European Commission

## Proceedings and programme

# JRC Summer School 2019: Non-Animal Approaches in Science

## *Challenges & Future Directions*

**JRC Ispra**
**21-24 May**
**2019**

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**The European Commission's**
**Science and Knowledge Service**
**Joint Research Centre**

Joint Research Centre

This publication has been produced by the Joint Research Centre (JRC), the European Commission's science and knowledge service, and presents the proceedings and programme of the JRC Summer School: Non-Animal Approaches in Science - Challenges & Future Directions, which took place between 21-24 May 2019 at the JRC Ispra, Italy.

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# JRC Summer School on Non-Animal Approaches in Science

## CHALLENGES & FUTURE DIRECTIONS

**Tuesday 21 May 12:00 to Friday 24 May 14.30**

The JRC's Chemical Safety and Alternative Methods Unit, incorporating the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), is proud to present the JRC Summer School on "Non-Animal Approaches in Science".

The aim of the JRC Summer School is to share knowledge and experience on the latest non-animal approaches used in research and testing including *in vitro* methods and computational modelling. In addition, the intention is to explore the role of the Three Rs (Replacement, Reduction and Refinement of animal experiments) in science today through discussion and debate.

The Summer School is specifically tailored for post-graduate students and early-career scientists working in the biosciences and will focus on non-animal methods and technologies and the opportunities and challenges associated with their application in various fields such as regulatory toxicology and biomedical research.

The programme will combine lectures from experts in the field with plenty of interactive sessions to encourage exchange of views and facilitate networking among participants. A visit to the EURL ECVAM laboratory is also planned. Participants will be asked to present a poster describing their own studies or interests related to the topics of the Summer School and to actively participate in a series of hot debates!

European Commission, Joint Research Centre (JRC)
Ispra, Italy
JRC-F3-SUMMER-SCHOOL@ec.europa.eu

## *A message from your hosts*

Dear Participants of this JRC Summer School!

Welcome!

We are truly delighted to host you all at our 2019 JRC Summer School on "Non-Animal Approaches in Science"!

Here at the European Commission's JRC, we believe in a Win-Win-Win. By applying the latest non-animal approaches in research and testing, we can combat disease and protect human health and the environment; avoid the use of animals for scientific purposes; and facilitate innovation, competiveness and economic growth.

To us, you represent key enablers and decision makers of the future. Young, smart, accomplished individuals that can make real positive change. Our aim with this Summer School is to provide you with essential knowledge and insights about alternative tools and thinking. We want to encourage and help you to become a champion in shifting the paradigm so that society can benefit from excellent, relevant and impactful science that does not need animals.

We have endeavoured to put together a broad, highly informative, and exciting programme for you. We count however on your very active participation to make these days that we spend together something really special and inspiring for all of us!

On behalf of the dedicated JRC Summer School Team, we hope you enjoy this unique experience and the great opportunity to network with the speakers and your peers!

Buon lavoro!

**Elke Anklam**
**Director - Health, Consumers and Reference Materials**

**Maurice Whelan**
**Head of Unit - Chemical Safety and Alternative Methods**

***We are grateful to the following organisations for providing travel support to 13 Summer School participants***

ESTIV

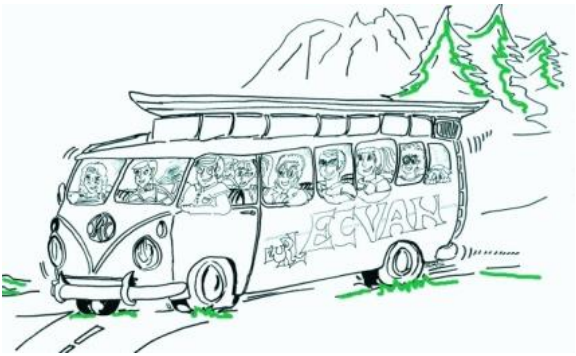
ESACT

HUMAN TOXICOLOGY PROJECT

IMM
Institute of Environmental Medicine
Institutet för miljömedicin

Karolinska Institutet

PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.



# Useful Links

**#ECVAM**
**@EU_ScienceHub**

**JRC Summer School Alumni**
**https://www.linkedin.com/groups/8610319**

**EURL ECVAM**
**https://ec.europa.eu/jrc/en/eurl/ecvam**

**Joint Research Centre**
**https://ec.europa.eu/jrc/en**

**Audience interactions**
**https://www.sli.do/ event code: #ecvam**

**App Kahoot for the Quiz Night**
**https://kahoot.com/mobile-app/**

**Free Wifi**
**JRC-IspraNET-Wifi-Guest**

# AGENDA

## Day 1 - Tuesday, 21 May 2019
## (Auditorium, bldg. 58c)

| | |
|---|---|
| 12:00-13:00 | Registration and welcome lunch |
| 13:00-13:30 | **Welcome and introduction**<br>Elke Anklam, Director - Health, Consumers and Reference Materials (JRC)<br>Maurice Whelan, Head of Unit - Chemical Safety and Alternative Methods (JRC) |
| 13:30-13:45 | **Summer school organisation**<br>Brigitte Landesmann (JRC) |
| **SESSION 1** | **Replacement of animals used for scientific purposes - legal obligations and state of play**<br>chairs: B. Landesmann & E. Berggren (JRC) |
| 13.45-14:30 | **Legal obligation and state of play in the European Union**<br>S. Louhimies (DG Environment) |
| 14.30-15:15 | **Legal obligation and state of play in North America**<br>C. Chandrasekera (CaCVAM, Canada) |
| 15:15-15:45 | Coffee Break |
| 15:45-16:30 | **Strenghts and limitations of animal and alternative approaches**<br>L. Gribaldo and F. Pistollato (JRC) |
| 16:30-17:30 | **Group meetings to prepare the Friday debates** |
| 17:30-19:00 | Aperitivo & poster exhibition (bldg. 100) |
| 19:00 | Bus to hotels |

## Day 2 - Wednesday, 22 May 2019
## (Auditorium, bldg 58c)

| | |
|---|---|
| **SESSION 2** | ***In vitro* methods and their applications**<br>chair: A. Hanberg (Karolinska Institutet, Sweden) |
| 09:00-09:20 | **Non-animal approaches for improving developmental neurotoxicity (DNT) testing**<br>A. Bal-Price & F. Pistollato (JRC) |
| 09:20-09:40 | **Are your non-animal cultures really animal-free?**<br>C. Chandrasekera (CaCVAM, Canada) & S. Coecke (JRC) |
| 09:40-10:00 | **Exploiting 3D cultures to model disease-specific cellular micro-environments**<br>C. Brito (IBET, Portugal) |
| 10:00-10:20 | **Organ-on-a-Chip systems: integrated microphysiological platforms recapitulating complex human biology**<br>P. Loskill (Fraunhofer Institute, Germany) |
| 10:20-10:30 | **Final thoughts & reflections by the chair** |
| 10:30-11:00 | Coffee Break |
| **SESSION 3** | **Computational methods and their applications**<br>chair: K. Dreij (Karolinska Institutet, Sweden) |
| 11:00-11:20 | **Overview of *in silico* methods used in predictive toxicology and drug development**<br>A. Paini & A. Worth (JRC) |
| 11:20-11:40 | **Thresholds of (eco-)toxicological concern for aquatic toxicity**<br>A. Kienzler (JRC) |
| 11:40-12:00 | **Biokinetic modelling to relate internal and external exposure**<br>N. Kramer (IRAS, Utrecht University, The Netherlands) |
| 12:00-12:20 | **Human *in silico* clinical trials in cardiology and pharmacology**<br>B. Rodriguez (University of Oxford, United Kingdom) |
| 12:20-12:30 | **Final thoughts & reflections by the chair** |
| 12:40-13:30 | Lunch |
| **13:30-15:00** | **WORLD CAFÉ**<br>**Have coffee and discuss together with the speakers from the morning sessions (bldg. 58c, 100 and 101)** |

| | |
|---|---|
| | **15:00 – 17:00 POSTER SESSION I** |
| | chairs: L. Wiklund (Karolinska Institutet) & A. Dura (JRC) |
| 15:00-15:45 | **Flash (3 min) poster presentations**, Auditorium |
| 15:45-17:00 | **Poster session (1-57)**, bldg. 100 |
| | |
| | **17:00 – 19:00 POSTER SESSION II** |
| | chairs: L. Wiklund (Karolinska Institutet) & A. Dura (JRC) |
| 17:00-17:45 | **Flash (3 min) poster presentations,** Auditorium |
| 17:45-19:00 | **Poster session (58-114),** bldg. 100 |
| | |
| 19:00 | Bus departure to Don Guanella, Barza |
| 19:15-22:00 | **Alternative solutions – more answers than questions** |
| | Quiz night at Don Guanella with finger food |
| 22:00 | Bus to hotels |

## Day 3 - Thursday, 23 May 2019
## (Auditorium, bldg 58c)

| | |
|---|---|
| **SESSION 4** | **From methods to integrated solutions**<br>chair: J. Barroso (JRC) |
| 09:00-09:20 | **Biomarkers and mode of action in pharma development**<br>I. Cotgreave (Research Institutes of Sweden-RISE, Sweden) |
| 09:20-09:40 | **High throughput methodologies at EURL ECVAM**<br>J. Sund, T. Palosaari & D. Carpi (JRC) |
| 09:40-10:00 | **Adverse Outcome Pathway (AOP) - a basis for integrated approaches**<br>E. Berggren & B. Landesmann (JRC) |
| 10:00-10:20 | **The regulatory uptake of combined methods for skin sensitisation**<br>S. Casati & D. Asturiol (JRC) |
| 10:20-10:30 | **Final thoughts and reflections by the chair** |
| 10:30-11:00 | Coffee break |
| **SESSION 5** | **Alternative methods and future challenges**<br>chair: P. Prieto-Peraita (JRC) |
| 11:00-11:20 | **Genomics data for public health and possible applications on chemicals safety**<br>M. Bale (Genomics England, United Kingdom) |
| 11:20-11:40 | **Alternatives to understand respiratory tract disease**<br>R. Gosens (University of Groningen, The Netherlands) & L. Gribaldo (JRC) |
| 11:40-12:00 | **Endocrine disruptors - impetus for change**<br>S. Munn & E. Grignard (JRC) |
| 12:00-12:20 | **Convergence between disease/toxicity and human/environment**<br>F. Madia & S. Bopp (JRC) |
| 12:20-12:30 | **Final thoughts and reflections by the chair** |
| 12:30-13:30 | Lunch |
| **13:30-15:30** | **WORLD CAFÉ**<br>**Have coffee and discuss together with the speakers from the morning sessions (bldg. 58c, 100 and 101)** |
| 15:30 | Group photo |
| 15:30-18:00 | **Laboratory visits and preparation for the Friday debates** |
| 18:45 | Bus departure to Villa Quassa |
| 19:00-22:00 | Social dinner at Villa Quassa, Ispra |
| 22:00 | Bus to hotels |

## Day 4 - Friday, 24 May 2019
## Don Guanella, Barza

| | |
|---|---|
| **SESSION 6** | **Debates**<br>chairs: E. Bernasconi & B. Landesmann (JRC) |
| 09:00-09:50 | **Debate I: Are legal obligations necessary to support the 3Rs?** |
| 10:00-10:50 | **Debate II: Can computational methods provide stand-alone solutions?** |
| 10:50-11:20 | Coffee break |
| 11:20-12:10 | **Debate III: Do Adverse Outcome Pathways have a future for regulatory toxicology?** |
| 12:20-13:10 | **Debate IV: Can we do science without animal experiments?** |
| 13:10-13:30 | **Poster awards ceremony & concluding remarks**<br>M. Whelan & E. Berggren (JRC) |
| 13:30-14:30 | Lunch |
| 14:45 | Bus departures to Milan airports, Milan Central Station and hotels |

# Meet the Speakers...

**Elke Anklam**

European Commission, Joint Research Centre

*Elke Anklam is a chemist by education with specialisation in food, organic and radiation chemistry. After having obtained her PhD from the University Hamburg, Germany, she worked in various European Research Institutions and was a teaching Professor at the Applied University of Fulda, Germany. Since 1991 she has been working in the European Commission's Joint Research Centre (JRC-EC); from 2006-2012 as Director of the JRC-Institute for Health and Consumer Protection (JRC-IHCP) in Ispra, Italy and then from January 2013, Director of the JRC-Institute for Reference Materials and Measurements (JRC-IRMM) in Geel, Belgium first and now Director of the JRC Directorate F - Health, Consumers and Reference Materials.*

**Maurice Whelan**

European Commission, Joint Research Centre, Ispra, Italy

*Prof. Maurice Whelan is head of the Chemical Safety and Alternative Methods Unit of the Directorate for Health, Consumers and Reference Materials of the European Commission's Joint Research Centre (JRC), based in Ispra, Italy. He also heads the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM). Maurice is the EU co-chair of the OECD Advisory Group on Molecular Screening and Toxicogenomics that is responsible for the OECD programme on Adverse Outcome Pathways, and he is a member of the Steering Committee of the European Partnership for Alternative Approaches to Animal Testing (EPAA). His publications include over 200 scientific papers and a recent book on the validation of alternative methods for toxicity testing. He has held a number of external appointments including the 2017-2018 Francqui Chair for alternative methods at the Vrije Universiteit Brussel (VUB, Belgium) and is currently visiting Professor of Bioengineering at the University of Liverpool (UK).*

**Susanna Louhimies**

Directorate-General for the Environment, European Commission, Brussels

**Legal obligation and state of play in the European Union**

Can modern policy advance the principle of the Three Rs – the replacement, reduction, and refinement of the use of animals in experiments? The Directive on the protection of animals used for scientific purposes took effect in 2013. The principle of the Three Rs is the cornerstone of the legislation and the requirement to use non-animal approaches, before resorting to animal use, is a firm legal obligation in the EU. The Directive review (2017) confirmed that the legislation was already contributing positively to animal use and care practices, including in the implementation of the Three Rs. The Directive has also provided new tools for the development and validation of non-animal alternatives.

*Susanna Louhimies works at the European Commission, the Directorate-General for the Environment since 1996. She is responsible for the EU Directive on the protection of animals used for scientific purposes and horizontal issues in relation to scientific, educational and regulatory use of animals. As an integral part of her portfolio, she follows closely the implementation of the principle of the Three Rs (Replacement, Reduction and Refinement of animal use) in different policy areas. She is also responsible for the co-ordination of the regulatory acceptance of new and updated testing methods in the EU legislative framework for chemicals (REACH).*

**Charu Chandrasekera**

Canadian Centre for Alternatives to Animal Methods (CaCVAM), Canada

**Legal obligation and state of play in North America**

This presentation will focus on science and legislative frameworks for Canada and the USA with limited reference to other countries in North America. The state of play in Canada covers the latest from the Canadian Chemicals Management Plan, a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. The U.S. initiatives include the ICCVAM inter-agency national strategy addressing the development, validation, and implementation of new approach methodologies for assessing the safety of chemicals and medical products as well as the Food and Drug Administration and Environmental Protection Agency roadmaps addressing specific chemical sectors and legislative mandates.

*Dr. Charu Chandrasekera is the founder and executive director of Canada's first and only centre dedicated exclusively to alternatives to animal testing, the Canadian Centre for Alternatives to Animal Methods (CCAAM) and the Canadian Centre for the Validation of Alternative Methods (CaCVAM) located at the University of Windsor. She obtained her Ph.D. in Biochemistry and Molecular Biology from the University of Calgary; she is an experienced scientist, former animal researcher, science policy expert, and an animal lover. Through her Centre, Dr. Chandrasekera promotes the replacement of animals in Canadian biomedical research, education, and regulatory testing through 21st century science, innovation, and ethics.*

**Laura Gribaldo & Francesca Pistollato**

European Commission, Joint Research Centre, Ispra, Italy

**Strenghts and limitations of animal and alternative approaches**

Which is the scientific justification for using animal models? The justification is based on Darwinian Theory of Evolution where, two species are the more similar to each other the less time has passed from the existence of their common ancestor. This Darwinian idea of common descent concerns all aspects of animal biology, including behaviour (thanks to Konrad Lorenz's intuition). The adoption of such perspective legitimises the use of a non-human animal to understand aspects of human biology. Despite conserved genotypes though, animal species have evolved over time, interacting with the environment, bearing distinct genomic imprinting and epigenetic backgrounds. In this perspective, the use of non-human animals to model and study human physiology and pathology is both scientifically and ethically questionable, whilst human relevant alternative models represent reliable tools for biomedical research and toxicology.

*MD, PhD in Microbiology and Virology, she has thirty years of experience in the field of testing for safety assessment. As leader of the Applied Molecular Biology and Genomics competence group she set up and managed a transcriptomics platform for the development of standardized assays in toxicology and she was responsible to support a programme ensuring harmonisation and validation of procedures in genetic testing for diagnostics purposes. For the Public Health Unit, she worked on Rare Diseases, representing JRC at the EUCERD meetings for the establishment of the European platform for rare diseases registry. Today, at the Chemical Safety and Alternative Methods Unit, she works in the field of knowledge dissemination, education and training.*

*Francesca Pistollato, Ph.D., is employed as contract agent at the EURL ECVAM, where she applies human induced pluripotent stem cell (hiPSC) culture models for developmental and adult neurotoxicity and cumulative risk assessment. She previously worked at the Physicians Committee for Responsible Medicine (PCRM), Washington DC, advocating for the use of human based approaches for Alzheimer's disease research. Dr Pistollato worked at the Department of Pediatrics of the University of Padua, where she was responsible of the research unit on brain cancers and the effects of microenvironmental hypoxia using in vitro approaches. At that time, she was professor of Biology, Genetics and Neuroscience at IUSVE, Venice, Italy.*

**Anna Bal-Price & Francesca Pistollato**
European Commission, Joint Research Centre, Ispra, Italy

**Non-animal approaches for improving developmental neurotoxicity (DNT) testing**

Despite the recognized need for a more systematic and rigorous evaluation of DNT at the regulatory level, DNT evaluation is not a mandatory requirement in the USA or the European Union. At the same time, recent societal concerns have been raised linking the increase in children's neurodevelopmental impairments (e.g., learning disabilities, autism and attention deficit hyperactivity disorder) to chemical exposures. Current, paradigm shift in toxicology has encouraged the use of human *in vitro* models, such as human induced pluripotent stem cell (iPSC)-derived neurons and glia, and the application of the adverse outcome pathway (AOP) framework to improve human risk assessment and gather mechanistic knowledge of chemical effects. During this presentation we will provide an overview of currently ongoing international activities in the context of DNT aimed at creating a DNT testing battery that relies on *in vitro* endpoints described as key events in several AOPs, and we will describe how human iPSC-neurons can be used to evaluate chemical mixtures with potential to cause learning and memory impairment in children.

*Anna Price is working at European Commission Joint Research Centre (JRC), the Unit of Chemicals Safety and Alternative Methods/EURL-ECVAM, Ispra (Italy) on developing testing strategy for developmental neurotoxicity (DNT) evaluation using in vitro models derived from human induced pluripotent stem. During the last years, she is also strongly involved in the development of Adverse Outcome Pathways relevant to neurotoxicity. Before JRC she worked at the Department of Biochemistry at Cambridge University (UK) studying mechanisms of neuronal cell death of neurodegenerative disorders. She published many papers in the peer reviewed journals, and she is a co-editor of two books "Cell Culture Techniques. Neuro-methods" (2011) and "In vitro Toxicology Systems" (2015) published by Springer.*

**Sandra Coecke & Charu Chandrasekera**
European Commission, Joint Research Centre, Ispra, Italy & Canadian Centre for Alternatives to Animal Methods (CaCVAM), Canada

**Are your non-animal cultures really animal-free?**

Many animal-derived products were of utmost importance in the development of cell culturing techniques. The way forward in the on-going high technology research where we can study biological processes with increasing accuracy is to strive towards fully defined systems with known composition. This strive clearly excludes the use of non-defined products such as those derived from animals, but also those from humans and plants. This will necessitate interdisciplinary efforts and increasing awareness at the political level, at the level of funding agencies, research as well as that of journals. It will be an effort ensuring reproducible, credible, and trustworthy scientific data generation in the 3Rs research and development arena.

*Dr Sandra Coecke is senior scientist at EURL ECVAM. In 1989 earned a Degree of University Engineer Biotechnology followed by PhD in Pharmaceutical Sciences. She joined in 1993 Janssen Pharmaceutica, Belgium, leading the In vitro Toxicology laboratory. In 1994 awarded the European Prize from Foundation for the Substitution of Animal Experimentation in Luxembourg. With more than 27 years' experience in in vitro toxicology; she was involved in many research & development and validation activities related to the development of new in vitro cell and tissue-based methods. She established and manages the regulatory required European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), which are 37 high quality laboratories across Europe.*

**Catarina Brito**
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB-NOVA) iBET, Instituto de Biologia Experimental e Tecnológica

**Exploiting 3D cultures to model disease-specific cellular microenviornments**

Cell microenvironment modulates physiological and pathological processes. Disease progression and therapeutic response are modulated by cellular components of the niche and by extracellular molecular components. A major challenge in studying the underlying mechanisms is the lack of human cell models in which cell types and dynamic interactions between cellular and extracellular spaces are recapitulated, without the confounding effects of heterologous matrices and soluble factors. To overcome these challenges we develop disease cell models, applying advanced cell culture approaches to human stem cells and other patient-derived cells. I will discuss recent data on the two of the main research lines in the lab: microenvironment dynamics in neuronal dysfunction and immunosuppressive microenvironments in carcinomas.

*I am the Head of the Advanced Cell Models Laboratory of the Animal Cell Technology Unit of iBET and ITQB-NOVA, since 2014. My current research is mostly translational, focused on development of advanced human cell models to target deregulation of cell microenvironment in disease progression and evaluate its role in therapeutic response. After a Biochemistry degree (University of Lisbon) and PhD in Biochemistry and Cell Biology (New University of Lisbon & Institut Jacques Monod), in the areas of glycobiology and intracellular trafficking, I joined iBET (Portugal) in 2007 for a postdoc in human Stem Cell bioprocessing. In 2019 I became a Senior Project Manager of academic and industrial collaborations, bridging cell biology and cellular biotechnology to develop cell models and bioassays.*

**Peter Loskill**

Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB Stuttgart and Faculty of Medicine, Eberhard Karls University Tübingen, Germany

**Organ-on-a-Chip systems**

Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem cells (hiPSC) has led to the emergence of a new paradigm of drug screening using human patient- and disease-specific organ/tissue-models. One promising approach to generate these models is by combining hiPSC technology with microfluidic devices tailored to create microphysiological environments and recapitulate 3D tissue structure and function. Such organ-on-a-chip or microphysiological systems combine human genetic background, *in vivo*-like tissue structure and functionality, and "vasculature-like" perfusion and pave the way for applications in drug development, personalized medicine, toxicity screening, and mechanistic research.

*Peter Loskill is Assistant Professor for Experimental Regenerative Medicine at Eberhard Karls University Tübingen and Organ-on-a-Chip Attract Group Leader at the Fraunhofer IGB in Stuttgart, Germany. Dr. Loskill graduated from Saarland University with a PhD in Physics and spent three years as a postdoctoral fellow / project leader at UC Berkeley developing Organ-on-a-chip (OoC) systems based on human iPS-cells. In 2015, he was named as one of Technology Review's "Innovators under 35 Germany" and awarded a Fraunhofer ATTRACT Grant. His µOrgano lab (http://loskill-lab.com/) combines approaches from engineering, biology, physics and medicine to generate next-generation tissue models recapitulating complex human biology in vitro. To advance European OoC research, he coordinates the MSCA-ITN EUROoC (https://www.eurooc-itn.eu/), is part of the EUROoC conference (http://www.eurooc2019.eu) organizing committee, and serves as vice-chair of the European OoC-Society (EUROoCS; https://www.euroocs.eu/).*

**Alicia Paini & Andrew Worth**

European Commission, Joint Research Centre, Ispra, Italy

**Overview of *in silico* methods used in predictive toxicology and drug development**

This presentation will explain "what is a model" and "why modelling is useful". We will introduce the basic concepts of *in silico* (computational) models (such as QSARs, PBK models, dynamic models) as well as their applications in research, chemical risk assessment and drug development. We will also offer insights into the challenges faced in using and translating *in silico* data in a regulatory setting, including the importance of model validation and the routes toward regulatory acceptance by assessors and decision makers.

*Dr Alicia Paini holds a degree in Food Science and Technology from the University of Parma, Italy. An MSc in Food Safety and a PhD in Toxicology from Wageningen University, The Netherlands, where she gained know-how in generation of data to develop physiologically based kinetic (PBK) models for genotoxic chemicals; she did her PhD research at the Nestlé Research Centre, Switzerland. Since 2012 she is working on implementing and promoting in silico tools (biokinetic and dynamic modelling, e-health, etc) for policy and application in regulatory decision making at the European Commission's Joint Research Centre (JRC) - EURL ECVAM; from 2013 she is a European Registered Toxicologist.*

*Andrew Worth is a senior scientific officer at the European Commission's Joint Research Centre (JRC), where he is a Group Leader and Project Leader within the Chemical Safety and Alternative Methods Unit. The unit incorporates the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), which promotes the development, validation and acceptance of non-animal methods in regulatory toxicology and biomedical science. The JRC provides independent scientific and technical support to the European Commission and other policy makers in the EU. Dr Worth has degrees in Physiological Sciences and in Linguistics from Oxford University, and a PhD in Computational Toxicology from Liverpool John Moores University. His current work is in the areas of chemical safety assessment and predictive toxicology.*

**Aude Kienzler**
European Commission, Joint Research Centre, Ispra, Italy

**Thresholds of (eco-)toxicological concern for aquatic toxicity**

An eco-TTC (Treshold of Toxicological Concern) approach has been recently developed for application in environmental risk assessment. In this context, an aquatic ecotoxicity database has been built, bringing together more than 91 000 data records for nearly 3 900 chemicals, on 1 563 different species. An accompanying analytical tool has also been developed, which allows the user to draw statistical distributions based on the underlying dataset. From those statistical distributions, two types of environmental concentrations of "no-concern" can be derived. A consensus approach for classifying the underlying data in a binary Mode of Action scheme (Narcotic/Specifically acting chemicals) has also been implemented, to help prioritizing chemicals for regulatory risk assessment.

*Dr Aude Kienzler is a Scientific Officer at the European Commission Joint Research Centre (JRC). Her work focuses on the risk assessment of chemical mixtures, the identification of endocrine disruptors and the development of alternative approaches to vertebrate testing, and more particularly to fish testing. Dr Kienzler has degrees in Agronomy and in Ecotoxicology and a PhD in Genetic Ecotoxicology. She previously worked in research institutions in France (INSA Lyon; French National Institute for Industrial Environment and Risks - INERIS), and as regulatory officer in the field of pesticides risk assessment.*

**Nynke Kramer**
Institute for Risk Assessment Sciences (IRAS), Utrecht University, The Netherlands

**Biokinetic modelling to relate internal and external exposure**

Kinetics *in vivo* (i.e. absorption, distribution, metabolism and excretion) play a central role in quantitative *in vitro-in vivo* extrapolation (QIVIVE) studies as these processes determine the concentration of a test chemical at the target organ where the toxic effect is initiated. Similar kinetic processes determine the target concentration and thus the level of bioactivity of chemicals in *in vitro* assays. Despite chemicals or assays eliciting similar effects at similar nominal concentrations, the bioavailable concentration may vary greatly between chemicals and assays. Here, studies are discussed illustrating tools and techniques that may be used to account for kinetics *in vitro* and *in vivo* and thereby improve QIVIVE for toxicological risk assessment.

*Nynke Kramer is assistant professor in toxicology of Utrecht University's Institute for Risk Assessment Sciences and heads its In vitro Toxicology group. Her research focusses on enhancing the uptake of in vitro models in toxicological risk assessment by developing models extrapolating effect concentrations obtained from in vitro cell assays to toxic doses relevant to humans and animals. She teaches toxicokinetics and (eco)toxicological risk assessment at undergraduate, graduate and postgraduate level across faculties. Her teaching and research neatly integrate the skills she aquired during her PhD in toxicology at Utrecht University, her MSc in environmental sciences at Oxford University, and her BSc in life sciences and economics at University College Utrecht.*

**Blanca Rodriguez**
Department of Computer Science, University of Oxford, United Kingdom

**Human *in silico* clinical trials in cardiology and pharmacology**

*In silico* clinical trials in medicine refer to the evaluation of a medical therapy using simulations with computer models. Already established in engineering applications (such as aeronautics), *in silico* trials are now starting to be more widely adopted in medicine with broad potential impact in academy, industry and regulatory bodies. The socio-economic potential in this area is thus huge. In my talk, I will describe our progress in computational modelling and simulation of human heart towards the realisation of *in silico* clinical trials for cardiac pharmacology and medicine. I will describe the causes of variability in the response of human hearts to pharmacological therapy, and their importance in assessing safety and efficacy during drug development. I will then address the synergies gained from combining modelling and simulation science with machine learning to unravel the causes of phenotypic variability in disease and drug response, and their implications for the advancement of human *in silico* trials in medicine. I will emphasize the strong collaborations underpinning this work with key partnerships in industry, regulatory agencies and experimental and clinical biomedicine. Through my talk, I will discuss the importance and challenges of inter-disciplinary, inter-sectoral collaborations in computational medicine.

