ABSTRACT BOOK

May 15th · 18th, 2023
Málaga (Spain)

EEMGS & SEMA

2023

51st European Environmental Mutagenesis and Genomics Society **(EEMGS)** & 27th Spanish Environmental Mutagenesis and Genomics Society **(SEMA)** meeting.

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eemgs EUROPEAN ENVIRONMENTAL MUTAGENESIS & GENOMICS SOCIETY

sema Sociedad Española de Mutagénesis y Genómica Ambiental

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PROGRAMME

MAY	MONDAY 15	TUESDAY 26	WEDNESDAY	THURSDAY 18
8:30	**REGISTRATION**			
9:00	HESI GTTC Workshop	IWGT Session	New Approach Methodologies (NAMs)	ECETOC	DNA Repair, Chromatin Structure and Genome Stability	Error-corrected Next-Generation Sequencing (ecNGS)
10:30				
11:00	Coffee Break			
11:30	HESI GTTC Workshop	ICAWG Session	Ecotoxicology	Machine Learning	New Challenges in Genotoxicity Assessment	Advances in Regulatory Genotoxicology	Human Biomonitoring
12:30				
13:00	Lunch			CLOSING CEREMONY
13:30				Lunch
14:00	HESI GTTC Workshop	Pitch-poster Presentation	Pitch-poster Presentation	
14:30		Poster Session 1 & Sponsor Visit (while coffee is served)	Fritz Sobels & Early Career Awards	Micronucleus Assay (HUMN) Workshop
15:00	**REGISTRATION**			
15:30	Open Ceremony			
16:00	Keynote Speaker **(Dr. Andrés Aguilera)**	Keynote Speaker **(Dr. Jordi Surralles)**	Poster Session 2 & Sponsor Visit (while coffee is served)	Coffee Break
16:30				Micronucleus Assay (HUMN) Workshop
17:00	New Investigators			
17:30			EEMGS General Assembly	
18:00				
18:30		Social Event	SEMA General Assembly	
19:00				
19:30	Welcome Reception			
20:30			Congress Dinner	
21:00				

HESI WORKSHOP
Monday 15th May: 09:00 - 15:00

08:00 – 09:00	**Registration & Breakfast (provided)** Quantitative Dose-response Analyses: State of the Science
09:00 – 09:10	**Welcome & Overview of Workshop Goals** *Co-chairs:* Andreas Zeller (Roche, SWITZERLAND), George Johnson (Swansea University, UK), Paul White (Health Canada, CANADA)
09:10 – 09:40	**Quantitative Interpretation of In Vivo Mutagenicity Dose Response Data for Risk Assessment and Regulatory Decision-Making** Paul White (Health Canada, CANADA) & Stefan Pfuhler (Procter and Gamble, USA)
09:40 – 10:10	**The interpretation of in vitro dose-response data for risk assessment and regulatory decision-making** Marc Beal (Health Canada, CANADA)
10:10 – 10:40	**Guided discussion**
10:40 – 11:10	**Coffee Break (provided)** Application of Quantitative Data Interpretation to Pharmaceutical Impurities
11:10 – 11:40	**Nitrosamine impurity issues and potential resolutions** George Johnson (Swansea University, UK)
11:40 – 12:10	**In vivo genetic toxicity assessments for nitrosamines** Maik Schuler (Pfizer, USA) or Shaofei Zhang (Pfizer, USA)
12:10 – 12:40	**Defining a NOGEL for mutation induction in Muta™Mouse following exposure to N-Nitrosodimethylamine (NDMA)** Anthony Lynch (GlaxoSmithKline, UK)
12:40 – 13:40	**Lunch (provided)**
13:40 – 14:10	**Regulatory considerations related to mutagenic impurities in pharmaceuticals** Roland Frotschl (BfArM, GERMANY)
14:10 – 14:50	**Guided Discussion**
14:50 – 15:00	**Concluding remarks**

01. NEW INVESTIGATORS

Monday 15th May: 17:00 - 18:30

Chairs: Fiona Chapman (Imperial Brands, UK)
Julen Sanz (Vrije Universiteit Brussel, BELGIUM)

17:00 – 17:30 **A journey in pursuit of marine genetic toxicology**
Helena Reinardy (Scottish Association for Marine Science). UK.
Invited speaker

17:30 – 17:45 **Mixtures of genotoxicants: does the principle of additivity applies?**
Julie Sanders (Sciensano) BELGIUM

17:45 – 18:00 **Estimating the mutation risks conferred by mutational processes**
Axel Rosendahl Huber (IRB Barcelona) SPAIN

18:00 – 18:15 **Negative data is still useful data: investigating and detecting non-genotoxic carcinogens *in vitro***
Demi Pritchard (Swansea University) UK

18:15 – 18:30 **Modeling human-derived in vitro barriers for environmental toxicology**
Alba García-Rodríguez (Autonomous University of Barcelona, SPAIN

02. IWGT SESSION
Tuesday 16th May: 9:00 - 11:00

Charis: Andreas Zeller (Roche, SWITZERLAND)
Hans-Joerg Martus (Novartis, SWITZERLAND)

09:00 – 9:30
Evaluation of the Standard Battery of In Vitro Genotoxicity Tests for Human Health Risk Assessment through Mathematical Modelling: A Report of the International Workshop on Genotoxicity Testing (IWGT)
Mirjam Luijten (National Institute for Public Health and the Environment) THE NETHERLANDS
Invited speaker

09:30 – 10:00
***In Vivo* Genotoxicity Testing Strategies: Report of the International Workshop on Genotoxicity Testing (IWGT)**
Carol Beevers (Corteva Agriscience) UK

