis.

Regulatory authorities might ask to demonstrate transfer of test compound to the pups in the milk during suckling. We will present the study designs and methods that can be used to include such evaluation in OECD 443 studies.

Despite the logistic challenge of this type of study, this poster should encourage to further drive refinement using molecular analysis, specific histological techniques and analytical methods to gather always more valuable information and finally develop new approaches for chemical risk evaluation.

P18-021
Effects of paroxetine on biochemical parameters and reproductive function in male rats

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Selective serotonin reuptake inhibitors (SSRI) are a class of molecules used in treating depression, anxiety,and mood disorders. Paroxetine (PRT) is one of the mostly prescribed antidepressant which has attracted great attention regarding its side effects in recent years**.** This study was planned to assess the adverse effects of PRT on the biochemical parameters and reproductive system. Fourteen male wistar rats were randomly allocated into two groups (7 rats or each): control and treated with PRT at dose of 5mg/kg.bw for two weeks. At the end of the experiment, blood was collected from retro orbital plexus for measuring the biochemical parameters, whereas the reproductive organs were removed for measuring semen quality and the histological investigations. Results showed that PRT induced significant changes in some biochemical parameters and alteration of semen quality including sperm count, spermatids number and sperm viability, motility and abnormalities. The histopathological examinations of testis and epididymis revealed an alteration of spermatogenesis, cellular disorganization and vacuolization, enlargement of interstitial space, shrinkage and degenerative changes in the epithelium of seminiferous and epididymal tubules with few to nil numbers of spermatozoa in their lumen. In conclusion, PRT treatment caused changes in some biochemical parameters and sperm profile as well as histopathologic effects of reproductive organs.

P18-022
Teratological evaluation of Artichoke leaf extract in rats

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Artichoke (*Cynara cardunculus* var scolymus) leaf extract have been studied intensively for its antioxidative, hepatoprotective and choler-

etic effects as well as lipid-lowering and anti-atherogenic activity with increased elimination of cholesterol and inhibition of hepatocellular de novo cholesterol biosynthesis. However there is a very little data about its toxicity in commercial preparations and no data is available about its effects on development. The aim of our study was to evaluate the possible teratogenic effect of the dry extract of artichoke leaves in Wistar rats. Intact females were treated, from gestation day (GD) 5 until GD15, with 0.0, 150, 400 or 1000 mg/kg body weight of extract of artichoke leaves. At GD20, a cesarean section was performed for evaluation of maternal and fetal parameters. Artichoke did not induce changes in food consumption, preimplantation or postimplantation losses, placental weight or biochemical profile. Experimental groups showed similar body weight gain during pregnancy. No reductions in fetal and placental weight were observed in experimental groups. The number of live pups per litter was not statistically significant. No fetal skeletal or visceral malformations were detected. Anogenital distance was not influenced. The results showed that the consumption of artichoke during pregnancy did not affect significantly either mother or fetus.

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P18-023
Effect of selective serotonin reuptake inhibitors on the serotonin system and junctional protein in human placenta

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Selective Serotonin Reuptake Inhibitors (SSRIs) are the most common pharmacological intervention for the treatment of antenatal depression. This type of antidepressant works by increasing the level of serotonin at the synaptic space by blocking the serotonin transporter (SERT; *SLC6A4*) on the post-synaptic neurons. The serotonin system, including SERT and monoamine oxidase A (MAOA), is expressed and functional in the placenta. Our group has shown that the exposure of human placenta to certain SSRIs affects the serotonin system and the trophoblast cell fusion and invasion. We hypothesized that the exposure of primary placental cells to the SSRIs alter the expression of genes involved in placental serotonergic system and the junctional proteins associated with trophoblast cells fusion. Thus, this study aims to assess the effects of most commonly used SSRIs in human trophoblasts. Primary villous trophoblasts were isolated from normal full-term human placentas and were exposed at two concentrations (0.3uM and 0.03uM) of fluoxetine, norfluoxetine, sertraline, venlafaxine, or citalopram for 24-h. The mRNA level of *SLC6A4*, *MAOA*, Connexin 43(GJA1), Tight junction protein-1(TJP-1) and Syncytin-1 (ERVW-1) were analysed by RT-qPCR. Overall, our preliminary data shows that all SSRIs tested tend to decrease the mRNA level of *SLC6A4*, *MAOA*, Connexin 43 and Zo-1 in primary trophoblasts, and were greater in the cells treated with the lower concentration (0.03uM) of Citalopram, Fluoxetine and Sertraline than the cells treated at the higher concentration (0.3uM). This preliminary study suggest that SSRI alters the mRNA expression of serotonin system and junctional proteins in human primary trophoblasts. This results need to be confirmed by further assessing the effect of SSRIs on proteins expression and placental function. The use of SSRIs during pregnancy poses adverse effect on the fetal development and may be associated with the pregnancy complications such as gestational hypertension. Thus pursuing the work is important to better understand the effect of SSRI on the placenta which is crucial for the maintenance of normal pregnancy and a healthy fetal development.

P19 – Systemic toxicology

P19-001
The toxicity of triptolide and mechanism involved

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Triptolide (TP) is the main active ingredients in Chinese medicinal herb *Tripterygium wilfordii Hook. F.* (TWHF) that is widely used in China. The toxicity of TP is the main factor limiting its clinical application. Acute toxicity and repeated dose toxicity showed that the LD_{50} of TP is 0.743 mg/kg•bw in mice, and it caused injuries in heart, liver, gastrointestine, male reproductive system. Further studies suggested oxidative stress as the main mechanism of TP-induced organ injuries. Our studies indicated that Nrf2-ARE defense response was involved in the cardiotoxicity, nephrotoxicity, hepatoxicity. Moreover, inhibition of SIRT3 deacetylase, activation of GSK-3β (glycogen synthase kinase-3β) and increased p53 nuclear translocation also contributed to mitochondrial damage of cardiomyocytes. In liver, TP blocked rescue system by inhibiting Notch1 signaling and activating PTEN/Akt/ Txnip (thioredoxin interacting protein), TP disrupted PKD1 (protein kinase D1)/NF-κb/SOD2 (superoxide dismutase 2) signaling, both of which led to oxidative damage in hepatocytes. In addition, accumulating evidences show that TP has obvious toxicity in reproductive system. Our study indicated that TP induced mitochondrial damage and led to cytotoxicity in mice sertoli cells by inhibition of SIRT1 and increased AMPK (adenosine monophosphate activated protein kinase) phosphorylation, which influencing PGC-1α (peroxisome proliferator-activated receptor coactivator-1α) activity and led to the suppression of glycolysis and overactivity of fatty acid β-oxidation. In summary, TP possesses multiple pharmaceutical effects while accompany with a series of toxicities. Its promising potency in therapeutics promotes accumulating molecular research of toxicity, which is beneficial for developing the strategy in ameliorating its toxicity.

P19-002
'Notch or Not' – mystery of an unexpected gastrointestinal toxicity of a gamma secretase modulator

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Gamma Secretase (GSEC) is a key enzyme in the metabolism of the transmembrane protein Amyloid precursor protein (APP). Proteolysis of APP generates beta amyloid (Aβ), whose amyloid fibrillar form is the primary component of amyloid plaques found in the brain of Alzheimer's disease (AD) patients. Therefore, GSEC is explored as a therapeutic target to decrease toxic Aβ formation in AD. GSEC also catalyzes the cleavage of Notch, receptors of a highly conserved cell signaling system with important regulatory function in cell differentiation. Initially developed inhibitors of GSEC showed safety profiles unacceptable for AD patients, mainly due to interaction with Notch signaling pathways. Modulators of GSEC can reduce Aβ formation via a conformational change of the binding site without inhibiting the enzymatic activity, thus entailing selectivity versus Notch and other GSEC substrates, and a higher likelihood for a beneficial safety profile. The preclinical development of a small molecule GSEC modulator included toxicity studies in mice and minipigs. While the 2-week dose-range finding studies in both species and the 4-week GLP toxic-

ity study in mice were uneventful, there were unexpected histopathology findings in the minipig 4-week GLP toxicity study raising concern for Notch-signaling (predominantly goblet cell hyperplasia/metaplasia in small intestine). Since GSEC modulators are not supposed to inhibit Notch signaling and the rodent study did not indicate any of otherwise typical Notch-related side effects, a mechanistic workup of the minipig findings was undertaken, including transcriptome profiling of affected intestinal tissue. The principal affected genes were identified as downstream Notch, involved in enterocyte differentiation (e.g., ATOH1 and SPDEF increased). Yet, the overall phenotype (pathology and transcriptome) was clearly different from typical signatures of GSEC inhibitors (e.g., HES1 remained unchanged for the modulator). These results underpin principal differences between modulators and inhibitors of GSEC. Seemingly overlapping pathological effects need to be understood at a molecular level to guide an improved screening and profiling of back-up molecules.

P19-003

Exposure to an aerosol generated by a novel electronic cigarette using *MESH*™ technology causes lower biological alterations than cigarette smoke on buccal organotypic epithelial cultures

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Electronic cigarettes (EC) are growing in popularity, although their impact on human health is still debated. It is therefore important to improve understanding of their effects, particularly in the context of tobacco harm reduction strategy. In this study, we investigated the impact of EC aerosol in comparison with that of cigarette smoke (CS) on human organotypic buccal epithelial cultures. Cultures were exposed to 112 puffs of undiluted aerosol generated from a variant of a novel EC device with *MESH*™ technology (Philip Morris International) or to diluted CS. Nine independent exposure repetitions were performed to ensure robust observations. A systems toxicology approach was applied to study the impact of exposure; a series of endpoints were analyzed, including histological modifications, global mRNA and miRNA expression, secreted miRNA and inflammatory mediator expression, and targeted and untargeted proteomic approaches. Histological evaluation showed minimal morphological changes in cultures exposed to undiluted EC aerosol but major damage in those exposed to CS. Lower alterations in mRNA, miRNA, and protein expression were detected in cultures exposed to EC aerosol than in those exposed to CS. The inflammatory mediators secreted following EC aerosol exposure were distinct from those following CS exposure: IL-1α secretion was enhanced following EC aerosol exposure, while IL-1β was highly induced following CS. The inflammatory mediators secreted following EC aerosol exposure were distinct from those following CS exposure: IL-1β secretion was highly induced following CS exposure, but IL-1α secretion was enhanced following EC aerosol exposure. RNAScope® technology was further used to localize the expression of *IL1A* gene, showing no difference in the expression and localization of *IL1A* in the EC aerosol-exposed cultures compared with the air-exposed control. Interestingly, increased apical expression of the *IL1A* gene was detected in the CS-exposed cultures. Overall, the results indicated that EC aerosol exposure did not elicit tissue damage in contrast to CS exposure at comparable (and higher) nicotine concentrations. Molecular changes were detected in the *in vitro* buccal epithelial cultures following EC aerosol exposure; however, the impact remained generally much lower than CS exposure.

P19-004

Predicting systemic concentrations following topical application using Physiologically Based Kinetic modelling

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Physiologically Based Kinetic (PBK) models are routinely used to predict human kinetic profiles of compounds. Using PBK models it is possible to predict systemic concentrations of xenobiotic compounds from different routes of exposure. Combined with dose-response toxicology data from appropriate *in vitro* studies this has application in assessing risk of systemic toxicity. The purpose of this study was to assess the accuracy of a PBK modelling approach for predicting plasma concentration time-profiles of topically applied compounds when the input data was generated using non-animal methods. Prediction of maximum plasma concentration (Cmax) and area under the curve (AUC) were compared to the observed kinetics from published clinical studies.

Plasma profiles following topical application were predicted using Gastroplus 9.0 PBK software for six compounds (diclofenac, salicylic acid, coumarin, nicotine, caffeine and N,N-diethyl-m-toluamide), and compared with existing clinical kinetic data from topical application studies. Dermal absorption was determined from *ex vivo* human skin penetration studies. Hepatic clearance was determined using primary human hepatocyte suspension cultures. Plasma protein binding data was taken from the literature or generated using rapid equilibrium dialysis. In all cases experimental data was used from the literature where available but generated if not.

The calculated and clinically observed Cmax values were in good agreement ($R^2 = 0.81$). The comparison between calculated and observed AUC_∞ are of similar accuracy ($R^2 = 0.84$).

The combination of *in vitro* data generation and *in silico* PBK modelling showed good accuracy for predicting human Cmax and AUC values within an order of magnitude for all six compounds. Given the small number of compounds and due to the lack of topical clinical studies reporting measured kinetics, additional data and approaches are required to improve the confidence in predicting systemic concentrations of topically applied compounds. However, the results of this study suggest that PBK modelling is a suitable approach for estimating the internal concentration of compounds applied to the skin.

P19-005

Risk assessment of methanol in consumer products

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Methanol also called methyl alcohol with chemical formula CH3OH. Methanol is classified as an alcoholic substance such as ethanol and have similar properties. It is a colorless liquid and has combustibility, volatility and toxicity. However, methanol contains less carbon and hydrogen than ethanol, and the boiling point of methanol is lower than ethanol. Methanol is converted from the liver to formaldehyde which may be fatal to humans. In addition, methanol produces metabolic acidosis (nausea**,** headache), central nervous system (CNS) depression, ocular toxicity and even death. Because of these toxicities, the Ministry of Food and Drug Safety (MFDS) is regulating methanol that is rapidly absorbed into the body (skin, mouth and lung) via consumer products. MFDS consequently set the limit concentration of methanol at 0.2 percent and 0.002 percent, in cosmetic products and wet wipes, respectively. To carry out risk assessment, systemic exposure dosage (SED) was estimated to be 0.0016 mg/kg/day and

0.0006 mg/kg/day respectively in cosmetics and wet wipes, with a total SED is 0.01606 mg/kg bw/day for adults. Also, SED was estimated to be 0.064 mg/kg bw/day and 0.00024 mg/kg bw/day respectively, with a total SED is 0.06424 mg/kg bw/day for children. NOAEL of methanol was found to be 500 mg/kg bw/day in rats, but the modified NOAEL was estimated to be 415 mg/kg bw/day (500 mg/kg bw/day x 0.83) because of the oral bioavailability of 83 percent. The margin of safety (MOS) for methanol in cosmetics and wet wipes was calculated to be 25841 and 6460 based on 415 mg/kg bw/day (NOAEL)/0.01606 mg/kg bw/day (SED) and 415 mg/kg bw/day (NOAEL)/0.06424 (SED) mg/kg bw/day, respectively. These data suggest that methanol has no risk to human when it is exposed to 0.2% and 0.002% of the finished cosmetics products and wet wipes, confirming its safety.

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P19-006
Next generation risk assessment of coumarin in personal care products

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Next Generation Risk Assessment (NGRA) is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates one or more new approach methodologies (NAMs) to ensure the safety of consumer products without the use of animal testing data. The International Cooperation on Cosmetics Regulation (ICCR) principles [1] were applied to a hypothetical safety assessment of 0.5% coumarin in face cream or shampoo. For the purpose of evaluating the use of NAMs, existing animal and human data on coumarin were excluded. Exposure calculations using specific consumer habits data were used to build a physiologically based kinetic model for dermally applied coumarin. For the systemic toxicity assessment, a battery of *in vitro* NAMs were used to identify points of departure (PoDs) for a variety of biological effects such as genotoxicity (ToxTracker®), receptor-mediated and immunomodulatory effects (Eurofins Safety44™ screen and BioSeek® Profiling, respectively), and non-specific pathways/general bioactivity [ToxCast data, *in vitro* cell stress panel and high-throughput transcriptomics (HTTr)]. A novel statistical Bayesian approach was applied to both the cell stress panel, HTTr and Toxcast dose-response data. The PoDs from the *in vitro* assays identified as demonstrating a dose response were plotted against the calculated *in vivo* exposure (C_{max} with associated uncertainty) in order to calculate a margin of safety (MoS). From these results, we concluded that coumarin is not genotoxic, does not bind to any of the 44 receptors or shows any immunomodulatory effects. The most sensitive PoD was the No-Observed-Transcriptional-Effect-Level (NOTEL) which ranged between 2.6-12.4 µM across different cell lines (MCF7, HepG2, HepaRG). The predicted C_{max} values for face cream and shampoo were lower than the all PoDs. However, the lower predicted C_{max} for shampoo (0.04 µM compared with 0.4 µM for face cream) results in a MoS that can be more confidently used to assure safety. Further refinements to the risk assessment are discussed. This case study demonstrates the value of integrating exposure science with computational modelling and *in vitro* bioactivity data that form the basis of non-animal safety assessments.

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P19-007
Development of *in vitro* hepatotoxicity assessment system to predict the toxicological potential of cosmetic raw materials

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Safety assessment system of cosmetic raw materials is required after animal test of cosmetic compounds was prohibited at 2009 in Europe. It is so important to construct that the proper assessment of systemic toxicity is needed. However, liver toxic data of human is absolutely lacked and animal test of cosmetic compounds is fully prohibited, it is difficult to examine the liver toxicity risk. Clinical information is abundant in pharmaceutical products compared to cosmetic material and some mechanisms of drug induced liver injury (DILI) are reported. The combination of mitochondrial toxicity and cholestasis is useful to predict DILI risk 1). In this study, we optimized the prediction system of DILI and applied this system to predict DILI risk of cosmetic raw materials. We constructed *In vitro* assay system using HepG2 and sandwich cultured human hepatocytes based on 1) the toxicity caused by mitochondria dysfunction, 2) cholestasis, 3) the inhibition of bile canaliculi formation, and 4) the accumulation of lipid droplet in 55 drugs (DILI classification; most concern 19 compounds, less concern 27 compounds and no concern 9 compounds). Next, we tested ANN analysis based on *in vitro* assay and optimized algorithmic program to predict liver toxicity in clinical. We preliminary applied these test systems and algorithmic program to cosmetic compounds.

From the inhibitory potency of drug in these four *in vitro* assays, the optimal algorithm was built by using artificial neural network (ANN) technology to give the highest accuracy of DILI concerns (overall accuracy: 73%). Although we excluded the assay of intrahepatic lipid accumulation from the final algorithm to avoid "overfitting" ,the accuracy of DILI concerns prediction in the algorithm applying the ANN (overall accuracy: 62%). Moreover, among 55 drugs, there was no false predictions ("Most concerned drugs" as "No concern" and "No concern drugs" as "Most concern") in the final algorism with three *in vitro* assays. In our mechanism-integrated *in vitro* prediction method is useful approach to predict the risk of DILI of drug candidates.

In conclusion, the combination of these cell-based assay is useful to recognize drugs classified high DILI risk. In addition, the predictability of liver injury is improved by using algorithmic program.

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P19-008

This abstract has been withdrawn.

P19-009
The UK Committee on Toxicity: Review of chemicals in the diets of infants and children aged 0 to 5 years

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As part of an ongoing review of scientific evidence that will inform the UK Government's updated dietary recommendations for infants and young children up to 5 years, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) are reviewing the risks of toxicity from chemicals in the diet of this population. Parallel work considering benefits of relevant chemicals is being undertaken by the Scientific Advisory Committee on Nutrition (SACN). The chemicals assessed in 2018 include alcohol, caffeine, food additives, legacy chemicals, soya phytoestrogen, vitamin A, trans fatty acids, perchlorate, chlorate, furan and methylfurans, chromium, selenium and zinc.

The toxicity of these chemicals was reviewed along with the basis of published health based guidance values (HBGVs) or other reference values. Where applicable, exposure assessments were undertaken using UK occurrence data, either from Total Diet Studies (TDS) or Food Standards Agency (FSA) surveys and UK consumption data from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey (NDNS). Calculated dietary, including breastmilk, exposures for the chemicals were either compared to the respective HBGVs or were used to calculate the margin of exposure (MOE) for risk characterisation.

The COT refers to and confirms its previous evaluations for legacy chemicals, soya phytoestrogens, vitamin A and for caffeine and alcohol in pregnant and breastfeeding women. Additives and trans fatty acids were outside the remit of the COT, exposures of chromium, selenium and zinc are not of toxicological concern. The data collected by the FSA on perchlorate and chlorate has been submitted to and forms part of EFSAs evaluations. In agreement with EFSA, the COT concluded that while there are considerable uncertainties in the assessment there is potential concern from dietary exposure to chlorate and perchlorate in infants and young children. The exposures to furan and methylfurans are of potential toxicological concern, however, there are numerous uncertainties in the assessment and the COT acknowledges that its assessment is based on worst case assumptions. Efforts to reduce concentrations of furan (and methylfurans) in the diets of infants and young children should continue.

P19-010
Estimation of Acceptable Ranges of Hematological Parameters in Wistar Rats for a better understanding of Adverse and Non-Adverse Effects of Test Substances in Toxicity Studies

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The aim of the present study was to define acceptable ranges for hematological control parameters which are essential for the evaluation of health effects and the derived impact of different test substances in toxicological studies. It can be shown that the health status of control animals after study completion could have much higher deviations than expected. Due to the differences in health status it is important to define acceptable ranges to generate a better understanding of adverse and non-adverse effects of test substances.

After generating a set of historical control data of two Wistar rat strains (RccHan^{TM} WIST and Crl:WI(Han)) from different breeders, the data sets were statistically analyzed using minitab. As far it was feasible in a first step, outliers were identified and afterwards both data sets were compared using t-test analysis.

It was noticed that in some cases outliers can affect the set of study control data thus the respective outliers were verified based on the available histopathological findings. Several of these outliers had corresponding histopathological findings such as pulmonary or sperm granuloma, and based on these were excluded from the control data set. Comparing both data sets it can be shown that also the different methods in blood sampling and anesthesia as well as the fact that the animals were derived from different breeders result in an offset between both hematological data sets.

It can be shown that even animals from control groups, which should be healthy, could have large differences in their current health status, and can alter, due to control group comparison, the whole study outcome. By excluding all the outliers a data set from animals with a presumably good status in health was generated. The acceptable ranges were defined as mean value ± 2 standard deviations. Values which were higher or lower than the defined acceptable range therefore can indicate adverse effects of test substance exposition.

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P19-011
Rat myocardium contractility changes associated with a subchronic lead intoxication

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Chronic lead intoxication in humans is known to induce arterial hypertension and thus can lead to some secondary disturbances of the heart function. However, no proof has yet been produced to show that this intoxication affects myocardium contractility. The purpose of this work is to investigate the effect of lead intoxication on myocardial contractility.