*Blanca Rodriguez is Professor of Computational Medicine, Head of the Computational Biology and Health Informatics Theme and Wellcome Trust Senior Research Fellow at the University of Oxford. She is interested in investigating the causes and modulators of variability in the response of the heart to disease and therapies. With her team, she embeds computational methods in cardiovascular research to augment experimental and clinical investigations (www.cs.ox.ac.uk/ccs). Their research is supported by an established network of collaborators in academia and industry, who are world-leading experts in in vivo and in vitro human cardiovascular medicine and pharmacology. Blanca has been in Oxford since 2004. Before her current post, she held Medical Research Council Career Development and Centenary Awards at Oxford (2007-2013) and worked in the USA as a postdoctoral research fellow at Tulane University in New Orleans (2002-2004). She is an Electronics Engineer by training from the Universidad Politecnica de Valencia, where she also conducted her PhD in Bioelectronics (1998-2001).*

**Ian Cotgreave**

Research Institutes of Sweden (RISE), Sweden

**Biomarkers and mode of action in pharma development**

The development of novel drug therapeutic approaches is a long and complex process involving an intertwined multidisciplinary approach. From disease target identification, through design of chemically or biologically-based, pharmacological molecules, through preclinical efficacy and safety testing and human clinical trialing, there is a contemporary trend to define biomarkers predicting both efficacy and safety in the patient population, and individual. The lecture will review, with a few examples, how the concept of "precision medicine" is developing at the cross-roads between use of modern technological advancements in understanding of molecular pathology, human genetics, translational biomarker identification and validation and clinical implementation, with our increasing knowledge of both pharmacological and adverse outcome pathways.

*Ian is a professor of toxicology from the Karolinska Institute and has held posts as head of molecular toxicology at AstraZeneca safety assessment for 10 years, where he was involved in progressing novel drug candidates over all stages of development, and senior research scientist and strategist at Swetox for 4 years. Ian is currently senior scientist and business developer at the department of Chemical and Pharmaceutical Safety within the Research Institutes of Sweden (RISE: www.ri.se). Ian has worked for many years academically, industrially and from a regulatory perspective on the development of mechanism-based safety assessment, in particular the development of new safety assessment methods, and partaken in research programs such as SEURAT-1 and EU-ToxRisk. Ian is currently a member of EURL ECVAM's Scientific Advisory Committee (ESAC).*

**Taina Palosaari, Jukka Sund, & Donatella Carpi**

European Commission, Joint Research Centre, Ispra, Italy

**High throughput methodologies at EURL ECVAM**

In High Throughput Screening (HTS), automated assays are used to screen large libraries (1000 – millions) of compounds such as medicines, small molecules or industrial chemicals. HTS is widely used in drug discovery but also in toxicology to identify hazardous chemicals. At EURL ECVAM we use a combination of robotics, High Content Imaging (HCI), electrophysiological measurements and OMICS to get a multi-layered view of toxicological pathways induced by chemical treatments

in different cellular systems (i.e. human hepatocytes HepaRG and cells derived from Human Induced Pluripotent Stem Cells). By integrating different lines of evidence we explore the reliability and biological relevance of such kind of approach for regulatory purposes.

*Taina Palosaari is a Medical Technologist with specialisation in Clinical Microbiology. She studied in the University Colleges of Oulu and Turku, Finland and has gained her work experience in Clinical Laboratory Sciences in Finland, Somalia and Italy before joining the EC's Joint Research Centre in 1997. Her main tasks have been in the field of in vitro functional neuro- and cardio-toxicology, in vitro nanotoxicology and automation of in vitro assays in High Throughput Screening / High Content Analysis. Actually, she is responsible for the HTS Facility of the EURL ECVAM laboratory.*

*I am a molecular biologist and biochemist from Helsinki, Finland. I did my Master's thesis on virology and my PhD on the health effects of engineered nanomaterials. I have a wide experience of different techniques and technologies in life sciences: e.g. molecular biology methods, cell culture, proteomics, electron microscopy and more recently high throughput technologies. I have been in EURL ECVAM for a year. Here, I am the main responsible of the liquid handling platforms. My goal is to automate everything, since the robot is more precise, more reliable, and much faster than a human. It does not sleep and creates a log of everything it has done.*

*Donatella Carpi has interdisciplinary competences, acquired during the degree in Environmental Science, the Specialization in Pharmacological Research (Mario Negri Research Institute) and the UK PhD in Life Science on endocrine disruptors. During the whole training she focused on the use of -omics approaches for the study of chemical toxicology and human diseases. From these experiences it cames a passion for data integration practiced working as post-doc in System Toxicology Unit (JRC) and as bioinformatician in a translational research context (INGM, Major Hospital of Milan). In 2017 she was recruited as a computational biologist for the Alternative Method and Chemical Safety Unit (JRC).*

**Elisabet Berggren & Brigitte Landesmann**
European Commission, Joint Research Centre, Ispra, Italy

**Adverse Outcome Pathway (AOP) - a basis for integrated approaches**

An AOP describes a sequential chain of causally linked events starting at molecular level, spanning multiple levels of biological organisation, to an adverse health or eco-toxicological outcome of regulatory relevance. AOPs facilitate the collection, integration and evaluation of underlying mechanistic information and provide increased level of confidence in the relationships between *in vitro* or *in silico* data and the probability of occurrence of adverse outcomes *in vivo*. This concept provides a framework for developing Integrated Approaches to Testing and Assessment (IATAs) using data derived from multiple methods and sources.

*Elisabet Berggren is Deputy Head of Unit of the Chemical Safety & Alternative Methods Unit, JRC. She is a passionate believer in new and better science to underpin a safe use of chemicals as well as faster progress in biomedical research. Elisabet started to work for the European Commission in 1996, first responsible for activities related to the hazard assessment of chemicals. She was involved in the negotiations of the Rotterdam Convention and the Globally Harmonised System of classification and labelling and their EU regulatory implementation. Elisabet made her PhD in physical chemistry at Stockholm's University 1991. In her academic career she focussed on the development of theoretical dynamic models.*

*Brigitte Landesmann is a medical doctor and has worked for many years in clinical medicine. She got the MD from the University of Vienna and the MSc in Public Health from the London University (LSHTM). She joined the Systems Toxicology Unit of the Institute for Health and Consumer Protection of the European Commission's Joint Research Centre in 2010 and got involved in the generation, dissemination and application of AOP knowledge. In the context of the FP7 SEURAT-1 research initiative she has developed an AOP to liver fibrosis which has been endorsed by OECD. As a member of the OECD AOP training committee she has experience in the organisation and execution of AOP training courses.*

**Silvia Casati & David Asturiol**

European Commission, Joint Research Centre, Ispra, Italy

**The regulatory uptake of combined methods for skin sensitisation**

Currently, for skin sensitisation assessment, the data generated by a single Key Event-based Test Guideline are not considered to provide equivalent information to the animal tests. Thus the data generated by individual methods should be used in conjunction with other relevant information. If the available data are considered in the context of a Weight-of-Evidence, this inevitably implies 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, thus reducing bias in subjective interpretations.

*Dr. Silvia Casati graduated with honours in Pharmacy and obtained a PhD in Biomedical Sciences at the University of Nottingham (UK). She has been working as scientific officer at the European Centre for the Validation of Alternative Methods (EURL ECVAM) within the European Commission since 2001. In 2003 she became responsible of the activities in the area of skin sensitisation. She has been involved in the coordination of international validation studies on alternative test methods for skin sensitisation and in providing support to their regulatory acceptance at OECD level. She served on several expert groups and lead OECD activities on the development of guidance documents on defined approaches to be used within IATA.*

*I studied Chemistry and obtained a PhD in Computational Chemistry in 2010 at the University of Girona (Spain). After a short postdoc experience in the Max Planck Institute für Kohlenforschung (Mulheim, Germany), I started working as data scientist/computational toxicologist at the Joint Research Centre of the European Commission (Italy) in 2012. The nature of my work is based on data science and their applications to extract knowledge and predict the toxicity of chemicals. I try to promote the use of in silico methods and QSARs for the risk assessment of substances and I am involved in several OECD projects aimed at providing a replacement to animal methods.*

**Mark Bale**

Genomics England, United Kingdom

**Genomics data for public health and possible applications on chemicals safety**

The 100,000 Genomes project is a national clinical / research project intended to provide key tools to deliver genomic medicine for the National Health Service. It offers a diagnostic service for patients with rare diseases and cancer. Participants are consented to join this as a research project with 100,000 genomes and linked clinical data from around 85,000 participants. Over 3000 researchers are organised into 45 Genomics England Clinical Interpretation Partnership domains. Key research aims include rare diseases, cancer, statistical and machine learning tools and population health to provide a better understanding on the impact of "environment" and genomic background on disease.

*Mark Bale is the senior genomics policy expert at the Department of Health & Social Care and Head of Science Partnerships at Genomics England. He has had responsibility for several emerging areas of science and their ethical, legal and policy implications. The current emphasis is on genomics (particularly the 100,000 Genomes Project) and emerging areas such as genome editing. At Genomics England, he co-ordinates the various partnerships within England, Scotland, Wales, and Northern Ireland, and links with other international partners. These include the EU 1 Million Genomes Initiative and representation at the Council of Europe and OECD.*

**Reinoud Gosens**

University of Groningen, The Netherlands

**Alternatives to understand respiratory tract disease**

Increasingly, animal studies are replaced by human disease modeling technology. I will focus on organoid technology in my lecture and its applicability in studying respiratory disease. Organoid technology is well suited for studies of epithelial biology, developmental biology, and pharmacology particularly as they pertain to the functional role of the stem cell in regeneration. Declared method of the year 2017, with applications all across medicine, the organoid model is now quickly conquering its position in academia and industry. In my view, it has one of the greatest potentials of truly replacing animal studies in specific areas. I will discuss the method, and its applicability to disease modeling and drug discovery.

*Reinoud Gosens is Associate Professor of Translational Pharmacology at the Faculty of Mathematics and Natural Sciences at the University of Groningen, the Netherlands. He has an MSc in Pharmaceutical Sciences from the University of Groningen and a PhD from the Department of Molecular Pharmacology at the University of Groningen. Dr Gosens has undertaken research fellowships at the University of Manitoba, Canada, and the University of Groningen. He is conferences and seminars director for ERS, board member of the Groningen Research Institute for Pharmacy (GRIP), programme leader of the Groningen Research Institute for Asthma and COPD (GRIAC) and chair of the scientific advisory board of the Netherlands Lung Foundation.*

**Sharon Munn & Elise Grignard**
European Commission, Joint Research Centre, Ispra, Italy

**Endocrine disruptors - impetus for change**

Endocrine disrupters are defined as substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism. The endocrine system regulates the body's development, growth, reproduction, metabolism, immunity and behaviour. The hormones actions on the body are precisely controlled, regarding both dose and timing, with specific windows of susceptibility during development. Disruption of the endocrine system can affect health, even with life-long effects. Recently, criteria for the determination of endocrine disrupting properties have been adopted, to be used for the regulation of pesticides and biocides. These criteria include the identification of a (endocrine) mode of action. This requirement highlights the change in regulatory toxicology, moving towards the need for a mechanistic understanding of how toxicity arises fitting well to efforts towards Adverse Outcome Pathway (AOP) development and Integrated Approaches to Testing and Assessment (IATA).

*Sharon Munn has an education in the field of human toxicology and risk assessment. She has been working for the last ten years in support of the Community Strategy for Endocrine Disrupters as well as on evaluating the utility and regulatory applicability of new approaches and technologies for chemical safety assessment, including AOPs and IATA, with the aim of placing less reliance on in vivo animal testing.*

*Dr Elise Grignard is a scientific officer of the Joint Research Centre. She received her PhD in Reproductive Biology from the Blaise Pascal University in France. She worked for many years in the field of reproductive toxicology, with an emphasis on endocrine disruption. Her main focuses are on the identification of gaps in knowledge and test methods for the identification of endocrine disruptors, and development of alternative methods.*

**Federica Madia & Stephanie Bopp**
European Commission, Joint Research Centre, Ispra, Italy

**Convergence between disease/toxicity and human/environment**

The development of disease and impacts on the ecosystem is a multifactorial process. What is the contribution of chemicals to this process compared to other factors? What evidence is there to link chemical exposure to development of specific diseases, and which methodology might be used to establish such links? Which role plays the combined exposure to multiple chemicals? How can we mutually learn from human and environmental toxicity assessments? Here, we will explore the state of the art and provide examples for these questions and related answers.

*Stephanie Bopp is a Scientific Officer at the European Commission Joint Research Centre (JRC) in Ispra (Italy). She works in the group on "Chemical Safety and Alternative Methods (F.3)", where she is leading the activities on the assessment of chemical mixtures. Before, she worked in the European Food Safety Authority (EFSA, Parma, Italy) on pesticide environmental risk assessment and in several other organisations in Germany and Switzerland in the area of ecotoxicology and environmental chemistry. She holds a MSc equivalent in Geoecology (University of Bayreuth) and a PhD in Environmental Toxicology and Chemistry (Helmholtz Centre for Environmental Research UFZ Leipzig / University of Rostock).*

*Federica Madia is a biologist and holds a Ph.D. in Pharmacology and Toxicology from University of Rome Italy (2000). Before joining the EC, she was research associate at the School of Pharmacy and had experience in the private sector, as a stagier and as toxicology study director. Moved by a keen interest in biology of cancer and aging, she joined the Longo's lab at the University of Southern California (US) where from 2004 to 2012, she was responsible of a number of studies aimed to elucidating effects of calorie restriction on the regulation of longevity pathways involved in aging, genomic instability and cancer. Since 2013, she works at JRC as a scientific officer dealing with the development of novel approaches to genotoxicity and carcinogenicity testing.*

# World Café
## 13:30 – 15:00 Wednesday, 22 May 2019

| | *Table host* | *Bldg/Room* |
|---|---|---|
| ***In vitro* methods and their applications** | | |
| Non-animal approaches for improving developmental neurotoxicity (DNT) testing | A. Price & F. Pistollato | 58c/11 |
| Are your non-animal cultures really animal-free? | C. Chandrasekera | 58c/12a+b |
| Exploiting 3D cultures to model disease-specific cellular microenvironments | C. Brito | 101/1003 1st floor |
| Organ-on-a-Chip systems: integrated microphysiological platforms recapitulating complex human biology | P. Loskill | 101/1302 1st floor |
| **Computational methods and their applications** | | |
| Overview of *in silico* methods used in predictive toxicology and drug development | A. Paini & A. Worth | 101/2002 2nd floor |
| Threshold of (eco-)toxicological concern for aquatic toxicity | A. Kienzler | 101/2302 2nd floor |
| Biokinetic modelling to relate internal and external exposure | N. Kramer | 100/1102 Terra 1st floor |
| Computational modelling in heart disease and therapies | B. Rodriguez | 100/ 2102 Aria 1st floor |

# World Café
## 13:30 – 15:30 Thursday, 23 May 2019

| | *Table host* | *Bldg/Room* |
|---|---|---|
| **From methods to integrated solutions** | | |
| Biomarkers and mode of action in pharma development | I. Cotgreave | 58/11 |
| High throughput methodologies at EURL ECVAM | J. Sund, T. Palosaari & D. Carpi | 58/12a+b |
| Adverse Outcome Pathway (AOP) - a basis for integrated approaches | E. Berggren & B. Landesmann | 101/1003 1st floor |
| The regulatory uptake of combined methods for skin sensitisation | S. Casati & D. Asturiol | 101/1302 1st floor |
| **Alternative methods and future challenges** | | |
| Genomics data for public health and possible applications on chemicals safety | M. Bale | 101/2002 2nd floor |
| Alternatives to understand respiratory tract disease | L. Gribaldo | 101/2302 2nd floor |
| Endocrine disruptors - impetus for change | S. Munn & E. Grignard | 100/1102 Terra 1st floor |
| Convergence between disease/toxicity and human/environment | F. Madia & S. Bopp | 100/2102 Aria 1st floor |

# Meet The Students: Flash Presentations
## Wednesday, 22 May 2019

Session I: 15:00 – 15:45

| # | Name | Presentation title |
|---|---|---|
| 1 | **Marialuisa BALDI** | Systematic assessment of air pollution induced pulmonary toxicity and new treatment strategies for chronic obstructive pulmonary disease (COPD) and chronic bronchitis using multi-cellular lung mucosa models |
| 2 | **João André ALVES BARBOSA** | The effect of emerging pollutants in the North Sea on fish growth: an *in silico-in vitro* approach |
| 3 | **Alison BAXLEY** | Developmental exposure to certain endocrine disruptors and Attention Deficit Hyperactivity Disorder (ADHD) – a structured literature review |
| 4 | **Danielle BRAIN** | Development of *in vitro* assays for the assessment of long-acting HIV therapy |
| 5 | **Katrin BRANDMAIR** | Successful technology transfer of a microphysiological system combining skin and liver models for extended and repeated exposure of chemicals |
| 6 | **Sophie CABLE** | Coumarin: A Next Generation Risk Assessment Case Study |
| 7 | **Edoardo CARNESECCHI** | Innovative open source QSAR models for human and ecological risk assessment of emerging contaminants and their mixtures |
| 8 | **Alexandra DAMERAU** | The *in vitro* multi-component 3D arthritic joint model |
| 9 | **Francesca FAGIANI** | CHD8 knockout BrainSpheres and chlorpyrifos to study gene environmental interactions in autism |
| 10 | **Irini FURXHI** | Application of Bayesian Networks in Determining Nanoparticle Induced Cellular Outcomes Using Transcriptomics |

# Meet The Students: Flash Presentations
## Wednesday, 22 May 2019

Session II: 17:00 – 17:45

| # | Name | Presentation title |
|---|---|---|
| 11 | **Valentina GARRAPA** | Animal-free monoclonal-like antibodies from a synthetic ribosome display selectable nanobody library |
| 12 | **Elizabeth GOYA JORGE** | Assessment of endocrine disrupting potential of chemical compounds via aryl hydrocarbon receptor (AhR) antagonism |
| 13 | **Emma GUSTAFSON** | Development and testing of a repeated dose toxicity ontology model for chemical risk assessment purposes: liver effects as a case study |
| 14 | **Jonathan JOSEPHS-SPAULDING** | Systems Medicine Approach to Deciphering Recurrent Urinary Tract Infections (rUTIs) |
| 15 | **Nika KHOSHAEIN** | Performance Verification and Application of a Cancer population for use in Physiologically Based Pharmacokinetic modelling |
| 16 | **Serhii KOLESNYK** | Prioritization of combined plant protection products for further risk assessment of endocrine disrupting properties using few *in silico* models |
| 17 | **Janine MCCARTY** | Increasing the Availability and Quality of Human Tissue in Science |
| 18 | **Thi Phuong TAO** | Microfluidic 4-Organ-on-a-chip for ADMET profiling |
| 19 | **Katarina ŽIVANČEVIĆ** | The influence of phthalates (diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP)) exposure on diabetes mellitus type 2 development – analysis of toxicogenomic data |

# Meet the Students: Poster Abstracts

**Poster 001**

**Ashraf Abdelkhaliq**

**Impact of *in vitro* digestion on the physicochemical properties of silver nanoparticles and their transport through the intestinal barrier**

Ashraf Abdelkhaliq[1,2], Meike van der Zande[1], Anna Undas[1], Ivonne, M.C.M. Rietjens[2], and Hans Bouwmeester[2]

[1]RIKILT - Wageningen Research - Wageningen; [2]Division of Toxicology, Wageningen University - Wageningen, The Netherlands.

Oral ingestion is an important exposure route for humans to silver nanoparticles (AgNPs) and ions. The study aims to investigate the effects of *in vitro* digestion (IVD) on size and dissolution of AgNPs with different surface chemistries, and on their cellular uptake/adhesion and transport. Caco-2/HT29-MTX cells, co-cultured on a transwell model were exposed to pristine or IVD 50nm citrate (-Cit) and/or lipoic acid (-LA) functionalized AgNPs, and silver nitrate ($AgNO_3$). AgNPs and $AgNO_3$ were characterized and quantified using inductively coupled plasma mass spectrometry (ICPMS) and single-particle-ICPMS before and after digestion. After digestion, the nominal size of (-LA) AgNPs was reduced, while no significant difference on the size distribution of (-Cit) ANPs. Number of particles detected in the cell fraction after exposure to IVD AgNPs was ~100 folds higher than the pristine ones. Silver detected in particulate form in the cell fraction of the IVD $AgNO_3$ exposure, suggesting -de novo-formation of particles. Cellular fraction of silver occurred mainly as particulates in the IVD AgNP groups, while about 60% was as particulates after exposure to pristine AgNPs. Cellular fraction of IVD (-LA) AgNPs was ~40% of the total mass balance, which was ~4 times higher than (-Cit) AgNPs. While ~15% as particulates in the cellular fraction for both pristine AgNPs. Transport of IVD and pristine AgNPs and $AgNO_3$ was lower than 5%. The IVD increased the uptake/adhesion of AgNPs, while no significant effect on transport. The surface chemistry of AgNPs plays a role in the stability/dissolution and the biological availability of AgNPs.

Keywords: Silver nanoparticles, *in vitro* digestion, single particle – ICPMS, dissolution, cellular transport, uptake and adhesion.

**Poster 002**

**Manon Bouwmeester**

**Biofabrication of human liver constructs**

Manon Bouwmeester[1], Maj-Britt Buchholz[1], Kerstin Schneeberger[1], Nynke Kramer[2], Bart Spee[1]

[1]Department of Companion Animals, Faculty of Veterinary Medicine, Utrecht University; [2]Institute for Risk Assessment Sciences, Utrecht University, The Netherlands.

Interindividual variation in *in vitro* models is difficult to model in single-donor derived tumorgenic cell lines. Liver organoids derived from adult stem cells represent a novel donor-specific model. Liver organoids are grown as hollow spheroid-like structures, able to differentiate towards cholangiocyte-like cells and hepatocyte-like cells. Although, current hepatic differentiation state expresses hepatic markers as well as metabolizing enzymes, expression levels are not yet at the level of cryopreserved primary human hepatocytes yet. By combining the organoid technology with tissue engineering, the complexity of the *in vitro* system can be increased. Improvement of the 3D microenvironment *in vitro* will probably

increase the hepatic differentiation state of the organoids. Here, we focus on bioprinting perfusable liver constructs containing human liver organoids. After bioprinting, organoids remain viable for at least eight days in a printed construct and retain hepatic characteristics. Co-culture with liver mesenchymal stem cells (LMSCs) or perfusion of the printed constructs can further improve complexity. Improved hepatic differentiation of adult stem cells derived liver organoids can improve the predictability of liver organoids as a toxicological model.
Keywords: Biofabrication, organoids, *in vitro*, hepatotoxicity, microfluidics.

**Poster 003**

**Maria Chiara Astuto**

**Safety assessment of vegetal mixtures using alternative methods**

Maria Chiara Astuto
Università degli Studi di Milano.

Recently, the increased interest in environmental sustainability, encouraged companies to introduce on the market 'biological pesticides' which also represents a profitable opportunity in organic farming. Biopesticides are those types of Plant Protection Product (PPP) derived from natural materials such asmicroorganisms and plants, the latter also known as botanicals. The tendency nowadays is to progressively replace synthetic or chemically derived PPP in favour of bio-pesticides, which are generallyconsidered of less toxicological concern due to their high selectivity and degradability. However, since natural does not necessary mean safe, an analysis of the current methods for the safety evaluation of vegetal mixtures is necessary, focusing on the need to implement and promote alternative methods. As a matter of fact, the risk assessment for products traded in the European market depends on their intended use and except for cosmetics and chemicals under the REACH regulation, animals are already widely overadopted. Overlooking the ethical perspective, relying completely on animals could lead, as reported in literature, to an over/under-estimation of risks due to the low biological relevance of the rat, which is the preferred species in the toxicological studies. Furthermore, vegetal mixtures present the issue of combined toxicity. *In vitro* methods could ensure a refinement of the old methods providing also precious information regarding the Mode of Action (MoA) of these promising substances.
Keywords: BioPesticides, Alternative Methods, Sustainable Agriculture, Risk Assessment.

**Poster 005**

**Moosa Awadha**

**Effects of lipid oxidation products on chronic inflammation in *ex vivo* systems and their inhibition**

Moosa Awadha, Johan Frostegard, Mizanur Rahman
Karolinska Institutet and IMM department of Inflammation and Chronic Disease, Sweden.

After recent studies conducted by Professor Johan's lab, new information regarding the action of oxidation in atherosclerotic plaques have been identified. Chronic inflammatory conditions including cardiovascular disease (CVD) and rheumatic disease represent major health problems. Lipid peroxidation and their products are implicated in these disease conditions. One example is Oxidized low density lipoprotein (OxLDL) which is of importance in atherosclerosis (and CVD) where it is abundant in the atherosclerotic plaques, through its toxic and inflammatory properties. During LDL-oxidation, different toxic and inflammatory compounds are generated, including protein (apoB)- and lipid derived ones. JF group has focused on two such epitopes, MDA and

phosphoryl choline (PC). An antibody response to PC and MDA (anti-PC and anti-MDA) is abundant in humans, and we have proposed that these antibodies can have atheroprotective properties and also may have such in rheumatic disease as SLE and RA. OxLDL and lipid peroxidation are implicated as an environmental factor with toxicologically relevant properties, being increased by smoking and in air pollution. In addition to anti-PC and anti-MDA, we have also identified another potential inhibitor, namely Annexin A5, which can also be used in experiments.
Keywords: Atherosclerosis, Plaques, Oxidation, Epitopes, MDA, PC, CVD, OxLDL, Antibody.

**Poster 006**

**Marialuisa Baldi**

**Systematic assessment of air pollution induced pulmonary toxicity and new treatment strategies for chronic obstructive pulmonary disease (COPD) and chronic bronchitis using multi-cellular lung mucosa models**

Marialuisa Baldi, Tania A. Thimraj, Lena Ernstgård, Gunnar Johanson, Lena Palmberg, Koustav Ganguly, Swapna Upadhyay
Karolinska Institutet, Stockholm, Sweden.

The adverse health effects related to the exposure to particulate matter (PM) in combination with gaseous pollutant such as nitrogen dioxide (NO2) and sulphur dioxide (SO2) are of increasing concern. Chronic obstructive pulmonary disease (COPD) is a global health problem, and it is the third leading cause of death. Chronic bronchitis (CB) is a separate disease entity but co-variates with COPD. It is therefore of utmost importance to explore the pathophysiological mechanisms behind COPD and CB. Particulate matter such as diesel particles are produced by vehicles emissions in the atmosphere leading to human exposure through inhalation. Depending on the size, PMs can reach different part of the lung: extra thoracic and upper respiratory tract (PM10) or deeper lung part – alveolar (PM2.5, PM0.1) triggering the inflammatory process that can be translated in lung toxicity outcomes such as COPD and CB. Hence, the main object of this study is to mimic and assess the potential pulmonary toxicity effects following repeated exposure to air pollutants (both particles and gases: diesel exhaust particles in combination with NO2 and SO2) using physiologically relevant multi-cellular bronchial mucosa model with primary bronchial epithelial cells (PBEC) as well as an alveolar mucosa model with NCI-H441 (alveolar type II cell line) cells co-cultured with HULEC-5a. The study will also focus on the evaluation of possible new treatment strategies for COPD and CB through *in vitro* studies.
Keywords: Lung, COPD, air pollutant, biofuels, diesel, ALI, 3Rs.

**Poster 007**

**Joao Andre Alves Barbosa***

**The effect of emerging pollutants in the North Sea on fish growth: an *in silico-in vitro* approach**

João Barbosa, Jana Asselman, Colin Janssen, Karel De Schamphelaere
Laboratory for Environmental Toxicology and Aquatic Ecology (GhEnToxLab), Ghent University.

Environmental risk assessment is of extreme importance to assure a safe, balanced and sustainable use of chemicals, while playing a key role in environmental regulation. However, it currently relies on ethically controversial, expensive and time-costly methods and experiments (e.g. animal testing). Moreover, quantification of chemical toxicity is commonly based on external concentrations in water, soil or air, even though it is the internal concentration in the

organism that gives raise to the biological effect. Recently, a combination of *in vitro* and *in silico* has been put forward as a viable alternative to the conventional *in vivo* testing. In this study, we have applied this concept to emerging pollutants detected in the North Sea, particularly pharmaceuticals and pesticides. We assessed the effect of these chemicals on growth impairment in fish through *in silico* methods, using predicted internal concentrations of these chemicals, based on environmental concentrations. In particular, we exposed gill cells of rainbow trout (Oncorhynchus mykiss), RTgill-W1 cell line, to the predicted internal concentrations of each chemical and then used the *in vitro* cell growth to predict *in vivo* growth. It is our believe that the adopted methodology allows the tackling of the previously described issues related to chemical risk assessment and encourages a shift of the current paradigm.
*travel grant recipient
Keywords: *In vitro*, *in silico*, RTgill-W1, growth impairment, pharmaceuticals, pesticides, North Sea.

**Poster 008**

**Magdalena Baricicova**

**Gold nanoparticle and macrophage interaction: dissolution and immune-modulation**

Magdalena Baricicova, Hanna Karlsson, Sarah Mc Carrick, Klara Midander
Unit of Biochemical Toxicology at IMM, Karolinska Institutet, Stockholm, Sweden.

Gold nanoparticles (AuNPs) have a wide range of applications in industry and medicine due to their physical and chemical properties. In a previous study an *in vitro* assay was developed in order to investigate the effect of dissolution of AuNPs in macrophages, where the AuNPs to high extent reside. The dissolution of 5 and 50 nm AuNPs coated with citrate was compared when in cell medium alone, in medium with macrophages and in medium with activated macrophages by lipopolysaccharide (LPS). The released gold was measured by the inductive coupled mass spectrometry (ICP-MS). In the assay murine macrophages (RAW 267.4 cells) were used, however due to their high replication potential the assessment of AuNP dissolution became more complex. For this project a different cell-line with slower replication cycle was selected, namely human monocytes (THP-1) differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA). Different sizes (5, 20 nm) and different coatings (citrate, PEG) of AuNPs were selected in order to compare the effect on the macrophages. In previous studies AuNPs have shown to immune-modulate the macrophage inflammatory response, however the mechanism behind this remains to high extent unclear. The aim of this project is to study the interaction of AuNPs with macrophages with a focus on the dissolution and immune-modulation.
Keywords: Dissolution, Nanoparticles, Macrophages, Inflammation, Gold.

**Poster 009**

**Alison Baxley**

**Developmental exposure to certain endocrine disruptors and Attention Deficit Hyperactivity Disorder (ADHD) – a structured literature review**

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Attention Deficit Hyperactivity Disorder (ADHD) is a debilitating and costly disease that is thought to affect between 3% and 12% of the world's children and 2.5% to 5% of adults. It has been suggested that developmental exposure to chemicals, such as some endocrine disruptors

(ED) commonly found in the environment can contribute to the risk of neurodevelopmental disorders, including ADHD. The purpose of this project is to systematically compile existing epidemiological and toxicological data in order to investigate the link between exposure to a few selected EDs (including plasticizers, surfactants, and flame retardants), during early development and ADHD in humans as well as to identify knowledge gaps. The aim is also to postulate a possible adverse outcome pathway (AOP) connecting endocrine activity to ADHD.
We will use systematic review methodology to create a transparent and reproducible search strategy to identify relevant epidemiology and toxicology literature. The identified studies will be organized into lines of evidence and the confidence (e.g. quality, consistency) in the evidence will be evaluated using methods such as the SciRAP tool and other systematic approaches developed in the field of environmental health. Data including population, animal model, exposure levels, effect measures and author's conclusions will be extracted and structured into a table format. The data will be used to draw conclusions about the link between exposure and the risk of ADHD, as well as for postulating an AOP.
Keywords: Attention Deficit Hyperactivity Disorder, Endocrine Disrupting Chemicals, Systematic review.

**Poster 010**

**Maila Becconi**

**Chemical control of cancer cell culture**

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The microenvironment in which cancer cells proliferate is a complex and heterogeneous system. Since Paget's seed and soil theory [A], tumor and its surrounding (tumor microenvironment) became object of lots of studies in order to understand completely this pathology and defeat it. Knowing in depth critical cancer processes e.g. development, angiogenesis, progression, resistance, relapse, would mean to impact clinical studies, clinical practice, drug discovery and drug delivery processes. Oxygen concentration held a pivotal role in malignant processes [B], is known that cancer cells proliferate in hypoxia and acidity conditions. The control of the chemical concentration of oxygen is the first step to imitate the real pathologic condition of the cancer tissue. We developed and patented a device that permits the chemical control of the culturing conditions. Scanning electrochemical microscopy (SECM) is used for the chemical characterization of the device, different cell lines were also tested. In accordance with the 3Rs rules, this device will open the door to the reduction and the replacement of animal model in both basic research and drug development fields. [A] Paget S., Distribution of secondary growths in cancer of the breast, Lancet 133,571-573 (1889). [B] B. Muz, P. Puente, F. Azab, and A. Azab, The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy, Hypoxia (Auckl), Vol. 3, 83–92, (2015).
Keywords: cellular microenvironment, animal alternatives, 3Rs rules.

**Poster 011**

**Sofia Beghi**

**Polymorphism rs7214723 in CAMKK1 and myocardial infarction risk**

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Myocardial infarction (MI) is the leading cause of death in industrialized countries. Several risk factors, like unhealthy lifestyle (tobacco usage, airpollution, unhealthy diet, harmful use of alcohol and physical inactivity), play an important role but it is increasingly suggestive to believe that the genetic factors and the molecular basis of excitation-contraction mechanisms contribute in modifying an individual's risk. It is assumed that among all the proteins involved in calcium signaling in the heart and the excitationcontraction coupling, calmodulin could be an important regulator because modulated Ca2+ channels. Since several works show how some polymorphic variants can be considered predisposing factors to complex pathologies, we hypothesize that the identification of some polymorphic variants of proteins involved in the calmodulin pathway, could be important to understand if the individual genetic background has a key role in the onset of myocardial infarction. The identification of the association of some polymorphisms to atherosclerotic pathology could be relevant both clinical, for the severity of the coronary pathology, and epidemiological, for the diffusion of the pathology.2 We decided first to focus on CAMKK1, Ca2+/calmodulin-dependent protein kinase kinase I, because recently it has been observed that CAMKKI, as part of the use of MSC cells, improves cardiac function after MI. We analyzed the polymorphism rs7214723 and we conducted a case-control study involving 300 patients.

Keywords: Myocardial infarction, prevention, polymorphism analysis.

**Poster 012**

**Omar Ben Mariem**

**Prospective of Quantum Computing in Animal Models Simulations**

Omar Ben Mariem
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Nowadays *in silico* simulations are widely used to study drugs and molecules, but in order to effectively analyse more complex systems, such as pharmacological interactions, cells or whole organisms, we are limited by the physical restrictions of classical computing, in which processing can make the most of only ones and zeros to solve the problem at hand. Furthermore, the smaller you go, the more relevant the effects of quantum phenomena become: they can be ignored while studying whole organisms' processes, but they can't be ignored while studying enzymatic interactions. An emerging technology that can help with the two issues presented is quantum computing, which may allow to study a far greater amount of data and can take into consideration the effects of quantum physics in molecules thanks to the quantum phenomena of entanglement and superposition used by these processors. Quantum superposition allows the "qubits" to work not only with ones and zeros, but with all states in between, so these computers, if presented with an apt algorithm, they can allow to compute vastly more complex problems than classical computers and faster by different orders of magnitude. The use of quantum computers to study biological or chemical systems may prove

limited in the first years, but as the number of qubits increases and quantum correction becomes more efficient, we can expect to build animal and molecular models that may one day completely substitute the living ones we use today, or at least decrease their number, for toxicological or biological studies.

Keywords: Animal Models, Quantum Computing, Quantum Processors, *In silico* Simulations.

**Poster 013**

**Rohit Bhatia***

**Latest advancements of *in vitro* evaluation of Pesticide Toxicology**

Rohit Bhatia

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Pesticides are used to protect crops from weeds, fungi and a variety of insect pests, resulting in increased agricultural output. However, exposure to pesticides may lead to acute and chronic health effects on non-target organisms, including bees, fish, and humans. Therefore, regulatory authorities around the world require that the toxicity and persistence of pesticides be assessed, which has historically involved tests on animals. These tests are expensive, time consuming and pose significant animal welfare and ethical concerns due to the pain and suffering experienced by the animals. Furthermore, data generated from tests on animals are limited when extrapolated to humans due to differences in anatomy, biochemistry and physiology. A number of validated animal-free methods are now available that avoid the drawbacks associated with testing on animals to predict the toxic effects of pesticides. Such methods are increasingly accepted by regulatory authorities, either alone or in a weight of evidence, and include read-across and in-silico modelling approaches, as well as in-chemico and in-vitro assays. For example, methods using reconstructed human epidermis can be used instead of Draize skin tests to predict the skin corrosion potential of pesticides. In addition to these, many more animal-free approaches are being developed to assess various toxicological effects including immunotoxicity, endocrine disruption, and ecotoxicity. In this presentation, I will provide an overview of recent developments in animal-free toxicological methods and discuss the degrees to which various pesticide regulatory agencies have accepted them with special emphasis on adoption by India regulatory agencies.

*travel grant recipient

Keywords: Pesticides, Read-across, In-silico, In-vitro assays, India.

**Poster 014**

**Antonella Bordin**

**Human Platelet Lysate-based preclinical preparations: a biological tool for boosting angiogenesis during cardiovascular insult**

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Cell free-based strategies represent a powerful strategy to enhance neoangiogenesis after vascular insult. Yet, standardized clinical formulations of soluble mediators suitable for vascular repair are still to be verified. Specifically, human platelets-derived preparations (Platelet lysate, PL) represent a promising relevant source to boost neonagiogenesis and vascular tissue

regeneration. Our patented PL (Mesengen®, No.WO2013042095), based on standardized concentration of soluble mediators with decreased immunogenic/inflammatory potential, enhances *in vitro* stromal clonogenic potential, migration, proliferation, cardiovascular-like commitment, nitric oxide bioavailability and restores oxidative states levels. Here, we conceive Mesengen® as a novel preclinical formulation to enpower endothelial regeneration in vascular diseases, hypothesizing a biological mechanism mediated by extracellular vesicles (EV) acting as modulators of homeostasis of inflammation and angiogenesis. Our preliminary results highlight that PL whithout intrinsic pro-aggregant activity, contains EV (~$8.5x10^9$/ml) of three main set sizes (>800, 450-800, <450nm). The selected filtration (0.8, 0.45, 0.22um) of PL is responsible of the migratory and angiogenic capacity but the smallest fraction in HUVEC. Hypoxia reverts this phenomenon and maximizes the pro-angiogenic effect. We also found that Mesengen® is enriched of miR126, known as angiomiRNA. After identifying the miRnome profile expression of PL, we will develop a 3D-bioprinted (bioink of alginate and polyethylene glycol monoacrylate-fibrinogen) model of functional vessels upon hypoxia, by employing HUVEC stimulated with PL, PL-derived EV or definite cardiovascular miRNAs. Newly formed vessels will be tested for angiogenic gene expression, spatial geometry and secretome. This study supports the proficiency of PL in fostering angiogenic network of endothelial cells after vascular damage.
Keywords: Platelet Lysate, cardovascular diseases, angiogenesis, extracellular vesicles, endothelial cells.

**Poster 015**

**Giulio Bracalente***

**Risks and implications for health and the environment associated with products and waste containing nanomaterials: regulatory and management issues in the European framework**

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Nanomaterials can revolutionize everyday products, but there are still many unanswered questions about the risks they may pose to human health and the environment. Inconsistent definitions and metrological issues are still of concern for manufacturers, importers and distributors who are demanded to comply with strict regulations. It is also likely that the increasing number of nanomaterial-containing products available on the market will vary the chemical and physical properties of the waste produced, which is currently treated in traditional plants without any particular differentiation. Treatment efficiency for nanomaterials-containing waste should then be addressed and the risks of uncontrolled emissions considered. Finally, the risks associated with the use of nanomaterials-containing products may not be sufficiently characterized as current exposure estimation models are not designed for estimating exposure to nanomaterials and they are likely to be affected by large uncertainties. Hence, it would be important for national and inter-national institutions to provide, as soon as possible, harmonized regulations covering all aspects of the life cycle of products and waste containing nanomaterials. This work is proposed as a starting point for reflection on the main regulatory

and management issues associated with products and waste containing nanomaterials, focusing mainly on the European framework.

*travel grant recipient

Keywords: nanomaterials; nanomaterials-containing products; waste management; nanomaterials-containing waste; risk assessment.

**Poster 016**

**Danielle Brian**

**Development of *in vitro* assays for the assessment of long-acting HIV therapy**

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Nanomedicines are renowned for activating the body's immune system and therefore there must be a way of screening potential nanomedicines to determine if they will be safe to use in humans. There is currently a battery of tests considered to be the standard for testing whether nanomedicines will cause an immune response. Although new formulations of nanomedicine drugs provide an additional challenge. Previously these formulations have not been extensively used and therefore the current assays must be adapted to better predict the body's immune response to these drugs. Adherence is a huge issue in the field of HIV therapy and in recent years the development of long-acting drug formulations has been a key aim in order to improve this. The future of these assays as they improve and can better reflect *in vivo* responses will provide alternatives to limit the use of animals in biocompatibility and toxicity testing. During my PhD I will develop assays to look at the response of the body's immune system to long-acting formulations of HIV drugs, predominantly Nucleoside Reverse Transcriptase Inhibitors (NRTIs). I will then use these assays to assess compounds made in the group. My recent work has been looking at the immune response of NRTIs and their role in activating the inflammasome. I have also begun to examine the role of exosomes in culture media on *in vitro* assay cell responses and the results will help to develop *in vitro* assays to ensure they reflect human response as closely as possible.

Keywords: Nanomedicine, *in vitro*, immune system and HIV.

**Poster 017**

**Brandmair Kathrin**

**Successful technology transfer of a microphysiological system combining skin and liver models for extended and repeated exposure of chemicals**

Brandmair K[1], Maschmeyer I[2], Tao TP[2], Gerlach S[1], Müller-Vieira U[3], Duplan H[4], Eilstein J[5], Genies C[4], Grégoire S[5], Jacques-Jamin C[4], Klaric M[6], Lange D[1]; Salhi S[7], Marx U[2], Schepky A[1], Hewitt N[6] and Kühnl J[1]

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The lack of *in vitro* methods for systemic toxicity testing is a major obstacle for the ab initio qualification of unknown compounds. Combinations of 3D tissue models in microphysiological systems are promising approaches to provide relevant data for risk assessment. Within a Cosmetics Europe-driven project, Beiersdorf and TissUse evaluated the transferability and robustness of TissUse´s Multi-Organ-Chip (MOC) technology, combining liver organoids and reconstructed human epidermis (RhE) models. In both labs, the bioavailability and metabolism

of two test compounds (hyperforin and permethrin) were analysed in either single or repeated dosing scenarios via the topical or systemic route for five days. For both case study chemicals, the results showed excellent intra- & inter-laboratory reproducibility. Our results show that 1) viability and metabolic capacity of both organ models is maintained over five days; 2) different application routes and frequency affect the systemic concentrations of both parent and metabolites in the chip throughout the experiment; 3) application route and frequency affects compound-specific gene induction. In summary, highly reproducible data of two labs indicated good transferability of the MOC technology and its capacity to provide information about the effects of different exposure scenarios on parent and metabolite disposition that may be relevant to risk assessment.

Keywords: Microphysiological system, Organ-on-chip, MOC, 3D cell models, metabolism, liver, skin.

**Poster 018**

**Gerrit Bredeck**

**Mucin profile and inflammatory feedback in nanoparticle-exposed human goblet-like cells and mice – an *in vitro*/*in vivo* comparison**

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Humans can be exposed orally to considerable amounts of nanoparticles that are contained in consumer products including foods and food contact materials. Thus, it is likely that nanoparticles reach the intestine and encounter the protective mucus layer - a network of highly glycosylated proteins called mucins - that shields the epithelium from harmful substances. The main objectives of our investigations were to study the impacts of nanoparticles on mucus-secreting epithelial cells, the mucus composition and integrity as well as to analyse their inflammatory potential. The cytotoxicity of Ag-PVP, $TiO_2$, $CeO_2$ and $SiO_2$ nanoparticles (NPs) on the mucus-secreting goblet-like cell line HT29-MTX-E12 (E12) was assessed by WST-1 assay. The gene expression of several mucins and IL-8 were evaluated by qRT-PCR. Using Alcian Blue, the presence of mucus was examined. In contrast to confluent E12 cells, pre-confluent cells secreted negligible amounts of mucus. Despite this fact, none of the investigated materials caused cytotoxic effects in pre-confluent cells at concentrations up to 80 µg/cm². Initial results showed that the transcription level of all investigated mucins was substantially increased in confluent cells. The level of mucin expression and secretion as well as the profile of E12 cells strongly depended on the cells' confluence. Following the thorough establishment and optimisation of the method including primer selection, the potential influence of nanoparticles on mucin expression and inflammatory markers will be investigated in pre-confluent and confluent E12 cells. An *in vitro*/*in vivo* comparison with material from murine feeding studies using the same nanoparticles will be performed.

Keywords: intestine, HT29-MTX-E12, nanoparticles, mucins, inflammation, feeding study.

**Poster 019**

**Marianne Gloria Brookman-Amissah**

**A toxicokinetic model for Zebrafish Embryo (ZFE): Understanding the influence of exposure systems for different chemicals**

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The Zebrafish (*Danio rerio*) embryo (ZFE) is increasingly used as alternative model for toxicity testing in higher vertebrates. This model is attractive in toxicology because of its easy detectable phenotypical changes, ex uterus development with optical transparency and potential use in high throughput screening. However, the toxicokinetic (TK) processes (absorption, biotransformation, distribution and elimination) of chemicals in the ZFE are not understood well. This is needed to develop a computational model to estimate internal concentrations of different chemicals, a much better dose metric to compare toxicity differences between chemicals, species and exposure routes. This project aims at providing some knowledge to better interpret time profiles of internal concentrations in the ZFE by developing a TK model. TK data were collected for different chemicals and exposure designs. Representative chemical classes (i.e. perfluorinated chemicals, pharmaceuticals, oxygenated PAHs and PAHs) differed in their lipophilicity and protein binding affinity that enables to develop a general TK model for ZFE by using the software Berkley Madonna. This study will aid to estimate internal concentrations of chemicals and improve the use of ZFE model as alternative to animal testing in toxicity testing and risk assessment.

Keywords: toxicokinetic model, zebrafish embryo (Danio rerio), internal concentrations, diffusion.

**Poster 020**

**Mathias Busch***

**Investigations of nanoparticle toxicity on the gastrointestinal tract using advanced *in vitro* and *in vivo* models**

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Engineered nanomaterials are widely used in consumer products that make their oral uptake highly likely, e.g. food products and toothpaste. To study the effects of their purposeful or incidental ingestion, toxicological testing can be performed *in vitro* using human intestinal cell lines. Here, DNA damage and cytotoxicity were investigated in basic and advanced intestinal *in vitro* models before and after artificial particle digestion. The *in vitro* results were validated against *in vivo* DNA damage in murine colonocytes from oral exposure studies. PVP-capped silver (Ag-PVP) and titanium dioxide (TiO2) nanoparticles (NPs) were incubated in artificial (1) gastric and (2) intestinal solution and characterised using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Monocultures and co-cultures of Caco-2 and HT29-MTX-E12 (E12) cells were exposed to undigested and digested NPs. Cytotoxicity and DNA damage were quantified via the WST-1 assays and alkaline comet assay, respectively. For the *in vivo*/*in vitro* comparison, DNA damage was assessed in freshly isolated colon epithelial cells from NP-exposed C57BL/6J mice (28-day feeding study). The simulated digestion caused formation of large particle agglomerates/aggregates. No differences were noted in the toxicity of pristine

and digested NPs. In both Caco-2 and E12 monocultures, Ag-PVP and $TiO_2$ NPs induced DNA damage, while no DNA-damaging effects were noted in the Caco-2/ E12 co-culture model. No DNA-damaging effects were found in murine intestinal epithelium after oral exposure to Ag-PVP and $TiO_2$ NPs. Altogether, the *in vivo* situation was better represented by a complex intestinal epithelial co-culture model, than by simple enterocyte monocultures.

*travel grant recipient

Keywords: Gastrointestinal tract, nanoparticles, DNA damage.

**Poster 021**

**Sophie Cable**

**Coumarin: A Next Generation Risk Assessment Case Study**

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Unilever.

There are very few examples of risk assessments for consumer products that rely solely on the use of new approach methodologies (NAMs). A hypothetical case study was developed to see if NAMs could be used to develop a consumer safety assessment for the use of 10% coumarin in a laundry liquid for launch in Vietnam. To explore the potential for a non-animal risk assessment, all existing animal, clinical and history of safe use data were excluded. Instead, a battery of *in silico*, in chemico and *in vitro* methods were used to calculate points of departure (PoD) for a variety of biological effects. These methods included advanced exposure calculations incorporating specific habits and practices data, non-animal sensitisation assays, a mechanistic genetic toxicology assay (ToxTracker), *in vitro* pharmacological profiling (Eurofins Safety44TM screen), BioSeek® Compound Profiling, an in-house *in vitro* Cell Stress Panel and transcriptomics (BioSpyder). A margin of exposure was calculated using the identified PoD. Key safety hazards identified were genotoxicity, skin sensitisation and off-target effects such as perturbations of biological pathways, including endocrine disruption. The risk of local effects was low and it was concluded that coumarin was not genotoxic; however the systemic effects of coumarin and its metabolites have not yet been fully elucidated. From this work, areas of uncertainty and data generation needs were identified. This case study illustrates how computational modelling, exposure science, *in vitro* models and transcriptomics can be used in safety decision making for consumer products.

**Poster 022**

**Sonia Carminati**

**Biocidal Product Family authorisation: data gap analysis approach**

Sonia Carminati

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Biocidal Products (BP) are substances or mixtures used to “control” harmful organisms by other means than just physical or mechanical action. Some examples are: antimicrobials, anti-fouling, preservatives, rodenticides, insecticides and repellents. Since they are widely used in households, hospitals, industry and institutional areas, and they might be harmful towards humans and the environment, their marketing is highly regulated (Regulation (EU)2012/528). The authorisation of a BP is a challenging procedure involving a considerable amount of time and resources. The first step to address is the definition of the test panel to which the product

must be subjected. Such panel depends on the product characteristics. For consumer products classified as irritant for the eyes it may be important to mitigate the classification calculated using CLP and to perform the tests relative to this end point. Since the BP Regulation (EU)2012/528 strongly recommends to reduce the tests on vertebrates, the tests performed with alternative methods are accepted, especially when described in an OECD Test Guideline. A number of *in vitro* tests are available to determine the severe eye irritation endpoint. The choice of the best method to get the correct classification depends upon the product characteristics. Usually a single method is not sufficient to obtain the correct classification for eye irritation and an IATA approach must be used. The present study shows a case study.

**Poster 023**

**Edoardo Carnesecchi**

**Innovative open source QSAR models for human and ecological risk assessment of emerging contaminants and their mixtures**

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Humans and ecosystems are continually exposed to very complex mixtures of chemicals, the composition of which is continuously changing. To date, current chemicals risk assessment is based on single substances. However, from a risk assessment perspective (RA), it is practically impossible to test all the possible mixtures experimentally, therefore it is needed to find smart strategies to assess the potential hazards using new strategies that incorporate alternative methods such as *in silico* tools. In 2017, EFSA published a report summarising the development of *in silico* QSAR models as tools to predict toxicity values or classify thresholds of single chemicals by using EFSA's OpenFoodTox database. Our research project aims at further developing QSAR models for hazard characterisation of binary mixtures in species of human health and of ecological relevance. Mathematical models such as Concentration Addition (CA) and Independent Action (IA) as well as Toxic Unit (TU) approach will be applied and tested for honeybee case studies. Preliminary results will be presented by applying CORAL QSAR model which will be based on the sum of toxic units (sTU) derived from single chemical. This work shows the potential use of alternative methods to animal testing such as QSAR models for the hazard assessment of chemical mixtures. In particular, when applying a tiered approach for ecological RA of multiple chemicals, QSARs models can be applied in order to a) predict (missing) information on individual compounds (tier 0) and (b) to predict directly or stepwise the combined effects and interactions of chemicals in the mixture (tier 1).

Keywords: Chemical mixtures, toxicity, risk assessment, concentration addition.

**Poster 024**

**Sarah-Sophia Carter***

**Towards the development of a microfluidic tool to assess the biological properties of biomaterials for bone regeneration**

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The longer life expectancy of the global population has increased the need of repairing bone injuries caused by fractures and local diseases. One appealing approach to restore this loss of function involves the use of biomaterials [1]. Before entering the clinic, these biomaterials need to be evaluated, which requires accurate *in vitro* models. In this work, the first steps were taken towards the development of a biomateral-on-chip system that could be used to assess the biological properties of biomaterials in a more reliable manner. Our prototype device consists of an polydimethylsiloxane (PDMS) microfluidic channel assembled on a titanium disc (i.e. Ti6Al4V). PDMS (Sylgard 184 silicone elastomer kit, Dow Corning) was prepared according to manufacturer's recommendations and cured for 24 hours at room temperature on a silicon wafer master, the latter prepared by standard lithographic processing. Afterwards, the PDMS channels (l = 3 mm, w = 2 mm, h = 100 µm) were stamped in uncured PDMS and assembled onto the titanium disc. The potential to culture cells in this device was confirmed by confining MC3T3-E1 cells (60,000 cells/cm$^2$) for a period of 6 hours. The biocompatibility of the device was confirmed by the presence of viable cells (calcein staining retained within living cells) with a similar morphology as the control, MC3T3-E1 cells seeded on titanium discs (10,000 cells/cm$^2$). This indicates no adverse effect of culturing cells in our microfluidic device and its potential for more accurate *in vitro* screening of biomaterials. [1] J. Henkel et al. Bone research 1, 216-248 (2013).

*travel grant recipient

Keywords: Organ-on-chip, microfluidics, biomaterials.

**Poster 025**

**Manuela Cassotta**

**An *in vitro* model for oxidative stress in Rett syndrome and CDKL5 encephalopathy**

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Rett syndrome is a rare X-linked progressive neurodevelopmental disease, representing the second cause of mental retardation in girls. In its typical form the disease is associated with methyl-CpG-binding protein 2 (MECP2) mutations. Rett syndrome is characterized by a period of 6-18 months of apparently normal development after which the affected girls present a developmental stagnation followed by regression in language and motor skills, loss of purposeful use of hands and develop characteristic repetitive hand stereotypies and autistic traits. The CDKL5 variant of RTT is characterized by early, intractable seizures, encephalopathy and Rett-like phenotype. It is caused by mutations in Cyclin-Dependent Kinase-Like 5 gene (CDKL5). Despite the great efforts, at present the pathogenesis of RTT remains enigmatic and there is no cure for Rett Syndrome and CDKL5-related encephalopathy. This may be largely due to the relative inaccessibility of human brain tissues, resulting in the lack of availability of human based models for research and to an over-reliance on animal models, which not always faithfully recapitulate human physiopathology. This contributes at

least in part to the high failure rate in translating preclinical studies into clinical arena. Several lines of evidence suggest a potential role of oxidative stress (OS) in the pathogenesis of Rett syndrome and therefore, OS might be considered to be a good potential target in developing new therapeutics. The present work has enabled the development of a method for a semi-quantitative automated evaluation of oxidative stress in cultured cells and the validation of a human based cellular model (based on human primary fibroblast cultures) for oxidative stress in RTT and CDKL5-encephalopathy, as proof of concept for drug discovery. Acknowledgments: O.S.A. Oltre la Sperimentazione Animale that provides travel grants for participation in JRC Summer School 2019.
Keywords: Rett Syndrome, CDKL5, oxidative stress, alternative to animal testing, *in vitro*, human cells.

**Poster 026**

**Jie-Hyun Choi**

**Development of a new cell-based assay for determination of skin sensitization potency of chemicals**

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Humans are exposed to many chemicals in their daily lives and research has shown that approximately 20% of the Europeans have acquired skin sensitization to at least one allergen. The murine local lymph node assay (LLNA) is currently the primary method to test the skin sensitizing potential and potency of chemicals. However, with the rapid growth of new chemicals and the EU ban on animal experimentation for cosmetics, there is a demand for new high throughput alternative testing methods to assess toxicological endpoints. The aim of this project is to develop an assay that can be used to determine the skin sensitization potential and potency of various chemicals. Our hypothesis is that keratinocytes secrete a specific enzyme that acts as a DAMP (danger-associated molecular patterns). In the extracellular space, this enzyme binds to receptors on dendritic cells, which then activates the adaptive immune system. In the project, we will test various chemicals with known skin sensitizing potencies (according to LLNA) and determine whether the secretion of this enzyme, or other well-known inflammatory cytokines, can be used to predict the skin sensitizing potencies of the selected chemicals.
Keywords: Skin sensitization, chemicals, alternative methods, *in vitro* assay.