Male outbred rats with initial body mass about 295 g were injected intraperitoneally with lead acetate (12.5 mg of Pb per kg body mass) 3 times a week for 5 weeks; the controls were receiving injections of sterile distilled water of the same volume. Cardiotoxic effects on myocardial contractility were studied by mechanical activity analysis of right ventricular trabeculae and papillary muscles isolated preparations that were put to contract in isometric, isotonic and physiological regimes of the contraction-relaxation cycle. Myocardial contractile function was also studied at the molecular level by measuring the sliding velocity of reconstructed thin filaments over myosin using an *in vitro* motility assay.

Rats developed an explicit, even if moderate, lead intoxication characterized by typical hematological and other impairments. Subchronic lead intoxication caused myocardial preparations of varying lengths to respond by decreased time and speed parameters of the isometric contraction while maintaining its amplitude and by a decreased passive stiffness of the trabecules. Lead intoxication led to a decrease in the maximal rate of isotonic shortening for all afterloads in papillary muscles and a decrease in thin filaments sliding velocity in *in vitro* motility assay. The later can be explained by the

established increase of slow myosin isoforms. Muscles of the same type collected from the lead-exposed rats displayed marked changes in most of the main characteristics of afterload contraction-relaxation cycles, but in trabecules were less pronounced. These changes were attenuated to some extent in lead-exposed rats treated with a Ca-containing bioprotector. The amount of work produced by both trabecules and papillary muscles preparations was unchanged by lead intoxication over the entire range of afterloads, which is evidence of adaptation to the production of adequate mechanical work despite resulting contractility disturbances.

P19-012
Prediction of endocrine disruption via QSAR modeling of androgen, estrogen, and aryl hydrocarbon receptor binding

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Endocrine disrupting chemicals (EDCs) are substances in food, environment, and consumer products that interfere with the body's endocrine system and cause various developmental, reproductive, and neurological effects. Estrogen and androgen receptors are nuclear hormone receptors responsible for some of these effects. OECD proposed various guidelines like (OECD TG 493), (OECD TG 455, ISO 19040-1 & 2), and OECD TG 458 for evaluation of endocrine disrupting chemicals. ECHA and EFSA, with the support of Joint Research Center (JRC), developed guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

Various QSAR models were built in the past for predicting binding affinity of endocrine disruptors to different receptors. Most of these models have smaller training sets and frequently have high predictivity but poor interpretability and low coverage. To investigate the ability of fragment based QSARs to effectively predict endocrine disruption with good interpretability, we developed models based on the interaction with different cell lines. The models are large and can be made publicly available. All the models can identify structural alerts in the query chemical.

Models related to various cell lines like HEK293, MDA-MB-453, BG1, HepG2 with alpha and beta receptors were built and evaluated using CASE Ultra software. Models were built to identify agonist, antagonist, and general binding activity. High throughput screening data related to endocrine, androgen, and aryl hydrocarbon receptors was collected from public sources. The data is based on rat and human kidney, breast, and ovarian cell types. The training data set size ranged from 885 to 20763 compounds. The ratio of positive and negative compounds is approximately 1:2. All models demonstrated external set validation metric in the range of 61–94% sensitivity, 61–87% specificity, 56–76% positive accuracy, 80–96% negative accuracy. With bootstrap cross validation, models exhibited 63–94% sensitivity, 56–92% specificity, 60–85% positive accuracy, 78–95% negative accuracy. Thus, we successfully built highly interpretable QSAR models for androgen, estrogen, and aryl hydrocarbon receptors to predict agonists, antagonists, and general binding activity.

P19-013
Biological safety evaluation of Ti-Nb-Zr dental implant fixture in rabbits

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BTS3S5018SW and its 31 types (Osstem, Korea) are dental implant fixtures to be used as a root for dental implants in restoring molar teeth. For human application, their toxicity must be assessed in *In vivo* model systems to ensure their biological safety. In this study, we tested systemic toxicity and local irritation of the dental implant fixtures by implanting a representative Titanium (Ti)-Niobium (Nb)-Zirconium (Zr) specimen into the tibias of New Zealand White rabbits. During the 13-week observation period, no abnormality was found in mortality, clinical symptoms, behavior, body weight, and feed/water consumption of all the animals implanted with the test specimen compared to the Titanium control specimen-implanted group. Also, no changes were observed in the hematological, serum biochemical, urinary, and ocular examinations performed during the test or at necropsy. Among the major organs examined, the absolute weight of lungs from the female test specimen-implanted group was significantly lower than the control group, but the change was found to be within the historically normal range, and there was no difference in the relative organ weight between the two groups. In gross examination, discolorization in the lungs, ovaries and adrenal glands was similarly noted in the two groups without histopathological significance. For all other organs, no specific legions related to the test specimen were observed in gross and histopathological examinations. When examining the implantation sites of the test specimen in non-decalcified bone tissue slices, the lesion score calculated according to ISO 10993-6: 2016 (E) was zero in both genders of rabbits, indicating that the test specimen is a non-irritant. Taken together, these findings demonstrate that the Ti-Nb-Zr test specimen did not cause systemic toxicity nor local irritation in rabbits under the test conditions in this study, suggesting that BTS3S5018SW and its 31 types are biologically safe for human application.

P19-014
CeleScreen: Innovative method of assessing toxicity in whole organism

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The nematode *C. elegans* has emerged as an important animal model for drug discovery. Nevertheless, it has been thought to be a poor candidate for drug testing due to the relatively inefficient drug uptake caused, *inter alia*, by the impermeability of the cuticle to non-water-soluble compounds. To circumvent this obstacle, *CeleScreen* implements specific carriers for testing the effect of drugs by bringing them directly into *C. elegans*. This method favors the ingestion of almost all drugs, whatever the appetence of the nematode for each, and moreover, at lower dose compared to earlier trials. This is very important as it allows for assaying molecules at a "physiological" condition. We have recently proved the concept by demonstrating that methotrexate (MTX), a potent teratogen, encapsulated into metal-organic frameworks (MOFs) was physiologically administrated into

the worm resulting in severe teratogenesis effect. Analytical analysis showed that the effective dose of MTX needed is far less using our technology compared to conventional delivery method. We are currently testing the efficiency of our protocol with other drugs eliciting toxic effect on different worm phenotypes. To this end, *CeleScreen* develops 2 different business areas: 1/ Provide services for molecule toxicity screening or activity testing using our proven and patented technology; 2/ Offer solutions for 'on-demand projects' by developing *C. elegans*-based assays for outcome investigations, quantitative analysis and biochemical analysis.

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P19-015
Acute and 90 day sub chronic oral toxicity study of herbal medicine containing *Abri folium*, *Licorice*, *Thymi herba*, *Chrysanthemi flos, and Imperatae rhizoma* in rats

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Herbal medicine containing *Abri folium*, *Licorice*, *Thymi herba*, *Chrysanthemi flos, and Imperatae rhizoma* has been used for aphthous stomatitis treatment empirically. Our previous study showed the anti-aphthous stomatitis activity of the herbal medicine in rat. However, there were not any toxicity data from the herbal medicine. This study was conducted to evaluate the acute and 90-day sub chronic oral toxicity of the herbal medicine in rats. The fixed dose method of acute toxicity study was conducted on 5 female Wistar rats. The preliminary study with dose of 2000 mg/kg did not show any toxicity signs and symptoms. So that, the study continued at dose of 2000 mg/kg on other 4 rats. There were no significant toxic effects and no death observed until the end of 14 day of the study, showed that the lethal dose 50% (LD50) of the herbal medicine was >2000 mg/kg. At the 90-day sub chronic oral toxicity study, 80 rats of both sexes were divided into 4 groups (n=20, 10 males and 10 females), 3 treatment groups and 1 control group. The 3 treatment groups received 204.7, 409.5, and 1000 mg/kg of herbal medicine respectively. The control group received aquadest 10 mL/kg. The herbal medicine or aquadest was given once daily for 90 days. The rats were observed for physical signs and symptoms to the possibility of the poisoning. The average daily gain was determined by measuring the body weight every week. The average food and beverage intake, the production of urine and feces was measured per day. The laboratory examination for routine blood tests, renal function, liver function, were performed on day-0, day-45th, and day-91. Macroscopic and microscopic examination for the vital organs was performed after the study or as soon as possible after the rat died during the study. There were no significant toxic effects observed at all doses on physical observation, macroscopic and microscopic examination. These findings showed that herbal medicine containing *Abri folium*, *Licorice*, *Thymi herba*, *Chrysanthemi flos, and Imperatae rhizoma* were included in unclassified criteria with the LD50 value was higher than 2000 mg/kg. The administration at dose up to 1000 mg/kg once daily for 90 consecutive days on sub chronic toxicity study did not result in death, did not cause toxic effect symptoms that could be observed based on clinical, laboratory, macroscopic and microscopic examination.

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P19-016
To the mechanism of combined action of the plant growth regulator of 2,6-dimethylpyridine-N-oxide (Ivin) and pesticides

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Today the actual problem of toxicology is the determination of the nature of the combined action of pesticides and plant growth regulators (PGR), the mechanisms of their biological action, which will contribute to the development of preventive measures on the harmful effects of pesticides for humans.

The acute isolated and combined oral toxicity of some pesticides and PGR 2,6-dimethylpyridine-N-oxide (Ivin) in Wistar Han rats was studied (OECD 425). The combined effect of Ivin and pesticides was determined at isotoxic doses corresponding 1LD50 and 1/2 LD50 (in ratios 1:1). The type of combined action was determined by the formula Finney D.J. Protective properties of Ivin (710–0.071 mg/kg bw) against cytotoxicity and clastogenicity of cyclophosphamide (40 mg/kg bw) by the degree of chromosome aberrations of in bone marrow cells of mice were studied (OECD 475). The level of hybridization of mRNA in blood and liver tissues of rats at isolated and co-actions of 2,4-D-EHE (540 mg/kg bw) and Ivin (13 mg/kg bw) was determined by modificated method of DOT-blot hybridization.

It`s established, antagonism was observed for acute action of Ivin combination with 2,4-D EHE, Tebuconazole, Difenoconazole, Thiamethoxam, Chlorpyrifos (coefficient of additivity C_{ad} = 0.43–0.83), additive toxicity – with Imidacloprid (C_{ad} =1.0), potentiation of toxicity – with Flutriafol (C_{ad} =1.49).

It's shown, Cyclophosphamide induces a frequency of chromosome aberrations in mice bone marrow cells (14.00 ± 1.55%, control of 0.20 ± 0.19%), Ivin does not exhibit mutagenic activity. At their combined effect in all studied doses the aberrant cell level caused by Cyclophosphamide was reduced by 64.3–75.9%.

With isolated ingestion, Ivin increases the level of hybridization of mRNA in the blood and liver of rats by 69% and 45% and 2,4-D-

2-EHE reduces its by 14 and 25%, respectively. When combined action, Ivin negates the effect of 2,4-D-2-EHE on the mRNA population, as evidenced by the tendency to normalize or completely restore the level of mRNA hybridization from rat liver and blood.

Thus, Ivin for a combined action with pesticides reduces their toxicity, contributes to a decrease in the clastogenic action of Cyclophosphamate, activates the mRNA-synthesis. One of the mechanisms of combined action can be the protective properties of Ivin at the genome level.

P19-017
Gaining insight into toxicity predicting machine learning algorithms

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Molecular initiating events (MIEs) are the chemical-biological interactions at the beginning of adverse outcome pathways (AOPs). [1] As they are, essentially, chemical interactions, MIEs provide a good target for the construction of computational models. [2] Models predicting the MIE do not jump over large amounts of biological complexity, nor incorporate many toxicity pathways when compared to predicting apical endpoints. This allows them to make more mechanistically sound predictions. However, some of the complex computational methodology used to make these predictions can add uncertainty to the procedure.

Machine learning algorithms are highly predictive computational approaches for making predictions based on existing data. [3] They are able to "learn" based on training data, and then make predictions for new scenarios by changing their internal parameters. These parameters are part of a complex web, which makes interpreting how they "learn" and "think" a challenge. Neural networks are one class of machine learning algorithms, and they are based on how the human brain works, with mathematical synapses connecting neural nodes.

We have constructed neural networks for the prediction of important human MIEs for use in safety assessment. Open source data from ChEMBL [4] and ToxCast [5] was used, providing a balance of positive and negative data points for several human MIEs, including G-protein coupled receptors, nuclear receptors, enzymes, ion channels and transporters. These networks show extremely high performance (accuracy >90% in most cases), as expected, and a similarity algorithm has been developed to assess how the signal in the network propagates through it when a chemical is introduced. This allows the model to provide activity predictions for new chemicals and training set molecules with high network similarity, meaning the prediction can be treated in a read-across style manner by the user, increasing their confidence in the computer's prediction.

The *in silico* prediction of MIEs is vital for the future of AOP based risk assessment. Powerful computational approaches, such as machine learning algorithms, with a solid understanding of their workings is extremely important in toxicology. With transparency, these efficient and inexpensive methods can be used in safety decision making.

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P19-018
A case study to leverage public resources to improve *in silico* chemical safety assessment

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The use of non-animal testing-based alternative methods in the safety assessment has made much progress in Europe. Whilst the replacement of animals still requires further developments in alternative methods for repeated-dose, reproductive and developmental toxicity, as well as in the understanding of toxicokinetics, the knowledge developed from other regulatory programs such as REACH is actively being leveraged. The formalised methods of QSAR, grouping and read-across are also, to some extent at least, applicable to cosmetics ingredients, agrochemicals and their metabolites and impurities as long as the applicability domains of the knowledgebase and the experimental data are covered for structures similar to those applications. COSMOS Next Generation (NG) provides assessment data relevant for cosmetics and food additives and this can be supplemented by the OpenFoodTox database (from EFSA) which covers pesticides and metabolites. They are similar in that the majority of the data are from assessment programs. The Cosmetics Safety Prediction (CSP) system of the Korean Cosmetics Institute of Industries (KCII) provides assessment results for cosmetics ingredients used in Korea, and is also augmented with COSMOS NG data. To expand the chemical domain, pipelining other large public toxicity database such OpenFoodTox is being performed through COSMOS NG. The *in silico* system implemented in various public systems can be applied to leverage possible public knowledge. Biological profilers were used from COSMOS NG, QSAR models from KCII CSP and Vega were combined. This concept of performing *in silico* analysis leveraging various systems by federation rather than tight integration has been tested via a case study with an industry example. A long alkyl chain (C12–15) benzoic ester used for skin conditioning was tested for the feasibility of applying QSAR, structural rules, and read-across for genetic toxicity and reproductive/developmental effects. This study addresses the strengths and weaknesses of each approach and strives to provide formulators with easy-to-understand workflows and empower them to leverage all available public knowledge.

P19-019
In silico prediction of respiratory complex I inhibitors

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Mitochondrial Complex I (also known as NADH dehydrogenas /NADH ubiquinone oxidoreductase, EC: 1.6.5.3) is a vital component of the respiratory chain and aerobically produces most of the energy required by mammalian cells. Complex I is the most complex protein in the electron transport chain (ETC). It acts as a regulatory enzyme as well as a proton pump in the cell membrane of bacteria and the inner mitochondrial membrane. Despite its important role in energy production, little is understood about Complex I even to this day due to its large size and complex structure. Because of the nature of Complex I, it has become the center of attention for drug research and genetics studies. Several pharmaceutical drugs and other small molecule compounds have been shown to inhibit complex I, leading to mitochondrial dysfunction and organ toxicity in human [1].

The aim of this study was to simulate binding of inhibitors to Complex I using protein-ligand docking into various sites, be able to predict the various inhibitors based on their structure and conformation. Interaction energies obtained from docking along with 2D and 3D molecular descriptors were used to develop quantitative structure-activity relationship models to predict inhibition potential by other (test set) compounds. IC_{50} values for the inhibition of bovine complex I were obtained for 114 compounds from ChEMBL (92 compounds) [2] and other literature. Complex I structures were obtained from protein databank; namely, 3IAM [3]and 5XTD [4] were used due to higher resolution of 3IAM which had NADH complexed to it, and 5XTD having a slightly lower resolution than 3IAM, but allowing investigation of the ubiquinone binding region which was not possible with 3IAM (due to it containing only the hydrophilic domain). Therefore, docking studies using 3IAM and 5XTD allowed docking into the binding sites for two of the most important substrates of Complex I: NADH and ubiquinone.

MOE software (Chemical Computing Group) was used for docking studies and molecular descriptor calculation. Weka (version 3.8) and Minitab statistical software were used for data mining and QSAR model development. The QSAR models were validated using the external test set comprising 20% of the compounds. The results indicated a good correlation between log IC_{50} values and docking scores obtained for the ubiquinone binding site of 5XTD ($r^2 = 0.648$). In addition, QSAR model ($r^2 = 0.86$) indicated the importance of conjugated double bonds (represented by the number of double bonds, or the energy of the highest occupied molecular orbital) and diameter of molecules for the inhibition of complex I. By analyzing this information, we were able to determine the relations between the molecular properties of compounds and their IC_{50} values.

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P19-020
Modelling of compounds interaction with P-glycoprotein: an *in silico* approach towards identification of safer chemicals

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P-glycoprotein (P-gp) is a transmembrane protein that actively transports a large variety of chemically diverse compounds out of the cells. It is highly associated with the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of drugs and toxins; this fact is evidenced by its expression in the small intestine, liver, colon, kidneys, placenta and the blood-brain-barrier (BBB) [1]. P-gp contributes to decrease toxicity by eliminating compounds from the cell and it is involved in the multidrug resistance (MDR) phenomenon [2,3], where drugs are pumped out of the cell and their concentration is lowered at the intracellular target site. Therefore, in order to obtain a more effective prediction of ligand's toxicity and safety, it is advisable to understand the ligand–P-gp interactions in the drug discovery and toxicological assessment process.

In this study, preliminary results are presented for an *in silico* modelling of compounds interaction with P-gp. Due to the lack of crystallographic structure of the human P-gp in the inward facing conformation – active conformation for binding ligands – a 3D model of human P-gp was generated by homology modelling, using the I-TASSER approach based on different structural templates (mouse P-gp) from the PDB library. The homology model was used to perform a docking analysis on a set of ten test compounds, from these: cyclosporine A, doxorubicin and amiodarone were screened as the ligands of most interest when applying a filter based on hydrophobic interactions and hydrogen bonding with some specific residues in the binding pocket; result that is in concordance with the experimental data related to their interaction with P-gp. The docking results showed as well that some interacting residues are the same when compared to the interacting residues found in a ligand (PDB-100) co-crystallized with the mouse P-gp. The post-docking complex was analyzed in order to evaluate the behavior of compounds with P-gp and to determine their potential role in toxicity. Our *in silico* approach confirmed available experimental results regarding affinity for binding P-gp, therefore it could help in designing *in vitro* experiments using P-gp in order to accurately predict the role of compounds in systemic toxicity.

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P19-021
Administration of 3,4-dimethylmethcathinone (3,4-DMMC) and methylone increases the release of antidiuretic hormone in female Wistar rats

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Synthetic cathinones and amphetamines are substituted phenethylamines and therefore share many toxicodynamic mechanisms. One of the potentially life-threatening consequences of amphetamine abuse, in particular of 3,4-methylenedioxymethamphetamine (MDMA), is serotonin-mediated hyponatraemia, which was also recently documented in synthetic cathinone intoxications. Most of these reported cases occurred in young women, suggesting a differential susceptibility according to gender.

Since altered release of antidiuretic hormone (ADH) has been implicated in MDMA-induced hyponatraemia, we hypothesised that synthetic cathinones may also disturb ADH secretion. Herein, we evaluated the impact of two cathinone analogues [3,4-dimethylmethcathinone (3,4-DMMC) and methylone] in the release of ADH.

Adult female Wistar rats weighting 250–300 g were administered with 20 or 40 mg/Kg 3,4-DMMC or methylone i.p (6 animals per group). A group of animals treated with 20 mg/Kg MDMA was also included for comparison. After 1h or 24h, animals were anesthetized and blood collected from the inferior vena cava into heparinized tubes, using an appropriate technique to avoid ADH oscillations attributed to hypovolaemia. Urine was also collected from animals exposed to the tested drugs for 24h. Quantification of ADH was performed on plasma and urine using a commercially available kit (Assay Designs, Michigan), according to the manufacturer's instructions.

Compared to controls (28.01 ± 6.31 pg/μL), administration of 20 mg/Kg 3,4-DMMC and methylone triggered an increase in plasma levels of ADH (367.6 ± 131.4 pg/μL and 285.0 ± 91.6 pg/μL, respectively; $p < 0.05$) similar to that observed for the same dose of MDMA (349.0 ± 73.8 pg/μL; $p < 0.001$), after 1h-treatments. This effect seems to be dose-independent (296.1 ± 63.3 pg/μL for 3,4-DMMC and 135.5 ± 34.8 pg/μL for methylone, at 40 mg/Kg). Although to a lesser extent, increased ADH plasma levels were still observed 24h after treatment (77.6 ± 13.4 pg/μL for 3,4-DMMC and 106.8 ± 19.6 pg/μL for methylone, at 20 mg/Kg; 17.8 ± 2.1 pg/μL for control; $p < 0.05$). ADH levels in 24h urine samples were also increased: 692.5 ± 111.0 pg/μL for 3,4-DMMC and 408.0 ± 53.1 pg/μL for methylone, at 20 mg/Kg *vs*146.8 ± 35.6 pg/μL for control ($p < 0.05$).

We report for the first time the increased release of ADH induced by cathinones, which may be related with the reported hyponatraemia in intoxicated users.

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P19-022
Administration of 3,4-dimethylmethcathinone (3,4-DMMC) and methylone to Wistar rats disturbs the energetic and antioxidant homeostasis in liver, brain, kidney and heart

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3,4-Dimethylmethcathinone (3,4-DMMC) and methylone are new psychoactive substances belonging to the group of synthetic cathinones, widely used in recreational settings by virtue of their stimulant, euphoric and empathogenic properties. The number of intoxications attributed to these drugs have been increasing in the scientific literature, but there is a lack of systematic studies concerning their *in vivo* toxicological mechanisms.

We aimed at contributing to the evaluation of the mechanisms of toxicity elicited by 3,4-DMMC and methylone in Wistar rats by measuring alterations in energetic content and oxidative stress parameters in the main target organs of cathinones' toxicity, specifically brain, liver, heart and kidney.

Adult Wistar rats, weighing 250–300 g, were injected i.p. with saline (0.9% NaCl), 20 mg/Kg 3,4-DMMC or 20 mg/Kg methylone (at least 6 animals per group). During the test period, animals were kept in metabolic cages and their behaviour carefully monitored. After 1 h or 24 h, rats were anaesthetized, euthanized, and brain, liver, heart and kidney were excised, weighed, and washed with 0.9% NaCl solution. Organs were homogenized (1:4 m/v) in ice cold 100 mM phosphate buffer (pH 7.4) and used for quantification of ATP, GSH and GSSG contents, and of activities of the antioxidant enzymes glutathione-S-transferase (GST), selenium-dependent glutathione peroxidase (GPx), and glutathione reductase (GR).

Methylone increased overall animal locomotion, salivation and piloerection. Piloerection was also observed in animals administered with 3,4-DMMC, which adopted a defensive posture and showed signs of confusion and imbalance. Overall, energetic content (ATP levels) decreased in liver after exposure to methylone at both timepoints, and in the heart and brain after 1h of exposure to 3,4-DMMC. The antioxidant GSH was also reduced after 3,4-DMMC treatment in brain (after 1 h) and liver (after 24 h); and after 24 h of exposure to methylone in the liver and kidney. Alterations induced by methylone and 3,4-DMMC in the antioxidant enzyme activities were highly dependent on the exposure time and organ analysed.

To our knowledge, this is the first *in vivo* study evidencing the alterations in stress parameters induced by exposure to 3,4-DMMC and methylone. These data will help elucidating the toxicological effects of these drugs on their main targets.

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P22 – Toxicology of the immune system

P22-001
Development of a zebrafish larvae screening assay to identify compounds with immunotoxicity and anti-inflammatory activity

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Zebrafish is a unique model for pharmacological manipulation of the innate and adaptive immune response. They are small and permeable to many small compounds and there are several transgenic lines available to visualize immune cells. Taking advantage of zebrafish embryo transparency, we can test the toxicity of pharmacological, agrochemical and cosmetic compounds to the immune system by quantifying these cell populations. Additionally, this model can also be used to identify new anti-inflammatory compounds, by following leukocyte recruitment to inflammation induced by sterile tissue injury.

We have developed an assay in zebrafish larvae to detect compounds with specific toxicity for the immune system and to screen and identify new anti-inflammatory drugs. For these purposes two transgenic lines have been used: neutrophil-specific Tg *(mpx:GFP)i114* and macrophage-specific Tg *(mpeg:mcherry)*. Several reference compounds (immunotoxic, anti-inflammatory, and no immunotoxic) were chosen and their doses selected after an MTC (Maximum Tolerated Concentration) assay carried out in 3 days post fertilization (dpf) embryos (when the innate immune system is already in place). To

assess the toxicity activity at the immune cells level, embryos were exposed to the compounds for 48 h and the population of neutrophils and macrophages was quantified by fluorescence microscopy. To determine compound effect on leukocyte activity, inflammation was induced by sterile injury of the tail fin and neutrophil recruitment to the wound site was evaluated at 4, 6 and 12 h post injury in the presence of reference compounds using a partially automated platform. Additionally, we also evaluated the ability of the compounds to suppress/trigger the expression of inflammatory genes (il1b, tnf-a) by quantitative PCR.

This zebrafish assay shows to be a cost-effective assay over mammalian models for the identification of new anti-inflammatory drugs as well as for the evaluation of immunotoxicity.

P22-002
Predicting the impact of immune interventions by a systems biology approach

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Background: Despite scientific advances it remains difficult to predict the risk and benefit balance of immune interventions. Over the last years, network models have been built based on comprehensive datasets at multiple molecular/cellular levels (genes, gene products, metabolic intermediates, macromolecules, cells) to illuminate functional and structural relationships. Here we used a systems biology approach to identify key immune pathways involved in immune health endpoints and rank crucial biomarkers to predict adverse and beneficial effects of nutritional immune interventions.

M&M: First, a literature search was performed to select the molecular and cellular dynamics involved in hypersensitivity, infection and resistance to autoimmunity and cancer. Thereafter, molecular interaction between molecules and immune health endpoints was defined by connecting their relations by using database information (Gene Ontology (GO), CTD and MeSH database). The resulting Immune network contains unique and overlapping genes among the different immune health endpoints and is the basis of the proposed screening tool.

Result: As a first step in generating the Immune network, MeSH terms related to the immune health endpoints were selected. This resulted in the following selection: hypersensitivity (D006967: 184 genes), infection (parasitic, bacterial, fungal and viral: 357 genes), resistance to autoimmunity (D001327: 564 genes) and cancer (D009369: 3173 genes). Next, a sequence of events was determined which drives the development of immune health disturbances resulting in the following selection: hypersensitivity (164 processes), infection (187 processes), autoimmunity (203 processes) and cancer (309 processes).

Finally, an evaluation of the genes for each of the immune health endpoints indicated that many genes played a role in multiple immune health endpoints but also unique genes were observed for each immune health endpoint. In all, this approach helped to build a screening/prediction tool which indicates the interaction of chemicals or food substances with immune health endpoint related genes and suggests candidate biomarkers to evaluate risk and benefits.

Conclusion: We provide a promising systems biology approach to predict genes that help to clarify, on one hand the relationships between immune interventions and the susceptibility to immune related disorders, and on the other hand, to identify interesting biomarkers to monitor for safety and efficacy in immune interventions.

P22-003
Developing a strategy for assessment of protein allergenicity using proteomic and bioinformatic (AllerCatPro) analyses

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Proteins in natural substances used in consumer products may pose a risk of IgE-mediated respiratory allergy when used in spray or wash-off products. For assessing the allergy risk of proteins, the standing assumption has been that 100% of the total protein content is allergenic unless data exist to demonstrate otherwise. Here we describe a novel approach using proteomic and bioinformatic analyses to determine the fraction of protein mixture that has allergenic potential. To illustrate this approach, we consider the case of a theoretical consumer product containing corn meal as a plant-derived raw material.

Corn proteins were analyzed by LC-MS/MS label free proteomic analysis on a QExactive HF mass spectrometer. Protein sequences were obtained from UniProt and matched with observed spectra and sum of fragment ion intensities per protein was used to calculate relative abundance. All identified proteins were analyzed by AllerCatPro, a new *in silico* tool which predicts the allergenic potential of proteins with high sensitivity and specificity. Predictions are based on comparison of 3D structure and amino acid sequence to a dataset of proteins associated with allergenicity comprising of 4180 unique sequences derived from the union of the major databases FARRP, COMPARE, WHO/IUIS, UniProtKB and Allergome.

Of the total 2009 proteins identified in corn, 429 individual proteins (61.6% of the total protein by weight) were predicted to have allergenic potential. The most abundant proteins per se (trypsin inhibitor and a second uncharacterized protein) comprise each 9.7% of total protein content.

The new information generated on the protein content and potential for allergenicity for natural substances can be used to refine the IgE-mediated respiratory allergy safety assessment. For example, the accumulated percent of proteins or the percent of the most abundant protein with predicted allergenic potential can be used instead of assuming 100% of the protein content is allergenic. Generating data on the abundance of proteins shown to have allergenic potential opens new opportunities to refine the current IgE-mediated respiratory allergy safety assessment process for proteins in consumer products.

P22-004
Chronic oral exposure to the food additive silicon dioxide (E551) induces food intolerance in mice

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Purpose: Food-grade synthetic amorphous silica (SiO_2, E551 in EU) is composed of aggregated nanoparticles (NPs) and used as anti-caking and antifoaming agent in foodstuffs. Oral exposure to E551 is chronic (0.8–74 mg/kg/day) [1], while SiO_2-NP models penetrate the intestinal barrier [2] as well as block induction of oral tolerance (OT) to dietary antigens in mice [3,4]. The current study aims at evaluating in mice the effect of oral chronic exposure to the food additive E551 at human relevant levels on the induction of OT to the food antigen model ovalbumin (OVA).

Methods: Mice were daily treated *per os* with E551 (1, 10 or 100 mg/kg/day) or water vehicle for 60 days. At day 41, OVA (20mg/mouse; OVA-tolerized mice) or PBS (controls) was orally administered for 3 days. All mice were subsequently immunized by subcutaneous injection of OVA (100µg/mouse) at day 48. Blood was collected 1 week after for anti-OVA IgG serum titers to evaluate OT induction in OVA-tolerized mice exposed or not to E551. In all groups, to further assess tolerance to food antigens, mice were orally challenged by OVA (25 µg/mouse) for 5 days before sacrifice. Secretion of pro- (IFN-γ) and anti-inflammatory (IL-10, TGF-β) cytokines by colon mucosa and mesenteric lymph node immune cells were assessed by ELISA. Fecal lipocalin (Lcn)-2 level used as inflammatory marker was also evaluated.

Results: In OVA-tolerized mice without E551 treatment, lower anti-OVA IgG production levels than immunized controls (oral PBS only) demonstrated OT induction. In contrast, a sharp increase of anti-OVA titers was observed in all E551-treated OVA-tolerized mice compared to mice not exposed to the food additive, showing a blockade of OT induction to OVA whatever the E551 dose. Moreover, when E551-treated OVA-tolerized mice were orally challenged with OVA, an increased intestinal level of the inflammatory markers IFN-γ and Lcn-2 was observed compared to mice not exposed to E551, showing OVA intolerance. We also observed a decreased production of IL-10 and TGF-β, both are crucial for OT induction. Altogether, these results showed that chronic oral exposure to E551 at human dietary levels impairs OT to dietary antigens, and promotes intestinal inflammation supporting food intolerance.

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P22-005
Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment (NGRA)

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Our aim is to develop, evaluate and apply next generation approaches to skin allergy risk assessment that do not require new animal test data, addresses novel exposure scenarios and better quantifies uncertainty. We have developed a Bayesian multi-level regression model to estimate the human sensitiser population threshold (defined as, the chemical-specific exposure level at which no individual in a population will experience induction of contact allergy) under the conditions of a human repeat insult patch test (HRIPT) [1]. This approach is built using dose response modelling of historical HRIPT data and allows predictions of human sensitiser potency to be made using historical murine local lymph node assay (LLNA, OECD TG 429) data and/or *in vitro* test method data [DPRA (OECD TG 442C), KeratinoSens™ (OECD TG 442D), h-CLAT (OECD TG 442E) and U-Sens™ (OECD TG 442E)]. A key feature of the approach is that the uncertainty in any prediction is explicitly quantified.

Our Bayesian probabilistic model is used to estimate population thresholds for 30 chemicals using a weight-of-evidence incorporating previously published HRIPT, LLNA, DPRA, KeratinoSens™, h-CLAT and U-Sens™ data. Estimates for a further 43 chemicals using *in vitro* test method data only are also presented. Comparisons are made with current risk assessment metrics and across data types. This analysis suggests that estimates of human potency generated from *in vitro* data alone have at least the same level of accuracy, on average, as estimates generated from LLNA data. Consequently, we propose that this approach can be used to derive a point of departure for next generation risk assessment and have submitted it for consideration by the OECD Defined Approach Skin Sensitisation (DASS) Expert Group as 'Skin Allergy Risk Assessment Defined Approach' or SARA DA. Application of the SARA DA to a theoretical, next generation skin allergy risk assessment case study (use of coumarin in face cream) will be presented to illustrate how the DA prediction can be used as part of a weight of evidence decision-making approach.

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P22-006
Effects of trichloroethylene exposure on the expression of genes involved in TAP-dependent antigen presentation pathway

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Trichloroethylene (TCE) is a widely used industrial solvent and a common environmental contaminant. TCE induced generalized hypersensitivity syndrome has become one of the critical health issues and requires intensive treatment because of it's characteristics of dose-independent and potentially life threaten. Previous studies found the disease is strongly correlated with HLA-B*1301, a subtype of HLA-B, which is part of a family of genes called the human leukocyte antigen (HLA) class I. HLA-B subtype molecules could present specific endogenic peptides to $CD8^+$ T cell for inducing immune response, and transporter for antigen presentation (TAP) – dependent antigen presentation pathway plays important role in the peptides production. To study the effects of TCE exposure on this pathway might be great importance for understanding of the correlation between the disease and HLA-B*1301. In this study, human B-lymphoblastoid cell line transfected with HLA-B*1301 and HLA-B*1302 (no correlated with the disease) were cultured with different concentration of TCE and it's metabolite trichloroethanol (TCOH), mRNA and protein expression of genes including ubiquitin, TAP1, TAP2, low molecular weight protease (LMP)2, LMP7 involved in TAP-dependent antigen presentation pathway were determined, HLA-B*1301 expression in the cell surface was detected by flow cytometry. Results showed that both TCE and TCOH treatments resulted in the higher HLA-B*1301 expression in the surface of HLA-B*1301 transfected cells, and TCOH induction was stronger than TCE. There was a significant increase in mRNA levels of TAP1, TAP2, LMP2, LMP7 and a significant decrease in ubiquitin mRNA level among TCE or TCOH treated cells vs. non-treated cells. The protein profile showed the same pattern and change in parallel to mRNA in all treated cells. There was no obvious difference in changes of gene expression associated with TCE or TCOH treatment between HLA-B*1301 and HLA-B*1302 cell line. Our results suggest

that TCE and TCOH treatment could activate TAP-dependent antigen presentation pathway and accelerate the degradation of endogenic protein and production of peptides. These effects of TCE or TCOH do not show the HLA molecular specificity.

P22-007
Common indoor air contaminating mycotoxin enniatin B potentiates proinflammatory repertoire of macrophages by microbial structural fragments

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Susceptibility to adverse health effects such as enhanced asthma risk has been associated with moisture-damaged buildings with poor air quality and presumed bioaerosol exposure. No other environmental exposure has yet gained as persistent attention in Finnish media as the level of concern today on public buildings 'loaded with harmful mold'. Similar to many of the symptoms related to the sick building syndrome, bioaerosols remain as poorly characterized, highly complex mixtures of microbial structural fragments and toxins. Our earlier preliminary data have indicated potent synergistic immunomodulatory interactions between microbial toxins and the structural components LPS (lipopolysaccharide from gram-negative bacteria) and BG (β-D-glucan of fungi) *in vitro*. We provide here detailed proinflammatory dose-response data for the mycotoxin enniatin B (EnB; 0.625–20 μM) from *Fusarium sp.* and its interaction with co-exposed LPS/BG in human THP-1 derived macrophages. Based on the metabolic formazan dye assay, co-treated THP-1 macrophages remained viable and were co-exposed at well-tolerable dose levels (5 μM EnB; 5 ng/ml LPS or 100 ng/ml BG). Characteristic proinflammatory cytokine patterns (TNFα and IL-1β mRNAs) were observed for EnB. However, EnB was capable of further amplifying the signaling cascades by LPS and BG but no similar effect observed in reverse order. The magnitude of inflammasome component NLRP3 mRNA induction revealed mechanistic cross-talk of the interacting factors behind intensified proinflammatory response. In our assays, differentiated THP-1 macrophages presented as a sensitive model for the study of subtle mechanistic interactions. Similar studies should proceed understanding of the immunomodulatory events in individuals susceptible to exposure settings at moisture-damaged buildings.

P22-008
Skin sensitisation potential: addressing concomitantly several events of the AOP using a 3D keratinocyte/THP-1 co-culture

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A co-culture system consisting of VitroDerm, an in-house RhE and THP-1 cells is developed for a use in the context of skin sensitisation. This streamlined approach enables the concomitant modelling of several events of the skin sensitization Adverse Outcome Pathway (AOP): the skin penetration and metabolism, the activation of keratinocytes and dendritic cells. Additional benefit is the preserved cross-talk between keratinocytes and dendritic cell surrogates in the co-culture system.

First, the VitroDerm RhE/THP-1 co-culture model was characterised (cell viability, histology and basal expression of CD54 and CD86 by flow cytometry). The design of the VitroDerm RhE/THP-1 co-culture and the readily disassemble allows the analyses of the different cell types specifically. The permeation of chemicals through VitroDerm RhE was assessed. The CD86 and CD54 were quantified by flow cytometry on THP-1 cells after topical treatment with chemicals. The prediction is compared to regular h-CLAT (THP-1 monoculture) and a correct classification is obtained. Then, a microarray analysis was performed to identify a gene signature following chemical treatment. Finally, the cellular interplay between the cell types of the co-culture was evaluated by analysis of the paracrine interaction using immunoassay. A differential extracellular release of cytokines was obtained (CCL3, CXCL8, CXCL10 and GM-CSF).

The presence of RhE within the co-culture allows closer replication of *in vivo*-like bioavailability and keratinocyte inflammation. Taking together with the preserved cellular interplay, it contributes to modelling the human skin exposure to sensitizer and activation of immune cells. Finally, the VitroDerm/THP-1 co-culture opens new horizon on assessing the skin sensitization potential of poorly soluble compounds or mixtures.

P22-009
The emerging mycotoxin alternariol modulates the immune response of gastrointestinal cells *in vitro*

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Scope: Produced by filamentous fungi of *Alternaria* species, the mycotoxin alternariol (AOH) is a ubiquitously occurring contaminant of a broad variety of food and feed commodities. Spoilage through its main producing mold, *Alternaria alternata*, does not only occur during culture but also during cooled transportation and storage. Still lacking in occurrence and hazard data, the emerging mycotoxin AOH is not regulated yet. Contaminating a broad variety of food and feed commodities, AOH might pose a risk to human and animal health. Beside its cytotoxic, genotoxic and estrogenic properties, several studies reported the potential of AOH to suppress the rich network of immune responses. The specific effect of AOH on inflammation-related signaling in non-immune cells of the intestinal epithelial layer has, however, not been investigated yet. Since intestinal epithelial cells (IECs) are, compared to underlying cells, exposed to higher concentrations of the ingested mycotoxin, the question was addressed whether immunomodulation by AOH must be considered.

Methods and results: The impact of AOH (0.02–40 μM) on inflammatory signaling in either IL-1β-stimulated or non-stimulated differentiated Caco-2 cells was determined. AOH significantly reduced IL-1β transcription after 5 h but showed an increasing tendency on IL-8 levels after long term exposure. In stimulated cells, AOH (20–40 μM) augmented TNF-α transcripts while repressing IL-8, IL-6 and IL-1β transcription as well as IL-8 secretion. Furthermore, inflammation related microRNAs miR-16, miR-146a, miR-125b and miR-155 were altered in response to AOH.

Conclusion: The obtained data indicates that AOH represses immune responses in an inflamed environment and it might be considered as a contributer to immune suppressive effects of mycotoxin mixtures.

P22-010

Pathway regulation of glyphosate, the co-formulant POEA and herbicide products in a dendritic cell model

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Several reports have stressed the effects of the herbicide glyphosate, marketed for e.g. agricultural, forestry and gardening, on human health. Few studies have been performed related to skin sensitization and immunotoxic effects on cellular and molecular mechanisms triggered by glyphosate, co-formulants and mixtures thereof. We have earlier investigated their skin sensitizing capacity using the GARD^{TM}skin assay, an *in vitro* assay for identification of contact allergens, and classified the mixtures, the co-formulant polyethylated tallow amine (POEA), as skin sensitizing formulations; however, glyphosate, per se, was classified as a non-sensitizing chemical. We have further analyzed their effect on protein regulation using a proteomic mass spectrometry-based approach. Here, we would like to present a detailed description of the regulated cellular pathways identified by proteomics, and follow-up studies of the regulation of selected pathways using biochemical assays.

A dendritic cell model was exposed to glyphosate, the surfactant POEA, and two commercial mixtures thereof, and protein and RNA was collected for proteomic analysis after 24 h stimulation. Pathway analysis based on the obtained dataset has been performed with the Key Pathway Advisor tool and Metacore^{TM} (both Clarivate Analytics). Additionally, the capacity of these substances to induce reactive oxygen species (ROS) production and to modify the autophagic flux in our cell model is investigated using flow cytometry-based CellRox® and Cyto-ID® assays, respectively.

The pathway analysis predicts cellular events linked to oxidant stress responses, immune responses, and cell cycle regulation. More detailed analysis revealed the regulation of the adapter protein p62/sequestosome in response to treatment of cells with POEA and an herbicide formulation, possibly linking autophagy processes to the oxidative stress response pathway KEAP1/Nrf2. Autophagy has previously been described as crucial for dendritic cell functions; however, its role in skin sensitization is largely unknown. We are currently following up these results with biochemical methods. In summary, this project will provide new insights into the molecular events and mechanisms leading to immunotoxic effects in response to herbicide formulations and the role of autophagy for skin sensitization in particular.

P22-011

TDAR and splenic lymphocyte subpopulation analysis in extended one-generation reproductive toxicity studies (OECD 443)

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OECD test guideline 443 provides a detailed description of the operational conduct of the Extended One-Generation Reproductive Toxicity Study (EOGRTS). The guideline describes three cohorts of F1 animals covering different developmental and reproductive endpoints. Among them, Cohort 3 is designed to assess the potential impact of chemical exposure on the developing immune system. Over the last 3 years Citoxlab has performed a series of OECD 443 studies in Sprague-Dawley (SD) rats. Herein we share background control data on the T cell dependent antibody response (TDAR) and splenic lymphocyte subpopulation analysis in this species. TDAR is assessed by immunization of all Cohort 3 animals with KLH as a model antigen on Day 56 post-partum. Anti-KLH IgM concentrations in serum are measured before and 5 days after immunization using a validated ELISA method. Splenic lymphocyte subpopulation analysis is performed on non-immunized male and female animals (Cohort 1A) using a validated flow cytometry panel for assessing the relative counts of the following splenic lymphocyte subpopulations: T lymphocytes, helper and cytotoxic T lymphocytes, B lymphocytes, natural killer (NK) cells and NKT cells. Data gathered so far show that before KLH immunization, samples displayed high background signals in terms of anti-KLH IgMs. This may be explained by the presence in rat serum of pre-existing antibodies directed against epitopes that are shared with KLH, such as polysaccharides. In control animals, KLH immunization induced a consistent and reproducible humoral primary immune response (anti-KLH IgM concentrations of 124 ± 23 µg/mL for males and 218 ± 67 µg/mL for females, respectively). Normal ranges of splenic lymphocyte subpopulations in male and female Sprague-Dawley rats, respectively, were as follows: T lymphocytes (35.3 ± 8.2% and 37.7 ± 10.7%) and the T-cell CD4+ (57.2 ± 9.2% and 58.2 ± 5.2%) and CD8+ (35.8 ± 9.5% and 37.7 ± 5.6%) lymphocyte subsets, B lymphocytes (37.6 ± 3.6% and 37.5 ± 2.6%), NK cells (4.0 ± 1.3% and 3.8 ± 1.3%) and NKT cells (5.8 ± 1.3% and 5.9 ± 1.1%). Reference control ranges were also generated for Wistar rats. In conclusion, these methods have been validated and successfully used at Citoxlab for assessment of the impact of chemical exposure on the developing immune system in the framework of OECD 443 studies.