**Poster 027**

**Valentina Citi**

**PIK3CA and MAPK molecular analysis in breast cancer cell lines after everolimus treatment as a possible method for reducing the animal use.**

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In cancer research, using animal xenografts is still the main method for characterizing the genetic profile that may predict resistance or sensitivity to anticancer drugs. Thus, in this field there is a strong need to develop valid, reliable and alternative methods to reduce the animal use. In this study, human breast cancer cell lines (MCF-7, T-47D, ZR-75-1, CAMA-1 and MCF-10A non-tumorigenic human epithelial cell line) were treated with everolimus (EVE) (mTOR inhibitor for the treatment of breast cancer) and the growth inhibitory effect of EVE was correlated to the genetic

profile and the phosphorylation status of mTOR, AKT and ERK1/2. EVE inhibited cancer cell growth in a concentration-dependent manner: ZR-75-1 and CAMA-1, characterize by PTEN loss, resulted to be good-responders (inhibition of cell viability of about 75%); differently, T47D and MCF-7, characterized by activating mutations in PI3KCA gene, showed less sensitivity to EVE (inhibition of cell viability of about 65%). Furthermore, EVE treatment evoked a significant increase of AKT and ERK1/2 phosphorylation in T47D, while in ZR-75-1, EVE treatment did not exert any significant changes in phosphorylation status. The analysis of the genetic profile of treated cancer cells may be viewed as an alternative strategy to the animal use to select responsive patients based on somatic mutations: indeed, BOLERO-2 showed that PIK3CA gene activating mutation (exon 9) represents a resistance biomarker for the treatment with EVE in breast cancer patients [1]. [1] N. Hortobagyi et al. J Clin Oncol. 2016; 34(5): 419–426.

Keywords: Breast cancer, everolimus, DNA analysis, reduction of animal use.

**Poster 028**

**Cristina Ana Constantinescu***

**Simulated blood flow to study interaction specificity of P-selectin targeted nanocarriers *in vitro***

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Introduction. An important feature of nanostructures is the possibility to customize them in order to target molecular structures specific for various pathological diseases. In the case of inflammation occurring during atherosclerosis, P-selectin, a cell adhesion molecule, is readily overexpressed on the activated endothelium, making it a potential target to specifically deliver drugs. Purpose. To predict the *in vivo* interaction between P-selectin targeted liposomes (Psel-lipo) carrying siRNA (Psel-lipo/siRNA lipoplexes) and activated endothelium using parallel-flow chamber to simulate blood flow. Materials and method. The culture medium containing Rhodamine-PE-labelled P-selectin targeted or non-targeted lipoplexes was recirculated over a monolayer of TNF-α activated murine EC (bEnd.3 cells) for 60 minutes at 37°C and at a shear rate of ~3.8 $s^{-1}$ (shear stress ~3.4 dynes/$cm^2$) using a perfusion pump. After 10, 30 and 60 minutes of perfusion, phase contrast and fluorescence images were acquired using a fluorescence microscope (Olympus IX81). At the end of the experiment, the cellular monolayer was rinsed with fresh medium, fixed with paraformaldehyde, counterstained with DAPI, and fluorescence micrographs using TRITC (red) and DAPI (blue) filters were captured. Results. The global association (binding and uptake) to the monolayer of activated bEnd.3 cells was higher for the P-selectin targeted lipoplexes compared with non-targeted ones, indicating the specificity of P-selectin mediated mechanism of interaction. Conclusion. P-selectin targeted lipoplexes are retained by the monolayer of activated endothelial cells, even under shear stress conditions similar to blood flow. Acknowledgements. This work was supported by the UEFISCDI, INTERA project contract no. 13PCCDI/2018.

*travel grant recipient

Keywords: lipoplexes, P-selectin, shear stress, siRNA, vascular wall.

**Poster 029**

**Lucie Čtveráčková**

**Effects of phthalates on liver homeostasis and tumor promotion**

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Esters of phthalate acid (phthalates) are used as major plasticizers, therefore humans are exposed to phthalates leaking from plastics from the environment and from cosmetics or food on a daily basis. Although phthalates are showing low acute toxicity, studies indicated other negative effects after chronic exposure even at low concentrations/doses. They can induce a variety of effects at the cellular and tissue level, including alterations of biochemical and signalling pathways by epigenetic mechanisms at non-cytotoxic doses of chemicals, which are also known to result in changes of cell physiology, gene expression and cell behaviour that contribute to adverse effects such as developmental and reproductive toxicity, inflammatory diseases or cancer. Gap junctional intercellular communication (GJIC) represents a key mechanism involved in the maintenance of tissue homeostasis and it is a central regulator of cell signalling and gene expression. Dysregulation of GJIC has been linked to various adverse health effects including tumor promotion. The effects of phthalates were investigated in a rat liver progenitor / liver epithelial cell line WB-F344 characterized by functional GJIC. Our results showed that modulation of GJIC by phthalates depends on the structure and molecular weight of the tested chemicals. The highest level of GJIC inhibition was observed after exposures to phthalic acid diesters with side chains made by 4-6 carbons. GJIC was affected even after short exposure times (<10 minutes), which implies, that apart from mechanisms of genomic signalling through nuclear receptors, the effects of phthalates can be mediated also through rapid mechanisms of non-genomic signalling.

Keywords: Phthalates, liver toxicity, oval cells, *in vitro*, GJIC.

**Poster 030**

**Alexandra Damerau**

**The *in vitro* multi-component 3D arthritic joint model**

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Although arthritis is a matter of research since more than 140 years, there is currently no valid 3D model available, which is able to mimic an inflamed joint. Thus, our ultimate goal is to develop a reliable human *in vitro* 3D joint model in order to simulate arthritis. Our *in vitro* 3D joint model consists of an osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane. Therefore, we have used human bone marrow derived mesenchymal stromal cells (hMSCs) to develop the different 3D tissue components that are characterized in detail (e.g. cell vitality, structural integrity) using histological, biochemical and molecular biological methods as well as µCT and scanning electron microscope. We assembled our developed osteogenic component by successful colonization of a mineralized bony scaffold (beta-tricalcium phosphate) with hMSCs, while the scaffold-free 3D cartilage component was produced by mechanical

stimulation. Non-animal stabilized hyaluronic acid was used to simulate the synovial fluid component. I order to model the synovial membrane, a confluent hMSC layer was formed on a polycarbonate membrane. Results from our single components confirming viability, integrity and morphology point towards an ultimately successful development of the anticipated *in vitro* 3D model. By combining the different components in a standard 96 well format, we aim to provide a high throughput system for preclinical drug testing as well as a valid *in vitro* human-based 3D disease model to study the pathogenesis of arthritis.

Keywords: *In vitro* 3D model, mesenchymal stromal cells, arthritis, bone, cartilage.

**Poster 031**

**Luisana Di Cristo**

**3D respiratory model and sub-chronic repeated exposure to aerosolized graphene oxide: preliminary considerations in precautionary contexts**

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Little knowledge is currently available on exposition limit value for humans for graphene family materials (GFMs). Also methods simulating realistic conditions for most of the exposure routes (e.g. lung, gut, liver, sewage, etc.) and the employment of realistic doses (long terms, sub-chronic) are currently missing. In the current study, we show an advanced *in vitro* tool that combines a 3D human bronchial tissue which is chronically exposed to aerosolized graphene oxide (GO) through the coupling of a nebulizer system (Vitrocell®Cloud). To allow for a sub-chronic repeated exposure of GO, low doses were selected based on exposure limit values for nanocarbon based materials currently available for humans. Different biological endpoints (cytotoxicity, barrier integrity, uptake, inflammation, oxidative stress) were assessed in relationto GO biotransformation, which, in turn, has been studied by TEM and SEM. Results showed that none of the investigated parameters was altered at cumulative doses of 10µg/cm2. Although no clear toxic effects were detected, chronic GO exposure elicited a significant autophagosomes accumulation (since ca. 2 weeks since the first exposure), a process resulting from blockade of autophagy flux, rather than induction of autophagy. This study highlights the potential toxic mechanism of sub-chronic doses of inhaled GO, indicating the importance of advanced exposure/toxicity testing methods for risk screening of NM. Importantly, the doses tested and their correlation to limit exposure values in occupational settings is expected to advance the functionality of the *in vitro* tool in precautionary context, providing information on “no adverse effect dose” and risk classification for humans.

Keywords: 3D lung model, graphene oxide, sub-chronic repeated exposure, human occupational exposure, aerosol system.

**Poster 032**

**Leticia Diez-Quijada Jiménez**

**Genotoxic and mutagenic assessment of a mixture of cyanotoxins, Cylindrospermopsin-Microcystin-LR by a battery of *in vitro* tests**

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At present, because of climate changes, an increase in the proliferation of toxic cyanotoxins is taking place. For this reason, the potential toxic effects of mixtures of cyanotoxins such as Cylindrospermopsin (CYN) and Microcystin-LR (MC-LR) are of interest due to their growing co-occurrence. Humans can be in contact with both biotoxins by different ways being the oral route the main one through contamined water and food consumption. Consequently, and following the recommendations of the European Food Safety Authority (EFSA), it is necessary to assess the toxicological profile of CYN-MC-LR mixtures. For this reason, in this work, the *in vitro* genotoxicity of CYN and MC-LR mixtures has been evaluated by the micronucleus test (MN) with presence/absence of the microsomal fraction S9 (OECD 487) in the L5178Y Tk +/- cell line, and by the standard and modified comet assay with restriction enzymes (Endonuclease III (Endo III) and Formamido pyrimidine glycosylase (Fpg)) to evaluate oxidative DNA damage in the Caco-2 cell line. Moreover, the mutagenicity of CYN-MC-LR mixtures was evaluated by The Bacterial Reverse Mutation Test (Ames test, OECD 471) in five Salmonella thyphimurium strains TA97A, TA98, TA100, TA102 and TA1535, and in mammals by the Mouse lymphoma thymidine-kinase assay (MLA, OECD 476) on L5178YTk+/− cells. Results obtained revealed that the mixtures CYN+MC-LR assayed didn´t show genotoxic and mutagenic effects in the different *in vitro* assays performed, although genotoxicity was shown in the MN test in the presence of S9 at high concentrations, possibly due to the metabolites of the cyanotoxins.

Keywords: *In vitro*, Cylindrospermopsin, Microcystin-LR, Genotoxicity, Mutagenicity.

**Poster 033**

**Maria João Bessa**

**Towards a reliable *in vitro* model to predict the toxicity of ceramic industry's engineered and airborne process-generated nanoparticles**

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In the field of the ceramic industry, it has not so far been possible to comprehensively assess the toxicity of nanoparticles used or derived from the production and manufacture processes. The use of *in vitro* toxicology assessment is an alternative approach to animal testing, which is ultimately used for decision-making on public health and the protection of the environment. This study aimed at evaluating the *in vitro* cyto- and genotoxicity of a set of engineered (ENPs; ZrO2 and Sb2O3•SnO2) and airborne nanoparticles (PGNPs; PM2.5 and UFP fractions) derived from processes used in the ceramic industry. Conventional submerged and polarized cell

cultures of A549 human alveolar epithelial cells along with 3D Human bronchial epithelium (MucilAir™) cultures at air-liquid interface (ALI) conditions were used as *in vitro* models. In submerged conditions, no cytotoxicity and a mild genotoxicity was observed after exposure to the ENPs, whereas at ALI, plasma membrane integrity and metabolic activity of A549 cells was affected, as well as cell's DNA integrity. Nonetheless, no effects on cell viability and DNA integrity were observed in 3D cultures after exposure to the aerosolized ENPs. Regarding the PGNPs, those induced cytotoxicity and DNA damage in A549 cells, particularly the UFP fraction. Moreover, a decrease in the metabolic activity of 3D cultures was evident following longer periods of exposure to PM2.5, while UFPs increased oxidative DNA damage. Thus, our data shows that different cellular models exhibit different sensitivity to inhalable nanoparticles, emphasizing the importance of these studies to fully address nanoparticles toxicity assessment.
Keywords: ceramic industry, engineered nanoparticles, airborne nanoparticles, *in vitro* toxicity, human cell models

**Poster 035**

**Malin Eriksson**

**Long-term T cell immunity against influenza A virus is primarily contained in airway-associated lymph nodes of infected mice**

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Aim: to study phenotype, function and localization of influenza virus antigen-presenting cells and virus-specific, memory T cells. Method: C57BL/6 and BALB/c mice were infected intranasally with a sublethal dose of H1N1 PR8 influenza virus followed by challenge with a lethal dose of homologous virus three months later. At 1 - 15 months post challenge the mice were euthanized followed by collection of mediastinal lymph nodes (MLN), lungs and spleens for analysis of cell proliferation, presence of viral replication and for measurement of cytokine production. For analysis of cytokine production purified T cells from MLN, lungs and spleen were co-cultured with syngeneic/autologous virus-infected, antigen-presenting cells (APCs). After 60 hours of co-culture cell-free supernatants were analyzed for cytokine content using Gyrolab Bioaffy. Results: Intranasal infection with H1N1 PR8 influenza virus resulted in infection only in airway-associated tissues. Furthermore, MLN APCs stimulated a higher proliferation and a higher production of pro-inflammatory cytokines of immune, splenic T-helper cells than APCs from lungs. It was also seen that production of IFN-γ was produced by T-helper cells in a proliferation-independent manner. Fifteen months after infection with influenza virus MLN CD4+ T cells were the most sensitive to stimulation by infected lung APCs reflecting locally residing, highly differentiated and long-lived, memory T cells. Conclusion: Airborne influenza virus only infects airway-associated tissues and generates long-term memory T cells in mediastinal lymph nodes (MLNs) due to interaction with highly efficient virus antigen-presenting dendritic cells. Thus, MLNs constitute an optimal environment for generating long-term local immunity against influenza virus infection.
Keywords: T cells, immunological memory, Gyros Bioaffy, cytokines.

**Poster 036**

**Lara Faccani**

**Dose Properties Characterization**

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Determining the parameters that influence the stability of dispersion of nanoparticle gives information for estimating effective doses in future modelling of toxicity. We compare values of z-potential and hydrodynamic diameter for freshly, aged and defrozen dispersion of eight materials. These materials belong a European Project, named PATROLS - Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment. In some cases, aged and defrozen sample showing a different behaviour for measurements of z-potential and hydrodynamic diameter. Understanding what treatment to reserve for samples during the project allows to kwon the nanoparticle characteristics. Why are the nanoparticles characteristic so important? Because the diameter and the charge affect the interaction nanoparticles-cells or nanoparticles-environment. Moreover, Patrols aims to develop *in silico* models to understand the effective dose of nanoparticles reaching the biological target and what dose could be toxic to human tissues and environment. Finally, a part of my work is addressing specifically dosimetry issues, providing inputs for the calculation of effective dose in different testing conditions.

Keywords: Characterization, Stability, Dosimetry.

**Poster 037**

**Francesca Fagiani**

**CHD8 knockout BrainSpheres and chlorpyrifos to study gene environmental interactions in autism**

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Autism spectrum disorders (ASD) is a complex developmental syndrome. Loss of function mutations of the chromodomain helicase DNA-binding protein 8 (CHD8) is one of the main genetic risk factors associated with ASD. In addition to genetic factors, a potential correlation between the exposure to environmental chemicals and the higher ASD risk was suggested. However, the specific mechanisms through which the environmental factors contribute to ASD are still unknown. The aim of our study is to investigate the synergistic effects of genetic and environmental factors in ASD, using a 3D BrainSphere model, derived from human induced pluripotent stem cells (iPSC). We analysed the sensitivity of BrainSpheres with known ASD genetic background (CRISPR-Cas9-induced heterozygous knockout of CHD8 gene) and control cultures from the same donor to environmental chemical (chlorpyrifos (CPF) and its metabolite - chlorpyrifos-oxon (CPO)). After 4 weeks of differentiation, we exposed BrainSpheres to CPF and CPO for 24 h. By performing confocal imaging, we observed an increase in reactive oxygen species production and a decrease in mitochondrial activity due to both mutation and exposure. Interestingly, we observed a significant increase in tyrosine hydroxylase expression in knockout BrainSpheres and due to exposure to CPF and CPO. To quantify dopamine and other

neurotransmitters in control and CHD8 knockout BrainSpheres upon exposure, we developed a method to detect neurotransmitters using mass spectrometry (LC-MS/MS). Finally, by performing untargeted metabolomics, we observed a strong perturbation of the metabolic network, after exposure to CPO than to CPF, with more profound effects in knockout line.
Keywords: Autism; BrainSpheres; CHD8; chlorpyrifos.

**Poster 038**

**Erika Ferrari**

**Microfluidic protein patterning methods to establish human liver *in vitro* models**

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Liver diseases represent a considerable public health burden. The development of efficient therapies is still largely based on animal models. However, there are several differences between animals and humans regarding disease pathways and drug metabolism. Therefore, there is the need of *in vitro* human-based models which enable high-throughput toxicity screening and are also predictive of human-specific clinical outcomes. Organizing cells on extracellular matrix (ECM) proteins is useful for optimizing cell-cell interactions and subsequently cell functions *in vitro*. Several techniques have been developed to micropattern ECM proteins on cell culture substrates such as microcontact printing and plasma ablation techniques. Both these techniques have been optimized on substrates of various stiffness (glass, hydrogel) with the aim of studying functionalities of primary human hepatocytes and liver sinusoidal endothelial cells by means of MPCC (micropatterned co-culture) model developed by Khetani and Bhatia. In MPCCs, hepatocytes are arranged on an ECM layer of defined circularity (domains of 500 µm in diameter and 1200 µm center-to-center spacing), surrounded by non-parenchymal cells. When hepatocytes are surrounded by 3T3 fibroblasts they produce the highest levels of albumin. Additionally, a deep characterization of the endothelial phenotype in MPCCs was assessed via immunofluorescence (CD31, SE-1) and ELISA (Factor VIII) assays. Moreover, building upon the MPCC platform, three polydimethylsiloxane (PDMS) devices for liver cells (HepG2) culture have been developed, combining micropatterning techniques and microfluidics in order to obtain high-throughput liver physiological human *in vitro* microscale models suitable for the application in the drug testing field.
Keywords: Organ-on-chip, Microfluidics, PDMS, microcontact printing, MPCC, PHH, HepG2, 3T3 fibroblasts.

**Poster 039**

**Irini Furxhi***

**Application of Bayesian Networks in Determining Nanoparticle Induced Cellular Outcomes Using Transcriptomics**

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This study bridges physicochemical properties of Nanoparticles (NPs), exposure conditions and *in vitro* characteristics with biological effects of NPs on a cellular level. The data is derived from transcriptomics studies and the bridging is achieved by implementing Bayesian

Networks (BNs) classifiers developed ad hoc. The BN structures are derived either automatically or methodologically and the data are classified either raw or pre-processed. The pre-processed data-driven BN has better performance compared to automatically structured BN and the raw data-driven BN. The pre-structured BN captures inter relationships between NPs properties, exposure conditions and *in vitro* characteristics and links those with cellular effects based on statistic correlation findings of the dataset. Exposure dose, NP and cell line variables are the most influential attributes. The BN methodology proposed in this study successfully predicts a number of cellular disrupted biological processes such as cell cycle and proliferation responses, cell adhesion and extracellular matrix, DNA damage and repair mechanisms etc., with a success rate >80%. The model validation from independent data shows a robust and promising methodology for incorporating transcriptomics outcomes in a hazard and, extendedly, risk assessment modelling framework by predicting affected cellular functions from experimental conditions.
*travel grant recipient
Keywords: Bayesian networks; machine learning; *in vitro*; transcriptomics; nanoparticles; information gain.

**Poster 040**

**Tianyi Li**

**The Role of CYP1/AHR Regulation in Steroid Hormone Signalling in Ovary**

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The aryl hydrocarbon receptor (AHR) is a transcription factor that mediates 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) –induced toxicity. Although the physiological functions of AHR signalling wtill remains elusive, it has been shown to regulate steroid hormone production in ovary. On the other hand, cytochrome P4501 (CYP1) enzymes are important regulators of AHR activation as they metabolize AHR ligands. Thereby, CYP1 enzymes and AHR form a feedback regulation loop which plays critical roles in maintaining the physiological functions of AHR. Aim: This study aims to determine the role of CYP1/AHR feedback regulation in ovary and analyse the effects of a compromised feedback loop in both mRNA and protein levels. Methods: Ovaries from C57BL/6 wild type (WT), AHR-knockout (AHR-KO), CYP1 triple-knockout (CYP1-KO, CYP1A1/1A2/1B1-/-) mice and mice with constitutive expression of CYP1A1 (CYP1A1-CA) were collected and stored at -80°C until further analysis. Total RNA was isolated from respective ovarian tissue and RNA integrity was determined using spectrophotometer and electrophoresis analysis and gene expression was analyzed by RNA sequencing. Subsequently, ovary-related genes significantly altered in the ovaries of genetic models compared to WT were identified with Ingenuity Pathway Analysis software. Expression of the identified gene-set was thereafter by RT-qPCR. UHPLC-MS/MS was used to analyse the functional changes of these genes.
Keywords: AHR; CYP1; Steroid Hormone; Ovary.

**Poster 041**

**Sergi Gomez Ganau**

**A biocide-like QSAR model for the prediction of acute aquatic toxicity in the algae *P. subcapitata***

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The evaluation of the ecotoxicological profile of chemicals and their harmful effects is highly relevant when a substance can impact the environment. Due to the high number of animal tests conducted each year and the ethical considerations that this entails, the requirement of alternative methods is growing and being more accepted. Within them, *in silico* tools stand out and could be applied as an alternative approach to test data. Here, a completely new quantitative structure-activity relationship model (QSAR) for biocides towards algae has been developed in the frame of the COMBASE project: http://www.life-combase.com, https://protoqsar.com/en/combase-project/. Firstly, a biocide-like chemical space was developed comparing 6512 chemical structures from Physprop database and 257 chemical structures of biocides from COMBASE database. Six molecular descriptors were selected as filters and were applied to the Japanese Ministry of Environment dataset for acute aquatic toxicity in algae (650 compounds). A dataset of 361 biocide-like structures was obtained. Secondly, an integrated model was arranged cascading a qualitative and a quantitative QSAR model. An automated neural networks (ANN) analysis was performed and the statistics for the training (254), validation (53) and external validation set (54) reached an accuracy of ≈ 80%. After, a Support Vector Machine (SVM) was performed. The statistics for the training set (253) and validation set (108) reached a $r^2$ of ≈ 75%. Thus, these models represent a completely new approach for the toxicity estimation in algae for biocides with great advantages due to their good statistics performance and the well-defined applicability domain.

Keywords: QSAR, *in silico*, alternative methods, biocides, algae.

**Poster 042**

**Michela Licciardello**

**A model of healthy and cancer lung on a chip microfluidic device**

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The lung cancer, also known as lung carcinoma, is a malignant lung tumour characterized by uncontrolled cell growth in lung tissue [1]. The study of new therapies to treat lung cancer requires experimental models that reproduce the behaviour of healthy and pathological tissues. In literature several examples of microfluidic lung-on-a-chip, devices were reported reproducing the multicellular architectures, environments and vascular perfusion of the body [2], [3]. However, reported devices did not mimic the extracellular environment due to the use of non-biomimetic materials (Polydimethylsiloxane microporous membranes). Here, we describe the implementation of a novel technological biomimetic platform to mimic both the multicellular composition of the lung and its vascular network as well as the composition and structure of extracellular matrixIn particular, our first aim is to design and create new structures composed by biomaterials that mimic the alveolar wall structure through electrospinning. The alveolus wall consists of an epithelium layer and a connective layer rich of capillaries. The alveolar

epithelium is composed by macrophages and two typical type of cells: the type I pneumocytes and type II pneumocytes. The connective layer is a fibroelastic layer that give elastic properties to the lung. To reproduce the basement membrane, nanofibrose collagen type I membranes were fabricated by electrospinning. Solution and electrospinning parameters were optimized in order to obtain defect-free nanofibers. The membranes morphology was evaluated by electronic scanning microscopy (SEM). Cell tests was carried out under static conditions (transwell) for both healthy and pathological models. References: [1] M. Mustafa, A. J. Azizi, E. IIIzam, A. Nazirah, S. Sharifa, and S. Abbas, "Lung Cancer: Risk Factors, Management, And Prognosis," IOSR J. Dent. Med. Sci., vol. 15, no. 10, pp. 94–101, 2016. [2] B. A. Hassell et al., "Human Organ Chip Models Recapitulate Orthotopic Lung Cancer Growth, Therapeutic Responses, and Tumor Dormancy *In Vitro*," Cell Rep., vol. 21, no. 2, pp. 508–516, 2017.
Keywords: Lung cancer, Lung on a chip, Microfluidic devices, Biomaterials.

**Poster 043**

**Lisa Grohmann**

**Charité 3R – the 3R Center of Charité Universitätsmedizin Berlin**

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Established in 2018, Charité 3R aims to bundle and coordinate the 3R research activities at Charité Universitätsmedizin Berlin for a better translation in biomedicine: finding the best therapies by using animal-free methods whenever possible, establishing meaningful human disease models, and increasing animal welfare. Giving a discipline and department overarching organisational structure, Charité 3R will promote researchers with their 3R projects, implement 3R in education and communicate the challenges and needs of 3R research to the public. To reach our goal to foster vigorously 3R research and better translation, we will join forces and consequently collaborate with local, national and international partners from public research institutions, and pharmaceutical and biomedical companies.

Keywords: 3R centre, translational medicine, university hospital.

**Poster 044**

**Alzbeta Liskova**

**Intra-laboratory reproducibility of the *in vitro* phototoxicity test using 3D reconstructed human epidermis model EpiDerm**

Alzbeta Liskova, Helena Kandarova

MatTek *In vitro* Life Science Laboratories.

Evaluation of the phototoxicity hazard and phototoxic potency (i.e. phototoxic risk) of compounds and mixtures is a crucial step in the safety assessment of cosmetic, pesticide and pharmaceutical products absorbing UV and visible light. The validated and regulatory accepted *in vitro* assay, the 3T3 NRU PT (OED TG 432), provides high level of sensitivity, however, it has been reported that it also generates high rate of false positive results due to the lack of barrier properties naturally appearing in the human skin or other targeted tissues. *In vitro* reconstituted human skin models are increasingly being investigated for their usability in hazard identification and safety testing, because of their organotypic structure with a functional stratum corneum that allows for assessment of bioavailability of topically applied compounds and mixtures. An *in vitro* phototoxicity test using the human reconstructed epidermis model

EpiDermTM (EpiDermTM H3D–PT) has been developed and pre-validated almost 20 years ago and can be used either as standalone method for the phototoxicity testing of topically applied materials, or in combination with the 3T3 NRU PT, to minimise the potentially false positive results from this assay. In the current study we internally validated the method with six reference substances, of which four were known phototoxins (chlorpromazine hydrochloride, two types of bergamot oil and anthracene) and two compounds were UV-absorbing, but without phototoxic potential (cinnamaldehyde, p-aminobenzoic acid). The high reproducibility of the predictions has confirmed the robustness of the protocol and the prediction model.
Keywords: phototoxicity, reconstructed human skin model, validation, EpiDerm, regulatory toxicology.

**Poster 045**

**Emilie Helte**

**Fluoride in food and drinking water and its association with bone health**

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Fluoride (F-) is an inorganic anion that is naturally occurring in the earth's crust and can be found in foods and drinking water. Following ingestion, F- is absorbed and excreted in urine, however, a part of the ingested F- can be stored in bone. For this reason, the relationship between F- and bone health has previously been investigated in a number of epidemiological studies and, although results are inconsistent, some suggests a positive association between F- exposure and bone fractures/osteoporosis. Thus, the objective of this project was 1) to assess the dietary intake of F- (DF) of women enrolled in a large cohort 2) to investigate its correlation with urinary F- (UF) and 3) to evaluate association between DF and UF, respectively, on bone mineral density (BMD), odds of osteoporosis at baseline as well as the incidence of bone fractures during follow-up. The data used in the project was collected within the Swedish Mammography Cohort and contained information on dietary patterns generated by food frequency questionnaires (FFQs), biomarkers for F- exposure and bone health in urine and blood as well bone mineral density measurements (DXA). DF was assessed by linking the answers from the FFQ to a database containing F- levels in various food products that was created based on existing data in the literature for the purpose of this project. Correlation between DF and UF was assessed by using Spearman´s correlation while associations between UF and bone parameters were investigated by using linear and logistic regression and survival analyses.
Keywords: Fluoride, diet, water, osteoporosis, bone mineral density, bone fractures, DXA.