P22-012

Validation of three flow cytometry panels for blood cell subpopulation analyses in Göttingen Minipigs

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The Göttingen Minipig is generating growing interest as a non-rodent alternative animal model for the immunological safety evaluation of drug candidates. New specific immunological tools, such as immunophenotyping on peripheral blood, are thus required. For this purpose, three flow cytometry-based methods were developed and validated for evaluation of the following cell subpopulations: total, cytotoxic and helper T cells, CD4+/CD8+ double positive T cells, regulatory T cells and natural killer (NK) cells (+/- activation marker) (panel 1: CD3, CD4, CD8a, CD25, FoxP3, CD335), B cells (panel 2: CD3, CD21, CD79a, SLA-DR) as well as mature and immature monocytes (panel 3: CD14, CD172a, CD163, SLA-DR). After antibody titration, samples were analyzed using the Miltenyi MACSQuant® Analyzer 10. Results were expressed as relative and absolute counts, taking into account the hematology results determined using an ADVIA® 120 System. Precision (within- and between-run), sample stability before/after staining, and carry-over were evaluated. Within- and between-run coefficient of variation (CV%) ranged from 0.57% to 17.54% and from 0.62% to 21.28%, respectively. There was no significant inter-sample contamination. Pre-staining stability assessment (expressed as % of recovery from "T0") demonstrated that blood samples could be stored at room temperature for up to 9 hours for all panel 1 subsets (85.4%–126.1%) except the following rare subsets: activated-helper and activated-cytotoxic T cells (134.4%–147.4%), up to 22 hours for panel 2 (86.5%–91.6%) and 4 hours for panel 3 (81.9%–116.4%). Post-

staining stability was also assessed and it was found that stained panel 2 and 3 samples could be stored at +5°C for 22 hours (87.3% – 116.5%), while panel 1 samples should be analyzed extemporaneously after staining. The three methods were thus considered as successfully validated for their intended use in immunotoxicology/immunopharmacology studies. Validation data combined with control group data have allowed us to obtain normal ranges for the different subsets of interest in Göttingen Minipigs.

P22-013
Assessment of alternative assay formats for assessment of the potential to initiate a cytokine storm response

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Following the serious adverse events that resulted in severe cytokine storm responses in clinical trial patients dosed with TGN1412 (anti-CD28 monoclonal antibody) it has become a regulatory authority expectation that any therapeutic with immune modulatory potential is assessed with in-vitro assays to predict the potential for activation of cytokine release. Over the last 10–12 years many investigators have assessed a variety of in-vitro cellular assays for evaluation of this response. Traditionally the standard approaches used are whole blood and PBMC based assays, using a compound which has been plate immobilized (dry or wet coated) or challenged in liquid phase. Other *in vitro* approaches mimicking the vascular microenvironment or the lymph node setting (High Density pre-culture assay) are also being adopted.

This poster will detail the optimisation and development of a high density PBMC pre-culture based cytokine release assay. It will also establish the physiological characteristics of the cells after culture at high density to give a greater understanding of how the assay responds to known activators of cytokine release. FACS analysis of the samples will be conducted to analyse any priming effect of high density PBMC pre-culture on key checkpoint and activation receptors, including: CD25, CD28, CD223,CD137, CD274,CD279 & CD366. These cluster of differentiation CD receptors has been chosen to establish if the method could be suitable to predict the cytokine release potential of checkpoint inhibitor therapeutic compounds. The assay will be assessed using positive controls (PHA, Anti-CD3 Monoclonal antibody, Anti-CD28 Monoclonal antibody, Anti-Her2 Monoclonal antibody and CD19&CD3 bi-specific T-cell engager) which are known to have different modes of action for induction of cytokine release. Utilising these data the poster will show the kinetics of the assay and how it can be used to accurately predict the potential of cytokine storm for novel biologics.

P22-014
Skin immune system in the juvenile Göttingen Minipig

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The development of new pharmaceuticals benefits from continuous advances in biomedical research for both adults and children. Some diseases are specific to childhood and others last lifelong but need to be treated early in childhood. Safety evaluation of new pediatric medicines is performed by the conduct of toxicology studies using juvenile animals. The minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels in many features of its anatomy, physiology and biochemistry make the minipig a good model for man. This is particularly true for the cardiovascular system, the digestive tract, the urogenital system, drug metabolism and the skin. For use in juvenile toxicology studies, the development of main organs or systems, including the immune system, of the minipig still requires further characterization. There is a real need to better understand the immune system organization and response in the Göttingen minipig to better evaluate the toxicological effect of new pharmaceuticals in development in this species. This project specifically focused on the skin immune system in the Göttingen minipig from birth to the adult age. Main skin immune cells (helper-T cells, cytotoxic-T cells, γδ-TCR cells, conventional dermal dendritic cells, Langherans cells) were characterized by flow cytometry and immunohistochemistry. A greater proportion of immune cell populations in the dermis and epidermis was generally observed from 2 weeks old animals than in neonatal and 7 days old piglets.

P24 – Emerging approaches in toxicology

P24-001
Molecular targets of Aflatoxin B1 in human primary trophoblasts

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Aflatoxin B1 (AFB1) is a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*. It contaminates crops and animal products causing acute and chronic toxicity in the liver as the major target organ. AFB1 can be transferred and metabolized through the placenta and can be found in breast milk, neonatal cord blood and serum of pregnant women. However, the effects and molecular targets of AFB1 in human placenta are almost unknown. In this study, AFB1 targeted gene expression profiles were determined in human primary trophoblast cells. Primary trophoblast were isolated from full term placenta after delivery, and exposed to 1 μM of AFB1 for 72 hours. Gene expression profiling was done by using Human HT-12 expression beadchips. Differential expression of selected genes was confirmed with quantitative RT-PCR. Ingenuity pathway analysis (IPA) software was used to identify AFB1 regulated gene networks and regulatory pathways within the gene expression data. AFB1 significantly dysregulated 165 genes (46 down- and 119 upregulated, ±1.5 fold, P-value<0.05) when compared to controls. The top three upregulated genes were chorionic somatomammotropin hormone 1 (CSH1), growth hormone 1 (GH1) and Pappalysin 1 (PAPPA1) that all have important roles during pregnancy. The top downregulated genes were involved in protein synthesis and regulation of cell cycle. The main canonical pathways identified by IPA were associated with translation of proteins (unfolded protein response and EIF2-signalling) and growth hormone signaling. Furthermore, the main upstream regulators of AFB1-regulated genes were beta-estradiol and follicle-stimulating hormone. As a conclusion, our findings indicate that AFB1 can disturb placental endocrine functions.

P24-002
Determination of barium in barium chloride poisoning samples by microwave digestion-inductively coupled plasma mass spectrometry

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Barium chloride is one of the most important watersoluble and toxic barium salt which exists. Health hazards of barium chloride often occur through ingestion, inhalation, and skin contact. Barium toxic-

ity is caused by the free cation and may causes severe gastrointestinal symptoms, hypokalemia leading to muscle weakness, cardiac arrhythmias, and respiratory failure. Many articles have reported accidental, iatrogenic, and suicidal modes of poisoning due to barium compounds. We describe 1 case of poisoning due to barium chloride. In witnessed cases, severe gastrointestinal symptoms, hypokalemia leading to muscle weakness, cardiac arrhythmias, and respiratory failure were noted. An analytical method was established to detect barium in rib, humerus, liver, kidney, blood and hair by improved microwave digestion-inductively inductively coupled plasma mass spectrometry(ICP-MS) method. The bone samples were frozen in liquid nitrogen for half an hour before being crushed. All the samples were digested by microwave digestion instrument. The barium concentrations have a good linear relationship($r \geq 0.999$) in the range of 0–20μg/L, the relative standard deviation(RSD) is 2.1% – 9.8% and the recoveries are 80.7% –102%. The limits of detection are 1.11μg/kg, which can meet the requirements of the existing standards.

P24-003
Cultivation and attachment of hRPTEC/TERT1 cells on silk fibroin membranes

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Introduction: Current membrane technology for cell growth and analysis of cell to cell metabolite transport relies on non-biological materials such as polyethylene terephthalate (PET). In order to create a more tissue-like environment, membranes made of biological materials are required to achieve a near physiological interface. Silk, a water insoluble biological material, which most importantly does not elicit an immune reaction, is made up of 2 main components, the inner fibroin and outer sericin. Thus silk fibroin (SF) would be amenable to membrane construction for *in vitro* cell culture.

Materials and Methods: To achieve water solubility of SF, sericin needs to be removed. For this, silk cocoons from *Bombyx mori* were degummed, i.e. boiled in 0.02 M Na_2CO_3 for 30 minutes and washed three times with H_2O, and the remaining silk fibrils air dried overnight. Subsequently, SF was packed tightly in a beaker and immersed in 9.3 M LiBr, the beaker covered with tin foil and heated in an oven at 60°C for 4 hours. The latter SF-LiBr solution was dialyzed against water for 48 hours with 6 x total water changes, followed by 2 consecutive centrifugation steps for 30 min at 3000 g, resulting in a clear amber-like SF solution. SF-membranes were cast into 12-well plate transwell inserts and dried overnight. Water annealing for 24 hours in a desiccator filled with water resulted in β-sheet formation, thereby rendering the resulting SF membranes water insoluble. Subsequently, membranes were sterilized with 70% ethanol. To monitor for attachment and growth, human renal proximal tubule epithelial cells (hRPTEC/TERT1) were seeded at 7.5×10^4 cells /ml on the SF-membranes. After reaching confluency, cells were fixed and prepared for imaging.

Results: Bright-field microscopy demonstrated cell adhesion to the SF membrane shortly after seeding, comparable proliferation rates as experienced in routine transwell applications, and the capability to maintain cultures for at least 3 weeks. SEM imaging showed that most of the SF membrane was covered with RPTECs. Upon closer inspection the RPTECs presented with a number of filipodia attached to the SF-membrane itself.

P24-004
Hepatocyte-like cells in microfluidic devices: a new platform for disease modelling, drug screening and toxicology

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Human-based *in vitro* systems mimicking human physiology could facilitate drug discovery. Organs-on-chips are a new class of *in vitro* models that have been introduced to combine the advantages of *in vivo* and *in vitro* models of tissues and organs. In particular, microfluidic devices (MDs) not only enable the potential replication of an *in vivo* environment, including the tissue–tissue interface, spatiotemporal gradients, and geometry, but also provide the possibility of studying the communication between different cell types. However, moving traditional 2D cultures to microfluidic devices (MDs) can be challenging. As such, this work is focused on the adaptation of human mesenchymal stem cell-derived hepatocyte-like cells (HLCs) from conventional monolayer cultures to double channel PDMS-based MDs and aims to evaluate cells hepatic function and energy metabolism. Primary human hepatocytes were used as controls. HLCs were obtained through a three-step differentiation protocol lasting 21 days. The two first differentiation steps were performed in static 2D cultures, whereas hepatic maturation (D17 onwards) was done either in 2D or in MDs. In MDs, both coating and cell inoculum were optimized. HLCs' albumin and urea production along with the expression of genes involved in glycolysis, gluconeogenesis, fatty acid metabolism, bile acid metabolism and mitochondrial function in response were also evaluated up to D34, upon exposure to 80 nM insulin, 100 nM of glucagon and fasting. Herein, HLCs were successfully adapted to MDs by inoculating 7.5×10^4 cells/channel using 0.2 mg/mL of type I collagen as coating. Moreover, HLCs presented an epithelial morphology and stable urea and albumin production. Contrary to fasting and glucagon stimuli, genes related to glycolysis, fatty acid and bile acid metabolism and mitochondrial function were downregulated in response to insulin. Importantly, for most genes, HLCs response to insulin and fasting was more accentuated in the MD than in 2D cultures. This work revealed metabolic responsive HLCs that may provide a relevant system for pre-clinical research.

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P24-005
An *in vitro-in silico* strategy to predict gut microbial metabolism of the isoflavone daidzein and resulting plasma concentrations of its metabolite *S*-equol

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The gut microbiome plays a significant role in the health of the host, among others due to a wide range of biochemical and metabolic activities that can affect the toxicity and bioavailability of xenobiotics.

Current *in vitro-in silico* based testing strategies used for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), however, do not include the gut microbiome. To predict gut microbial metabolism of xenobiotics in rats and the resulting plasma concentrations of the metabolites formed, an *in vitro* method was developed to quantify the kinetics of gut microbial metabolism, with a physiologically based kinetic (PBK) model developed including the gut microbiome. The isoflavone daidzein was used as model compound, and the plasma concentrations of its intestinal microbial metabolite *S*-equol were predicted. The experimental model was based on anaerobic incubations of rat fecal samples, and was optimized to allow definition of the maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) of intestinal microbial metabolism of daidzein. To this end, fecal slurry concentration and incubation time were selected from their respective linear ranges before testing a range of substrate concentrations. Apparent V_{max} and K_m for daidzein degradation and formation of the metabolites dihydrodaidzein (DHD), *S*-equol and *O*-desmethylangolensin (*O*-DMA) were quantified. They were used as input parameters for PBK modelling, which includes an intestinal microbial compartment and enables prediction of both daidzein and *S*-equol plasma concentrations. Predicted plasma concentrations of daidzein and *S*-equol were comparable to reported plasma concentrations in *in vivo* studies. The described *in vitro-in silico* strategy allows prediction of *in vivo* consequences of intestinal microbial metabolism of xenobiotics, thereby contributing to 3Rs (Replacement, Reduction and Refinement) principles and 21st century toxicity testing strategies.

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P24-006
High-throughput platform for rapid TEER measurement of organ-on-a-chip endothelial and epithelial tubules

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Organ-on-a-chip technology has rapidly grown in the past decade, driven by the need for better predictive *in vitro* models for drug efficacy and toxicity assessment. These systems enable the formation of endothelial and epithelial tubules that are used to mimic *in vivo* cues such as flow exposure, mixed co-culture, and overall microenvironment. In conventional barrier transport studies, Trans-Epithelial/Endothelial Electrical resistance (TEER) is used to determine the integrity of barrier tissues. However, current approaches to TEER measurement involve the use of chopstick electrodes, incompatible with high-throughput Organ-On-a-Chip platforms.

Using the OrganoPlate®, a microfluidic platform for perfused 3D cell culture developed by MIMETAS (The Netherlands), one can replicate ECM-supported tubular structures and study the transport of drugs across a cellular barrier [1]. The format of the OrganoPlate makes it very suitable for high-content imaging [2], but the need for TEER-based measurements of cell models in the OrganoPlate still needs to be addressed.

To this end, we developed a fully automated TEER measurement platform capable of addressing up to 96 tubules in an OrganoPlate. The developed system makes use of an electrode interface compatible with the OrganoPlate microfluidics layouts. The system is lightweight, fits in an incubator and can be used in combination with a rocker platform to provide perfusion in parallel to long-term TEER experiments. The device can read out an entire 3 lane OrganoPlate with 40 perfused tubules within 60 seconds and allows programmable measurements over the entire duration of an epithelial/endothelial study. We quantified TEER values in multiple epithelial/endothelial tubules, including widely used primary and immortalized cell lines such as Caco2, Huvec, and RPTEC, and developed an automated signal analysis solution, suited for high-throughput assays in the OrganoPlate. To validate the system for compound exposure studies, experiments were conducted on established OrganoPlate Caco2 gut model. Collagen I at 4mg/ml was layered in the gel compartment of the OrganoPlate. A suspension of Caco2 cells at 10e6 cells/ml was added to the perfusion compartment and incubated at 37 degrees on a perfusion rocker. TEER was monitored for 11 days until a plateau was reached at 490 Ohm.cm² +- 87 Ohm. Additionally, inflammatory cytokines were added on a subset of the tubules at day4. A subsequent change in TEER at 72h was quantified showing compound dependent effect in TEER decrease.

Complementing the OrganoPlate scope of application, we developed a novel technique for on-a-Chip epithelial/endothelial tissue TEER investigation with very fast measurement times, automated signal extraction and compatible with tissue culture incubator environments. This provides a valuable tool for drug toxicity and transport studies in Organ-on-a-Chip.

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P24-007
The *in vitro* study of the metabolism of zearalenone (ZEN) by intestinal microbiota from three species

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Zearalenone (ZEN) is a mycotoxin produced by *Fusarium* species which can contaminate crops, mainly wheat and maize. The toxicity of ZEN has been associated with reproductive disorders due to the ability to exert estrogenic effects through the binding to estrogen receptors (ERs). Species differences in sensitivity to ZEN exposure have been observed, and pigs are considered the most sensitive species. The reduction of ZEN to the major phase I metabolites, α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), is considered to play a role in the differences in sensitivity. α-ZEL and β-ZEL have60-fold higher and 5-fold lower estrogenic potency than ZEN, respectively. Hence, the preference for the formation of α-ZEL may contribute to the higher sensitivity of pigs. While hepatic metabolism of ZEN is generally considered the major source of α-ZEL and β-ZEL, intestinal microbial metabolism has been suggested to also yield these metabolites, which may contribute to the species differences.

To assess the contribution of the intestinal microbiome to ZEN metabolism, an *in vitro* model for intestinal microbial metabolism in

different species was developed. To this end, anaerobic incubations with fecal samples from rat, pig and human were optimized to define the reaction kinetics for the formation of α-ZEL and β-ZEL from ZEN. In all species tested, α-ZEL was formed to a higher extent than β-ZEL; the ratios of these metabolites were 2:1 in rats and pigs and 6:1 in humans. To facilitate interspecies comparison, the *in vitro* catalytic efficiencies (K_{cat}) for α-ZEL and β-ZEL were scaled up to relevant *in vivo* K_{cat} values, considering species differences in fecal production. Pigs had the highest *in vivo* K_{cat} for α- and β-ZEL formation of 234 and 157 mL/h, respectively, and rats the lowest K_{cat} of 0.6 and 0.16 mL/h respectively. The *in vivo* K_{cat} for α-ZEL and β-ZEL in humans were 51 and 7 mL/h, showing the highest relative preference for conversion to α-ZEL. A comparison to liver K_{cat} based on published data for pigs and rats indicates that in these species the K_{cat} of the microbiome is comparable to that of the liver, underlining the important role of the microbiome in toxicology. The developed model is an indispensable tool to study intestinal microbial metabolism of xenobiotics, and can be applied to derive rates of metabolism in different species.

P24-008
A comparative study on risk characterization methods for combined inhalation exposures to biocide mixtures in consumer products

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Biocides have been broadly developed and used in different sectors, manufacturing, construction, and service industries. Consumer products can include one or more biocides for maintaining the function of original products without any biological contamination. Many scientific studies have shown that mixture toxicity can be caused by cocktail effects (also known as joint effect or combined effect) among mixture components even at their no observed effect concentrations (NOECs).

Global biocidal product regulations (BPRs), e.g., European Union BPR, and Korean 'Consumer Chemical Products and Biocides Safety Act (also known as K-BPR)', have been newly adopted and updated for improving the authorization process of biocides. The mixture toxicity is taken into consideration in the risk assessment of biocidal products under those BPRs. In general, chemical risk assessment can be determined by considering both hazard and exposure data concerning target chemical products. Risk characterization is a final step in the risk assessment to assess if risks are adequately controlled by estimating the risk characterization ratio (RCR) indicating the ratio of the estimated exposure and the derived no-effect levels (e.g. DNELs). If the RCR does not exceed one, it can be considered that the risk is properly controlled within given exposure conditions. However, studies on estimating the RCR of combined exposures to different mixture components are still lack for developing a reliable mixture risk assessment despite a need for developing appropriate risk assessment methods for mixtures.

Therefore, the objectives of this study were i) to conduct a comparative case study on different risk characterization methods for combined inhalation exposures to different biocide mixtures; and ii) to examine possible deviations between RCRs calculated by different methods so that future challenges in the mixture risk assessment could be derived.

P24-009
In vitro fermentation of *pleurotus ostreatus* and *ganoderma lucidum* by human gut microbiota: cytotoxic, genotoxic and metabolomic analysis of the products

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Edible basidiomycetes are known for their health-promoting properties. Growing evidence supports that their immune-modulating and anti-cancer effects are mediated by their prebiotic capacity. β-glucans, a group of β-D-glucose polysaccharides abundant in the fungal cell walls, are considered responsible for their potential prebiotic effects. The use of indigenous fungal genetic resources to develop nutraceuticals is, thus, of great importance.

In the present study, the prebiotic activity of *Pleurotus ostreatus* and *Ganoderma lucidum* cultivated mushrooms deriving from Greek habitats with high β-glucan content and the **cytotoxic, genotoxic and metabolomic analysis of their fermentation products** is being investigated. Hence, the whole fungus as well as β-glucan enriched extracts were tested for their ability to alter the composition of the intestinal microbe following their *in vitro* fermentation by fecal slurry of healthy volunteers. Lyophilized fungal substrates and inulin, an established prebiotic, at appropriate concentrations, were *in vitro* fermented for 24 hours. *In vitro* fermentation without any additional carbon source was in parallel carried out to be used as reference.

The fermentation products were found to be cytotoxic in hematopoietic U937, colorectal CaCo2 cell lines as well as Peripheral Blood MonoCytes (PBMCs) cells in a dose-dependent manner. The global metabolic profiling of fermented products was assessed by the use of ^{1}H NMR spectroscopy, and metabolites resonances were assigned guided by Chenomx NMR Suite and literature data. Preliminary results revealed variations in the profile of the products as a result of the *in vitro* fermentation of *P.ostreatus* and *G.lucidum* derived substrates.A comparative survey between the above substrates, using chemometrics in combination with 2D NMR spectroscopy will be further applied and discussed, in order to identify biomarkers associated with the health promoting effects and the biological activities of *P. ostreatus* and *G. lucidum*.

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P24-010
Two new approaches for the risk characterisation of chemicals: The Source Related Hazard Quotient and Hazard Index (HQs, HIs) and the Adversity Specific Hazard Index for mixtures (HI_A).

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A hazard quotient (HQ) for a single chemical and hazard index (HI) for a mixture of chemicals were first described as approaches for risk characterisation by the EPA. HQ is defined as the ratio between exposure and an appropriate reference dose (e.g. ADI, RC, etc). HI is the sum of the HQs of the chemicals in a mixture. HQ and HI were used by many researchers in their effort to characterise risk after various exposure scenarios. However, both approaches have at least one serious limitation. The accurate use of HQ or HI requires estimation of aggregate exposure meaning the exposure to a given chemical(s) from all possible sources. In many studies, risk is assessed assuming exposure from a specific source such as, consumption of water or a specific food stuff, in which chemical(s) concentration(s) have been measured. In this case the classic HQ/HI approach cannot be used. For this purpose, we developed an alternative approach, named as Source Related HQ/HI (HQs, HIs) where a correction factor is calculated based on the permitted contribution from the specific source in the aggregated exposure, and its application to the classic HQ/HI value of one. A second serious limitation relating specifically to the HI approach is the use of chemical specific ADIs which do not correspond to the same critical effect. In this study, we describe an analysis based on the individual critical effects, in order to derive the critical effect for the whole mixture and an adversity specific Hazard Indices (HI_A) and risk characterisation.

P24-011
Candida albicans increases inflammatory responses through ER stress in human colorectal epithelial cells

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Candida albicans (*C. albicans*) is known to cause invasive candidiasis in humans when hyphae are formed. Although imbalance of microbiota and compromised immune function are known to be associated, there is lack of data how *C. albicans* induce inflammation in human intestinal barrier. Thus, the aim of this study was to investigate inflammatory responses and the underlying mechanisms induced by *C. albicans* in human colorectal epithelial cells. Human colorectal epithelial cells (Caco-2) were infected with *C. albicans* (6 h, $1x10^4$–$1x10^7$). Results showed that cell adhesion and cell invasion through extracellular matrix was significantly increased at $1x10^6$–$1x10^7$ CFU/mL of *C. albicans*. The expression of tight junction proteins (claudin-1, claudin-5, occludin and E-cadherin) were significantly decreased in cells infected with $1x10^6$–$1x10^7$ CFU/mL of *C. albicans*. Furthermore, cells infected with *C. albicans* ($1x10^6$–$1x10^7$ CFU/mL) increased inflammatory responses, such as upregulation of cyclooxygenase-2, IL-1β, IL-6, TNF-α and NF-κB. Cells infected with *C. albicans* also induced endoplasmic reticulum (ER) stress through increased expression of glucose regulate protein 78 and phosphorylation of eukaryotic translation initiation factor 2α. The level of intracellular reactive oxygen species was significantly higher in cells infected with *C. albicans* ($1x10^5$–$1x10^7$ CFU/mL). These results demonstrated that *C. albicans* induce inflammatory responses, particularly through ER stress in human colorectal epithelial cells when hyphae are formed. Our data suggest that *C. albicans* is an important risk factor for the integrity of intestinal barrier function.

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P24-012
The risk management strategy in chemicals risk area of Thailand

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Purpose: According to the various sources of chemicals in occupations and environment, the health surveillance program should be dealt with risk assessment of all kinds of exposed chemicals. Therefore, this study is aimed to establish the effective of risk management strategy in chemicals risk area of Thailand.

Methods: The literature of chemicals risk area, particular in heavy metal mining, petrochemicals, and agriculture, were studied in following the economic growth under the National Economic and Social Development Plan and international cooperation on health during three decades (1988 –2018). The systematic reviews on methodology of risk assessment, environmental and health impact assessment (EHIA), and selected safety value were proceeded.

Results: The chemicals were released from the production process, mineral ores, ambient air pollution, and daily activities. The sign and symptom of health effects resulted from the unexpected combined effects of chemicals. For occupations, all workers exposed to chemicals in the production process and the environmental pollution. The risk management strategy regularly processed on risk assessment of one focused chemical. Besides, the linkage between environment and health impact was not concerned. It was shown that the health surveillance program was deviated from the actual situation caused by insufficient information.

Conclusions and suggestions: The chemicals from various sources are able to enter to the human body. Therefore, the effective risk management strategy should be processed on community risk mapping and the linkage between environment and health accompanied with in-depth interview to justify the risk group for health surveillance program.

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P24-013
Microbial biotransformation of azo dyes to carcinogenic aromatic amines

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Natural and synthetic dyes are used to enhance the appearance of food. Some of them are applied indirectly, as feed additives given, e.g., to salmon or laying hens. Several azo dyes, including Sudans I-IV and Para Red, are forbidden to use in food and feed because they are found to be genotoxic. One of the proposed mechanisms is through their biotransformation to aromatic amines. Because of the reports of the illegal application of azo dyes to laying hens, we have decided to verify the potential of poultry gut bacteria to enzymatically cleave azo bounds of selected dyes.

The experiment was performed with reference strains of *Bacillus subtilis*, *Clostridium perfringens*, *Enterococcus spp.*, *Escherichia coli*; as well as the isolates of the same species from gut flora of laying hens. The total non-specified microflora was tested in parallel. Its composition was characterized by high throughput sequencing metataxonomics on the Illumina MiSeq platform. Bacteria were incubated with a respective dye for 24 hours. Then the culture medium was analyzed for the presence of the dyes and their degradation products using target liquid chromatography-tandem mass spectrometry method on Shimadzu Nexera/8050 system.

Only monocyclic amines were detected after incubation with bacteria. These were: aniline for Sudan I and III, 2,4-dimethylaniline for Sudan II, o-toluidine for Sudan IV, and 4-nitroaniline for Para Red. The 1-amino-2-naphtol, selected by *in silico* approach as a potential product of all tested azo dyes, was not found in any conditions, possibly because of further biotransformation. Within one species, the degree of biodegradation was highly reproducible. *Enterococcus* was the only bacteria that metabolized all the dyes. *Bacillus*, *Clostridium* and *E.coli* biotransformed four (Sudan I, III, IV and Para Red), two (Sudan I and IV) and one (Para Red) dyes, respectively. In some samples, the signal with the transitions and retention time of 2,4-dimethylaniline was present, although chemically such product was not feasible.

The results confirm that the extent of biodegradation of azo dyes depends on bacteria species. The high degree of decoloration of dyes in the gut questions their purpose as feed additives. At the same time, it means that consumers may be exposed to both parent compounds and their toxic biotransformation products when azo dyes are illegally administered to hens. Further research on the kinetics of azo dyes in animals is needed.

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P24-014
Smoked meat products: assessment of hormonal activity using estrogen receptor transactivation assay and immature hamster uterotrophic responses

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Background: High intake of processed meat has been associated with increased risk of hormonally-dependent cancers [1]. Smoked meat products (SMPs) are a prevalent dietary source of polycyclic aromatic hydrocarbon (PAH) carcinogens that possess hormonal activity and thus may increase the risk of cancer [2,3]. However, no studies have examined whether intake of smoked meat influences hormonal balance.

Methods: PAHs fractions extracted from 12 SMPs were tested for estrogenic and antiestrogenic activity in an *in vitro* yeast-based estrogen receptor (ER) reporter gene assay [4]. The estrogenicity of extracts was also investigated in uterotrophic assay in immature female hamsters (OECD TG 440) [5]. The effects on weights of ovaries, adrenals, liver and kidneys were also studied.

Results: *In vitro* estrogenic activity was observed in case of four extracts in the range from 1.06 to 27.24 ng EEQ/g. All extracts showed antiestrogenic activity in the presence of 1 and 2 nM of 17β-estradiol (E2). Seven of them strongly reduced E2 response by more than 75%. Statistically significant uterotrophic response ($p < 0.05$) was observed for three extracts with the highest relative uterine wet weight increase of 26% in comparison with a negative control. The statistically significant hypouterotrophic effects observed for four extracts suggest antiestrogenic activity of SMPs. Relative ovaries weights were elevated after exposure to eleven SMPs extracts, but statistically significant increase (in the range from 39 up to 72%) was shown for five extracts. Although exposure to all extracts decreased relative adrenal weights (4–28%), significant adrenal atrophy ($p < 0.05$) was shown in case of four extracts. Five of 11 SMPs extracts caused significant liver hypertrophy (31–44%) and increase in kidneys weights (27–48%).

Conclusions: *In vitro* estrogenic and antiestrogenic activity, uterotrophic and hypouterotrophic effects as well as increased weights of ovaries indicate influence of SMPs on hypothalamic–pituitary-gonadal (HPG) axis, whereas adrenal atrophy suggests influence on hypothalamic–pituitary–adrenal (HPA) axis. Increased kidney and liver weights should also be considered as potentially adverse effects. High intake of SMPs may disturb hormonal balance as well as negatively affect liver and kidney functions.

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P24-015
AOP-based experimental models to evaluate effects of azole mixtures

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The effects of binary mixture of cyproconazole (CYPRO) and triadimefon (FON) sharing the same adverse outcome pathway (AOP) for craniofacial malformation were studied using different experimental models. The proposed AOP is based on the inhibition of CYP26, the retinoic acid (RA) local increase and key events leading to branchial defects in embryos and cranio-facial malformations at term of gestation. The activity of the two molecules on CYP26 enzymes was evaluated by an *in silico* method (docking), while teratogenic effects were evaluated both *in vitro* (postimplantation rat whole embryo culture at E9.5, WEC) and after *in utero* exposure. WECs were exposed for 48 hours to CYPRO (7.8–250 μM), FON 6.25–125 μM or mixtures. CD1 mouse females were treated at E8 (comparable to rat E9.5) by gavage with CYPRO (25–100 mg/kg), FON (37.5–500) or mixtures. Maternal and foetal outcomes were evaluated at term of gestation (E18). Some dams were sacrificed at E9 (midgestation, comparable to the rat stage at term of WEC) to evaluate embryonic morphology and compare with WEC results. Malformation data were modelled by PROAST 65.2 software. Docking results show a CYP26 inhibitory potential for both molecules. Both *in vitro* and *in vivo* results showed a clear dose–response for single fungicides, better defined by WEC, co-exposure resulted in an additive effect. Cranio-facial malformations recorded at E18 were related at midgestation to branchial defects similar to those observed in WEC experiments.

The obtained data support the hypothesized AOP and suggest that WEC results could be a simple but predictive alternative method applicable to the hazard evaluation of mixtures' exposure. On these bases, we suggest the use of WEC in order to test azole mixtures *in vitro* and their effects on cranio-facial morphogenesis.

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P24-016
Microbiome modification as possible way to reduce toxic load in agriculture (on the example of cereal spiked crops treatments)

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Receiving good yields of cereal spiked crops as major contributors of nutrients nowadays accompanies with sometimes enormous applications of pesticides (fungicides, herbicides etc.) and fertilizers (especially toxic in particular conditions nitrogen fertilizers). Farmers perform treatments of crops 3 to 5 times per vegetative periods and being exposed to various types of pesticides and fertilizers mixtures.

Purpose of our **research**: To evaluate efficiency of microbiome modification in relation to reduce toxic load in agriculture (on the example of cereal spiked crops treatments).

Methods: Full-scale in-field hygienic experiment (2 series), microbiological and statistical (Mann–Whitney *U*-test) methods were used in our study. Cultures of *A.chroococcum* and representatives of *Pseudomonas, Rhizobium, Lactobacillus, Bacillus* genera and *T. aestivum, H. vulgare* were materials of research.

Results: Presowing prepared grains treated with microquantities of 2,4-D (as callus inductor), cytokinine (kinetin) and studied microbial cultures were planted and treated second time with microbial cultures after approx. 7 weeks in Ukrainian argoclimatic conditions. Amount of fungicides applied in this case was reduced twice, nitrogen fertilizers were totally excluded from the standard treatment procedure, and potassium-phosphorus fertilizers ($N_{16}P_{16}K_{16}$) reduced to $^2/_3$.

Biological parameters of plants' root system indicate its resistance to *X. Campestris* (common cereal crops pathogen).

Content of protein in grains was 16.08% in average samples and gluten content – 29.3%. Grains in harvest time did not contain the residue amounts of applied fungicides. Correspondent levels of them were below relevant LODs.

Fungicides level in the working zone air did not exceed 25% of AOEL.

Conclusion: Suggested approach helped to decrease toxic load on farmers during the treatment (absence of nitrogen fertilizers and decreased fungicides levels in working zone air), harvest did not contain fungicides and agriculture environment was protected. Microbiome modification in cereal spiked crops treatment reduces the amount of possible toxic substances applied and thus decreases toxic load on farmers, consumers and environment.

P24-017
Establishment of a multi-organ-chip based identification platform for endocrine disruptors

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The extrapolation of toxicities observed in rodent studies to human health still represents a major issue for toxicologists. Especially thyroid hormone disrupting compounds present a reoccurring problem for pharmaceutical and agrochemical industry. The levels of thyroid hormones are not only regulated via synthesis and secretion by the thyroid gland, but also by its metabolism and clearance via the liver. To this end, it is a pre-requisite to consider direct effects on the thyroid gland as well as indirect effects mediated by the liver when it comes to safety assessment of potential thyroid hormone disrupters. Furthermore, species differences between rodents and humans have to be taken into account and the extrapolation of data derived from rodent testings have to be critically evaluated.

Here, we describe the establishment of a human three-dimensional (3D) liver and thyroid co-culture model and its integration into a Multi-Organ-Chip (MOC) platform. Our organ chip model is composed of two culture compartments connected by microfluidic channels which allow an inter-organ specific cross-talk. On chip micropumps enable a near to physiological tissue-to-fluid ratio and enhance oxygen and nutrition supply of the integrated thyroid and liver organoids resulting in a long-term culture of at least 14 days. Our study presents first results of our MOC-based liver-thyroid assay including data which demonstrate the long-term viability of the co-culture model as well as required functionality of the assay. Thus, we

show for the first time the attempt to model the human hepatic-thyroid axis within a single *in vitro* assay. It is expected that the assay will provide a better understanding of thyroid hormone disrupting toxicities in humans and will serve as a highly predictive tool for human risk assessment of drugs and agrochemicals.

P24-018
Streptococcus pneumoniae inhibits Pseudomonas aeruginosa growth on nasal human epithelium *in vitro*

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Pathogens colonizing the respiratory tract compete with a range of other bacteria and may interact with xenobiotics upon inhalation. *Pseudomonas aeruginosa* (PA) infection are increasingly associated with acute exacerbations in chronic obstructive pulmonary disease. *Streptococcus pneumoniae* (SP), meanwhile is a main cause of pneumonia, meningitis, it can leads to infections and other respiratory diseases such as bronchitis.

We report herein the use of 3D airway epithelia reconstituted *in vitro* to study interactions of PA and SP on nasal mucosa. MucilAir™, a fully differentiated human airway epithelium made of a mixture of primary nasal cells from 14 donors, was used to study the effects and behaviour of PA and SP (inoculated at $3E^{+02}$ and $3E^{+11}$ CFU/cm^2 respectively) cultivated separately or together over 24 hours.

Apical, basolateral and intratissular PA and SP growth were quantified by Colony Forming Unit (CFU). Impairment of epithelial homeostatic barrier function was evaluated through monitoring of tissue integrity (Trans Epithelial Electrical Resistance – TEER); cytotoxicity (LDH), cilia activity, mucin and IL-8 release.

PA infection induces a loss of TEER, 20% cytotoxicity and an increase of Il-8 (+100 ng/ml). On the contrary, SP strongly increases the mucin and H2O2 production. While inoculated together, a lower apical PA growth is observed (-$3E^{+3}$ CFU/cm^2) suggesting an inhibition due to the presence of SP.

These results suggest that *in vitro* human airway epithelia is a useful model to study bacterial interaction on the human nasal mucosa and to understand how microbiome modifies local toxicity.

P24-019
High-throughput teratogenicity screening validation in zebrafish embryos

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There is an increasing demand for a rapid, reliable and cost-effective methodology for detecting developmental toxicity, i. e. teratogenicity, of chemical substances, particularly new drugs and medications. Zebrafish has gained popularity in the field of safety pharmacology and ecotoxicology due to its unique biological qualities to such an extent that now is considered a potential alternative to experimentation in higher vertebrates. Here, in this study, we aim to validate a high-throughput system in zebrafish for the appraisal of teratogenicity of a set of 30 compounds reported as teratogenic or non-teratogenic in humans. Non-dechorionated zebrafish embryos are exposed to test compounds from 6 to 96-hour post-fertilization. In a first studdy phase, we perform a dose range finding assay in order to determine the Benchmark Dose 10 (BMD10; lethal concentration for 10% population) for each compound. The BMD10 is subsequently used to establish a narrower exposure range of concentrations (BMD10/2, BMD10, BMD10x2, BMDx4, BMDx8) that permits, after automated imaging through a capillary-based system, to determine and score morphological changes and functional abnormalities according to 15 physiological teratogenic endpoints, namely body deformity, heart edema, otic vesicle, yolk size, length, eye size, head anomalies, pigmentation, developmental delay, body axis, scoliosis, fin absence, necrotic tissue, kidney cyst and hatching rate. By following this new approach, we are able to detect even the most subtle teratogenic effects, including those manifested at concentrations close to lethality. Our study shows clear dose-response teratogenic effects for those compounds previously reported as teratogens in humans, including thalidomide, a substance usually reported as non-teratogen in zebrafish, while non-teratogenic compounds induced no phenotype alterations. The good correlation between our results and the results previously published in zebrafish and higher vertebrates, including humans, indicates the predictive potential of the model for high-throughput teratogenicity screening, and supports its use as experimental alternative.

P24-020
Development of a rodent liver-thyroid-2-organ-chip for thyroid toxicity testing

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Thyroid hormones are essential for many cellular processes such as the basal energy metabolism, cell proliferation and differentiation. Consequently, thyroid dysregulation can have tremendous effects on many organs. Rodents, which are widely used for regulatory toxicity testing, are particularly sensitive to perturbations of the thyroid homeostasis and respond with hypertrophic, hyperplastic or even neoplastic alterations of the thyroid gland. Chemicals can induce thyroid toxicity directly through effects on the thyroid gland or indirectly by accelerating the turnover of thyroid hormones secondary to liver activation. Differentiation between direct endocrine disruption and indirect liver-mediated thyroid toxicity has major impact on the safety risk assessment of chemicals, as only the latter is a generally accepted threshold mechanism. However, an integrated *in vitro* model to test and distinguish direct and indirect thyroid toxicity is currently not available.

Here we describe the development of a micro-physiological system (MPS) for co-cultivation of three-dimensional (3D) rat liver and thyroid tissue. The on-chip micro-pump and microfluidic channels interconnect the two tissues and support their lifelike behavior. Liver and thyroid 3D-cell cultures were obtained from freshly isolated primary rat tissue and maintain key physiological features. Liver and thyroid 3D-cell cultures can simultaneously be cultivated in a media perfusion circuit within a 2-Organ-Chip, which results in maintaining the organ specific architecture and functional activities for at least 14 days. This 2-Organ-Chip will serve as useful tool to investigate interactions of the two target organs leading to liver-mediated thyroid toxicities and support the safety risk assessment thereby contributing to the 3R principles (replacement, reduction, refinement). In parallel, this project also aims to develop a 2-Organ-Chip for the co-cultivation of human liver and thyroid tissue, which together allows to study potential species specificity of chemical-induced perturbation of the thyroid homeostasis.

P24-021

Risk assessment of EDCs in Europe based on human biomonitoring data

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A major advantage of human biomonitoring (HBM) data is that they provide an integrated overview of the body burden to xenobiotics that an individual is exposed to. However, quantification of exposure based on HBM data poses significant challenges that are worth facing, given the opportunities that HBM provides in terms of informing and effectively supporting risk assessment. Based on the above, the aim of this within the HBM4EU project was to derive EU-wide external exposure estimates starting from HBM data and to derive and risk characterization ratio (RCR) by comparing these estimates with existing regulatory thresholds. For the reconstruction of exposure the INTEGRA computational platform was properly parameterised for the compounds of interest, namely bisphenol-A (BPA), phthalates (DEHP, DiNP and DnBP) and DINCH, emerging flame retardants (TCEP) and Perfluorinated compounds (PFOA and PFOS).

The results indicated that for the majority of the examined compounds, daily intake levels are below the existing regulatory thresholds. For BPA, mean daily intake is almost 2 orders of magnitude below the respective threshold proposed by EFSA. For phthalates, daily intake estimates are usually one or two orders of magnitude below the respective TDI, with the exception of BBzP, for which intake estimates of the upper part of the exposure distribution is close to the threshold of 10 μg/kg_bw/d. Regarding TCEP, which is a typical emerging flame retardant, the mean daily intake estimate is below 0.1 μg/kg_bw/d, which is far below the calculated 'provisional' TDI of 13 μg/kg_bw/d, however, at the moment very few HBM data were available and these exposure levels are rather indicative than representative of the European countries. Finally, regarding the estimated intakes of PFCs, intake levels of PFOS are very close to TDI (0.15 μg/kg_bw/d proposed by the CONTAM Panel), while the calculated levels for PFOA are one order of magnitude below the respective TDI of 1.5 μg/kg_bw/d.