**Poster 046**

**Oscar Herrero**

**Evaluation of the toxic response, through ecological and molecular biomarkers, in model and non-model invertebrate species**

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Traditional approaches for the assessment of the toxic effect of environmental pollutants include mainly ecological endpoints. In recent years, the use of molecular techniques has brought a good opportunity to achieve an in-depth knowledge of mechanisms underlying those harmful effects. Those tools include gene expression and enzyme activity studies, among other targets. Invertebrates are a group of animals of great importance in ecosystems and their use can be very useful for the evaluation of their health status. Molecular techniques allow us to

obtain a large amount of data in a short time, and several studies have demonstrated that for different physiological effects there is a certain correlation between vertebrate and invertebrate species, as for example in the case of endocrine disruption effects or genotoxicity. Furthe study and refinement of this techniques and with these invertebrate test models could serve to reduce the number of animal experiments currently needed to carry out the toxicological evaluation of chemicals, especially after under the REACH Regulation. By analyzing toxicogenetic effects (changes induced in the transcriptional regulation) in of genes directly or indirectly involved in hormonal pathways, reproduction and development, the mechanisms of action of environmental pollutants could be better understood, improving the environmental risk assessments.

Keywords: molecular biomarkers, gene expression, qPCR, Chironomus riparius, genotoxicity, endocrine disruption, REACH.

**Poster 047**

**Kathrin Herrmann**

## Teaching animal-free approaches in basic and applied biomedical research

Kathrin Herrmann

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Directive 2010/63/EU, with its goal of replacing all procedures on live animals, is fundamental not only for furthering the implementation of the 3Rs but for helping to change the current research paradigm. Unfortunately, the Directive does not provide a plan of action on how to achieve this important goal. It is not unrealistic that this crucial shift can be accomplished, given the scientific and ethical shortcomings of animal modelling, which forms the basis of the prevailing paradigm (1-4). However, these limitations are not yet widely taught to future scientists, and there is evidence that they are not appreciated or simply ignored, especially by experimenters who currently base their work on animal use (5,6). In the EU, over 65% of laboratory animals are used in basic and applied research (7) which account for 68% of all animal experiments (8). An EU requirement to use animals in these fields is to complete a FELASA-accredited course, which is generally 40-hour long and includes only a 1-hour lecture on non-animal methods, mostly only applicable for regulatory testing. Clearly, there is further scope for additional education in non-animal, human-relevant approaches. This poster will describe such a comprehensive course. Its eight modules cover the main shortcomings of animal use in science, how to fully apply the 3Rs principles, how to properly conduct literature searches, and how to plan, conduct, analyse and report research studies. The course further teaches how to critically appraise the validity of animal and non-animal models and methods in order to choose the best means for particular research interests. References: (1) Archibald, K. (2018). Journal of Animal Ethics, 8(1), pp. 1-11. (2) Archibald, K., et al (2018). Journal of the Royal Society of Medicine, p. 0141076818812783. (3) Herrmann, K. and Jayne, K. (eds.) (forthcoming March 2019). Animal Experimentation: Working Towards a ParadigmChange. Vol. 22, Leiden: Brill. Table of Contents available at: https://brill.com/view/title/35072 (4) Pound, P. and Ritskes-Hoitinga, M. (2018). Journal of translational medicine, 16(1), p. 304. (5) Franco, N.H., et al. (2018). PloS One, 13(8), p. e0200895. (6) Daneshian, M., et al. (2015). Alternatives to Animal Experimentation, 32, pp. 261-274. (7) Fitzpatrick, B.G., et al. (2018). Lab Animal, 47(7), p. 175–177. (8) Taylor, K and Rego Alvarez, L. (in press). Letter: A summary of EU national

statistical reports of animal experiments in 2014-2016. Alternatives to Animal Experimentation.
Keywords: Non-animal methods, human-relevant approaches, basic research, applied research, teaching, education.

**Poster 48**

**Jennifer Hochmuth**

**Non-Animal Approaches under REACH – A retrospective validation approach of the robustness of a human QSAR to replace acute oral toxicity testing**

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According to the REACH (Registration, Evaluation, and Authorization of Chemicals) legislation, a registration dossier has to be submitted for every chemical substance produced in or imported to the EU at or above 1 tons/year. While REACH requires animal testing for the human health and environmental risk assessment, at the same time, regulators encourage alternatives to animal testing wherever feasible, according to the 3Rs principles (Replacement, Refinement and Reduction). For the endpoint of acute oral toxicity, toxicity tests on animals have primarily used mortality as the main observational endpoint, usually in order to determine LD50 or LC50 values. While *in vitro* (NRU3T3 test) and *in silico* non-animal approaches exist, this data can currently only be used for hazard identification and risk assessment purposes as part of a weight of evidence approach and not as a fully endorsed replacement for the *in vivo* tests. Here we would like to present an automated framework for a validated acute oral toxicity QSAR model based on human (and not animal) data. We conducted a meta-analysis, comparing the available in-vivo REACH data on acute oral toxicity against predictions made with both, a human toxicity data based QSAR model and an animal toxicity data based QSAR model, with the specific aim to assess the conservativeness of both models and to add extend the existing knowledge to a wide range of chemicals, while at the same time addressing the limitations and highlighting the regulatory potential of QSAR models for replacing acute oral toxicity tests with animals.
Keywords: *In silico*, REACH, QSARs, acute oral toxicity, 3Rs.

**Poster 049**

**Yan Huang**

**Evolution of structures in major facilitator superfamily: A group of multidrug transporters**

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The multidrug efflux transporters are integral membrane proteins that confer multidrug resistance in bacterial pathogens, tumor cells etc. This is due to the action of the transport protein that catalyzes the active extrusion of drugs. One of the typical examples is the major facilitator superfamily (MFS). MFS is composed of 23 Pfam (Protein domain database) families and it's one of the largest and most well-studied families of membrane transporters. The functions of MFS members include transporting a huge variety of organic and inorganic compounds, including a variety of drugs. An evolutionary study of structures of related transporters from different organisms can be very important in understanding their mechanistic function and modulation of drug availability and toxicity.
Keywords: Bioinformatics, Major Facilitator Superfamily, Topology Modelling.

**Poster 050**

**Nathalia Indolfo de Carvalho***

**Evaluation of cytotoxic effects on corneal epithelium model by MTT reduction or multiparametric high content analysis - a comparative STE based study**

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The development of non-animal models with better predictive capacity is a worldwide need. The assessment of eye irritation is a regulatory requirement for safety characterization of several chemicals or products. Although many *in vitro* eye irritation tests exist, they are not capable alone to fully categorize chemicals because each one reproduces partially the complex *in vivo* response. A strategic combination of *in vitro* methods in an integrated approach has better chances to completely replace animal test. Short Time Exposure test, used to assess ocular irritation/corrosion, is not effective to classify 100% of all tested substances in Category 1 or No category, according to GHS. The Adverse Outcome Pathways are promising tools, which links the molecular initiating event and the adverse outcome at a level of biological organization, relevant to risk evaluation. AOPs can be applied in integrated approaches development process. It is important to evolve the use of mechanistic toxicological data for risk assessment and regulatory affairs. After showing proficiency in STE method and performing it on an experimental basis, we modified the test implementing a multiparametric High Content Analysis as an endpoint, instead of tetrazolium reduction assay. This new approach has potential to be an analytical strategy for cytotoxicity assessment, by AOPs elements identification, which assesses cell viability and functionality after treatment with a chemical. In addition, seeking an increase in accuracy and therefore an improvement in prediction power, this modification aims to contribute significantly to the construction of a robust integrated approach to ocular risk testing and assessment.

*travel grant recipient

Keywords: Eye irritation, ocular toxicity, STE, HCA, AOP.

**Poster 051**

**Luca Isacco**

**The use of non-animal testing in the pesticide regulatory framework**

Luca Isacco

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Regulation (EC) 1107/2009 supports the use of in-vitro and in-silicon tests in the evaluation process of pesticide active substances. In art. 18, the Commission includes the use of intelligent strategies instead of standard animal testing as key element for the future work programme. However, the general perception about the acceptability of non animal testing by Member State experts involved in the evaluation of technical dossiers is limited to few specific aspects. Moreover, these cases are usually related to the hazard assessment of metabolites and degradation products, not for the active substance, for which standard animal testing is still considered essential. Within this project, the full data-requirement list is analysed, section by section, taking into account the possibility of different level of complexity. The aim is to identify where alternative approaches can successfully implemented in order to minimise animal testing.

Keywords: Pesticide, plant protection product, alternative strategy, non-animal testing.

**Poster 052**

**Yanfei Lu**

**Model of fractured bone regeneration in presence of bioresorbable material**

Yanfei Lu

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A growing number bioresorbable bone substitute materials are being used in orthopaedics and dentistry. Among advantages of such material, one can mention no-need for secondary surgery, easy to synthesize, no-need for application of autogenic or allogenic grafts as well as exogenic from animals, and the potential of drug delivery. However, material composition and microstructure of the implants and mechanical environment at surgery site increase the complexity and unpredictability in practice. Aim of the work was to develop a new model of bone regeneration after implementation of such bone substitute material. The mathematical model was focused on the cell-cell and cell-structure interaction. Effects such as biomechanical signalling, nutrients supply, influence of microstructure and cellular mediated resorption of biomaterial were taken into consideration. This mathematical model was implemented in numerical simulation using FEM software Multiphysics COMSOL. A 2D computational model has been developed to simulate the creeping substitution of bioresorbable material by living bone tissue. Simulation results reflected several typical clinically observed reactions after implantation and revealed the possibility of using computational approach in designing of orthopaedic or dental implants as well as in planning of the surgeries. The next planed step of investigation requires determination of the values of the parameters used in the proposed model by using *in vitro* cell culture experiments.

Keywords: Mathematical modelling, bone healing, bone substitute material.

**Poster 053**

**Fatematuj Juhara**

**Probing glutaredoxin activities of thioredoxin related protein of 14 kDa**

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The protein named thioredoxin related protein of 14 kDa (TRP14), also called TXNDC17 for thioredoxin domain containing protein 17.It seems to be a key enzyme for redox control of cell signaling and responses to oxidative insults to cells. It is ubiquitously expressed and well conserved between species. Recently found that it is a highly efficient substrate for the selenoprotein thioredoxin reductase 1 (TrxR1, TXNRD1) and that TRP14 has denitrosylase activity, efficiently reduces L-cystine, reduces polysulfides and protein persulfide moieties, and regulated the protein tyrosine phosphatase PTP1B, which is a key enzyme for control of protein phosphorylation cascades as triggered by several cellular growth factors including EGF and PDGF. It is noted that the unusual active site motif of human TRP14 and that of most other species Trp-Gly-Pro-Asp-Cys (WGPDC) is in some species instead found as WGPYC, which is thus very similar to that found in most glutaredoxins (XCPYC). In this project we thereby wish to assess whether TRP14 indeed has glutaredoxin activity, which in that case would suggest that the protein is a unique hybrid between thioredoxins and glutaredoxins. That, in turn, would provide a new and hitherto unknown direct link between the two reductive enzyme pathways in cells, i.e. the thioredoxin and glutathione pathway that would have major importance for the understanding of cellular redox pathways.

Keywords: Thioredoxin related protein (TRP14), glutaredoxin like activity, cellular redox pathway.

**Poster 054**

**Diana Kättström**

**Multifunctional additives with biocidal function: their use and potential negative effects on human health and environment**

Diana Kättström

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Multifunctional additives are chemicals that are characterized by having multiple functions in a product. These additives can be found in a variety of consumer products such as cosmetics, and chemical products where they act as e.g. moisturizers or stabilizers. Some of these additives also have biocidal function. Due to their multi-functionality, these substances are not subjected to authorization through Biocidal Product Regulation (regulation (EU) No 528/2012) or Annex V of Cosmetic Regulation (Regulation (EC) 1223/2009) that lists preservatives allowed in cosmetic products. By using multifunctional additives, a biocidal function can be achieved without following the time-consuming process related to authorization or evaluation. The limited data on use and effects of multifunctional additives on human health and the environment is a reason for concern. Long-term low-level exposure of bacteria to substances with biocidal function may ultimately be related to development of antibiotic resistance. The main goal of the project was to identify and collect information on multifunctional additives in cosmetic and chemical products and prioritize them based on their toxicological profile. Preliminary results indicate that the approximately 25% of the identified substances are harmful to aquatic life with long-lasting effects and approx. 10% are very toxic to aquatic life as classified according to CLP (Regulation (EG) No 1271/2008). Final findings may be useful as a basis for further investigation and potential regulatory actions to limit the use of multifunctional additives.

Keywords: Multifunctional additives, chemical products, cosmetics, antibiotic resistance, biocides, antimicrobials.

**Poster 055**

**Maren Lück**

**Creating the perfect target cell: Differentiation of motor neurons for *in vitro* potency testing of Botulinum neurotoxin**

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Botulinum neurotoxins (BoNTs) inhibit the release of neurotransmitters, causing a paralysis which can result in death due to asphyxia. In very low concentrations, BoNTs are currently being used for pharmaceutical and medical applications for which the potency of the toxin needs to be determined accurately. Lacking a universal *in vitro* method, an ethically questionable mouse lethality assay is still used commonly. The natural target cells of BoNTs are motor neurons (MNs), which can be derived from induced pluripotent stem cells (iPSCs) *in vitro*. BoNTs exert their toxicity by cleaving SNARE proteins inside the MNs after entering the cell by attaching to specific receptors. The aim of this project is to generate MNs *in vitro* for a cell based test system for BoNTs. Several protocols for the differentiation of MNs from human iPSCs have been published. Three of these protocols were compared for their capacity to generate MNs and their potential suitability to be used in BoNT potency testing, by analyzing the expression of

BoNT receptors and substrates. The differentiation protocols compared in this study generated significantly different amounts of MNs and BoNT targets. For the selected protocols, there seems to be an inverse correlation: When the proportion of MNs was higher, the expression of BoNT targets was lower and vice versa. In following studies including the toxin, we hope to answer which aspect is more important for BoNT sensitivity. Acknowledgement: The MoNLightBoNT-Assay development is funded by a grant from the German Federal Ministry of Education and Research (FKZ 031L0132A/B).
Keywords: Botulinum neurotoxins, motor neurons, *in vitro* assay.

**Poster 056**

**Juliane Klare**

**Requirements relating to important endpoints for the classification of dangerous goods**

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Plenty of well-developed non-animal testing procedures have been published in recent years. While some methods are easier in implementation such as corrosivity tests some are more complex and challenging like tests for systemic or long-term toxicity, for instance. Nevertheless just a small amount is validated and consequently obtained regulatory acceptance. Experiments mostly based on simple screening methods used in the industries often do not run through validation due to diverse reasons: missing access to regulatory requirements, demanding functionality in terms of comparability with clinical test results and not least because of lengthy procedures, human resources and high costs. Additionally, legal regulations regarding *in vitro* approaches for toxicological assessment of hazardous chemicals are not always as uniform and easily applicable as requested by test developers, researchers and the industries. Therefore, the methods often are not feasible due to the missing access to specific regulatory conditions and requirements. Especially regarding the classification of dangerous chemicals there are several differences within the standard regulations, e. g. between the GHS (Globally Harmonized System of Classification and Labelling of Chemicals) and the TDG (Recommendations on the Transport of Dangerous Goods). For that reason an insight into the requirements relating to important endpoints for the classification of dangerous goods is represented in this poster. Thus, users such as researchers, regulatory bodies and the industries can benefit from it as a common base and hence, support the constantly developing welfare movement.
Keywords: *in vitro* testing, toxicological assessment, toxicity, corrosivity.

**Poster 057**

**Sebastian Lungu-Mitea**

**Potentials and pitfalls of using transient bioassays in ecotoxicological screenings**

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Reporter gene assays in transfected mammalian cells for analyzing toxicity-related biomarkers are a valuable tool. Development of fish reporter assays has been proposed as a promising strategy to reduce and replace the use of fish in aquatic toxicology. The widespread assumption that transgene integration into a host preserves the genotypic, epigenetic, and phenotypic traits of the latter is not universally valid. Instead, transgenesis and experimental conditions are inflicting systemic stress, leading to an impact beyond the function of the manipulated gene and thus to non-specific effects. Here, we report an in-depth investigation on how to deal with

potentially intervening cis- and trans-effects and thus increase the sensitivity and reliability of the assay. In D. rerio fibroblast (ZF4) and liver (ZFL) cell lines exposed to known inducers (SFN, tBHQ, metazachlo) the Nrf2-responsive Firefly luciferase plasmid pGL4.37 was co-transfected with a variety of Renilla normalization plasmids, bearing different backbones and regulatory promoter elements (null, TK, SV40, CMV). By alternating conditions, the sensitivity in transient reporter-gene bioassays might drastically differ. Highest sensitivity was achieved by using a pRL backbone with a CMV promoter. To confirm results and neglect artifacts, alternating transfectional set-ups and viability test on different endpoints (protein mass, membrane stability, energy metabolism, cell proliferation, apoptosis) were conducted. From this study, we conclude that a number of factors, including the vector backbone and the constitutive promoter of the normalization plasmid, can have vast effects on the sensitivity of reporter gene assays and need to be optimized when a new assay is established.

Keywords: Alternative methods, *in vitro* assays, reporter gene assays, environmental screening.

**Poster 058**

**Anna Jacobsen Lauvås & Gabriela Gorczyca**

**A Human induced pluripotent stem cell-derived neuronal model to assess developmental neurotoxicity upon exposure to environmental chemical mixtures**

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European Commission, Joint Research Centre, Ispra, Italy. *equal.contributing authors

Human induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes represent unique *in vitro* cellular models to study key brain developmental processes, such as synaptogenesis, neurite outgrowth and neuronal network formation. These models are nowadays considered for the assessment of developmental neurotoxicity (DNT) *in vitro*, enabling mechanistic understanding of chemically-induced adverse effects. Moreover, since infants and children are co-exposed to more than one chemical at a time, novel mixture risk assessment (MRA) strategies for the evaluation of DNT should be implemented. Here, we used hiPSC-derived neural progenitors differentiated into mixed cultures of neurons and astrocytes to assess the effects of single chemicals and in mixtures on DNT specific endpoints (i.e., synaptogenesis, neurite outgrowth, and brain derived neurotrophic factor (BDNF) protein levels). Our data suggest that chemicals, individually present at non-toxic concentrations, can have neurotoxic effects in mixtures. As a follow up, neuronal electrical activity will be evaluated using microelectrode arrays to determine whether changes observed in the above endpoints (synaptogenesis, neurite outgrowth and BDNF levels) will cause impairment of neuronal network function.

Keywords: developmental neurotoxicity; hiPSC; neuronal network function.

**Poster 059**

**Valentina Garrapa**

**Animal-free monoclonal-like antibodies from a synthetic ribosome display selectable nanobody library**

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Nanobodies or VHHs are taking their place in medical research as new single variable binding units derived from camelids heavy chain antibodies. Since they naturally bind the target without the corresponding variable light domain, they can be singularly expressed preserving high target

affinity and high solubility with the concurrence of a really small size. Here we developed a new scaffold suitable for artificial nanobodies expression in Escherichia coli: from the alignment of more than 750 natural nanobodies we derived a consensus sequence consisting of nanobodies framework regions suitable as a scaffold. In order to permit the selection of new diagnostic VHHs avoiding animal manipulation/immunization, we built an artificial nanobody library introducing semi-random complementary determining regions in the scaffold and we isolated new Maltose Binding Protein binders through Ribosome Display technique. To render newly selected nanobodies suitable for the most common diagnostic applications we also developed a quick and easy method to fuse them to immunoglobulins Fc regions derive from different animals. Exploiting SpyTag and SpyCatcher technology, two polypeptides which can form a covalent bond between them, we demonstrated that by conjugating any expressed SpyTag-fused-VHH to different SpyCatcher-fused-Fc regions, with a simple *in vitro* reaction, totally functional monoclonal-like antibodies can be obtained. Thus, taking advantage of our artificial nanobody scaffold, we built a functional synthetic VHH library that permits the isolation of potentially any binder of interest avoiding animal manipulation and, additionally, any such selected nanobody can be easily converted *in vitro* in a functional monoclonal-like antibody.
Keywords: Nanobody, Ribosome Display, Monoclonal Antibody, Diagnosis.

**Poster 060**

**Elizabeth Goya Jorge**

**Assessment of endocrine disrupting potential of chemical compounds via aryl hydrocarbon receptor (AhR) antagonism**

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The endocrine disruptors (EDs) have gained increasing interest due to the diversity of mechanisms by which they could interfere with the endocrine system, coupled with the lack of standardized norms for their regulation and risk assessment. The ED could interrupt hormonal synthesis, metabolism and transport or directly interact with nuclear receptors. Therefore, the endocrine disrupting activity is a complex endpoint related to multiple biochemical pathways, some of them still unknown. The aryl hydrocarbon receptor (AhR) is a ligand-activated receptor/transcription factor with an essential role in regulating xenobiotic metabolism, development, immunology and homeostasis. The endocrine disruption via AhR has been reported mostly through agonistic mechanisms. Thus, the antagonistic effects on this receptor have been mainly studied from a pharmacological perspective with limited toxicity estimations. Additionally, few studies have focused on the theoretical and computational analysis of structural features that could be influence the antagonistic potency of chemical compounds. Therefore, in this research *in vitro* measurements of AhR antagonistic activity using cell-based screening methods were combined with *in silico* predictive models. Original experimental results and reports from literature allowed the construction of a diverse and broad database and its use in the modelling of the AhR antagonistic activity based on chemical structural fingerprints. All determinations complied with the OECD principles of validation, and were thus used to virtually screen non-tested compounds.
Keywords: AhR antagonism, endocrine disruptor, QSAR.

**Poster 061**

**Emma Gustafson***

**Development and testing of a repeated dose toxicity ontology model for chemical risk assessment purposes: liver effects as a case study**

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The scientific community is encouraged to develop animal-free methods, in particular *in vitro* and *in silico*-systems, to assess chemical safety based on mechanistic toxicology. An important tool in this regard is the adverse outcome pathway (AOP), which portrays existing knowledge regarding the linkage between a single molecular initiating event (MIE) and an adverse outcome via a linear series of key events (KE) at a biological level of organization relevant to risk assessment. Although very valuable for hazard identification, AOPs cannot be used as stand-alone frameworks for risk assessment purposes. In fact, AOPs only constitute one of four pillars of a generic strategy to enable sound safety evaluation of chemicals or so-called ontology model. Other pillars relate to aspects of the chemistry to allow for read-across and *in silico* modeling, kinetics to understand the exposure process through the use of quantitative *in vitro-in vivo* extrapolation (QIVIVE), reverse dosimetry and physiologically-based pharmacokinetic (PBPK) models, and the toxicological profile of the chemical through collection of available *in vivo* data obtained from animal testing, clinical data and/or epidemiological data. This doctoral thesis project aims to test the relevance and practical applicability of the ontology model in a repeated dose exposure scenario with hepatotoxicity as a case study. This is aligned with Cosmetics Europe's Task Force on Systemic Toxicity which focuses on the mode-of-action and adverse effects induced by chemicals relevant to the cosmetics industry, thereby using *in vitro* and *in silico* tools.

*travel grant recipient

**Poster 062**

**Jonathan Josephs-Spaulding**

**Systems Medicine Approach to Deciphering Recurrent Urinary Tract Infections (rUTIs)**

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*Escherichia coli* (*E. coli*) are a highly flexible group of microbes that thrive in various niches and environments by rapidly tuning gene expression at the transcriptional level to dynamically acclimate to tolerate stressful environments or rapidly grow for successive colonization. Metabolic requirements and trade-offs to maintain homeostasis which fluctuate environmental

phenotypes of *E. coli* can be taken advantage of for the treatment of human disease. We reanalyzed NGS patient data on rUTIs and bacterial response to antibiotics to ascertain the preliminary hypothesis of which differential expressed genes are being regulated towards the host pathology of a rUTI. From this preliminary information, we sequenced both urinary 16s rRNA and metatranscriptome case and control from a patient cohort. To support clinical postulations outside of the human model system, confirmatory experiments are presently being implemented to bolster the translational significance of this project. Replication and replacement of the human renal system *in vitro* will be employed by modeling the infectious behavior of *E. coli* colonization with an artificial human bladder-like environment for phenotype and gene expression. Finally, *in silico* metabolic modeling of a bladder-like model will be developed based on various multi-omics workflows from accumulated data to predict rUTI *E.coli* phenotypes via metabolite interactions of the bladder community during infection through the BacArena package. By identifying novel pathogen signatures that indicate growth (short intensive antibiotic treatment) or resistance (long sequential treatment), we hypothesize that these results will provide new insight into the treatment of antimicrobial resistant rUTIs.

Keywords: rUTIs, Systems Medicine, Antibiotic Resistance.

**Poster 063**

**Nika Khoshaein**

**Performance Verification and Application of a Cancer population for use in Physiologically Based Pharmacokinetic modelling**

Nika Khoshaein, Udoamaka Ezuruike, Oliver Hatley, Kate Gill, Iain Gardner

Certara UK Limited, Simcyp Division .

Background: Physiologically based pharmacokinetic (PBPK) modelling can predict the pharmacokinetics of drugs. By combining PBPK models with *in vitro-in vivo* extrapolation approaches, it is possible to parameterise human PBPK models as an alternative to animal testing. A further strength of PBPK modelling is that by describing the demographics, physiology, biochemistry and drug metabolism/transporter levels in different sub-populations, it is possible to explore differences in pharmacokinetics between different groups of individuals. Herein we describe an approach to develop a PBPK model describing pharmacokinetics in cancer patients. Methodology: Literature searches were conducted to find references describing changes in physiological parameters in patients with cancer, which were incorporated into a virtual cancer population. The performance of the population was verified by simulating the pharmacokinetics of 6 drugs (Midazolam, Caffeine, Rosiglitazone, S-Warfarin, Tolbutamide, and Digoxin) and 3 anti-cancer agents (Methotrexate, Docetaxel, and Paclitaxel) and the results compared with *in vivo* clinical studies. Result: Cancer patients were found to have increased AAG and decreased albumin levels compared to healthy volunteers. These changes were verified with the simulated unbound plasma AUC of digoxin and paclitaxel, which were in line with observed data. In addition, the simulated AUC and clearance of the 6 drugs mentioned above in cancer patients were within 1.5 fold of the observed values. Finally, the pharmacokinetics of the 3 anti-cancer drugs were successfully predicted, with the observed data lying within the 5th and 95th percentiles of the simulated concentration-time profiles of the population. Conclusion: The utility of the developed population in predicting the pharmacokinetics of cancer patients was demonstrated.

Keywords: Alternative methods to animal testing, pharmacokinetics, anti-cancer drugs, PBPK modelling.

**Poster 064**

**Serhii Kolesnyk**

**Prioritization of combined plant protection products for further risk assessment of endocrine disrupting properties using few *in silico* models**

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Humans are exposed to mixture of chemicals both through diet and in occupational environments. Currently many plant protection products are complex mixture of few active ingredients and other ingredients, here called adjuvants. Every year in Ukraine number new plant protection products and biocides containing from 2 to 5 active ingredients from different chemical groups are authorized. Furthermore, some adjuvants and impurities may be more hazardous, than active ingredients, and can enchance toxicity of plant protection product. As whole mixture testing is not feasible as regular practice for risk assessment of mixtures, we explore possibilities to use *in silico* methods to guide further studies and risk assessment. Given high attention to issue of endocrine disruption connected to pesticides, we use two *in silico* tools to model binding affinity to nuclear receptors of components and impurities of eight formulations containing 2-5 active ingredients using OpenVirtualToxLab software and EndocrineDisruptome on-line tool. According to estimated by OpenVirtualToxLab toxic potential of active ingredients formulations No 1 (tebuconazole, triadimefon), 7 (spiroxamine, tebuconazole, difenoconazole), 4 (tebuconazole, triadimefon, spiroxamine) and 3 (propiconazole, azoxystrobin, cyproconazole) require attention concerning their endocrine disruption potential. Considering results from OpenVirtualToxLab and Endocrine Disruptome together endocrine disruption effects that require attention in further assessment of these formulations may be antiandrogenic effects and thyroid function disruption.

**Poster 065**

**Xinxin Luo**

**Immunomodulatory properties of perfluoroalkyl compounds during chemically-induced intestinal inflammation in zebrafish**

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Inflammatory bowel disease (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are disorders characterized by chronic inflammation of the gastrointestinal tract. Although the etiology of IBD is yet to be elucidated, it is regarded as a complex disease where an active immune response is triggered by environmental factors in genetically susceptible hosts. Environmental factors, particularly, per- and polyfluoroalkyl compounds (PFASs) are substances widely found in consumer products due to its useful physicochemical properties like water and oil repellency. Despite their value in industry, they have been reported to be persistent not only in the environment but also in humans as world-wide environmental contaminants. Epidemiology studies have also revealed that PFASs in serum with slow degradation can further exert harmful impacts on the next generation growth through maternal exposure. Recently an emerging concern about PFASs affecting intestinal immunity has been raised by few studies. Villablanca lab have shown that perfluorooctane sulfonic acid (PFOS) exacerbates intestinal inflammation in zebrafish and mice. This study is performed in order to investigate the inherent immunomodulatory

properties of other PFASs,for example PFHxS in chemically-induced models of intestinal inflammation in zebrafish. We investigated the aberrant immunological responses caused by PFHxS in zebrafish larvae undergoing chemically-induced intestinal inflammation. To address if intestinal neutrophil recruitment is altered by PFOS neutrophil-specific transgenic Tg(lysC:dsRed) zebrafish larvae was used to monitor their recruitment to the inflamed intestine. Additionally, by RT-qPCR, expression of proinflammatory markers were anylized, such as il1b and tnfa transcripts.
Keywords: PFHxS, Inflammatory bowel disease (IBD), Zebrafish larvae.

**Poster 066**

**Ambra Maddalon**

**Understanding chemical allergen potency: an *in vitro* approach**

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Allergic contact dermatitis (ACD) is a T-cell mediated skin inflammation provoked by repeated skin exposure to low MW chemicals. There is a pressing need for alternative non-animal methods to reduce and ultimately replace animal tests for this endpoint, as also required by some European regulations. While is possible to discriminate contact sensitizers from non-sensitizers with *in vitro* methods, currently it is not possible to estimate the sensitizing potency without using animals. Using THP-1 cell line, a model for T-cells, we have conducted a study using allergens of different potency. Up-regulation of CD80, CD86 and HLA-DR, and the release of several cytokines were evaluated. Results suggest that allergens of different potency differently activate DCs; extreme allergens induce a higher degree of maturation compared to moderate and weak ones. Based on these results, we moved to the analysis of miRNAs expression in response to chemical allergens to understand allergenic potency. MicroRNAs are non-coding RNAs that regulate gene expression at post-translational level. Recent findings indicate that miRNAs contained in micro-vesicles may determine reprogramming of gene expression in target cells. We identified few miRNAs involved in ACD and these include let-7, miR-142 and miR-155. We are currently investigating the possible correlation between miRNA expression and the potency of the selected contact allergens; the extreme sensitizer DNCB and the strong PPD. Furthermore, we have used a keratinocyte cell line (NCTC-2544) to identify differentially expressed miRNAs after contact allergen exposure. We will focus on the down-regulation of miR-155 and miR-21 upon treatment with DNCB and PPD.
Keywords: *In vitro*, ACD, chemical allergens, sensitizing potency, miRNA.

**Poster 067**

**Julia Maria Malinowska**

**Optimising direct infusion mass spectrometry metabolomics for compatibility with a 96-well based *in vitro*, high-throughput screening (HTS) platform**

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High throughput screening (HTS) is becoming a recognised approach for supporting decision-making in chemical safety assessment. In parallel, *in vitro* metabolomics is a promising tool to

accelerate a departure from the use of animal models in toxicity testing and provide mechanistic understanding of the biological responses in target systems exposed to chemicals. However, traditional metabolomics methods lack the sensitivity and sample throughput to successfully integrate into an *in vitro* HTS program. The objective of this study is to seek compatibility of high-resolution spectral-stitching nanoelectrospray direct infusion mass spectrometry (nanoDIMS) metabolomics (University of Birmingham) and a high-throughput screening platform (Joint Research Centre, EU Reference Laboratory for Alternatives to Animal Testing). Low biomass cell samples (50,000 hepatocytes of HepaRG per well) were prepared for metabolomics analyses using a newly established automated protocol and solvent system (Biomek FXp laboratory automated workstation) and analysed using a modified nanoDIMS method (Thermo Scientific Orbitrap Elite). The method was assessed with respect to sensitivity and reproducibility of the entire workflow. It has been demonstrated that the optimised nanoDIMS metabolomics method provides acceptable sensitivity (>3,500 non-background peaks detected in positive ion mode) as well as acceptable intra- and inter-plate variability (median relative standard deviation <30%). Baseline characterisation of HepaRG endogenous metabolism over time and a pilot study with a model toxicant (cadmium chloride) are underway. This newly established method shows that *in vitro* metabolomics may be readily applied as an additional high content assay in 96-well HTS, complementing HT measures of the transcriptome and image phenotype.

Keywords: high-throughput screening, non-animal approaches, *in vitro* methods, toxicity testing, metabolomics.

**Poster 070**

**Janine McCarthy**

**Increasing the Availability and Quality of Human Tissue in Science**

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Human tissues are invaluable resources for medical research, diagnostic tests, biomarker discovery, drug development, product testing, chemical exposure, and much more. Fresh tissue samples are, for many applications, the preferred model environment to conduct in-depth analyses of human biological processes. As interest grows in using more human tissues for numerous applications, policies and guidelines are needed to ensure an uninterrupted supply of a variety of human tissues. Variations in tissue handling, processing, and characterization protocols often compromise reproducibility and effectiveness of donor tissues for research applications. Harmonizing methods and developing cross-industry standardization of best practices will improve their integrity and maximize their potential in research. Supporting biorepositories, the development of new human tissue modelling technology, and raising awareness in the scientific and regulatory communities are key ways in which the barriers to greater uptake of human tissue models can be overcome. Key challenges have been identified in the areas of policy, scientific development, and public engagement with respect to the provision and application of human tissues and cells for scientific purposes. Following working group recommendations, stakeholders are working to facilitate the increased availability and quality of human tissues and cells available to the basic and translational research communities as well as provide a framework for the education of the public and researchers to foster human tissue and organ donation and utilization in place of animal studies. The pursuit of these

recommendations will facilitate greater access to and use of high quality human tissues for biomedical and translational research.
Keywords: Public health, Human tissues.

**Poster 071**

**Angela Miccoli**

**Cosmetic safety assessment of a so-called green shampoo and a common shampoo, a TTC approach**

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This study consisted of a safety assessment of two shampoos, a common one and a so-called green one. The choice to assess two different shampoos depends on the recent trend of considering any product bearing the words "green" as automatically safer than the others. The word green has become a powerful concept in cosmetics, but with a much less certain definition. A cosmetic safety assessment is been conducted following the protocols. After calculating SED and MoS values, a TTC approach has been evaluated using COSMOS TTC workflow. The Threshold of Toxicological Concern (TTC) is a pragmatic risk assessment based approach that has gained regulatory acceptance for food and has been adapted to address cosmetic ingredient safety. MoS was evaluated only for those ingredients that have a NOAEL, resulting for all of them way more 100. Unfortunately, it was not possible to apply the TTC for some ingredients, due to the lack of a precise SMILE. It was missing for 6/24 (25%) and 6/14 (43%) ingredients of common and green shampoo, respectively. Based on TTC approach, the remaining ones do not need any other evaluation because they have a negligible concern with the exception of sodium benzoate and potassium benzoate. Both shampoos were assessed safe and the TTC approach proved to be valid for their safety assessment, especially for the common shampoo. Its limitation depends, among other things, on the absence of SMILE, above all absence for the ingredients of the green shampoo due to the presence of many oils.
Keywords: Safety assessment; cosmetic; hair; green products.

**Poster 072**

**David Miguel-Vilumbrales**

**Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment**

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Our aim is to develop and apply next generation approaches to skin allergy risk assessment that do not require new animal test data, address novel exposure scenarios and better quantify 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). 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), KeratinoSensTM (OECD TG 442D), h-CLAT (OECD TG 442E) and U-SensTM (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, KeratinoSensTM, h-CLAT and U-SensTM 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 four theoretical skin allergy risk assessment case studies (caffeine, coumarin, curcumin and sulforaphane) will be presented, each addressing a different product exposure scenario, to illustrate how the DA prediction can be used as part of a weight of evidence decision-making approach.

Keywords: Risk assessment, skin sensitisation, sensitiser potency.

**Poster 073**

**Erika Molica Colella**

**Alternative *in vitro* methods for the toxicity test of autogenous vaccines**

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According to the 3R principle (Replacement, Refinement, Reduction), this study aims to find alternative methods to evaluate the toxicity of autogenous vaccines. Currently in Italy the II.ZZ.SS. must perform the *in vivo* toxicity test for each lot of autogenous vaccine produced as laid down in the Decree of 17 March 1994. This paper describes two *in vitro* methods for assessing the toxicity of autogenous vaccines. The first is the MTT test based on the metabolic reaction of the tetrazolium salt in vital cells. For this test the L-929 cell line was used, seeded at the concentration of $1.0x10^5$ cells/ml in 96-well plates in MEM culture medium added with 10% of SFB, and incubated at +37 °C ± 1 °C in 5% of CO2. After an overnight incubation, the vaccine antigens were diluted and distributed 100 µl/well. The absorbance of the yellow tetrazole reduced to purple formazan was measured by a spectrophotometer. The second method is the test for measurement of IL-1ß production by macrophages. In this assay, macrophages were obtained after differentiation from pig monocytes in peripheral blood mononuclear cells (PBMC), using 10 ng/ml of Macrophage-Colony Stimulating Factor (M-CSF). Differentiated macrophages were reacted with the same antigens at different dilutions for 24 hours, followed by quantification of released IL-1ß by "Duo set ELISA for Porcine IL-1ß/IL-1F2" (R&D System). Preliminary results seem to be promising to present the potential of this methodology for replacement of the current *in vivo* test to get an overview of the potential toxicity of the vaccines.

Keywords: Autogenous vaccines, toxicity tests, *in vitro* methods.

**Poster 074**

**Serena Montalbano**

**A biotechnological approach for the development of new antifungal compounds to protect the environment and the human health**

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Nowadays, mycotoxin contamination represents a challenge to food safety. Aflatoxins are a group of secondary fungal metabolites: they can accumulate in food during agricultural, storage, and processing practices and are not easily eliminated during food processing because of their stability against heat, physical, and chemical treatments. Several strategies have been proposed to prevent the toxic effects of mycotoxins and in particular of aflatoxins; however, no clear-cut solutions exist (Sarma et al., 2017). The Aflatox project (http://aflatox.unibs.it/) aims to develop an innovative multi-step approach to design and synthesis new typologies of inhibitors of aflatoxigenic fungi (Zani et al., 2015). These compounds could represent a new generation pesticides, responding to “greener” and environmentally sustainable agricultural strategies. The project can be divided in three section. The first was the design and synthesis of some parent compounds from natural molecules; the second one was the study of their biological effect on aflatoxigenic fungi and their cytotoxicity and genotoxicity on healthy human cells and on bacteria and plant cells; the last one was the chemical modification of the most active compounds in order to study the mechanism of action and to improve the biological activity. In conclusion, this approach allows us to obtain 180 compounds tested for antifungal and antiaflatoxigenic properties. The most promising molecules were evaluated for their toxicological and genotoxicological profile. All these results have contributed to the creation of a QSAR (Quantitative Structure-Activity Relationship) database correlating chemical structures and biological/toxicological activities. Financial support: Fondazione Cariplo-Project N. 2014-0555, http://aflatox.unibs.it/

Keywords: Food safety; innovative approach; antifungal and antiaflatoxigenic compounds.

**Poster 075**

**Andrea Montano Montes**

**Toxicokinetics of ochratoxin A and its association to food intake and other background characteristics in Riksmaten Adolescents 2016-2017**

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Ochratoxin A (OTA) is a ubiquitous but nephrotoxic mycotoxin that has been classified as a group 2B agent; possibly carcinogenic to humans, by IARC. Previous studies have found comparably high OTA levels in the adult Swedish population. The aim of this project was to study the correlation of serum OTA concentration with food intake, dietary patterns and socioeconomic background of Swedish adolescents. In the Swedish national dietary survey Riksmaten Adolescents 2016-2017, adolescent students were recruited class-wise from 5th, 8th and 11$^{th}$ grade. Participants (n=3099) filled out a web-based questionnaire and a 3 days dietary

recall, and their weight, height and physical activity was measured. From a subsample (n=1305) blood and urine samples were collected. Concentrations of OTA and 2´R ochratoxin A (2´R-OTA), an isomer formed from OTA at high temperatures, were measured by HPLC-MS/MS.Preliminary results show that OTA was found in all participants serum and 2´R-OTA found in 8,3% of participants serum, however urine OTA concentration was undetectable in all urine samples. The toxicokinetics of OTA is not yet fully understood, the long half-life of serum OTA introduces high uncertainty in exposure assessment through biomonitoring in urine and blood. Still, biomonitoring of OTA proves useful as marker of exposure.

Keywords: Ochratoxin A, Toxicokinetics, Biomonitoring, Diet, Socioeconomic Factors, Adolescents, Mycotoxins, Biomarkers.

**Poster 076**

**Jessica Monterosso**

***In vitro* assessment of HDAC inhibitors effects**

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HDAC inhibitors (HDACi) are a heterogeneous group of molecules, with different therapeutic applications, widely used in human therapies. Their teratogenic potential is known, and some evidences correlate their acetylation impairment to developmental abnormalities. Hence, for evaluating acetylation fine-tuning and possible toxicity we tested these compounds *in vitro* using immortalized lymphoblastoid cell lines from healthy donors. We assessed valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA) and butyric acid (NaB) compared to vehicles (i.e. water and DMSO) by AlphaLisa Assay for measuring histone H3 acetylation levels. We performed Ki67 immunocytochemistry for proliferation analyses and TUNEL Assay for quantifying apoptotic cells. We found acetylation level significantly different among compounds and vehicles. These *in vitro* screening tests could be a good alternative to limit the animal approaches in the study of new compounds with HDAC inhibitory effects and their possible toxic effects.

Keywords: HDACi, teratogenic, acetylation.

**Poster 077**

**Dania Movia**

**Multilayered Cultures of NSCLC cells grown at the Air-Liquid Interface allow the efficacy testing of inhaled anti-cancer drugs**

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Evidence supports the advantages of inhalation over other drug-administration routes in the treatment of lung diseases, including cancer. Although data obtained from animal models and conventional *in vitro* cultures are informative, testing the efficacy of inhaled chemotherapeutic agents requires human-relevant preclinical tools. Such tools are currently unavailable. Here, we developed and characterized *in vitro* models for the efficacy testing of inhaled

chemotherapeutic agents against non-small-cell lung cancer (NSCLC). These models recapitulated key elements of both the lung epithelium and the tumour tissue, namely the direct contact with the gas phase and the three-dimensional (3D) architecture. Our *in vitro* models were formed by growing, for the first time, human adenocarcinoma (A549) cells as multilayered mono-cultures at the Air-Liquid Interface (ALI). The *in vitro* models were tested for their response to four benchmarking chemotherapeutics, currently in use in clinics, demonstrating an increased resistance to these drugs as compared to sub-confluent monolayered 2D cell cultures. Chemoresistance was comparable to that detected in 3D hypoxic tumour spheroids. Being cultured in ALI conditions, the multilayered monocultures demonstrated to be compatible with testing drugs administered as a liquid aerosol by a clinical nebulizer, offering an advantage over 3D tumour spheroids. In conclusion, we demonstrated that our *in vitro* models provide new human-relevant tools allowing for the efficacy screening of inhaled anti-cancer drugs. Reference: Movia et al., Sci Rep (2018), 8,12920. DOI:10.1038/s41598-018-31332-6.

Keywords: ALI cultures; lung cancer; 3D *in vitro* model.

**Poster 078**

**Joel Nava Palacios**

**The role of nuclear receptors in dampening PFOS-exacerbated chemically-induced intestinal inflammation in zebrafish**

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Inflammatory bowel disease is a disorder that affects 0.5% of the Western population and is influenced by many factors that include host genetics and environmental exposures. Among the latter, we can find dietary compounds, and pollutants such as perfluorooctanesulfonate (PFOS), from the perfluorinated compounds (PFCs) group, with persistent, bioaccumulative, and toxic properties. Humans are exposed to PFOS mainly through diet and an increase in serum levels of PFOS has been detected in people even without occupational exposure. On the other hand, dietary metabolites can regulate intestinal homeostasis through activation of nuclear receptors (NRs) LXR, PPARs and RAR, among others. Although their individual axes are well-characterized, the effects of their simultaneous activation are not entirely understood, and a beneficial interaction between NRs and PFCs is possible, considering that NRs may counteract the proinflammatory effect of PFOS. Zebrafish are a powerful *in vitro* tool to study inflammatory responses in a high-throughput fashion, useful for the evaluation of the combinatorial effect of PFCs and NRs activators on the immune response in connection with intestinal inflammation. The experimental procedure consists of exposing zebrafish embryos to both PFOS and NRs agonists from 3 days post fertilization (dpf) to 5 dpf. The inflammatory response is analyzed *in vivo* using the transgenic zebrafish reporter for neutrophils Tg(lysc:DsRed), allowing to track and quantify the process over time. The analysis of the expression of proinflammatory cytokines, including IL-1ß and TNF-α, using qRT-PCR is also performed.

Keywords: Zebrafish embryo; perfluorooctanesulfonate; nuclear receptor; inflammatory bowel disease.

**Poster 079**

**Katarzyna Nawrotek**

***In vitro* evaluation of degradation of implants intended for peripheral nerve regeneration**

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The formation of correct neural circuits during development or regeneration is dependent on the ability of growing axons to accurately locate their targets. The accurate path is sensed by growth cones, which detect distributions of molecular guidance cues in the extracellular environment. Chemotaxis based on both growth cones' detection and response to concentration gradients of appropriate cues is regarded as a key mechanism for guidance. Calcium ions take part in growth cone turning as well as outgrowth. In this study, we developed implants releasing calcium ions in a controlled manner. The implants are intended for peripheral nerve regeneration. The structure of implants is made of naturally derived mimetics of glycosaminoglycans. Moreover, it is hydrogelic and contains reservoirs of calcium ions. Five structures with different properties of mimetics of glycosaminoglycans are incubated in a phosphate buffered solution (PBS, pH 7.4) and in phosphate-buffered solution (PBS, pH 7.4) containing lysozyme (enzyme responsible for degradation of mimetics of glycosaminoglycans in living organism) for specified periods of 1, 7, 14, 21, 28, and 56 days under continuous shaking. Subsequently, cytotoxicity of samples is evaluated towards nerve cells cultured *in vitro* in MTT reduction assay according to ISO-10993-5-2009 protocol.

Keywords: chitosan, implants, peripheral nerve regeneration, degradation, *in vitro* studies.

**Poster 080**

**Axel Nordström**

**Potential pulmonary toxicity and risk due to multi-flavored electronic cigarette use: Evaluation through physiologically relevant *in vitro* models and identification of protective bioactive compounds**

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Increased usage of E-cigarettes (E-cig) is of high concern, especially for respiratory health. Long-term exposure toxicity data is lacking and uncertainty regarding E-cigarette vapor is high. An E-cig device consists of a cartridge filled with E-liquid, which gets aerosolized in a vaporization chamber by a heating unit running on a battery. Different sizes of aerosolized particles are generated, where smaller particles have the potential to reach deeper in the lung. E-liquid can vary greatly in its composition and proportions of constituents; glycerol and/or Propylene glycol, diacetyl and other flavouring substances, and commonly nicotine. The flavours used in E-liquids are intended as food additives, where regulation only requires oral toxicity testing. However, proper toxicity testing following inhalation exposure is lacking and emerging evidence from cell and animal models suggests chronic lung disease (CLD)-like effects; oxidative stress, inflammation, airway hyperactivity, damaged epithelial cilia and excessive mucus secretion. The

aim with this study was to assess potential pulmonary toxicity caused by multi-flavoured E-cig vapour exposure to a physiologically-relevant air-liquid interphase 3D cell model cultured with human primary bronchial epithelial cells and alveolar epithelial cells (NCI-H441 cell line). Cytotoxicity-, tissue injury- inflammatory- and oxidative stress response at gene and protein level was investigated via qRT-PCR, ELISA and LDH assays. Protective properties of bioactive (anti-oxidants) compounds was also investigated via pre-treatment of the bronchial and alveolar mucosa models before E-cig vapour exposure, compared to untreated models following E-cig vapour exposure.

Keywords: E-cigarette, multi-flavored vapor, pulmonary toxicity, Bronchial and alveolar mucosa model.

**Poster 081**

**Linda Ok**

**The effect of SSRIs antidepressant on serotonergic, glucocorticoid, and junction systems in human primary placental cells**

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Selective Serotonin Reuptake Inhibitors (SSRIs) are common antidepressant which works by increasing the level of serotonin at the synaptic space by blocking the serotonin transporter (SERT) on the post-synaptic neurons. Generally, non-pharmacological interventions are recommended as the first line of treatment for pregnant women with depression. However, in a severe case of depression, SSRIs treatment is necessary to prevent the harmful effect of depression per se. Studies suggest that the use of SSRIs during pregnancy is associated with adverse outcome in fetal development and pregnancy complications such as gestational hypertension and preeclampsia. Recently, our group has shown that the exposure of human primary placenta cells to SSRIs affects the trophoblast cell fusion and invasion. However, the effect of SSRIs on the placental function, which is crucial for the maternal health and fetal development, remains poorly understand. In order to test the hypothesis that the SSRIs exposure on the human primary placenta cells alter the expression of genes and proteins involved in serotonergic, glucocorticoid and junction systems in placenta, human primary trophoblast cells obtained from the full-term placenta were exposed to Fluoxetine, Norfluoxetine, Sertraline, Venlafaxine, and Citalopram at two concentrations: 0.3uM and 0.03uM for 24-h. mRNA of SERT, Monoamine oxidase A, Tryptophan hydroxylase, 11β-Hydroxysteroid dehydrogenase, Connexins -43,-40,-32, Tight junction protein-1 and Syncytin-1 will be extracted and the cDNA will be synthesized for the amplification with RT-qPCR. Their protein levels will be analysed by Western Blot.

Keywords: Selective Serotonin Reuptake Inhibitors, Placenta, Prenatal depression, Cytotrophoblast, 5-HT, primary cell.

**Poster 082**

**Eleftheria Maria Panagiotou**

**Effects of phthalates on human ovarian health and function**

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The study focuses on the effects of DEHP, a banned phthalate, and its replacement, DiNP, on human ovarian health and function. Phthalates are compounds mainly used as plasticizers but have also widespread use in other common products such as cosmetics, food and beverage packaging and clothing. Women, especially of reproductive age, exhibit the highest exposure levels due to their patterns of product use. Phthalates have been found to act as endocrine disrupting chemicals with potential target organ the ovary. Since females are born with a finite number of follicles, it is crucial to study their effects on human female reproductive biology and elucidate the mechanisms underlying any adverse effects.Initially, the cytotoxicity of the compounds will be assessed using the human granulosa tumour cell line KGN. Next, gene expression studies will be conducted using qPCR after exposure of the same cell line to the compounds. The genes of interest will include hormone receptors, steroidogenesis enzymes and important ovarian growth factors and receptors. The concentrations that will be used will be determined based on the composition of a phthalate mixture which is part of the EU study EDC-MixRisk, in order to better reflect the real-life exposure levels. Next step will be the study of the compounds' effects *ex vivo*, using human ovarian cortical tissue and having as endpoint the preantral follicular growth and survival, assessed via histomorphometric analysis of the tissue. Lastly, levels of anti-mullerian hormone and estradiol in the conditioned media will be measured using ELISA.

Keywords: Phthalates, reproductive toxicity, endocrine disruptors, ovarian toxicity.

**Poster 083**

**Martina Panzarea**

**Validation study of *in vitro* methods for the detection of thyroid disruptors**

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Endocrine disruptors chemicals are defined as substances present in environment, food and consumer products that can disrupt the hormonal balance in humans and wildlife; one important endocrine system is mediated by the thyroid hormones which are metabolized by different pathways (glucuronidation, sulfation and deionization). An impairment of these hormones can lead to very severe health complications and for this reason the requirement to identify chemicals with the ability to disrupt thyroid hormones is compelling. Currently, there are lot of tests able to identify thyroid disruptors chemicals, but only few of them are performed *in vitro*. In line with the 3Rs principles (Replacement, Refinement and Reduction) the EU member states are required to increase the efforts to validate alternative methods to detect TDs chemicals. A total of 17 methods have been selected from EURL ECVAM as candidates for this validation study project and will be carried out in collaboration with EU – NETVAL. Two of the seventeen methods have been evaluated in the context of this work.Validation of toxicological methods, as in this case, is an important part in the context of risk assessment evaluation. If those methods perform well, they may be selected for their use for regulatory

purposes and in particular for evaluate a potential risk, for human and for environment, concerning the exposure to thyroid disruptors chemicals.

Keywords: Thyroid Disruptors, Alternative Methods, Risk assessment.

**Poster 084**

**Camilla Paoletti**

**Direct reprogramming of human cardiac fibroblasts to cardiomyocytes using microRNA mimics**

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The combination of four different microRNAs (miR-1, 133, 208 and 499), named "miRcombo", has been used for the direct reprogramming of murine fibroblasts into cardiomyocytes (CMs) for myocardial infarction (MI) treatments.[1,2] Here, we evaluated miRcombo mediated reprogramming of human adult cardiac fibroblasts (AHCFs) into CMs in 2D and 3D culture. After 4 days in 2D culture, ddPCR analysis showed significantly increased expression of early cardiac transcription factors (TFs) Hand2 and Mef2c ($p < 0.005$), slightly increased expression of Tbx5 and Nkx2.5 although non-significant ($p > 0.05$), and reduced Vimentin expression ($p < 0.05$) in miRcombo-transfected AHCFs compared to controls. ICC analysis showed increased expression of late cardiac markers α-sarcomeric actinin and cardiac Troponin T (cTnT) in miRcombo-transfected AHCFs after 10 and 20 days of culture in 2D. However, ddPCR showed no significant differences of late cardiac markers Myosin heavy chain 6 (Mhy6) and cardiac Troponin I (cTnI) expression between the groups after 15 days in 2D culture. On the other hand, non-transfected AHCFs cultured in 3D fibrin-based hydrogels showed enhanced cardiac TFs expression compared to 2D cultures after 4 days, while, miRcombo transfection did not significantly increase cardiac gene expression of AHCFs cultured in 3D hydrogels for 4 days. After 15 days, AHCFs cultured in 3D hydrogels showed a strongly enhanced expression of cardiac genes such as cTnI and Myh6 compared to 2D cultures. In conclusion, results showed that a 3D environment plays a key role in enhancing direct reprogramming of AHCFs into CMs. References: [1] Jayawardena TM et al. Circ Res. 2013, 110, 1465–1473. [2] Li Y et al. Sci. Rep. 2016, 6, 1–11. Acknowledgement: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 772168).

Keywords: Cardiomyocytes, Myocardial infarction, microRNAs, cardiac fibroblasts.

**Poster 085**

**Moritz Pfeiffenberger**

**Modelling the initial phase of fracture healing *in vitro***

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Fracture healing disorders (mostly nonunion and delayed union) occur in up to 10% of human patients. There are only a few human based *in vitro* models addressing this pathophysiological situation. Therefore, we aim at developing a valid 3D-model to simulate the initial phase of fracture healing *in vitro*. To this end, we validated a fracture hematoma model consisting of a

defined concentration of human blood cells and mesenchymal stromal cells (MSCs). To evaluate this model, we compared our data to *ex vivo* data obtained from human patients and discovered striking similarities with regard to cell composition, typical markers on mRNA-level as well as the protein pattern of secreted proteins. In parallel, we created a 3D scaffold-free osteogenic construct only consisting of MSCs, patented by the fzmb GmbH. The bone-like characteristics of these constructs were confirmed by qRT-PCR, histology, µCt and immunohistochemistry. In order to create a more realistic 3D-model with regard to fracture healing processes, we co-cultivated the fracture hematoma with the 3D scaffold-free construct. This model will give us the opportunity to study the early phase of fracture healing *in vitro*, and as a result to test new strategies and to simulate models of disease.

Keywords: Fracture healing, fracture hematoma, 3D model.

**Poster 086**

**Gelsomina Pillo**

**Comparative evaluation of systemic endpoints: the case of carcinogenicity**

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In the context of the activities carried out to develop and evaluate scientifically robust and innovative approaches for safety assessment of chemicals across multiple regulatory sectors, a project was started to explore how information from regulatory toxicities studies can be re-used in novel way in the safety assessment process. The overall goal is to avoid redundant *in vivo* studies, to minimise the reliance on those and ultimately, to have a 3Rs impact. A comparative analysis of different toxicity endpoints, measured in different species and/or different models was initiated. Mechanistic and data-driven approaches were identified and a combination of both was explored further. Here I present, as part of my traineeship project, a mapping exercise in the area of carcinogenicity where, we have identified effects and/or mechanisms related to cancer, using as reference the "10 characteristics of carcinogens. This approach has been then applied for a particular key study. This allowed us to check possible overlaps with observations made in the current regulatory *in vivo* and *in vitro* studies, and to identify eventual information gaps.

Keywords: carcinogenicity; *in vivo*; *in vitro*; mechanisms; integrated approaches.