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LP | Late Posters

P-Late-01
Prediction of non-genotoxic carcinogenic potential of agrochemicals

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To protect man and the environment from health risks, chemical substances are subject to hazard and risk assessments before being marketed, including assessment of carcinogenic potential. Non-genotoxic carcinogens (NGTXC) do not directly interact with DNA and therefore cannot be detected based on genetic toxicity assays. Consequently, the risk of NGTXC may remain largely undetected unless carcinogenicity studies are performed. From a scientific perspective, prominent weaknesses in rodent carcinogenicity studies have been identified related to the translation to humans and the reproducibility of the results. In recent years, alternative approaches have been introduced for industrial chemicals and pharmaceuticals, while for agrochemicals the 2-year rodent carcinogenicity study is still required. Our study aims to provide a concept for the development of a science-based and mode of action (MOA)-driven approach for predicting the carcinogenic potential of non-genotoxic agrochemicals. To this end, from a database of 411 substances we collected information on the MOAs of 171 non-genotoxic agrochemicals. Among the nine identified MOA networks are liver enzyme induction, endocrine disruption, receptor activation, oxidative stress and sustained cytotoxicity. Nevertheless, for a substantial number of agrochemicals, the MOA leading to a specific tumour type remains unknown. These substances are of concern, as we aim for an inclusive approach avoiding false negatives that is also in line with the needs of regulators. Our long-term goal is to provide an approach for detecting non-genotoxic carcinogenic potential of agrochemicals without performing a 2-year carcinogenicity study, based on MOA information collected via dedicated batteries of *in vitro* studies complemented with information from the 90-day repeated dose toxicity studies.

P-Late-02
Cosmetic Safety Assessment: *in silico* contribution in practice

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Changes imposed by the international regulation (Reach and 7th Cosmetics Amendment) and the introduction of safety assessment notions in the early development stages of new ingredients, have strongly accelerated the development of *in vitro* and *in silico* alternative methods for assessing the safety of ingredients. Developing these alternative methods was an early objective of our Research group to resolve such challenges. As compared to other industries, the cosmetic domain has to take into account major specificities, owing to the large diversity of its products and the various ways of application. These include, among others, a large variety of ingredients of different physicochemical properties, with major toxicological endpoints related to skin.

Official guidelines are available on *in vitro* methods, and an increasing amount of recommendations are available with regard to *in silico* methods for assessing the safety of cosmetic ingredients. Some of our findings are presented here, based on in house examples of applications of *in silico* methods for such assessments. For example:

Ex. 1 – Our Defined Approach (DA) for skin sensitization of ingredients, combining *in silico* methods with *in vitro* tests was shown to provide a more satisfying response, especially when these are based on notions of adverse outcome pathways.

Ex. 2 – *In silico* methods may also be part of read-across strategies for the safety assessment of ingredients. As shown, derived outcomes from different quantitative structure property/activity relationships (QSPR/QSAR) models can be used as parameters to help establish the "similarity" of the investigated ingredient with another ingredient of known toxicity.

This task remains however difficult, especially in the case of complex endpoints. In addition, further *in silico* developments should probably integrate even more the exposure(s) (i.e. ADME parameters) of ingredients.

P-Late-03

This abstract has been withdrawn.

P-Late-04
Copy number variants in silver nanoparticles-primed hyperactive rats

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For long time, we had believed that the fetus would be guaranteed by placenta against foreign materials until thalidomide and diethylstilbestrol (DES) had been found to exert harmful effects on fetus. After then, reproductive and developmental testing for chemicals is legally carried out with obligation. However, recent research shows evidence that some chemical effects were inherited through the next generation: even that is a single exposure. Standing on this fact, we examined if hyperactivity seen in ADHD or autism would be inherited in the rat.

We exposed pregnant rat (E7 day) to silver nanoparticle (4mg/kg), after which we never exposed it, again. Then, we got hyperactive rats at next generation by outcross. Also, at F3 generation, we got hyperactive rats by mating with female control rats. We developed two lines of the model. They were 1.4~1.5 fold higher than that of control in the spontaneous motor activity. They were not soft inheritance.

Possible etiology of autism might come from genetic factors and/or maternal life style in pregnancy. Particularly, much attention has been paid to copy number variants (CNVs) in patients with autism. There are many CNVs reported, in particular, 16p11.2 has much attention, because it was reported in many psychological disorders, not only autism but also ADHD, schizophrenia, and bipolar disorders. Therefore, we examined CNV in our hyperactive rats. There were many CNVs found, including chromosomes 1 to 20, except chromosomes 5, 7 12 19. Both amplification and/or deletion occur. Intense fluoresce signals were found in chromosomes 1,2 3,6, and 20. We are now examining if these CNVs is pathogenic or not.

P-Late-05

This abstract has been withdrawn.

P-Late-06
Dietary exposure of Finnish children and adults to inorganic arsenic

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Inorganic arsenic is an environmental carcinogen, and it enters the food chain through plants taking up the heavy metal from the soil as well as through water. International expert organizations have determined that there is no safe threshold value for inorganic arsenic exposure (EFSA, 2009), and therefore, the margin of exposure to a benchmark dose is used to estimate the risk to consumers. Benchmark doses have been determined by e.g. the FAO/WHO expert group (JECFA, 2011) based on cancer risk increase, particularly that of lung cancer, with dietary exposure via food and water.

The dietary exposure to inorganic arsenic was determined probabilistically using governmental data on arsenic occurrence in foodstuffs and nationally collected food consumption data from studies DIPP [Kyttälä *et al.* 2008] and FINDIET 2012 [Helldán *et al.* 2013]. Most of the occurrence data were available as total arsenic, and the inorganic arsenic content was calculated from total arsenic using fixed percentages for the portion of inorganic arsenic in water, fish and seafood, and all of the other foodstuffs. The food consumption data were already calculated from 3-day food diaries (DIPP) and 48-h recall interviews (FINDIET 2012) to ingredient level for each individual. The online program MCRA was used to assess the dietary exposure probabilistically from the dataset on concentrations in foodstuffs and the datasets on individual food consumption data.

The margin of exposure for Finnish children and adults in the age groups 1 to 6 years and 25 to 74 years is presented. The margin of exposure was lowest for 1-year-old girls, for whom it was slightly above 9, and highest for 65–74-year-old men, for whom it was nearly 33. These values show low to moderate risk. The sources of dietary exposure to inorganic arsenic in the different age groups are also presented. Due to the higher inorganic arsenic levels in rice, compared with other grains, the relative importance of rice products as a dietary source of arsenic is higher than their consumption would suggest.

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P-Late-07
Validation and use of *in vitro* 3D Skin genotoxicity assays in a tiered strategy to support the safety assessment of cosmetic ingredients

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The EU Cosmetics Directive has banned the use of *in vivo* genotoxicity models and, while the *in vitro* 2-test battery has a high sensitivity for prediction of *in vivo* genotoxic/carcinogenic agents, it tends to result in misleading positive results. Therefore, there is a need for refined *in vitro* models that are more predictive for the risk assessment of cosmetic ingredients. To address this, new *in vitro* 3D human reconstructed skin (RS) models have been established as follow up assays to improve the prediction in the absence of *in vivo* data. These more complex assays also consider the most relevant route of exposure to cosmetics, namely topical. Here, we report on the validation of two assays combining human 3D RS tissues with classical genotoxicity readout-parameters.

The testing of coded chemicals (55 across both assays) is now complete and the results have been evaluated by an independent statistician. Each assay exhibited a good sensitivity and specificity (being finalized): 77% and 85% for 3D Skin Comet and 80% and 87% for RSMN. The strategy of their use is based on an endpoint-triggered follow up of positive results from the 2-test battery. For topically applied chemicals, the reconstructed skin micronucleus test (RSMN) assay is recommended as a follow-up for *in vitro* micronucleus (MNvit) positive chemicals; whereas, Ames positives should be followed-up with a 3D

Skin Comet assay. A combination of the two skin-based assays enables all three types of DNA damage (mutation, clastogenicity and aneugenicity) to be addressed. Since most of the 'true positive' chemicals (*in vivo* genotoxic rodent carcinogens) tested in these assays were positive in Ames and MNvit, the sensitivity increases to 89% if the endpoint-triggered strategy is applied (both 3D skin comet and RSMN would be performed). Importantly, the specificity remains high (above 80%).

In conclusion, the excellent sensitivity and specificity of these *in-vitro* assays supports their use as a follow-up tests to the standard 2-test battery. Both assays can be used as a follow up for the testing of topically applied chemicals, depending on the result of the 2-test battery. Moreover, this tiered strategy shows great promise as an *in vitro*-only approach for genotoxicity testing of cosmetic ingredients.

P-Late-08
A novel cell-based high-throughput screening assay to identify and characterize potential (anti-)estrogenic substances

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A particular strength of alternative *in vitro* methods is the ability to efficiently study effects of a large number of different chemicals and to provide mechanistic insights into the processes mediating toxicity. Given the enormous number of chemicals marketed worldwide and the more or less infinite number of possible combinations, high-throughput screening (HTS) approaches are playing a central role.

Especially chemicals with the potential to disrupt the endocrine system, thereby causing adverse health effects, have been of increasing concern. Therefore, we established a screening library of 440 toxicologically relevant industrial chemicals, biocides and plant protection products that have been proposed to act on different nuclear receptors (estrogen receptor, androgen receptor, glucocorticoid receptor and thyroid-stimulating hormone receptor). We used this library to test the predictive capacity of a novel phenotypic HTS assay for the identification of estrogenic and anti-estrogenic substances. This functional cell-based screening method quantifies the changes in E-cadherin membrane levels, which we showed to be mediated by the estrogen receptor (ER) signaling pathway. Screening of the 440 substances identified 22 estrogenic substances with EC50 values that correlate with the ER bioactivity score published by the US EPA Endocrine Disruptor Screening Program [1]. Moreover, we identified 10 substances with apparent estrogenic activity that have not been described as those before. Additionally, the assay identified two known anti-estrogenic substances, i.e. Tamoxifen and Raloxifene, demonstrating its applicability to screen a large number of chemicals for both estrogenic as well as anti-estrogenic activity.

These data will eventually help to further advance our understanding of molecular modes of action of chemicals that act on the ER pathway and their potential adverse health effects in humans.

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P-Late-09
A new functional assay to identify chemicals with estrogenic potential

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Adverse health effects caused by endocrine disrupting chemicals (EDC) in our environment, food or consumer products are of high concern. Available *in vitro* assays provide information on mechanisms and pathways of endocrine activity such as receptor binding affinity and receptor transactivation capabilities of EDCs but largely do not cover functional endpoints such as hormone-related tumor formation and progression.

Here, we describe the development of such an *in vitro* assay for identification and characterization of test substances with estrogenic activity by phenotypic screening for estrogen-dependent changes in cell membrane morphology.

We found that estrogen signaling modulates the organization of adherens junctions in human breast cancer cells. Inhibiting estrogen signaling by knock down of estrogen receptor a or treatment with various anti-estrogens caused a clustering of adherens junctions and a distinct change in membrane morphology. This effect seems to have also clinical relevance since a similar (re)organization was also found in breast cancer tissue samples. Thus, we hypothesize that this particular estrogen-dependent change in membrane morphology might be a predictive and functionally relevant endpoint for the establishment of an *in vitro* assay.

We developed a robust high-content-based assay set-up and a pipeline for automated image acquisition and quantitative image analysis. Treatment of breast cancer cells with the anti-estrogen Fulvestrant resulted in an efficient inhibition of estrogen signaling activity and reorganization of adherens junctions that was prevented by co-treatment with substances of known estrogenic activity in a dose-dependent manner. Using a test set of 17 reference chemicals with known estrogenic activities, we could successfully confirm a high predictivity of adherens junctions reorganization as a readout for estrogenic activity.

In conclusion, this study introduces a novel robust and predictive assay for the identification of chemicals with estrogenic activities using a functional endpoint.

P-Late-10
Characterizing the low dose effects of methylmercury in early developmental stages using cultured human embryonic stem cells

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Methylmercury (MeHg) is a ubiquitous environmental contaminant. The body of evidence available to date suggests that neurodevelopment is the most sensitive health outcome and development in utero the most sensitive period of MeHg exposure. While most *in vitro* studies have focused on the effects of MeHg exposure during neural differentiation using differentiated cells, the effects of embryonic exposure to low dose MeHg at pre- and during implantation stages remain unclear. In this study, we used undifferentiated human em-

bryonic stem cells (hESC) as an *in vitro* model to determine the effects of MeHg exposure at pre- and during implantation stages. The hESC were exposed to Na_2CO_3 as vehicle control and 5–200 nM MeHg in fresh Essential 8™ Flex Medium on matrigel at 37°C, 4% O_2 and 10% CO_2 for 24 h or 7 days. Cell morphology and colony formation were examined under microscope. Cell viability, proliferation, apoptosis, autophagy, cell cycle, and stress response were measured at the end of exposures to MeHg. Our results revealed that exposure to nano molar concentrations of MeHg decreased cell viability and colony formation, increased apoptosis, oxidative stress and spontaneous differentiation, and altered expression of marker genes for cell fate. These results suggest that embryonic exposure to low concentrations of MeHg at pre- and during implantation stages may affect pregnancy outcome and fetal development if it occurs *in vivo*.

P-Late-11
Are Generic PBK Models the Panacea for QIVIVE?

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With the increasing application of *in vitro* cell culture models as primary tools for predicting chemical safety, the extrapolation of effective concentrations *in vitro* to adverse exposures *in vivo* has become increasingly important. Generic PBK models could potentially be the tool for the integration of kinetics into *in vitro* to *in vivo* extrapolations. Such generic models shall be user-friendly, open-access, and able to predict the kinetics of many different chemicals. Even if an effort has been put on the construction of such models, their calibration with real kinetic data is still lagging behind. Moreover, their applicability domains have not been explored extensively. Consequently, we explore here the applicability domain of a generic PBK model, the IndusChemFate. The model contains incorporated QSARs for the prediction of the distribution partition coefficients. For determining applicability domains, compounds were chosen based on a series of physicochemical characteristics, which were used for parameterization. Major determinants of kinetic predictions included lipophilicity and ionization state at physiological pH. In addition to these, certain ADME properties, such as metabolism and excretion pathways, play an important role. The results were analyzed with a principle component analysis. This study illustrates the added value of generic models as well as their limitations for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) as a tool in chemical safety assessment.

P-Late-12
Effect of a high-fat diet on factors related to energy balance and inflammation in AH receptor-deficient rats

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Previous studies conducted in C57BL/6 mice have revealed that global AH receptor (AHR) deficiency protects them against high-fat diet (HFD)-induced obesity and associated co-morbidities including glucose intolerance, diminished insulin sensitivity and hepatic steatosis & inflammation. However, it is currently unknown whether this a mouse-specific or more general phenomenon. Thus, the present study set out to address that point. Young adult (7–8-week-old) male rats on Sprague-Dawley background and harboring deletion of exon 2 in their *Ahr* gene (AHR-knockout, AHRKO), along with their wildtype (WT) littermates, were assigned to either a standard diet (SD, 10% of energy from fat) or HFD (45% of energy from fat) feeding group. In addition, the rats on HFD were provided a choice of either a 10% sucrose solution or water for drink, while the rats on SD obtained only water. In total, there were 32 rats in the study (10 + 10 on HFD and 7 [AHRKO] or 5 [WT] on SD). The feeding regimen was continued for 24 weeks. Food intake and sucrose & water consumption over 24 h were measured in weeks 6, 10, 11 and 21. At termination, serum, liver, interscapular brown adipose tissue (BAT), epididymal white adipose tissue (WAT) and skeletal muscle were harvested for analysis.

As the HFD was exactly the same brand as the one that had previously been reported to cause obesity in WT mice within 12 weeks, it was surprising that it failed to do so in WT rats over 24 weeks; body weight gain was only slightly greater on HFD vs. SD with no difference between the genotypes. Yet, rats on HFD consumed more energy than those on SD throughout the study. The lack of obesity may have been due to enhanced BAT thermogenesis, since BAT relative weight was or tended to be increased in both genotypes on HFD. Expression of the BAT key thermogenic gene, *Ucp1*, was elevated in AHRKO rats on HFD whether compared with their genotype or diet controls. No changes were seen in expression levels of the hepatic lipogenic enzyme genes *Acaca*, *Scd1* or *Fasn* or the key ketogenic enzyme gene *Hmgcs2*, in contrast to the fatty acid transporter *Cd36*, whose expression was increased in AHRKO rats on HFD. No differences among the groups were further recorded in the hepatic expression of *Fgf21*, whose gene product was previously suggested to mediate the enhanced BAT thermogenesis in AHRKO mice. Liver retinoid concentrations were lower in rats on HFD vs. SD. Interestingly, the liver and WAT transcript abundances of the cytokines IL-1β, TNF-1α and IL-10 were elevated by HFD in AHRKO rats alone. These findings thus suggest that the background strain of AHRKO rats is quite resistant to development of dietary overweight and would require an energy-denser diet to get obese. Despite this, the AHRKO rats still exhibited some unique metabolic and inflammatory responses, in particular induction of liver cytokine expression by HFD, which is in contrast to the reported mitigation of hepatic inflammation in AHRKO mice on HFD.

P-Late-13
Can inducing Phase II metabolism in the liver perturb thyroid homeostasis enough to cause adverse foetal neurodevelopment?

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Thyroid hormones (TH) are essential for the control of metabolism and nervous system development, and altered TH levels during critical periods of development result in adverse outcomes in the human foetus [1].

Exogenous compounds can exert thyroid effects through disruption of homeostasis and thereby TH metabolism dysfunction which can contribute to childhood neurological impairments [2]. Many of these chemicals can cause the induction of Phase II metabolism. This picture becomes more complicated however given that Phase II metabolism involves the clearance of the endogenous thyroid hormones triiodothyronine (T3) and its prohormone, thyroxine (T4) [3].

We have developed a multi-compartment model of TH balance in mother and foetus covering key developmental stages of the human foetus when critical neurodevelopmental effects of the TH metabolic network occur, using two modelling approaches. As well as a fully-parameterised ordinary differential equation based physiologically-based pharmacokinetic (PBPK) model, we are also pursuing Petri nets as a parallel technique. Petri nets have well defined mathematical foundations that allow characterisation and analysis of concurrent systems such as metabolic networks [4]. Being parameter agnostic, they are concerned solely with network connections, and not the values of the parameters in that network [5]. Thus Petri nets allow quick, initial modelling and can be used to gap-fill missing parameters in the PBPK model.

Our model uses a hybrid of deterministic and stochastic methods to quantitatively model the metabolic processes involved, including those in the liver. This approach has already shown promise in providing key input to Adverse Outcome Pathways (AOPs) [6].

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P-Late-14
Assessing safety concern of food contact chemicals in absence of toxicological data

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Thousands of chemicals contained in food packaging or used in food production, processing, storage and transportation may potentially migrate into foods and result in unexpected consumer exposure. There is an increasing alarm about their potential toxicological effects since most of them lack experimental data. There have been already initiatives to screen food contact chemicals using *in silico* toxicology, but many of them use qualitative approaches, mainly mutagenicity predictions, suitable for hazard identification. However they do not provide information about hazard characterization (how much is needed for triggering a toxic effect) and even less about health risks. We developed an *in silico* strategy to assess rapidly, cost-efficiently and without animal toxicity testing, safety concern of packaging chemicals. A number of toxicity endpoints relevant for risk assessment were sequentially screened using *in silico* predictive models and read across, i.e. mutagenicity, developmental, reproductive and chronic toxicity [lowest-observed-adverse-effect level (LOAEL)]. Individual predictions were integrated in order to identify the most relevant toxicological value to be compared with exposure through a margin of exposure approach (MoE, the ratio between predicted toxicity value and exposure estimate). To address the actual value of this approach, a pilot study was run using a compiled list of 195 food contact chemicals and structural analogues. About 17% of these chemicals were predicted as mutagenic, 14% being experimentally characterized structural analogues and 3% toxicologically uncharacterized chemicals [1,2]. For non-mutagenic chemicals, the lowest quantitative predicted toxicity values were compared to exposure resulting from a theoretical migration level in food of 10 ppb and a food intake of 1 kg for a 60 kg individual [3]. This level of 10 ppb is widely used as a pragmatic cut-off to prioritize management of migrating chemicals without toxicological information. For 99.97% of these chemicals, a MoE compatible with safety was obtained. We are currently applying the same approach on a larger set of ~3,500 curated food packaging chemicals. Preliminary results on this new set have shown similar percentages of chemicals predicted to exhibit genotoxicity alerts. For the non-genotoxic chemicals, 99.99% would be considered of no chronic toxicological concern at a level in food < 10 ppb.

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P-Late-15
Genomics analysis reveals the molecular mechanisms underlying the hepatotoxicity associated with oral azole drugs

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Ketoconazole (KTZ) and itraconazole (ITZ) are clinically prescribed antifungal drugs; however, their uses can be associated with serious adverse drug reactions, most notably hepatotoxicity. In particular, an increasing number of studies have reported liver injury by oral KTZ, leading to the recommendation of a ban on the prescription of oral KTZ. However, the cause of hepatotoxicity and molecular mechanisms induced by oral azole drugs remains unclear. We carried out comprehensive genomic investigations, and this study is the first to compare the gene expression profiles in liver or hepatocytes treated with the antifungal azole drugs KTZ and ITZ in *in vivo* and *in vitro* systems. The results revealed that genes related to cholesterol synthesis were overexpressed in the liver in the KTZ-treated group, whereas expression of those related to acute phase injury was significantly altered in the ITZ-treated group. Our data suggest that oral KTZ and ITZ act differently in the liver and have different hepatotoxic effects. Toxicological function analyses of the *in vivo*/*in vitro* KTZ-treated groups revealed DEGs that were significantly associated with liver tumors, hepatic steatosis, and cell death in the liver. Several transcription factors including *HNF4A*, *PPARA*, and *SREBF1/2* were identified as upstream regulators in the *in vivo*/*in vitro* KTZ treatment groups. KTZ may cause hepatotoxicity by inhibiting cholesterol following activation of transcription factors, such as *SREBFs*, which induce subsequent cholesterol synthesis, inflammation, or oxidative stress. We hope this study increases our understanding of hepatotoxicity induced by oral azole drugs. This work was supported by a grant (2016M3A9C4953144) from the Ministry of Science, ICT, and Future Planning and a general research grant from the Korea Institute of Toxicology.

P-Late-16
Development of three dimensional bio-mimetic hepatic zonation system

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Hepatic function is highly specialized according to the spatial location along the portal-central vein axis, and this hepatic zonation is a large hurdle in predicting hepatotoxicity. We designed a simple and efficient hepatic zonal system by generating a gradient of CHIR99021 (CHIR), an inducer of Wnt/β-catenin signalling, through an agarose hydrogel channel containing 3 dimensional (3D) HepaRG cells. The enzymatic activity revealed that CYP2E1, CYP1A2, and CYP3A4 were activated by CHIR and we introduced CHIR at the end of polylefin tube containing 3D HepaRG cells. The distribution profile of CHIR after 7 days in the 3D hepatic zonal channel showed that CHIR diffused in a manner that led to a concentration gradient The 3D hepatic zonal system enables long-term exposure to hepatotoxic drugs over several days, and we confirmed that zone-specific hepatotoxic compounds, including bromobenzene and acetaminophen, could be screened by direct imaging analysis. Here, we suggest that a 3D hepatic zonal system can be generated by modulating Wnt/β-catenin signalling. This system provides a simple and robust method to generate the zonal distribution of drug metabolism and permit screening of zonal hepatotoxic drugs as well as sub-acute toxicity over several days. The zonal toxicity profiles can give information to explain the conflicting data on heterogeneous drug metabolism and to clarify the spatial heterogeneity of toxicological responses.

This work was supported by a grant (NRF-2017R1D1A1B03035898, 2016M3A9C4953144, and 2014M3A7B6020163) from the Ministry of Science, ICT, and Future Planning and a general research grant from the Korea Institute of Toxicology.

P-Late-17
Cross-species comparison of CAR-mediated procarcinogenic key events in a 3D liver microtissue model

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Characterisation of the mode of action (MOA) of constitutive androstane receptor (CAR)-mediated rodent liver tumours involves measurement 5 key events including activation of the CAR receptor, altered gene expression, hepatocellular proliferation, clonal expansion and increased hepatocellular adenomas/carcinomas. To test whether or not liver 3D microtissues (LiMTs) recapitulate CAR- mediated procarcinogenic key events in response to the prototypical CAR activator phenobarbital (PB) we performed hepatocyte proliferation (LI%) analysis in rat and human LiMTs using a microTMA technology in conjunction with integrated transcriptomics (microarray) and proteomics analysis. The rationale for this approach was that LiMTs containing parenchymal and non-parenchymal cells (NPCs) are more physiologically representative of liver and thus would generate data more relevant to the *in vivo* situation. Rat and human LiMTs were treated with PB over a range of concentrations (500 uM–2000 uM) and times (24 hr – 96 hr) in a dose-response/time-course analysis. There was a dose-dependent induction of LI% in rat LiMTs, however there was little or no effect of PB on LI% in human LiMTs. ATP levels in the rat and human LiMTs were similar to control in all of the PB treatments. There was also a dose- and time-dependent PB-mediated RNA induction of CAR regulated genes CYP2B6/Cyp2b2, CYP3A7/Cyp3a9 and UGT1A6/Ugt1a6 in human and rat LiMTs, respectively. These CAR regulated genes were also upregulated at the protein level. Ingenuity pathways analysis (IPA) indicated that there was a significant (Z score >2.0;-log p value >) activation of CAR by PB in both human and rat LiMTs. These results indicate that human and rat LiMTs showed the expected responses at the level of PB-induced hepatocyte proliferation and enzyme induction with rat LiMTs showing significant dose-dependent effects while human LiMTs showed no proliferation response but did show dose-dependent enzyme induction at the RNA and protein levels. In conclusion LiMTs serve as a model to provide mechanistic data for 3 of the 5 key events considered necessary to establish a CAR-mediated MOA for liver tumourigenesis and thus can potentially reduce the use of animals when compiling mechanistic data packages.

P-Late-18
Absence of Mutagenic and Clastogenic Effects of Decolorized *Aloe Vera* Whole Leaf Juice Concentrate in Mammalian Cells by the L5178Y/TK+/- Mouse Lymphoma Assay

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Hydroxyanthracene derivatives (HADs) are naturally occurring components in commonly consumed vegetables, spices and other botanicals, including *Aloe vera*. Research has shown that HAD compounds such as aloin A, B and aloe-emodin found in *Aloe vera* latex cause genotoxicity in bacteria and mammalian cells, possibly attributing to the carcinogenicity observed in a 2-year rodent cancer bioassay of orally administered *Aloe vera* whole leaf extract. The purpose of this study was to evaluate mutagenic and clastogenic potential of a purified *Aloe vera* whole leaf juice concentrate dry powder (hereafter referred to as test article [TA]), in which HADs are removed through activated charcoal filtration process, also known as decolorization.

Methods: *In vitro* L5178Y mouse lymphoma assay (MLA: OECD 490) was performed to test for mutagenic activity of TA at the *tk* locus in the presence and absence of metabolic activation. Stock formulations were prepared in purified water and the concentrations of TA administered to the test system ranged from 250 to 5,000 µg/mL.

Results: HPLC analysis showed that the purified *Aloe vera* whole leaf juice concentrate contained less than 0.1 ppm aloins A and B, and no detachable aloe-emodin (LOQ of 0.2 ppm). Mutant frequencies in vehicle control cultures fell within acceptable ranges and clear increases in mutation were induced by the positive control chemicals methyl methane sulphonate (without S-9) and benzo[a]pyrene (with S-9). Post-treatment precipitation was observed at 3,000 µg/mL and above in the presence of S-9. The highest concentrations analyzed at 3-hour (with and without S-9) and 24-hour (without S-9) had relative total growth (RTG) values ranging from 64 to 133%. TA did not induce mutation at the *tk* locus when tested up to 5,000 µg/mL (the maximum concentration required for testing mixtures according to OECD guidance) for 3 and 24 hours without S-9 and when tested up to a precipitating concentration of 3,000 µg/mL for 3 hours with S-9.

Conclusion: The evidence supports that purified *Aloe vera* whole leaf juice concentrate with *de minis* HADs did not induce mutation under the experimental conditions described. Furthermore, an *in vivo* comet assay (OECD 489) is being conducted to evaluate the potential of orally administered TA in inducing DNA damages in the colon of male F344 rats.

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P-Late-19
Mechanistic studies in cadmium-induced carcinogenesis using the Cell Transformation Assay.

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Carcinogenesis is one of the areas of major concern in the context of 3Rs and alternative approaches to the mouse bioassay are needed. Among these, the Cell Transformation Assays (CTAs) are one of the *in vitro* models for the identification of potential human carcinogens [1,2], especially in the context of an integrated approach to testing and assessment (IATA) [3]. These assays, limited to the screening of compounds, actually, are employed for studying the process of transformation. In this context, we exploited the use of CTAs for mechanistic studies of cadmium-induced carcinogenesis. We carried out a whole-genome analysis to evidence deregulated pathways in C3H10T1/2Cl8 after 24 h of cadmium treatment or in foci-derived transformed cells. Consequently, according to *in silico* analyses, we focused on metabolic rewiring and mitochondrial structure and function. In more details, we applied seahorse methods, spectrophotometric enzymatic assays, laser scanning confocal fluorescence microscopy and flow cytometry technique. The essential aspect of this approach was considering many variables at once [4] by integrating bioinformatics tools combined with laboratory work. We are confident that the joint use of many techniques could develop a mechanistic-based method for improving the reliability of CTAs, leading to the Reduction of animal used.

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P-Late-20
Evaluation of the toxic effects of aluminum containing nanomaterials *in vitro* and *in vivo*.

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The incorporation of aluminum-containing nanomaterials (Al NMs) into consumer products is expected to increase in the years to come, despite considerable gaps remaining in the toxicological evaluation of Al NMs. This represents an area of concern for consumers and public health agencies, and further investigation of the human health risks is therefore necessary. The present study aimed to evaluate the fate and the toxicological effects of aluminum containing nanomaterials in the liver *in vivo* and *in vitro*. Two forms of aluminum containing nanomaterials (Al^0 and Al_2O_3) of the same size were used and compared with the ionic form $AlCl_3$. In this context, rats were treated by gavage for 28 days with concentrations of aluminum nanomaterials ranging from 6.25 to 25 mg/kg bw/day. Following treatment, organs were harvested for quantification of aluminum content, genotoxicity assays as well as for RNA extraction and quantitative RT-PCR analysis. In addition, in order to examine the potential for *in vitro - in vivo* extrapolation, the toxic effects of these aluminum containing nanomaterials were also investigated in a long term repeated dose study *in vitro* in differentiated human HepaRG hepatic cells.

Significant aluminum accumulation was observed in the kidney, spleen, and to a lesser extent, the liver of rats treated for 28 days with Al-NMs. Interestingly, Al_2O_3 NMs resulted in significantly higher levels of aluminum in these tissues when compared to rats treated with Al^0 NMs. Despite the accumulation of aluminum in organs, with the exception of spleen in rats treated with Al^0 NMs, no genotoxic effects were observed when assessed with the comet assay. Interestingly, several genes involved in the DNA Damage Response were up-regulated in liver in rats treated with Al^0 NMs.

Very little cytotoxicity was observed in HepaRG cells treated for up to two weeks with aluminum containing NMs. However, cytotoxicity was observed in cells treated with $AlCl_3$ after one or two weeks of treatment. This increase in cytotoxicity was accompanied by a significant increase in the secretion of IL-8, and increases in endosomal and lysosomal markers, as well as an increase in the autophagy marker LC3B.

The results of this study indicate that further investigation is necessary in order to assess the toxicity of aluminum-containing nanomaterials, and in particular, long-term repeated dose studies both *in vivo* and *in vitro* are required to clarify the chronic effects of exposure to NMs.

P-Late-21
Is the current Biocidal Regulation in Europe protective enough with human health and the environment? An evaluation case of wood preservatives.

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The commercialisation of biocidal products in Europe requires to undertake a risk assessment for human health and environmental safety. Biocides were first settled under Directive 98/8 repelled by the current Regulation 528/2012 (BPR). After more than 20 years of their implementation, most exposure estimations and default values for the risk assessment of the different product types and uses remain unchanged.

To determine the adequacy of the current methodology for the biocidal products risk assessment under BPR, we have selected product type 8: wood preservatives.

In an attempt to have a global overview, we evaluate industrial and secondary exposures. Moreover, both perspectives on human health and environmental exposures have been considered.

Within the different exposure scenarios, we have revised the ori-

gin, relevance, reliability, representativity and accuracy of the main default values used for the exposure assessment, according to the latest guidelines.

The results of the evaluation of the current risk assessment for wood preservatives show unrealistic and incoherent figures for wood absorption with a disproportion between human health and environmental exposure calculations, wrong conversion formulae, statistically unreliable default values for the frequency of industrial product application and the amount of treated wood per day per treatment type. Furthermore, the information on the models to be used is inconsistent throughout the latest guidance, with incomplete information regarding the original document for these exposure models. Moreover, the high dispersion of information on the right methodology to be used may disorient both the risk assessor and the evaluating authority.

The work presented in this article ultimately raises a question: is the current risk assessment methodology for biocides in Europe protective enough for human health and the environment?

We describe a number of lines of evidence illustrated by the evaluation of wood preservatives as an example of biocidal products that a thorough revision is needed.

- Revaluation of the default values should be done in order to include statistically representative data while using updated methodologies for exposure assessment;
- A shocking misconception of unit conversion was found;
- Harmonisation within other regulations such as REACH would be desirable to make a risk assessment of combined exposures of individual substances by uses under the scope of different regulations possible. Different regulatory bodies should establish common working groups.
- Regulators should focus on the human and environmental exposures to multiple chemicals to calculate the risks of mixtures currently evaluated under different regulations.

As a final conclusion, an in-deep revision of the risk assessment process for biocidal products within a broad strategy across the EU regulations would be encouraged by regulators in the near future to ensure adequate human health and environmental protection.

P-Late-22
A 3D-tetraculture system at the air-liquid interface as valuable tool for hazard assessment of respiratory irritants and sensitizers

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Purpose: The aim of the research was developing an *in vitro* system for reduction, replacement and refinement of animal models commonly used in the hazard and risk assessment procedure for potential respiratory irritants and sensitizers.

Methods: A 3D-coculture system representative of the alveolar region was set up by Chary *et al.*, 2019. This *in vitro* model was obtained modifying the original model set up by Klein *et al.* (2013) combining alveolar epithelial cells (A549 cells) and macrophage-like cells (seeded on the apical side of a Transwell™ insert) with endothelial cells (EA.hy926 cells) and dendritic cells (THP-1 cells) placed on the basolateral side. This orientation allows growing the apical side at the air-liquid interface (ALI) while maintaining the basolateral side in submerged conditions. The co-cultures were exposed through the 6-w Vitrocell™Cloud System to increasing concentrations of chemicals representative for different categories of compounds. Acrolein (Acr), Methyl Salicilate (MeSa), TriMellitic Anhydride (TMA), Phthalic Anhydride (PA), were selected as reference compounds representative respectively for potential respiratory irritants and respiratory sensitizers (Patent WO2018/122219 A1). A dose response curve for each compound was calculated and CV_{75} was used as exposure dose for further analyses (cytokine release, THP-1 surface marker expression, gene expression).

Results: The exposure to respiratory sensitizers induced dendritic cells activation and a specific cytokine release pattern, while the irritants did not. In particular, increased expression of CD54 and CD86 was observed after exposure to the chemical respiratory sensitizers TMA end PA while Acrolein induced a decrease in CD54 expression. Increase of TSLPr expression and secretion of specific cytokines were observed after exposure to the chemical respiratory sensitizers TMA and PA.

The selected markers thus represent promising parameters to discriminate between respiratory irritants and sensitizers making the model a potential *in vitro* tool to be used for hazard assessment of unknown compounds.

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Patent WO2018/122219 A1

P-Late-23
Assessing the suitability of advanced 3D *in vitro* hepatic spheroid models as potential *in vivo* substitute models for acute and long-term engineered nanomaterial genotoxicity and hazard assessment.

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The liver serves a vital role in metabolic homeostasis and detoxification thus it is imperative that robust and physiologically representative models for liver hazard assessment *in vitro* are established. 3D *in vitro* liver models have been found to better mimic *in vivo* complexities and intricate multi-cellular interactions than their 2D counterparts. The PATROLS (Physiologically Anchored Tools for hazaRd assessment of nanOmateriaLS; EU Grant Agreement #:760813) project is partly aimed at advancing the existing 3D *in vitro* liver models to create more physiologically relevant ones, whilst using the known *in vivo* adverse outcome pathways to better understand hazards associated with long-term exposure to engineered nanomaterials (ENM). To achieve this *in vitro* 3D liver models are developed based on an immortalised cell line HepG2, which are viable for long-term culture (>14 days) and able to support both long-term and repeated ENM exposures. Their ability to predict a range of toxicological endpoints (*e.g.* liver function, (pro-)inflammatory response, cytotoxicity and genotoxicity) has been characterised using a range of ENMs (*e.g.* TiO_2 and ZnO) across both short- (24 hr) and long-term (120 hr) exposure regimes. It was found that neither acute nor long-term exposure to (2.50, 5.00, 10.00 and 20.00μg/mL) both TiO_2 or ZnO ENMs significantly ($p \geq 0.05$) reduced albumin or urea production in 3D HepG2 spheroids. Both ENMs exhibited a similar effect on albumin and urea pro-

duction, although liver functionality was shown to be lower post TiO_2 exposure then ZnO. (Pro)-inflammatory mediators indicated a significant ($p \leq 0.05$) increase in IL-8 production between the acute and long-term ZnO exposures, whilst IL-8 production following TiO_2 exposure remained fairly consistent ($p \geq 0.05$) across both regimes. Further work is needed to fully assess the capability of each liver model system and associated bioassays when evaluating a range of different ENMs, thereby providing easily accessible and robust alternative technologies to better support ENM hazard assessment *in vitro*.

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P-Late-24
In vitro-in silico-based prediction of peroxisome proliferator-activated receptor γ (PPARγ) activation by bixin and crocetin in humans

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Bixin and crocetin are carotenoids, present in among others annatto and saffron, and regarded as potential functional food-ingredients exerting beneficial health effects in type II diabetes mellitus. These effects are suggested to be mediated via activation of peroxisome proliferator-activated receptor γ (PPARγ). However, it remains unclear whether PPARγ activation can be achieved at realistic human estimated daily intake levels of bixin and crocetin. The purpose of the present study was the prediction of the effective dose levels of bixin and crocetin in humans for PPARγ activation using an *in vitro-in silico* approach. The effects of bixin and crocetin on PPARγ-mediated gene expression were quantified *in vitro* using a PPARγ reporter gene assay. Hepatic metabolism was assessed using primary human hepatocytes, and physiologically based kinetic (PBK) models were defined to describe the kinetics of bixin and crocetin in humans. Using PBK modeling-based reverse dosimetry, the *in vitro* concentration-response curves for PPARγ activation were translated to predicted *in vivo* dose-response curves from which benchmark dose for 50% response (BMD_{50}) values for bixin- and crocetin-mediated PPARγ activation were derived, which were subsequently compared to dietary exposure levels. Bixin and crocetin activated PPARγ-mediated gene transcription *in vitro* in a concentration-dependent manner with similar potencies. Due to differences in kinetics, showing more efficient clearance of bixin than of crocetin, the doses required to reach equimolar plasma concentrations were estimated to be higher for bixin than for crocetin, and the predicted BMD_{50} value for PPARγ activation was 32 times higher for bixin than for crocetin. Comparison of the BMD_{50} values to human dietary and supplementary intake revealed that human dietary and/or supplementary estimated daily intakes may reach these BMD_{50} values for crocetin, pointing at possibilities for *in vivo* PPARγ activation, while bixin intake was predicted to be ineffective. The study provides a proof-of-principle to predict effects of functional food ingredients in human without the need for a human intervention study.

P-Late-25
Use of generic reference values for estimation of the sensitization/hypersensitivity potential of substances extracted from medical devices

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Generic reference values, such as the threshold of toxicological concern (TTC) have been established for risk assessment for various endpoints. To assess hypersensitivity of substances without specific data, for cosmetics, dermal sensitization thresholds (DSTs) were established. Similarly, the Product Quality Reasearch Institute (PQRI) established a qualification threshold (QT) for parenteral and ophthalmic applications of drugs. Compared to these product types, medical devices (MD) are special due to their big range of application sites and contact with different tissues. During the biological safety evaluation of a MD, as first step, extractables are toxicologically characterized according to10993-1:2018; biological tests should be conducted only to fill data gaps. In case no reliable sensitization data are available for the detected substances, further evaluation is necessary. In accordance to the 3R principle the use of generic reference values might be suitable to assess the sensitization risk of these substances. As existing values (DST or QT values) were not established for exposure after implantation or contact with breached/compromised skin their applicability for MD is questionable. This was evaluated in a tired approach: 1. comparison of non-proteinogen substances extracted from medical devices with substances present in cosmetics and fragrances used for estimation of DST value, 2. comparison of hypersensitivity mechanisms in skin and deep tissue, 3. evaluation of application sites' impact on hypersensitivity.

The evaluation showed, that the most substances used for DST value derivation are not known to be contained in MD, that the mechanism in deep tissue is less comparable to mechanism in healthy skin and the implantation side impacts body reaction.

Thus, it can be concluded, that the DST value is suitable for MD in contact with healthy skin while for other application sites a case by case evaluation is required.

P-Late-26
Impedance spectroscopy as a method to discriminate between all GHS categories for eye irritation *in vitro*

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For the toxicological endpoint of eye irritation, the first alternative test systems based on ex vivo or *in vitro* models have been developed and validated. However, besides all efforts, the Draize eye test is still not completely replaced by alternative animal-free methods because the alternative methods cannot distinguish between the globally harmonized system for the classification and labelling of chemicals (GHS) category 1 serious eye damage and category 2 eye irritation [1]. To develop a single *in vitro* test to identify all GHS categories for eye irritation, we combined organotypic cornea models based on primary human cells with an electrical readout system that measures the impedance of the test model. First, we showed that employing a primary human cornea epithelial cell based models is advantageous

in native marker expression such as cytokeratin 3 and 12 to the primary human epidermal keratinocytes derived models. Secondly, by employing a non-destructive measuring system based on impedance spectroscopy, we could increase the sensitivity of the test system. Moreover, the impedance measurement allowed for the first time to detect the persistence of irritative effects by repeated measurements in an *in vitro* model and thus to distinguish between all GHS categories. Substances that do not need to be labeled stayed above 60% normalized to the negative control. Category 1 substances reduced the tissue integrity after application below 6% and the effect did persist over a period of 7 days. Category 2 substances however, could be identified by a decrease below 60% after the application of a category 2 chemical such as ethanol and increased again above 50% after 7 days. Thereby, all GHS categories of eye irritation could be identified by repeated measurements over a period of 7 days. Based on a novel prediction model we achieved an accuracy of 78% with a reproducibility of 88.9% to determine all three categories of eye irritation in one single test. This could pave the way according to the 3R principle to replace the Draize eye test.

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P-Late-27
Toxicological approach in the safety assessment of novel foods in the European Union (EU)

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According to the European legislation, novel foods (NF) are foods that were not consumed to a significant degree by humans within the European Union before 15 May 1997. NF are foods consisting of, isolated from or produced from different sources (e.g. microorganisms, fungi, plants or animals and their parts), produced by new processes or technologies or newly synthetized compounds which have not been previously consumed in our diet to a significant degree. Although the history of safe use within a third country may be relevant, the safety of such foodstuff has to be assessed before the marketing authorisation can be granted.

The role of EFSA is to assess the safety of NF and provide scientific advice to the competent EU regulatory bodies. Since the entry into force of the relevant regulation ((EU) No 2015/2283) of January 2018 more than two hundred applications for NF have been received by the European Commission (EC) and depending on the complexity of the dossier and the characteristics of the NF an assessment by EFSA has been requested. The Authority shall complete a safety assessment within nine months. The scientific opinion of EFSA is then considered by the EC during the authorization process of the NF.