**Poster 087**

**Debora Polla**

**Mechanism of LAT1 amino acid antiport: a molecular dynamics simulation of the behaviour of a solute and of an inhibitor**

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The L-type amino acid transporter 1 (LAT1) is an ubiquitous Na+- and pH-independent antiporter, involved in the cellular uptake of essential amino acids. Being over-expressed in many human cancers, LAT1 was recently acknowledged as a novel target for cancer therapy. Here, the unknown mechanism of transport mediated by LAT1 was investigated by molecular

modelling approaches, by comparing the transport of its solute tyrosine (Tyr) with the behaviour of the well-known inhibitor 3,5-diiodo-L-tyrosine (DIT). First, the outward-facing (OF) and the inward-facing (IF) LAT1 structures were built by comparative modelling. Then, a series of targeted molecular dynamics simulations (tMD) were carried out in an explicit membrane-like bilayer, driving LAT1 structure from the initial to the final state of the transport. A different behaviour was observed between Tyr and DIT. Only at the highest spring value both Tyr and DIT could pass through the LAT1 transport channel, the solute Tyr being faster that the inhibitor DIT. Under these simulation conditions, an additional putative inner gate was identified. Decreasing the spring constant, Tyr and DIT progressively lost the ability to pass across the LAT1 transport channel. Tyr appeared to interact with some specific residues; conversely, DIT established only transient interactions with residues located in the external part of the transport channel. Overall, these tMD simulations allowed us to describe for the first time the amino acid transport mechanism of LAT1. This approach can be proposed for developing novel inhibitors, useful for further pharmacological applications in cancer therapy (Palazzolo et al, Front Chem 2018, 6:350).

Keywords: LAT1; amino acid transporters; molecular docking; targeted molecular dynamic.

**Poster 088**

**Roxane Prieux***

**How is cigarette smoke involved in the development and/or exacerbation of inflammatory skin disorders?**

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Cutaneous tissue is the largest organ of our body directly exposed to environmental stressors of which cigarette smoke (CS) is one of the most toxic. During the last years, it has been shown that CS and more specifically the oxidative compounds derived from incomplete combustion of the cigarette can affect the skin. Indeed, CS is not only associated with pulmonary and cardio vascular diseases but also contributes to numerous skin diseases and conditions. Cigarette smoking is linked to various dermatological conditions and pathologies: poor wound healing, squamous cell carcinoma, melanoma, acne, psoriasis, eczema, and hair loss. CS can induce an inflammatory response upon interaction with skin, thus leading to inflammatory skin conditions. This dysregulation of the immune system is expressed by an increased production of inflammatory mediators leading to a downregulation the expression of epidermal proteins, including filaggrin, loricrin, and involucrin. As a result, keratinocytes hyperproliferate and incompletely differentiate, hence creating an impaired skin barrier more prone to pollutants penetration. At the University of Ferrara, we aim at understanding how cigarette smoke is involved in the development and/or exacerbation of skin pathologies such as psoriasis and atopic dermatitis. To better understand the link between skin disorders and cigarette smoke exposure, we are working with both healthy and pathological *in vitro* skin models such as reconstructed Human Epidermis (RHE) and Full Thickness (FT) which have been exposed to cigarette smoke. Post exposure cytotoxicity, inflammation, oxidative stress as well as molecular and morphological change have been assessed.

*travel grant recipient

Keywords: Cigarette smoke exposure, inflammation, oxidative stress, *in vitro* skin model, skin pathologies.

**Poster 089**

**Linda Reilly**

**Testing the TTC using EFSA's OpenFoodTox database**

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Improving analytical methodologies have led to detection of an increasing number of compounds present at low concentrations, with insufficient toxicity data for risk assessment. Alternative methods such as the Threshold of Toxicological Concern (TTC) approach could be used as a prioritisation tool for a large cohort of compounds requiring toxicity testing. The TTC concept integrates data on exposure, structure, and metabolism to identify a safe exposure threshold value. There is an expressed need to expand the current TTC dataset therefore, this study aims to test the TTC concept using EFSA's OpenFoodTox database. The TTC concept was applied *in silico* to categorise over 300 compounds from EFSA's database into one of three classes, under the Cramer decision tree. These three classes are reflective of toxicity and identify low, moderate, and high toxicity. The lognormal cumulative distributions of reference points for compounds were plotted for each of the three classes. The 5th percentile of each cumulative distribution was used to derive a TTC value by applying an uncertainty factor of 100, and factoring in average human weight. Results showed the threshold value derived for Cramer class III was protective of the original threshold value. However, Cramer class I fell below the original threshold value, while Cramer class II had too few compounds to carry statistical weight. Our analysis shows that although the TTC approach is protective also for chemicals pertinent to food safety further expansion of the TTC compound dataset would be beneficial for potential refining of the TTC values.

Keywords: Threshold of Toxicological Concern; TTC; food ingredients; Cramer classification.

**Poster 090**

**Selina Rinaldi**

**Assessment of biomass particle induced lung toxicity using bronchial and alveolar mucosa models along with screening for protective naturally occurring bioactive compounds**

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Inhalation of biomass smoke (BMS) is known to be a risk factor for developing chronic obstructive pulmonary diseases (COPD) which is among the major causes for death world-wide. BMS arises from combustion of wood, coal or other biomass fuels, which are used for heating or cooking in developing countries. Therefore, the sensitive group affected by BMS, includes mainly women living in poverty. BMS exposure along with malnutrition or coinfection exacerbate the situation. Therefore, supplementing phytochemicals with anti-oxidative and anti-inflammatory properties might be a future prophylactic treatment to prevent the women from developing chronic lung diseases. Since research and data on this topic is still missing, we aim to investigate mechanisms and effects of phytochemicals as an affordable alternative to reduce susceptibility on developing COPD following exposure to BMS. To accomplish this, we will use bronchial (human primary bronchial epithelial cells, PBEC) and alveolar (NCI-H441 cells) mucosa models developed in air-liquid interface (ALI). The model will be pre-treated with phytochemicals about 24h prior to BMS exposure. The following endpoints will be in focus:

cytotoxicity, oxidative stress and inflammation. Cytotoxicity will be assessed by using trypan blue staining and LDH-assay. To detect the reversal or therapeutic effect of phytochemicals, phytochemical pre-treated biomass group will be compared to untreated biomass group. Anti-inflammatory responses, anti-oxidative stress, tissue injury and repair will be analysed at protein and gene expression level by ELISA and qRT-PCR respectively.
Keywords: Biomass smoke, COPD, Air-liquid interface, ALI, 3R.

**Poster 091**

**Francesco Salvi**

**Identification of target proteins potentially related to muscle and metabolic adverse effects of statins by the combination of *in silico* and *in vitro* approaches**

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Background. Statins are commonly used to prevent cardiovascular disease (CVD) and to treat patients with hypercholesterolaemia since they control the level of plasma total and LDL-cholesterol by inhibiting HMG-CoA reductase, the rate limiting enzyme of the mevalonate pathway for the cholesterol biosynthesis. The use of these drugs is associated with adverse effects due to their interaction with off-targets in liver, muscle and brain. In particular, they can cause statin-associated muscle symptoms (SAMS) and de novo type II diabetes mellitus (T2DM). However, the molecular targets behind the pathophysiology of these side effects still wait to be clarified. Aim of the study. We aim at assessing potential off-target proteins responsible for the above-mentioned adverse effects of statins, through a combination of *in silico* plus *in vitro* approaches. Studi design. *In silico* phase: taking advantage of the SPILLO-PBSS *in silico* tool, we will identify potential off-target proteins, based on the ability of statins to interact with their orthosteric site. *In vitro* phase: using appropriate *in vitro* cell-based systems, including human HepG2 cells, we will validate the findings obtained from the *in silico* approach, by means of enzymatic activity assay, modulation of gene and protein expression and related intra-cellular signalling pathways, as well as metabolic changes. Potential outcomes. The identification of specific off-target proteins for statins may help to define a personalized therapeutical approach for patients, preventing the occurrence of statin-related adverse effects.
Keywords: Statins, off-target protein, *in silico*, *in vitro*, adverse effects.

**Poster 092**

**Pilar Samperio Ventayol**

**Deciphering Salmonella early invasion events in an *ex vivo* mammalian intestinal epithelium model**

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*Salmonella enterica* infections constitute a globally prevalent cause of foodborne intestinal disease. To prevent the infection, it is essential to better understand the initial steps of enterobacterial invasion and the intestinal epithelium response. In the past, most of the cellular and molecular host-pathogen interactions have been studied in simplistic culture settings, using tumor-derived cell lines. These models may not represent the arrangement and physiology of the intestinal epithelium and may hence have limited physiological relevance. On the other hand, *in*

*vivo* infection experiments lack precision for cell biology and single event studies, while also standing the risk of being questionably cruel. The aim of our work is to transfer the cell biology of *Salmonella enterica* Typhimurium (*S.* Tm) infection from simple cell culture settings into a model that enables cell biology studies in a more natural infection context, but still avoiding animal experimentation. For that, our research relies on recent progress in *ex vivo* culture of primary intestinal epithelium in the form of so called organoids, which we adapted for the study or S. Tm early invasion events. The combination of this breakthrough culture model with state-of-the-art microscopy enables the study of cellular/subcellular events during the first minutes of epithelium invasion, what would be unfeasible in animal models. In response to S. Tm stimulation, we observed both single cell and tissue-scale intestinal epithelium responses. Our present work aims to uncover the cell biological mechanisms underlying S.Tm invasion of the physiologically arranged epithelium and the link to specific S. Tm effectors.

Keywords: Organoid; epithelium; primary cell; intestine; Salmonella; host-pathogen interaction; microscopy.

**Poster 093**

**Maria Sampieri**

**Zebrafish as an alternative method for toxicological studies**

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According to the Directive 2010/63/EU on protection of animals used for scientific purposes, fish embryos do not fall into regulatory frameworks dealing with animal experimentation. Therefore, according to the 3Rs principles, zebrafish (Danio rerio) embryos are considered as replacement or refinement methods. Since different industrial chemicals, plant protection products, biocides, pharmaceuticals and other chemical products are recognized causes of skin sensitization, it is needed a thorough understanding of the toxic mechanisms to make predictions of the toxic potential of novel compounds. Thus, because of its small size, zebrafish is emerged as a feasible vertebrate organism for chemical risk testing. Fish acute toxicity tests are standardized by OECD guidelines as the Fish Embryo Toxicity (FET) test that provides exposure of zebrafish embryos to chemicals for a total of 96 hours. Every 24 hours, under the microscope, up to four apical observations are recorded as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail bud from the yolk sac and lack of heartbeat. Besides, other developmental toxicity endpoints (malformations) may be observed such as a pronounced yolk sac oedema, a pericardial oedema or also a spinal curvature (scoliosis). Then, in order to assess whether the skin sensitization given by incubation with several chemicals was really measurable, the Fish Interleukin 8 (IL8) ELISA Kit was performed. The preliminary results obtained so far seem encouraging. However, they need to be deepened and confirmed through further ELISA assays and by comparison with other *in vitro* methods.

Keywords: Zebrafish, skin sensitization, toxicology.

**Poster 094**

**Nicoletta Santori**

**An integrated *in vitro* approach to study Microcystyn detoxification by Glutathione-S-Transferases: from toxicokinetic parameters to the possible identification of differently susceptible population groups**

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Daily we are exposed to xenobiotics via the environment, water and food which can be toxic, in relation to the exposure dose. These substances are generally lipophilic, therefore organisms, as a defence, promote their excretion, through biotransformation or conjugation catalysed by specific enzymes as Glutathione-S-Transferases (GSTs) characterized by different polymorphic isoforms. Our work has been focused on the application of an integrated approach to study the *in vitro* detoxification (that is conjugation with glutathione mediated by GSTs) of two variants of Microcystins (MC), a group of natural hepatotoxic compounds with more than 100 congeners. MC are characterized by very similar structure but the difference in some amino-acidic groups leads to different *in vivo* toxicity. The *in vitro* inhibition potency of protein phosphatase by single MC congener, the first step of their mechanism of toxicity is comparable: therefore the toxicokinetic of MC seems to be the critical point to explain congener-dependent toxicity. Human hepatic recombinant isoforms (GST A1, A2, A4, M1, T1 T2, P1, and O1) were used followed by human hepatic cytosol, where all the isoforms are present. The different affinity of the single recombinant isoforms was evidenced and the kinetic parameters Vmax, Km and Cli, were derived. Differences in GSTs contribution at low and high MC and/or GSH concentrations have been evidenced. Considering that some GSTs, as T1 and M1 are highly polymorphic (these enzymes are lacking in the 50% of Caucasian population) the toxicokinetic information can suggest different susceptibility of population groups to MC toxic effects.

Keywords: Microcystin, GST, toxicokinetics.

**Poster 095**

**Lena Schaller**

**The genetic landscape of human SLC transporters**

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The rise of Next-Generation Sequencing (NGS) techniques has groundbreaking applications in the field of pharmacogenomics, the branch of genetics focused on understanding how variations within the human genome can affect an individual's response to pharmaceutics. The majority of research using NGS data has focused primarily on the effects of common polymorphisms, with some findings translating into pharmacogenomic drug labels designed to promote therapeutic efficacy while limiting adverse effects. However, recent studies have revealed that rare genetic variants greatly outnumber common variants, and that these rare variants contribute substantially to the functional variability of most pharmacogenes. The resulting changes in protein functionality are a major cause of adverse drug reactions and lowered therapeutic effect. The future of precision medicine demands the characterization of the extent of genetic variation within human pharmacogenes. The solute carrier (SLC) gene superfamily makes up 48% of known transport-related genes, and encodes nearly 400 membrane-bound proteins. SLC transporters play a significant role in the cellular uptake of a

variety of pharmaceuticals, including chemotherapeutics (i.e. topoisomerase inhibitors, antimetabolites, and taxanes). The SLC protein family is also an emerging drug target class in itself, with selective serotonin reuptake inhibitors (SSRIs) and sodium/glucose co-transporter inhibitors already approved for the treatment of depression and diabetes, respectively. Despite its pharmacological relevance, the genetic variability within the SLC gene family has not been characterized in a large-scale, multi-population analysis. This project will map the genetic variability of the SLC superfamily in a systematic analysis through the computational assessment of NGS data.

Keywords: SLC; precision medicine; targeted therapy; rare variants; variant frequencies.

**Poster 096**

**Julia Scheinpflug***

**A Developmental Model for Intramembranous Ossification Based on a "Bone-on-a-Chip" for Toxicity Testing and Basic Science**

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Conventional 2D cell culture is limited when cellular composition and physical parameters (pH, pO2 or mechanical forces) of a certain tissue need to be imitated. Possible technologies to physiologically recreate tissues *in vitro* are advanced 'Organ-on-a-chip' systems. In our project a micro-physiological 'Bone-on-a-chip' is developed to model the process of intramembranous ossification. For this, key parameters that influence bone development and function like hypoxia and mechanical load are monitored and regulated by a microfluidic platform. Further, the platform will house a scaffold-free organoid, initially generated from primary bone-forming osteoblasts. Therefore, osteoblasts are isolated and expanded from primary human bone tissue. Since samples from different donors are used, a high inter-individual variability is expected. Consequently, all donated cell samples have to be characterized in advance for their viability, proliferation and phenotype in order to group cells according to these parameters, thus minimizing variability. After successful generation of organoids from osteoblasts and their adaptation to the micro-physiological system, co-culture systems that also include mesenchymal stromal cells will be generated and evaluated. To simulate bone remodeling, the orchestrated process of bone formation and bone resorption, bone-resorbing osteoclasts will be introduced into the organoid. Further, vascularization of the organoid is planned to ensure an optimized supply of oxygen and nutrients throughout the organoid. This project might enable the implementation of a developmental model for intramembranous ossification for applications in basic science and toxicology and has thus a high potential to reduce and replace animal testing in the context of bone biology.

*travel grant recipient

Keywords: Bone-on-a-chip, osteoblasts, organoid.

**Poster 097**

**Johannes Schimming**

**Application Of Imaging-based Nrf2 Pathway Activation To Support A Read Across Of Phenolic Compounds**

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Biological support for read across approaches is gaining major attention. Damage caused by oxidative stress and soft electrophiles are common during chemical induced liver injury. The correct identification of the liability of a compound to cause such damage is essential for mechanism-based testing and adversity prediction. Various structural similar phenolic compounds are liable for causing oxidative stress-mediated cytotoxicity. The NRF2-mediated antioxidant stress response is activated after oxidative and electrophile-derived cell stress. Here we evaluated whether fluorescent protein reporter cell lines for the Nrf2 pathway complemented by TempOSeq targeted RNAseq could support a read a cross for structural similar phenolic compounds. BAC HepG2 GFP-NRF2 and GFP-SRXN1 reporter cell lines were exposed to 6 hydroquinone like compounds with redox-cycling potential, 12 redox-cycling negative compounds with alkyl side chain and 3 phenols without an alkyl side chain. Both HepG2 BAC-GFP reporter cell lines allowed identification of redox-cycling positive hydroquinone like compounds. A concentration- and time-dependent compound specific activation of SRXN1-GFP was observed, and all compounds that induced SRXN1 also induced stabilization and nuclear translocation of NRF2-GFP. Cell death was induced at high concentrations for all redox-cycling phenols. Dual-exposure of hydroquinones showed an additive dose effect. The redox-cycling negative phenols were inactive in both NRF2-GFP activation as well as SRXN1-GFP induction. Alkylated phenols induced onset of cell death, likely independent from oxidative stress induction. To deepen our mechanistic understanding of the phenol-induced toxicity, we also used TempOSeq targeted RNAseq of ~3000 toxicity related genes, including Nrf2 target genes, in HepG2 and cryopreserved primary human hepatocytes. The transcriptomics analysis confirmed the selective activation of NRF2 target genes by redox-cycling phenols and did provide further insight in the differential pathway activation by the three different phenol groups. In conclusion, our data indicate that our NRF2 GFP-BAC reporter cell models are a valid test system to provide biological support for read across in cases of anticipated oxidative stress activation. This work was supported through the EU H2020 EU-ToxRisk project (grant agreement 681002).

Keywords: Oxidative injury.

**Poster 098**

**Trushenkumar Maganlal Shah**

**Implementation of alternatives to animals in academic research**

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In the whole world, the use of animals in research has increased with the advancement of research and development in Biomedical science. Every year, millions of animals are used all over the world. In 2011 around 3.71 million animals were used for research in UK. In the USA, in

the year 2009 the total number of animals used were 1,131,076. However, there are so many countries which official statistical data do not have to justify use of animals in various kind of research. As per the available data, it is clear that, huge numbers of animal are used in research either in academia or commercial organizations without submitting data of animal usage to national regulatory authorities. Moreover, in most of drug study we obtained inaccurate result because of vast physiological variation of animal and human so for this instance, to control unethical practice and reduce animals in research, there is keen need to establish legislation and regulatory policies to restrict use of laboratory animals and to promote use of alternatives to justify research work. In addition, huge numbers of animal are used in academia for master's research, and among the all academic research work, only few explorations are considered for further investigations. So, I strongly believe that there is best scope to reduce use of animals in academic level by promoting use of several non-animal testing and they can't provide accurate result but provide comparable information about testing of drug. In addition, the data obtained from studies performed on alternatives must be complied and statistical analysis must be submitted to regulatory authorities to make comparative evaluation for justification of use of alternative to animals. Furthermore, awareness and training programme must be arranged to promote use of alternatives in academia. Implementation of the Three Rs (replacement, reduction and refinement of animal experiments) should be expected to result in a decline in animal use, but without regular, accurate statistics, this cannot be monitored.

Keywords: mammalian models, drug discovery, drug toxicity, novel therapy.

**Poster 099**

**Kristina Shchitko**

**Comparation of NAM's costs and their risks**

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In the last years on the scientific "podium" we can see more and more appearance of the terms as *in vitro* assays, *in silico* models, virtual approaches, computer modelling, in other words NAM (non animal methodologies). In parallel to the ethical and scientific considerations, it will be useful to compare the costs of animal models and NAM, both in term of resources employed (money and time) and of results obtained, considering also the cost, for the society, of a missed or retarded hazard/risk characterization. The aim of my work will be quantitating procedure of the risk characterization in different context (drugs, pesticides etc.) using traditional and new approaches.

Keywords: NAM, costs, hazard/risk.

**Poster 100**

**Julijana Simonović**

**Bone cell population models and their advantages: simplicity, purposefulness and exactness**

Julijana Simonović

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Equations of bone turnover balance between bone resorption and formation together with time changes of bone resorbing and forming cells numbers form the mathematical model of system of ordinary differential equations (ODEs) capable to describe process of bone remodelling inside one bone multicellular unit (BMU) or compartment (BMC). The level of complexity depends of number of cell lineages involved in signalling processes and the number of parameters that describe

biochemical changes in mechanotransduction of the signals. The bottom-line goal of the parameter's importance defines the appropriate model to be used. Two models will be discussed and presented in this paper in order to compare them and to underline the necessities of complex representations and simultaneous multi-parametric analysis. The first model comprises of the system of coupled ODEs with power-law nonlinearity terms that describe autocrine and paracrine signalling of the cell lineages inside BMU. The second contains the cooperative binding Hill`s equation terms that describe decoy-receptor linking processes of realised molecules inside BMC. The former is simpler and more elegant however still has a power to describe importance of involved process. The second model is more detailed and complex with an increased number of equations and of introduced parameters. In both models the multi-parametric analysis give the opportunity to decide, which one of the parameters is the more important depending of the purpose of the model. The models are explored in numerous numerical (in-silico) experiments also provide more possibilities for interpreting the development of interventions for possible bone trauma and diseases.

Keywords: Bone cell population model, system of ordinary differential equations, cell signalling, and nonlinear multi-parametric analysis.

**Poster 101**

**Saskia Sperber**

**Characterization of drug induced liver injury using metabolomics *in vitro***

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Analogous the *in vivo* metabolome database MetaMap®Tox (containing the profiles of > 900 compounds), an *in vitro* liver metabolome platform using the liver carcinoma-derived HepG2 cell line has been established. This *in vitro* database contains the intracellular metabolite profiles of more than 80 toxicants with different modes of action (MOA) as well as negative substances. The MOA for liver toxicity covered within the database range from e.g., peroxisome proliferation, liver enzyme induction, steatosis to general liver toxicity. Within the BMBF- and ZonMW-funded project SysBioToP, different liver toxicants are currently being tested in different *in vitro* liver cell systems using imaging technologies, transcriptomics and metabolomics. We have analysed the metabolome of HepG2 cells treated with 9 substances known to induce drug induced liver injury in a human clinical setting and a negative control. The metabolome consisted of 236 unique metabolites, including amino acids, carbohydrates, lipids, energy metabolites, nucleobases, vitamines and cofactors. Our data show that valproic acid, paracetamol, and ciprofloxazine did significantly change the intracellular HepG2 metabolome, whereas diclofenac is only hardly distinguishable from the controls. A principal component analyses shows that valproic acid and paracetamol build clusters of samples separate from control but close to each other, whereas ciprofloxacine builds a cluster which clearly separates from control, but also from the other treatments. The differences in the *in vitro* metabolome response might reflect the *in vivo* effects both in terms of the underlying mode of action as well as the potency and frequency of liver injuries in the clinical application.

Keywords: Metabolomics, Hepatotoxicity, DILI, *in vitro* prediction.

**Poster 102**

**Thi Phuong Tao**

**Microfluidic 4-Organ-on-a-chip for ADMET profiling**

Ilka Maschmeyer[1], Alexandra Lorenz[1], Anja Ramme[1], Juliane Hübner[2], Tobias Hasenberg[1], Sophie Bauer[1], Uwe Marx[1] and Thi Phuong Tao[1]

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Current *in vitro* models are lacking the systemic approach and therefore fail to predict the crosstalk between different organ cultures. Here, we present a new multi-organ approach to overcome this problem. Four human organ equivalents are combined by a microfluidic flow in a bioreactor the size of a microscopic slide: A primary human small intestine model cultured on a membrane forms a barrier function to the first circuit for absorption analysis. An on-chip micro-pump enables distribution from the basolateral side of the intestine model to a liver equivalent, where potential substances can be metabolised. The microfluidic channel further passes a membrane, seeded with renal proximal tubule cells, which separate the first circuit from a second circuit on the apical side of the kidney model, which allows excretion. The addition of a forth organ (e.g. a skin model) enables the analysis of potential toxicity. The combination of the four organs was cultured for up to 28 days and results showed constant expression of constitutive phase I and II enzyme expression on protein and mRNA level in liver tissues, constant expressions of glucose transporters and a constant close to physiologic TEER value for intestinal tissues. Renal proximal tubule cells showed polarisation and a steady expression of tight junctions and metabolic activity. Thus, a potential new tool for sub-systemic substance testing with a potential for ADMET profiling has been developed.

Keywords: Multi-Organ-Chip, ADMET, Microphysiological system.

**Poster 103**

**Belinda Trachsel**

**Short cell-penetrating peptides as inhibitors of amyloid protein aggregation**

Belinda Trachsel, Sebastian Wärmländer

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Oligomeric aggregates of amyloid proteins are considered cell-toxic and are part of the pathology of multifold health conditions such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Type 2 Diabetes mellitus (T2DM). Studies in cells have already confirmed that short cell-penetrating peptides (CPP:s) can inhibit amyloid toxicity and AFM images have demonstrated that amyloid fibers change their structure in their presence. These results suggest that CPP:s and amyloid proteins can co-aggregate leading to lower toxicity of the amyloid fibers. Tests in animals and humans have furthermore shown that the studied CPP:s are well tolerable with little to no side-effects. All in all, these properties make them promising drug candidates for the treatment of amyloid diseases. This project aims at investigating the amyloid-inhibiting properties of CPP:s with the help of *in vitro* techniques such as NMR, circular dichroism (CD), fluorescence spectroscopy, TEM, AFM and thereby further elucidate their mechanism of action.

Keywords: cell-penetrating peptides, amyloid protein aggregation, amylin, β-amyloid.

**Poster 104**

**Mieke Van Mulders**

**RE-Place: a database compiling New Approach Methodologies**

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In Belgium, about half a million laboratory animals are being used on a yearly basis for several scientific and educational purposes1. Importantly, an increasing number of alternative methods to animal testing such as in chemico, *in silico* and *in vitro* are being developed. These are also referred to as 'New Approach Methodologies' (NAMs). Unfortunately, the expertise on NAMs is extremely scattered and there is a lack of communication between different laboratories. In 2017, the Flemish and Brussels regions initiated the project 'RE-Place' which aims to centralize the available knowledge in a database and make it more accessible to the public. An online tool has been developed in order to collect specific information on the NAMs that are currently being used. The requested information was originally based upon the template of 'EURL ECVAM DataBase service on ALternative Methods to animal experimentation' (DB-ALM), but was extensively adapted according to the feedback of the RE-Place steering committee members. These are experts from academia, industry and government institutions with extensive know-how in the field of NAMs. By centralising the available expertise, it will be possible to identify the existing gaps and stimulate the development of new techniques, methods and strategies. The database can evolve into a broader platform where researchers will be able to connect with peers and partners to engage in new collaborations. RE-Place will allow knowledge sharing between different parties (scientists, regulators, industry, ethical committees, animal welfare bodies and the general public), support education and training, and promote the use of NAMs. Reference: 1. Animal Welfare, Department Omgeving, "Statistieken Proefdieren (België)", consulted on 09/01/2019, https://www.lne.be/cijfers-en-statistieken-dierenwelzijn.

Keywords: 3R, replacement, alternative methods, New Approach Methodologies, *in vitro*, *in silico*, in chemico, database.

**Poster 105**

**Melita Videja**

**Metformin decreases trimethylamine N-oxide level in db/db mice without improving insulin sensitivity**

Videja Melita[1], Kuka Janis[1], Liepins Edgars[1], Makrecka-Kuka Marina[1], Liepins Janis[2], Makarova Elina[1], Grinberga Solveiga[1], Dambrova Maija [1,3]

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Trimethylamine N-oxide (TMAO) is a gut microbiota-derived metabolite, which is associated with cardiovascular risks and incidence of diabetes in clinical studies. However, it remains unclear whether TMAO is just a biomarker of diet-derived detrimental processes or it induces direct detrimental cardiometabolic effects. The aim of the present study was to investigate the effects of metformin on microbial metabolism of choline *in vitro*, TMAO plasma levels *in vivo* and the relation between typical markers of type 2 diabetes and increased TMAO levels in plasma. Metformin was administered to db/db mice at a dose of 250 mg/kg for 8 weeks and plasma samples were collected 4 and 8 weeks after the start of the treatment. Diabetic db/db

mice when compared to non-diabetic db/L mice presented 10-13-fold higher TMAO plasma concentrations. Metformin administration significantly decreased TMAO levels 1.8-2.0-fold when compared to db/db control mice and was also found to decrease production of TMAO precursor trimethylamine (TMA) *in vitro* by gut bacteria and TMAO production *in vivo* when choline was administered to facilitate the gut microbiota-dependent TMA/TMAO production. At the same time metformin treatment had no effect on insulin sensitivity and also on glucose and insulin plasma concentrations in db/db mice in both fed and fasted states. Our data provide evidence that metformin can significantly decrease TMAO level in diabetes. These data support the hypothesis of TMAO as a metabolic disease risk marker and warrant further investigation of TMAO for diabetes research applications.