The experts of EFSA's Panel on Nutrition, Novel Foods and Food Allergens (NDA) follow a multifaceted approach to carry out the safety assessment of the NF under the proposed uses and use levels. The assessment is based on dossiers provided by applicants. Dossiers need to contain data on the compositional, nutritional, toxicological and allergenic properties of the NF as well as information on respective production processes, and the proposed uses and use levels, as specified in the relevant EFSA guidance (EFSA NDA 2016). The toxicological assessment is based on the whole set of data provided and particularly on ADME and *in vitro* and *in vivo* toxicity studies that shall provide insight on kinetics, genotoxicity, sub-chronic/chronic toxicity, and reproductive and developmental toxicity. A tiered toxicity testing approach is implemented with the aim of limiting the use of animals and resources. The results provided may trigger the need for further specific testing.

A thorough assessment by EFSA and its experts on the full set of data of the NF with particular focus on the available toxicological information helps to ensure a high level of food safety for the consumers within the European Union.

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P-Late-28
Assay-Ready Use of KeratinoSens® Cells in Skin Sensitization

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The reproducibility of cell-based assays strongly depends on the cell quality, which in turn is influenced by multiple factors such as the choice of the culture media and sera, the source and passage number of the cell line, or even slight differences in cell handling by different operators. Thereby, all these parameters need to be optimally standardized. For this, the use of pre-made and pre-qualified assay-ready cells, which can be applied in a cellular assay basically like a reagent without prior cultivation or passaging, can minimize the variability related to cell culture.

To evaluate the skin sensitizing potential of chemicals, reporter skin cell lines are used to measure the activation of the ARE/Nrf2 pathway, which is one of the key events of this complex cascade. Within the context of the keratinocyte activation, the KeratinoSens® cell line has been developed by Givaudan and validated by the ECCVAM. Here we demonstrate that the use of these in an assay-ready format to test the proficiency substances according to the OECD guideline 442D leads to equivalent results as compared to continuously cultured cells.

P-Late-29
A two-year carcinogenicity study of the new opioid receptor antagonist ondelopran in rats

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Purpose: New drug Odelepran (INN: ondelopran) with a unique binding profile to all three types of human opioid receptors (μ, κ, δ) is being developed by R-Pharm. Ondelopran is intended for the treatment of alcohol dependence [1,2]. Since the drug is intended for the long-term treatment, the study of carcinogenic potential of chronic (two years) administration to rats is required to be approved by regulatory authority.

Method: The study was performed in male and female Wistar rats at the age of 8-10 weeks at the start of experiment. There were four groups of male and four groups of female, 50 animals each. Test item (ondelopran film-coated tablets, 125 mg), was administered to the animals intragastrically (vechicle - 1% starch solution) daily, 5 days a week for 24 months in two doses: 10 mg/kg (equivalent therapeutic dose for humans) and 100 mg/kg. The amount of placebo (tablet excipients) administered intragastrically equated to the amount of excipients contained in the tablet mass proportional to the 100 mg/kg ondeloprane dose. The control group was administered with the

vehicle (1% starch solution). Clinical observation and examination were conducted weekly to detect any signs of toxicity; mortality; dynamics of the body weight. At the end of the treatment period all animals in the study had been subjected to a full, detailed gross necropsy with subsequent histopathological study.

Results: During the study the mortality rates did not differ between the groups. Changes in the body weight ranged within the normal values. There were no any signs of toxicity in groups treated with tested items. Neoplastic lesions were found in all groups of animals. More than 30 types of neoplasms were identified upon pathomorphological examination, icluding follicular thyroid cancer (11/164 in males and 10/169 in females) and malignant non-Hodgkin's lung tissue lymphoma (17/164 in males and 20/169 in females) as the most frequent cases. The identified tumors are typical for rats and considered as spontaneous age-related pathology. There was no statistically significant differences between groups in the total incidence of tumors and the incidence of specific types of tumors. To conclude the above said, the test item of the ondelopran film-coated tablets, 125 mg, has no carcinogenic potencial.

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P-Late-30
Assessing reactive oxygen species produced by nanomaterials and their consequences for cells: contribution to a testing strategy for grouping approaches

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The large variety of nanomaterials (NMs) entering the EU market poses the issue of performing a robust risk assessment without performing a huge amount of time-consuming and costly animal experiments. The use of alternative approaches to overcome the case-by-case risk assessment would permit not only the reduction of work load, but also allow a targeted, prioritized and more reliable risk assessment. Grouping approaches represent a valid alternative to the case-by-case assessment and several approaches have already been proposed. The existing grouping approaches would benefit greatly from the inclusion of the toxicity mode of actions in the framework.

The observed toxicity of NMs can often be evaluated considering the production of reactive oxygen species at the surface of NMs, which can trigger oxidative stress and thus irreversible modifications of proteins, DNA and lipid oxidation and further lead to apoptosis and inflammation. Assessing the oxidative potential of NMs using functional assays would permit grouping of NMs, provide a deeper insight into the mode of action of the cellular toxicity, and support prioritization of NMs for further testing. This strategy could moreover represent a first step into a safer–by-design approach.

Within the GRACIOUS project we tested and optimized several assays to assess the oxidative potential of NMs in different environments of increasing complexity: Electron Paramagnetic Resonance (EPR), dichlorodihydrofluorescin diacetate ($DCFH_2$-DA) assay, Ferric Reduction Ability of Serum (FRAS) assay and protein carbonylation, focusing on proposed benchmark materials and on different variants of several classes of NMs. The results from each assay were compared.

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P-Late-31
The development of an *inhouse* reconstructed human epidermis (RhE) and performance as a skin irritation model.

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Reconstructed Human Epidermis (RhE) as recommended by OECD TG 439 is one of the best alternatives for *in vitro* skin irritation evaluation, since it mimics skin barrier function and is histologically similar to native human epidermis. However, in some countries it is difficult and expensive to import the commercially available models recommended by the guideline. To overcome this limitation and increase the accessibility of *in vitro* skin irritation testing, we developed a novel *in-house* RhE model and verified its potential use based on the OECD TG 439. For RhE construction, primary keratinocytes (KCs) derived from neonatal donors were seeded upon collagen IV-coated inserts and kept under submerged condition for cell proliferation, followed by an air-liquid interface condition for differentiation and stratification. RhE was characterized regarding morphological and biochemical features by standard H&E staining and immunofluorescence against cytokeratin-10, cytokeratin-14, filaggrin and involucrin. Quality control was verified by quantification of cell viability in the control RhEs (570nm O.D.) and evaluation of barrier function integrity after RhE exposure to four different SDS (sodium dodecyl sulfate) concentrations. To validate our RhE model, the irritation potential of different chemicals listed in OECD TG 439 was evaluated by topical application for 42 min followed by a 42 h post-incubation. Cell viability was subsequently measured by the MTT assay. Our *in-house* RhE model presented a multilayered epidermis with a mature *stratum corneum* and a pattern of differentiation markers similar to that of the native human epidermis. The developed model presented a mean O.D. value of 1,57 and barrier function parameters in accordance to OECD TG 439. Moreover, the *in-house* RhE-based skin irritant test was able to discriminate between skin irritating and non-irritating substances. Taken together, our data pointed out the promise use of the *in-house* RhE on OECD TG 439 in countries in which validated RhEs are not available for purchase due to customs barriers.

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P-Late-32
Immune response of the sea urchin *Paracentrotus lividus* to contaminated marine sediments

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Decommissioned industrial activities cause an accumulation of different xenobiotic inquinants, especially into the water bodies, with a tremendous impact on ecosystem functioning and human health. The Site of National Interest (SIN) Bagnoli-Coroglio, a post-industrial iron and steel activity site, represents a paradigmatic case study in the South of Italy. A severe pollution from heavy metals and hydrocarbons has been reported to occur in this site. In the frame of the restoration project of the site, ABBACO, in this work we have investigated the effects of contaminated sediments on the immune system of the Mediterranean sea urchin *Paracentrotus lividus*. The sea urchin immune system is formed by a heterogeneous population of cells, coelomocytes, present in the coelomic fluid, including phagocytes, red and white amoebocytes and vibratile cells. These cells are considered to be the sentinels of environmental stress, to which they respond by changing phagocytes' morphology, increasing the number of red amoebocytes or activating stress-responsive genes and pathways. *P. lividus* specimen have been exposed to Bagnoli-Coroglio sediments in closed flow-through tanks for 34 days. Different frequencies of water turbolence events have also been applied, mimicking the wave motion at the sea. Coelomic fluid of the animals has been collected at several times of exposition and the number of immune cells and the morphology of the different cell types have been examined by optical microscopy. In addition, the oxidative status of the cells has been assessed through biochemical assays, including measurements of the reactive oxygen species (ROS) and total antioxidant capacity. The results showed that in the sediment-exposed animals there are changes in the percentage of different immune cells, resulting in phagocytes decrease and red amoebocytes increase, together with an increase of ROS and antioxidant capacity. These studies are relevant considering that the dangerous effect of polluted marine water on local species represents one of the key points to be investigated for the environmental restoration of critical contaminated areas.

P-Late-33
Comparison of suspension method vs. sandwich culture method for the generation of human alveolar lung organoids

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Organoid, a miniaturized three-dimensional mini-organ, are derived from adult stem cell or pluripotent stem cells. Organoids can be self-organize and recapitulate the structural and physiological characteristics of *in vivo* organ, which are very useful for disease modeling studies and drug screening. In particular, lung organoids can be a useful tool for toxicity test for the environmental risk factor, such as particulate matter. Recent studies demonstrated the generation of lung organoid from human pluripotent stem cells. However, the generation efficiency of surfactant protein-expressing alveolar lung organoids is low. Therefore, this study aims to efficiently produce alveolar organoids for use as a model of alternative lung toxicity testing.

Here, we compared two culture methods, matrigel sandwich culture method and suspension culture method, to generate alveolar lung organoids derived from human ES cells through qPCR and flow cytometry analysis. The gene expression level of the lung-specific marker, *Nkx2.1, Sox9, Mucin5AC*, and *P63* was 1.3-3 times higher in the lung organoids generated by suspension culture method compared to the sandwich method. Interestingly, expression of *surfactant protein A*, a typical marker of alveolar epithelial type II cells, was 4 times higher in lung organoid generated by suspension culture method compared to sandwich method. Moreover, flow cytometry data showed that Prosurfactant C protein expression level of the alveolar organoids, generated by suspension culture method was 50% higher than that of lung organoids generated by the sandwich method.

These data indicate that suspension culture method is more efficient to generate surfactant protein expression alveolar lung organoid. Therefore, our optimized suspension culture condition for generating alveolar lung organoids are very useful for the development of alternative pulmonary toxicity test platform.

P-Late-34
Testicular toxicity of nanosilver and extrapolation to non-nano forms of silver

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Simple salts of silver and more complex silver-containing active substances (SCAS) are widely used in biocidal products due to the antibacterial properties of the silver ion; silver nanoparticles (nanosilver) have similar widespread biocidal uses. Based on an assumption that the toxicity of the different (nano and non-nano) forms of silver is attributable to the solubilised silver ion, there is a tendency to use data generated with one form of silver to predict the toxic hazard of other forms. The validity of this approach is assessed with a specific focus on testicular toxicity, taking into account the available toxicity and toxicokinetic data. High quality guideline-compliant regulatory studies performed with silver salts or SCAS do not identify testicular toxicity or adverse effects on fertility as a feature of silver toxicity. Published data for nanosilver are inconsistent, with some studies reporting no effects at high dose levels and others reporting significant testicular toxicity at very low dose levels. Nevertheless, the testicular toxicity reported in some published nanosilver studies are cited by regulatory authorities as raising concerns for the testicular toxicity, and consequently for effects on fertility and reproduction, of other forms of silver. Comparative oral toxicity studies with nano and non-nano forms of silver have led some workers to conclude that nanosilver is not absorbed and that nanosilver toxicity is due to silver ions solubilised in the gastrointestinal tract. In contrast, a number of other workers have demonstrated the presence of silver nanoparticles in tissues (including the testes) following oral dosing with nanosilver. This has led to the alternative conclusion that silver nanoparticles are absorbed (and are able to traverse the blood-testes barrier) intact. It is also reported that silver nanoparticles may be formed in the gastrointestinal tract following oral dosing with silver salts, and that methods used to visualise nanoparticles in tissues may result in the artefactual formation of nanosilver from ionic silver. As a consequence of the high level of variability in toxic responses reported for different forms of silver and considerable uncertainty relating to toxicokinetic aspects, the extrapolation of toxicity data generated using nanosilver to non-nano forms of silver is concluded not to be scientifically valid.

P-Late-35
Toxicity Assessment of graphene oxide in zebrafish as a model organism

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Graphene-based nanoparticles (GNs) constitute one of the most promising types of nanomaterials used in biomedicine and nanotechnology, due to their unique physicochemical properties and applications. Due to their extensive use, GNs released into the environment would probably pose a threat to living organisms and ultimately to human health. Their accumulation in the aquatic environment creates problems not only in aquatic habitats, but also other food chains. Thus, to

assess its potential toxic effects the following study was undertaken. We have evaluated the adverse effects of graphene (GO) in zebrafish (*Danio rerio*) embryos at various endpoints, such as mortality rate, heart rate, hatching rate. We also asked questions like whether GO affects cardiovascular development by affecting cardiac looping, apoptosis and globin expression. We used various trace concentrations of GO (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/mL) for our study and effects were observed in an embryos of 24, 48, 72, 96 and 120 hpf. Interestingly, GO has induced significant embryonic mortality, increased heartbeat, delayed hatching, cardiotoxicity, cardiovascular defects, retardation of cardiac looping, increased apoptosis and decreased hemoglobinization and all these effects were observed at a higher concentration (0.4–1 mg/mL GO). Surprisingly, the lower concentration was found to be safe enough (0.1 – 0.3 mg/mL GO). In brief, this study provided deep insights on the adverse effects of GO exposure and the specific mechanisms of GO toxicity are needed to elucidate its potential biomedical use. In order to get complete picture of GO toxicity the study need to be further extended by employing varying physical characteristics like sizes and oxidation state of GOs and similarly, exposure concentrations and sensitivity of the animal model.

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P-Late-36
Establishment of a tolerable daily intake (TDI) for hydroxyanthracene derivatives (HAD) in Aloe vera by benchmark dose modelling (BMD)

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HADs are substances present naturally in botanical species and are used in food supplements and drugs for the laxative effects in many countries. HADs present in *Aloe vera* leaf are aloins A, B and aloe-emodin, which can be removed through activated charcoal filtration, known as “decolorization”. Purified “decolorized” *Aloe vera* whole leaf juice contains only trace levels of HADs and is commonly used in foods and supplements. In a 2-year carcinogenicity study by the US National Toxicology Program, HAD containing “non-decolorized” *Aloe* leaf extract *via* drinking water increased the incidence of tumors and hyperplasia in the large intestine mucosa. Varying incidences of hyperplasia in the large intestine were also reported in the NTP 90-day studies on “non-decolorized” *Aloe* leaf extract and HAD aloins, however, no hyperplasia or pre-neoplastic lesions were observed with purified *Aloe vera* juiceconcentrate with < 100 ppb HADs, suggesting a threshold point of departure (POD) may be identified for HADs in *Aloe vera*. In the present research, the tumor and hyperplasia data from the NTP studies were subject to benchmark dose modelling using the PROAST model to derive a 95th percentile lower bound on the benchmark dose ($BMDL_{10}$) as the POD, from which a “tolerable daily intake” (TDI) could be calculated. $BMDL_{10}$ values of 3.76 and 4.93 mg/kg bw/day were established based on the tumor data in males (2-year NTP study) and hyperplasia incidence in females (13-week NTP study), respectively. Based on a minimum 10,000-fold margin-of-exposure (MOE) as per EFSA guidance for genotoxic/carcinogenic impurities to be considered of “low concern”, a TDI of 0.376 µg/kg bw/day was established. Given the ability of BMD modelling to incorporate multiple data sets, the hyperplasia incidence data from all 3 subchronic studies combined were also assessed. These data yielded similar $BMDL_{10}$ values as the NTP 2-year and 90-day data sets providing corroborative evidence of the robustness of these data. As an example of how the TDI can be applied to products in the marketplace, we compared the TDI and anticipated intake of HADs from daily consumption of an 8 oz purified *Aloe vera* wholeleaf juice and determined that a maximum residual level of 111 ppb HAD would not result in an exposure exceeding the TDI.

P-Late-37
Comparison of negative control historic data of the Bacterial Reverse Mutation Test (Ames Test): Implications in assays acceptance and results evaluation.

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Regulatory requirements for registration of plant protection products (PPP) comprise rigorous safety assessment during development, including for genotoxicity. The genotoxic potential of the product active ingredients, impurities and metabolites, is primarily determined using *in vitro* approaches.

For this purpose, ASCENZA AGRO Microbiology and Cellular Biology Laboratory, produces independent results of the Bacterial Reverse Mutation Test (Ames Test) (OECD TG 471), the *in vitro* Mammalian Cell Micronucleus Test (MN) (OECD TG 487) and the *in vitro* Mammalian Chromosomal Aberration Test (CA) (OECD TG 473), compliant with the Good Laboratory Practices (GLP) of OCDE.

Regarding the Ames Test, we present the comparison of negative control historic data of our lab with recommended/literature values, this acceptance criteria is mandatory so that the results obtained can be accepted, showing the proficiency of the laboratory.

Over the years, diverse authors have recommended different acceptable ranges for specific strains, based on their experience, and the observed ranges in individual publications did not always fall absolutely, or even partially, within the recommended values.

In general, the ranges that we have observed in our lab with using Ames *Salmonella Typhimurium* TA98, TA100, TA102, TA1535 and TA1537, are in accordance with the recommended.

P-Late-38
Investigation into the effects of metabolism on the cytotoxicity of a subset of cosmetically-relevant compounds using an animal-product-free assay

A. J. P. Edwards, F. P. Jacobs, C. Longmore, *L. Roscoe, C. Taylor, B. Sim, C. Treasure

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The toxicity profiles of many chemicals are known to change following metabolism in the liver. This is often overlooked when using non-

hepatic *in vitro* cytotoxicity assays and the effects of metabolic breakdown of compounds, either detoxification or the generation of toxic metabolic by-products, are seldom taken into account. Some toxicity models use metabolically competent cell lines or addition of exogenous metabolic components e.g. induced mammalian liver S9. However, these models rarely use human derived S9 even though this is widely available.

The effect of metabolism (through addition of human liver S9 to the system) upon cytotoxicity to TK6 cells was investigated using a thiazole orange-based animal-product-free cytotoxicity assay for cosmetically-relevant, but often cytotoxic, ingredients that are widely used in cosmetics and fragrances. These chemicals were categorised based upon their structure or function.

We have demonstrated that the majority of the toxicity profiles generated for cytotoxic raw materials in the ketone (100%), absolutes (94.44%), aldehyde (92.9%), dye/colourant (92.6%) and essential oil (76.4%) subgroups are altered by expanding the *in vitro* cytotoxicity test to encompass metabolic functionality.

Incorporation of metabolic functionality, in the form of human S9, into an animal-product-free cytotoxicity assay provides the basis of a more comprehensive assessment of cytotoxicity. The identification of raw materials that may yield cytotoxic metabolic-breakdown products, is useful in informing product safety.

P-Late-39
Development of a 3D Genotoxicity Model for Assessment of Cosmetic Formulations

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3D tissue models can be effectively combined with a genotoxicity screening assay in order to expand the type of samples that can be tested *in vitro*. Use of the 3D tissue model mimics the skin barrier and allows for absorption to be taken into account when assessing genotoxic potential. This facilitates investigation of whether a positive result in a standard 2D cell-based assay is relevant to products with respect to penetration of the skin barrier. Use of the animal free BlueScreen test as the genotoxicity endpoint allows for identification of all 3 classes of genotoxins; mutagens, clastogens and aneugens.

TK6 cells (with GLuc reporter) were seeded into 24 well plates. EpiDerm 3D tissue models were placed into the 24 well plates so that the basolateral side of the insert made contact with the TK6 cell suspension forming a co-culture system. The same experiments were carried out using TK6 cells only as a control. DMSO and Paraphenylenediamine (PPD) were added to the apical side of the tissue models or the cells directly across a dosing range for 24 or 48 hrs. Cells were collected at the end of the dosing periods and added to a 96 well plate alongside assay controls for the endpoint measurements. Endpoint measurements for luminescent (genotoxicity) and fluorescent (cytotoxicity) endpoints were collected.

Results show that the 3D tissue models and TK6 cells can be effectively combined within a co-culture system and that both DMSO and PPD were able to penetrate through the 3D tissue layer as expected. Cytotoxic dose response effects were observed for PPD and DMSO at both 24 hr and 48 hr timepoints. As expected, greater cytotoxicity was observed at 48 hrs compared to 24 hrs. PPD elicited an inverted genotoxic dose response effect at 48 hrs in the co-culture system and upon the TK6 cells alone with higher doses of PPD causing more cytotoxicity. The genotoxic induction was increased compared to control at the lowest dose. No change in genotoxic induction was observed at 24 hrs suggesting that the 48 hr timepoint was needed in order to elicit a genotoxic induction. The addition of the 3D model also dampened the cytotoxic effects of the compounds indicating that the skin barrier is effective at preventing a proportion of the PPD from being absorbed, or is detoxified by the skin's metabolic enzymes. This system provides a physiologically relevant model for investigation of genotoxicity for topically applied substances.

P-Late-40
Association of blood lead and mercury with thyroid function in Korean National Health and Nutrition Examination Survey 2013

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Background/Aim: The relationship of heavy metals to the thyroid function have been evaluated inconsistently. This study is aimed to investigate the association between lead, cadmium, mercury and thyroid function.

Methods: In this study, we used the data from Korean National Health and Nutrition Examination survey VI (2013). We analyzed 1812 subject with the blood lead, cadmium, mercury and thyroid function examination. Patient with thyroid cancer and other thyroid diseases were excluded. We estimate associations after adjusting age, urine iodine/creatinine, BMI, house income, education, smoking, alcohol drinking, physical activity, and occupation and stratification of sex.

Results: In men, there was no significant relationship between heavy metals and thyroid function. In women, lead was related with free T4 positively in linear regression. The highest quartile group of blood lead and mercury was significantly associated with increased free T4. Thyroid stimulating hormone was not related with heavy metals. TPO antibody was associated with Blood lead in linear regression.

Conclusion: These results suggest that blood lead and mercury was related with thyroid function.

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