Keywords: Trimethylamine N-oxide, diabetes, metformin.

**Poster 106**

**Roberta Visone**

**Beating organs-on-chips as advanced platforms for drug screening and disease modelling**

Roberta Visone[1,2], Paola Occhetta[1,3], Stefano Ugolini[1], Daniela Cruz-Moreira[1], Andrea Mainardi[1,3], Andrea Barbero[3], Marco Rasponi[1,2]

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Drug discovery is a long and expensive process, whose efficiency is often limited due to the poor predictivity of *in vitro* models currently exploited in the pre-clinical phases. Here we present new beating organ-on-chip (OOC) platforms mimicking the mechanical stimulation that tissues sense *in vivo*, to enhance the functionality of the reproduced human engineered microtissues. We developed two platforms: i) uHeart, a spontaneously beating heart-on-chip and ii) uKnee, the first model of osteoarthritic (OA) cartilage. Human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) were embedded in fibrin hydrogel and cultured within uHeart for 7 days under mechanical stimulation (cyclic uniaxial strain, 10-12% at 1Hz). Once synchronous beating was reached, the electrophysiological signals (i.e. field potential) were recorded through paired electrodes specifically inserted. The effects of compounds known to alter the cardiac electrical activity were evaluated at incremental concentrations. Terfenadine and Sotalol prolonged the repolarization time of cardiac microtissues at 100-1000 nM and 10-60 µM, respectively. Primary human articular chondrocytes were embeded in a poly(ethylene-glycol)-based hydrogel and matured under chondrogenic conditions within uKnee for 2 weeks. Then, constructs were subjected for 7 days to a cyclical compression at hyperphysiological levels (i.e. 30%) to elicit an OA-correlated biological response. Anti-inflammatory and anti-catabolic responses of four drugs clinically used (Rapamacyn, Celecoxib, IL-1Ra and dexamethasone) and one compound currently under investigation were measured and resulted consistent with data from animal studies. The proposed microfluidic reliable pre-clinical models represent new powerful tools for efficient *in vitro* drug screening and disease modelling.

Keywords: Cardiac model, electrophysiology, cardiotoxicity, QT syndrome, cartilage model, osteoarthritis, DMOADs.

**Poster 107**

**Yafan Wang**

**Early-life exposure to cobalt and child development**

Yafan Wang, Klara Gustin, and Maria Kippler
Metals and Health, Institute of Environmental Medicine, Karolinska Institutet.

Cobalt compounds are widely distributed in the environment and they are used in various anthropogenic activities. In the general population, exposure occurs predominately via the diet, but is some cases also via the environment and medical applications (drugs and prostheses). Excessive exposure, in both experimental and observational studies, has been associated with several adverse health effects, primarily on the neurological, cardiovascular, and endocrine systems. In pregnant women, some previous studies indicated that cobalt would partly transfer across the placental barrier. However, studies concerning cobalt exposure during pregnancy and potential implications on pregnancy outcomes and later child health are limited. The aim of the present study is to determine the exposure to cobalt in pregnant women, identify potential sources of exposure, and evaluate the associations with adverse pregnancy outcomes and infant sensitization towards common allergens as well as child cognition. This is explored using three different mother-child cohorts in different geographical locations (i.e. northern Sweden, Greece, and Bangladesh). The exposure to cobalt has already been determined by measurements in maternal blood and urine during pregnancy using inductively coupled plasma mass spectrometry (ICP-MS). In the Swedish mother-child cohort detailed questionnaires concerning environmental exposures and diet will enable identification of important exposure sources using principal component analysis. Furthermore, the exposure biomarkers will be related to various child health outcomes in the different cohorts, i.e. pregnancy outcomes (preterm birth and birth anthropometry), sensitization towards common allergens, and cognitive function and behaviour. The latter evaluations will primarily be done using various non-parametric test followed by multivariable-adjusted linear or logistic regression analyses.

Keywords: Cobalt, adverse pregnancy outcomes, infant sensitization, child cognition.

**Poster 108**

**Paulina Werner**

**Does AMPK deficiency promote the toxicity of environmental risk factors in Parkinson's disease?**

Paulina Werner
National University of Singapore/Karolinska Institute.

Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by a progressive and selective loss of dopaminergic neurons in the substantia nigra of the midbrain. Currently it is estimated that more than 10 million people worldwide are diagnosed with PD. Despite great advances in PD research, the etiology of PD is still not fully understood. Nevertheless, several described mechanisms of neuronal death in PD pathogenesis converge and implies oxidative stress having a major role. The main risk factor for PD is unquestionably age, however epidemiological studies have suggested that exposure to environmental toxicants such as pesticides might increase the risk of developing the disease. Alongside this, emerging evidence suggests that energy dysregulation may underlie neurodegeneration in PD. A key cellular regulator of energy metabolism is AMP-kinase (AMPK). Notably, the level of active AMPK is selectively downregulated in the ventral midbrain of PD animal models (unpublished

results from host's laboratory), suggesting that deficient AMPK activity could predispose midbrain dopaminergic to neurodegeneration. In this study, the potential link between exposure to environmental toxicants and AMPK will be investigated in order to investigate whether AMPK deficiency promotes toxic effects of environmental toxicants. This will be done through analysis of patterns of neurotoxicity in AMPK-KO cell cultures and AMPK-KO mice exposed to environmental toxins.

Keywords: Environmental toxins, AMPK deficiency, Parkinson's disease.

**Poster 109**

**Ilaria Zanoni**

**Silver 300K characterization in biological compartments for high and low exposure range**

Ilaria Zanoni [1,2], Carlo Baldisserri[1], Simona Ortelli[1], Felice Carlo Simeone[1], Magda Blosi[1], Lara Faccani[1], Jamie Lead[3], Anna Luisa Costa[1]

[1]CNR-ISTEC-National Research Council of Italy, Institute of Science and Technology for Ceramics, Faenza, RA, Italy; [2]Clinical Unit of Occupational Medicine, Department of Medical and Surgical Sciences, University of Trieste, Trieste, Italy; [3]SmartState Center for Environmental Nanoscience and Risk (CENR), Arnold School of Public Health, University of South Carolina, South Carolina 29088, USA.

Silver nanoparticles are well known for their very promising antibacterial properties that are exploited to produce a great number materials and nanomaterials already on the market. At the same time, aggregation and dissolution ability of silver are the main driving forces that determine final antibacterial outcomes and any possible negative interactions with the final biological target. Nevertheless, their dependence on concentration is still not well investigated, hindering any prediction on biological activity in different testing conditions. For these reasons, the aim of my work is to characterize PATROLS silver nanoparticles (Ag 300K NPs) at high and low exposure concentrations in water and in RPMI media, trying to mimic the behaviour respectively at short and long term exposure. To reach this aim, we used different techniques in function of concentration range, obtaining a complete view of nanoparticles behaviour. Size, dissolution and redox activity are the main results obtained for high concentration and short exposure time, while for low concentration and long exposure time Single Particle ICP-MS was adopted to provide data on size distribution, dissolution and number of nanoparticles. All these results are shared within PATROLS network and partners to provide useful knowledge for the development and implementation of toxicity *in vitro* test and of computational tools for modelling evaluation, at the final aim to minimise animal testing.

Keywords: Silver nanoparticles; (eco)toxicity; long exposition; SP-ICP-MS.

**Poster 110**

**Anna Zettergren**

**Exposure to Phthalates and Obesity in young adults**

Anna Zettergren (Supervisor and co-author Anna Bergström)

Karolinska Institutet, Sweden.

Phthalates are a group of chemicals that constitutes the most common plasticisers on a global scale. They are abundantly found in everyday products including general purpose PVC, cosmetics, wall coverings, cables and synthetic leather products. Human exposure to phthalates include eating food or drinking beverages that have been in contact with phthalates in packaging, breathing in household dust containing phthalates or skin contact with phthalate products. Due to

their widespread exposure to humans, phthalates have been extensively studied in recent years, and their health effects are still being revealed. It has been established that phthalates cause various types of toxicity, most prominently being endocrine and reproductive effects. However, there are also indications of phthalates having metabolic effects, including increasing the risk of developing obesity. To investigate phthalates role as obesogens, data from the Stockholm birth cohort BAMSE is being analysed. Spot urine samples (n=1000) from participants at the 24-year follow up are being analysed for a number of phthalate metabolites using LC/MS/S technique. This data will be compared to clinical data collected at the same timepoint regarding weight, height and adiposity (from bioelectrical impedance analysis data). Associations between phthalate concentrations in urine and obesity will be investigated using multiple logistic regression.

Keywords: Phthalates, Obesity, BMI, Bioelectrical impedance analysis, Cohort, Epidemiology, BAMSE.

**Poster 111**

**Boyao Zhang**

**Effect of microbe-derived AHR ligands on mouse neurodevelopment**

Boyao Zhang

Karolinska Institutet, Sweden.

Aryl hydrocarbon receptor (AHR) has been known as a signal transducer that dimerises with its nuclear translocator ARNT upon binding of its ligands. Dioxins are the classic AHR ligands that induce adverse effects in the body but now there is increasing evidence showing that endogenous AHR ligands generated by the body and gut microbiome can have a wide range of effects in mice. In this study we aimed to understand the role of AHR in different cell types when they are exposed to potential AHR ligands.

Keywords: Aryl hydrocarbon receptor, mouse neurodevelopment, gut microbiome.

**Poster 112**

**Katarina Živančević**

**The influence of phthalates (diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP)) exposure on diabetes mellitus type 2 development – analysis of toxicogenomic data**

Katarina Živančević

Department of Toxicology “Akademik Danilo Soldatović”, University of Belgrade - Faculty of Pharmacy, Belgrade.

Diabetes mellitus type 2 is one of the significant public health issues and is considered a risk factor for development of many diseases. An important pathogen mechanism in the onset of diabetes mellitus type 2 is change in the expression and activity of genes caused by chemicals that interfere with the functions of the endocrine system. These chemicals also include phthalates. The aim of this study was to examine a correlation between the exposure to most common phthalates (diethylhexyl-phthalate (DEHP) and dibutyl-phthalate (DBP)) and development of diabetes mellitus type 2 by analyzing toxicogenomic data. The Comparative Toxicogenomic Database (CTD) was used to obtain the information on phthalate and gene interactions and their associations with diabetes mellitus type 2. Query tools such as Chemical-Gene interactions, MyVenn and Set Analyzer were used in this study. Obtain results indicate that DEHP and DBP correlate with the development of diabetes mellitus type 2 –DEHP by interacting with 65 genes involved in 94 moleular pathways, and DBP by interacting with 60

genes involved in 84 molecular pathways. Among these genes, INS, INS1, INSL3 and ADCY5 stand out askey factors in the regulation of insulin sensitivity and glucose homeostasis. Additionally, both phthalates interact with PPARA and PPARG, which effect on carbohydrate and lipid metabolism can lead to metabolic dysfunction. The toxicogenomic analysis indicates that exposure to examined phthalates can be linked to the development of diabetes mellitus type 2, while further *in vitro* and *in vivo* studies are necessary to confirm these findings.
Keywords: diethylhexyl-phthalate, dibutyl-phthalate, diabetes, toxicogenomics, genes.

**Poster 113**

**Patrícia Zoio***

**Fully-humanized Skin-on-a-chip for medical applications**

Zoio, P.A[1,2], Oliva, A.G. [1], [2]Conde, J.P. [2]
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*In vitro* human skin models are gaining attention for their importance as tools for basic research and for the pharmaceutical and cosmetic industries. However, current commercially available human skin models are only suitable for short-term studies, include non-human extracellular matrix components and lack dynamical flow systems and mechanical forces. In recent years, advances in biomaterials and microfluidics technology made it possible for the culture of artificial skin to move a step ahead giving rise to the development of microfluidic skin-on-chip platforms. These systems are able to reproduce key aspects of the *in vivo* cellular microenvironment by including fluid flow and finely tuned forces. This project aims at developing an innovative microfluidic system to grow and sustain a physiologically relevant human skin model. This approach begins with the production of a fully-humanized skin model by combining the production of a fibroblast derived matrix and the use of an inert porous scaffolds for long-term, stable cultivation, without using animal components. This technique is then combined with the use of a biomimetic “organ-on-a-chip” system which includes dynamic perfusion for continuous supply of nutrients and metabolites. Also, we present a reversibly sealed chip with a module-based architecture that provides an easy to use workflow, an efficient and precise cell seeding and a removable culture insert that can be transferred between modules. We anticipate that this innovative platform will reduce the dependence on animal models and provide a new *in vitro* tissue to evaluate the safety and efficacy of novel drugs and technologies.

*travel grant recipient

Keywords: Skin-on-a-chip, Microfluidics, *In vitro* skin models, 3D cell culture.

**Poster 114**

**Daniela Patricia Pacheco Peneda**

## Engineering *in vitro* Lung Microbiota for Antimicrobial Treatment

Daniela P. Pacheco[1], Anna Ziccarelli[1], Federico Bertoglio[2,3], Natalia Suarez Vargas[1], Francesco Briatico-Vangosa[1], Sebastião van Uden[1], Sonja Visentin[4], Livia Visai[2,3],Paola Petrini[1]

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Cystic fibrosis (CF) mucus exhibits altered chemical and viscoelastic features, limiting its clearance and leading to chronic bacterial infections. Current bacterial culture fails to recreate bacteria communities and microenvironments of lung microbiota. Additionally, it is difficult to induce representative human multibacterial infections in animals. Three-dimensional hydrogels (Bac3Gel) were engineered to recreate lung microbiota by modelling the physico-chemical properties of CF mucus, and supporting bacteria growth. Bac3Gel is mainly composed of alginate, a polysaccharide produced by P. aeruginosa, and mucin. Bac3Gel exhibits similar viscoelastic properties alterations to those reported for CF sputum. Bac3Gel successfully sustains growth of *P.aeruginosa* and *S.aureus*, the prevalent bacteria colonizing the airway CF mucus, with a bacterial concentration of 109 CFU/mL after 24h of “infection”. Bac3Gel, infected for 24h with *P. aeruginosa*, were treated for 24h with three different antibiotics, to which P. aeruginosa is sensitive, and compared in effectiveness to standard bacteria cultures. Bacteria resulted more susceptible to antibiotic treatment under planktonic conditions than when cultured within Bac3Gel, where these instead displayed increased antibiotic tolerances even at high antibiotic 2 concentrations (10 MIC). The sensibility difference between Bac3Gel and planktonic confirmed the wellreported mismatch between planktonic conditions and clinical outcomes. These results indicate that Bac3Gel is a promising substrate to recreate lung microbiota for antimicrobial screening. The versatile production process of Bac3Gel allows to generate microgradients of viscoelastic properties, nutrients, and gases, which are typical of lung microbiota. Overall, Bac3Gel holds the potential to recreate relevant microbiota environments, including the intestinal microbiota.

Keywords: Lung, Microbiota, Cystic Fibrosis, Mucus Model.

# Debates
## 09:00 – 14:30 Friday, 24 May 2019

| | *Groups* | *Coach* | *Bldg/Room* |
|---|---|---|---|
| *1* | Debate I<br>YES: Are legal obligations necessary to support the 3Rs? | S. Louhimies,<br>M. Holloway | 58/11 |
| *2* | Debate I<br>NO: Are legal obligations necessary to support the 3Rs? | C. Chandrasekera,<br>A. Dura | 58/12a+b |
| *3* | Debate II<br>YES: Can non-testing methods provide stand-alone solutions? | N. Kramer,<br>A. Franco | 101/1003<br>1st floor |
| *4* | Debate II<br>NO: Can non-testing methods provide stand-alone solutions? | L.Wiklund,<br>D. Asturiol | 101/1302<br>1st floor |
| 5 | Debate III<br>YES: Do Adverse Outcome Pathways have a future for regulatory toxicology? | A. Hanberg,<br>B. Landesmann | 101/2002<br>2nd floor |
| 6 | Debate III<br>NO: Do Adverse Outcome Pathways have a future for regulatory toxicology? | I. Cotgraeve,<br>A. Price | 101/2302<br>2nd floor |
| 7 | Debate IV<br>YES: Can we do science without animal experiments? | J. Zilliacus,<br>S. Coecke | 100/1102 Terra<br>1st floor |
| 8 | Debate IV<br>NO: Can we do science without animal experiments? | M. Bale,<br>L. Gribaldo | 100/ 2102 Aria<br>2nd floor |

# Keep in contact: students

**#: Poster number**
**Colour background: colour Debate Team**

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# The JRC Summer School Organising Team

**Eva Åhs**

Eva Åhs is an administrative assistant dedicated to the coordination and organisation of events and communication activities. She joined the EURL ECVAM team in 2011 and has been working at the European Commission's Joint Research Centre since year 2000. She organised numerous meetings and conferences inside the JRC and off-site with official JRC presence. Eva has a Bachelor Degree of Social Sciences with a major in Psychology at the University of Stockholm.

**Elisabet Berggren**

see pag. 19

**Elisa Bernasconi**

Elisa Bernasconi is the Unit Secretary, dedicated to assist the staff and to support the organisation of events and communication activities. She joined the EURL ECVAM team in 2017 after having worked many years as senior executive assistant sectors within international private companies. Elisa has a Bachelor Degree in Public Relations and Communication from University of Milan.

**Adelaide Dura**

Adelaide is a science communication specialist with a great belief that change is always possible! She joined EURL ECVAM in 2016 to support the promotion of alternatives to animal testing and is involved in communication, dissemination and education & training projects. Adelaide is currently coordinating the ECVAM Stakeholder Forum (ESTAF) and a co-editor of the annual EURL ECVAM Status Report, while she really enjoys PR activities. She has a master's degree in Environmental Science and a PhD in Electrochemistry. She had previously worked as a science communicator for the Italian Consortium for the Recovery and Recycling of Cellulose-based Packaging (Comieco).

**Enzo Genco**

I joined the JRC in 2003 and as since I am working to support all the activities of EURL ECVAM. I am involved mainly in the unit's events organisation. I am also responsible for purchase orders, and I deal with the activities of the Laboratories, such as receiving of material, inventory and organization of the spaces. I am also a financial operational initiating agent for the Unit as well. I love helping others and I am always available for any task and project.

**Laura Gribaldo**

see pag. 12

**Marcelle Holloway**

Marcelle Holloway has been employed at the European Commission's Joint Research Centre, EURL ECVAM in Ispra, Italy since September 2017. She is involved in and also leads a number of projects directly supporting and advancing the impact of Directive 2010/63/EU on the protection of animals used for scientific purposes. Formerly employed at the European Commission's Directorate General Environment in Brussels between 2007 and 2013, she worked assisting with the coordination, preparation and adoption of Directive 2010/63/EU and related projects and legislative instruments. She has worked at the European Commission for 14 years based in Luxembourg, Brussels and now Ispra. She has a background in constitutional law and politics of the European Union, economics, publishing and communications.

**Brigitte Landesmann**

see pag. 19

**Sofia Batista Leite**

Sofia Batista Leite is a Scientific Project Officer working on the validation of alternative methods at EURL ECVAM. She has a special interested on complex *in vitro* systems ranging from 3D/spheroids to organ-on-chip, technologies used in her research experience. She developed her PhD at IBET/ITQB in Oeiras, Portugal, on strategies to culture hepatocytes as 3D spheroids, using stirred bioreactors. From 2012 to 2016 she worked as a Post-Doc at VUB, Belgium where she explored different strategies for *in vitro* studies of liver fibrosis. Her work has contributed to several national and EU projects on the development of alternative methods to animal testing, with special highlight to SEURAT-1.

**Federica Madia**

see pag. 22

**Alicia Paini**

see pag. 12

**Nikolaos Parissis**

Dr Nikolaos Parissis is a Scientific Officer at the European Commission, Joint Research Centre (JRC). His main areas of work are in the risk assessment of chemical mixtures. Dr Parissis has degrees in Pharmacy and a PhD in Toxicology. He was previously working in University of Ghent, Belgium in the area of analytical and forensic Toxicology, and also in the laboratory of General Biochemistry, Centre for Standards of the International Commission on Pharmaceutical Enzymes, F.I.P., University of Ghent, Belgium. Lecturer of the postgraduate specialisation summer course entitled “Pharmaceutical Technology” of the United Nations Industrial Development Organisation and the World Health Organisation, (UNIDO). Lecturer of the postgraduate specialisation summer course entitled "Environmental Assessment and Management Aspects of Air and Water Pollution from Industry" of the United Nations Industrial Development Organisation and the World Health Organisation, (UNIDO).

**Clemens Wittwehr**

Clemens Wittwehr is responsible for the "Protection of Animals Used for Scientific Purposes" project in the JRC. Protecting test animals is an important and highly visible goal of EU policy, also made explicit by Directive 2010/63/EU, which is one of the most stringent animal welfare standards worldwide. Making a cutting edge tool-set available to policy makers to both boost and monitor Directive 2010/63/EU impact is one of the aims of this project. Earlier in his JRC career, Clemens was also manager of the IUCLID project, which delivered the standard software now used by chemical industry to submit their registration dossiers under REACH, the EU chemicals legislation.

# The JRC Summer School Coaches & Chairs

**David Asturiol**
see pag. 20

**Elisa Bernasconi**
see pag. 101

**Mark Bale**
see pag. 20

**Anna Bal-Price**
see pag. 12

**João Barroso**

João holds a PhD in Biochemistry. He joined EURL ECVAM in 2008, where he coordinates the validation of alternative methods (AMs) towards regulatory acceptance and global use, including the identification of promising AMs for regulatory and biomedical applications; the assessment of incoming test submissions; the peer review of AMs by the ESAC; the issuing of EURL ECVAM Recommendations on validated AMs; and the engagement with regulatory experts, stakeholders and international partners. João also acted as Cosmetics Europe Project Manager on Alternatives to Animal Testing, being responsible for managing

research activities and implementation of new AMs for multiple endpoints. João is the vice-president of the European Society of Toxicology *In vitro* (ESTIV).

**Charu Chandrasekera**
see pag. 1111

**Sandra Coecke**
see pag. 13

**Ian Cotgreave**
see pag. 17

**Kristian Dreij**

Associate Professor of Toxicology and Programme Study Director at the Master's programme in Toxicology at the Institute of Environmental Medicine, Karolinska Institutet, Sweden. A chemist and toxicologist by training, his research interests are mixture toxicology with focus on genetic toxicology and development of new approaches for testing and risk assessment of mixtures. His research spans from experimental *in vitro* toxicology to population studies with main focus on PAHs and pesticides. He is currently member of a PAH working group within a UNECE/WHO Task Force with the mission to evaluate the current knowledge on PAHs and identify critical gaps in relation to their risk assessment as air pollutants.

**Adelaide Dura**
see pag. 101

**Antonio Franco**

Antonio Franco is an environmental engineer (MSc University of Padova, Italy) specialised in environmental chemistry, fate and risk modelling (PhD, Technical University of Denmark). His role at JRC is to translate scientific advances across human and environmental health sciences into improved, cross-sectorial approaches to chemical policy. In his previous role at Unilever Safety and Environmental Assurance Centre (UK) he led the development and evaluation of spatial and probabilistic models applied to ecological risk assessment of chemicals. He collaborated globally with various research and dissemination activities working with industry (CEFIC) and professional societies (SETAC) to promote risk-based approaches to chemical management.

**Laura Gribaldo**
see pag. 12

**Annika Hanberg**

Annika Hanberg, ERT and professor of toxicology at the Institute of Environmental Medicine (IMM), Karolinska Institutet (KI), Sweden. She is responsible for the global master's programme in toxicology and also organises short courses in health risk assessment. Annika Hanberg is active in giving health risk assessment support for national agencies, EU Agencies and the WHO. Focus of the risk assessment activities is in the areas of endocrine disrupters (ED), POPs and contaminated areas. Her research was previously in experimental toxicology, but has changed to development of risk assessment methodology, such as systematic review, mixtures, ED and dose-response. Recently she got involved in the Swedish National Platform for Nanosafety, SweNanoSafe.

**Marcelle Holloway**
see pag. 102

**Nynke Kramer**
see pag. 16

**Brigitte Landesmann**
see pag. 19

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see pag. 11

**Pilar Prieto**

Pilar Prieto is a Senior Scientific Officer at the European Commission's Joint Research Centre (JRC) where she contributes to the development and evaluation of integrated approaches for systemic toxicity of regulatory concern, within the Chemical Safety and Alternative Methods Unit. The unit incorporates the EU Reference Laboratory for alternative to animal testing (EURL ECVAM). She holds a PhD degree in Pharmacy from Salamanca University (Spain). During her postdoctoral position at Novartis Pharma AG (Basel, Switzerland) she investigated *in vitro* the mechanism of pathogenesis of Cyclosporine A. In 1996 she joined the JRC where she established activities in the area of acute oral toxicity testing, including the evaluation of epithelial/endothelial *in vitro* barriers models.

**Lars Wiklund**

Lars Wiklund, ERT, is a Senior Toxicologist/Safety Assessor at RegSafe with more than 35 years of experience in chemical, pharmaceutical and consumer products safety assessments. Lars previously had a position at Pharmacia World Wide Toxicology. He is also highly involved as a teacher at international programmes and courses, e.g. Global Master Programme in Toxicology, Karolinska Institutet. Lars is engaged in animal ethics/welfare (3R) issues, and was appointed by the Swedish Government as a member of the National Board for Laboratory Animals (CFN), Ministry of Agriculture, 1992 – 2004. Lars has been a board member of the Swedish Society of Toxicology (SFT) and a member of the EUROTOX Registration Sub-Committee.

**Johanna Zilliacus**

Dr Johanna Zilliacus is associate professor and senior lecturer in experimental endocrinology at the Institute of Environmental Medicine, Karolinska Institutet, Sweden. She has performed research in molecular endocrinology, endocrine disruption and health risk assessment and has a specific expertise in *in vitro* methods and in molecular endocrinology. Her on-going research focuses on risk assessment methodology to support decision-making, including research on systematic review methodology, adverse outcome pathways and risk assessment of combined exposures. She is extensive experience in organising training in the area of risk assessment.

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| 04 | Servizio medico | Medical Service |
| 08 | Mensa | Canteen |
| 08A | Nuova mensa | New Canteen |
| 08D | Cafeteria | Cafeteria |
| 46 | Visitors' Centre | Visitors' Centre |
| 58C | Nuovo Auditorio | New Auditorium |
| 100 | Ricerca ambientale | Environmental Research |
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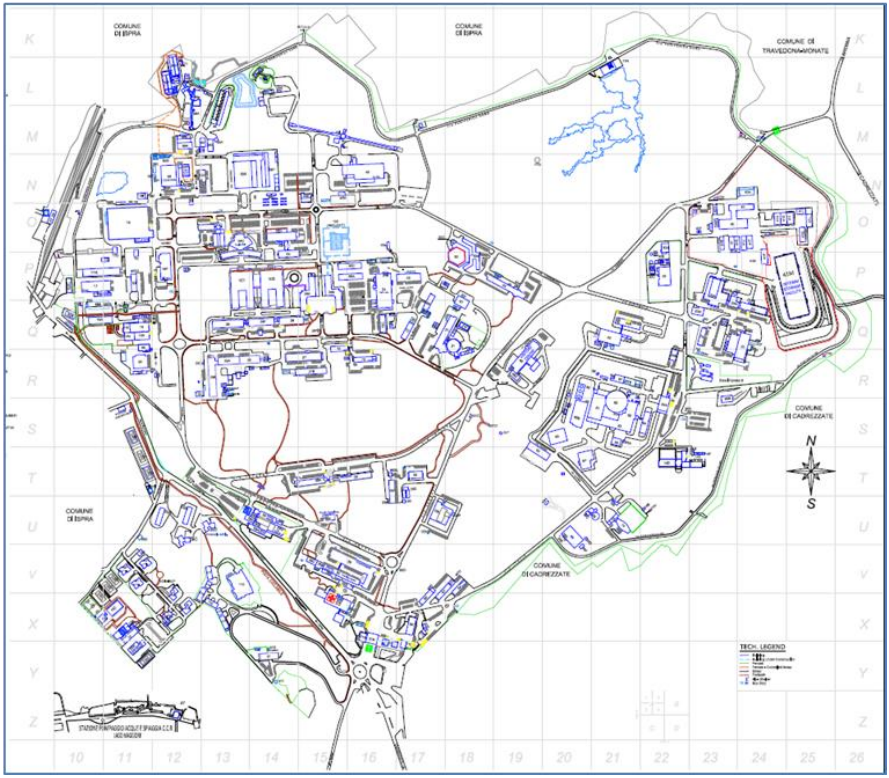

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