10:00 – 10:30
Assessing the Quality and Making Appropriate Use of Historical Negative Control Data: A Report of the International Workshop on Genotoxicity Testing (IWGT)
Stephen Dertinger (Litron Laboratories) USA

10:30 – 11:00
Summary of 8th IWGT in Ottawa, Canada
Hans-Joerg Martus (Novartis) SWITZERLAND

03. NAMs SESSION
Tuesday 16th May: 9:00 - 11:00

Chairs: Guillermo Repetto (Universidad Pablo de Olavide de Sevilla, SPAIN)
Isabel Gaivão (Universidade de Trás-os-Montes e Alto Douro, PORTUGAL)

09:00 – 09:45
3Rs: from complexity to predictivity
Francesca Caloni (University of Milan) ITALY
Invited speaker

09:45 – 10:00
The human HepaRG cell line: a brief history of its use in genetic toxicology: advantages, limits and future directions
Ludovic Le Hégarat (French Agency for Food, Environmental and Occupational Health & Safety - Fougeres Laboratory, Toxicology of Contaminants Unit) FRANCE

10:00 – 10:15
***In vitro* cell transforming capacity of different types of nano-plastics**
Julia Catalán (Finnish Institute of Occupational Health) FINLAND

10:15 – 10:30
In silico modelling of crosstalk between DNA damage and oxidative stress for prediction of cellular adversity
Elsje Burgers (Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research - Leiden University) THE NETHERLANDS

10:30 – 10:45
Perspectives for DNA adductomics in large-scale exposomics: upscaling sample preparation and preprocessing data
Lieselot Hemeryck (Laboratory of Integrative Metabolomics, Ghent University) BELGIUM

10:45 – 11:00
Oxidative Stress Disrupt Differentiation of Human Induced Pluripotent Stem Cells
Ann-Karin Olsen (Norwegian Institute of Public Health, Division of Climate and Environmental Health) NORWAY

04. ICAWG SESSION
Tuesday 16th May: 11:30 - 13:00

Chairs: Goran Gajski (Institute for Medical Research and Occupational Health, CROATIA)
Amaya Azqueta (Universidad de Navarra, SPAIN)

11:30 – 12:00
Advanced 3D cell models and zebrafish embryos: A bridge between in vitro and *in vivo*
Bojana Žegura (Department of Genetic Toxicology and Cancer Biology, National Institute of Biology) SLOVENIA
Invited speaker

12:00 – 12:15
Utility of 3D HepaRG spheroid model for testing genotoxicity using high-throughput CometChip platform
Mugimane Manjanatha (Division of Genetic and Molecular Toxicology, US FDA, National Center for Toxicological Research) USA

12:15 – 12:30
Use of the CometChip® for the in vitro, in vivo and Fpg-modifed assay
Miguel Collía (Department of Pharmacology and Toxicology, School of Pharmacy and Nutrition, Universidad de Navarra) SPAIN

12:30 – 12:45
Evaluation of antioxidative, antigenotoxic, and anticancer activities of commercial medical mushrooms products: Agaricus blazei, Cordyceps Sinensis and Immune Assist
Lada Zivkovic (Department of Pathobiology, Faculty of Pharmacy, University of Belgrade) SERBIA

12:45 – 13:00
Maternal exercise during pregnancy modulates genetic and biochemical damage caused by high consumption of fructose in offspring
Vanessa Andrade (Laboratory of Translational Biomedicine, Graduate Program of Health Sciences, University of Southern Santa Catarina – UNESC) BRAZIL

05. ECOTOXICOLOGY SESSION
Tuesday 16th May: 11:30 - 13:00

Chairs: Awadhesh Jha (University of Plymouth, UK)
Óscar Herrero (Universidad Nacional de Educación a Distancia, SPAIN)

11:30 – 12:15 **The challenge of assessing contaminants of emerging concern and microplastics**
Marinella Farré (Institute of Environmental Assessment and Water Research) SPAIN
Invited speaker

12:15 – 12:30 **Assessing the Impact of Persistent and Emerging Contaminants on Aquatic Organisms**
Awadhesh N. Jha (School of Biological and Marine Sciences, University of Plymouth) UK

12:30 – 12:45 **Hydrogen peroxide-induced oxidative damage in sea urchin (Paracentrotus lividus) DNA**
Fengjia Liu (The Scottish Association for Marine Science) UK

12:45 – 13:00 **Plant comet assay in biomonitoring of air pollution in Sarajevo, Bosnia and Herzegovina**
Mujo Hasanović (University of Sarajevo - Institute for Genetic Engineering and Biotechnology) BOSNIA & HERZEGOVINA

06. ECETOC SESSION
Wednesday 17th May: 9:00 - 11:00

Chairs: Roland Frötschl (BfArM, GERMANY)
Bennard van Ravenzwaay (Environmental Sciences Consulting, GERMANY)

09:00 – 09:15 **An Introduction to ECETOC's workshops on Point of Departure and IVIVE modelling**
Bennard van Ravenzwaay (Environmental Sciences Consulting) GERMANY
Invited speaker

09:15 – 09:40 **Physiologically Based Kinetic Modelling applications in Chemical Risk Assessment**
Stephan Schaller (esqLABS) GERMANY

09:40 – 10:05 **Applying transcriptomic benchmark modelling for toxicological decision making**
Richard Currie (Syngenta International Research Center) UK

10:05 – 10:30 **Determination of point of departures based on high throughput in vitro metabolomics**
Franziska Zickgraf (BASF) GERMANY

10:30 – 10:50 **Summary of the ECETOC workshop from IVIVE to PODS – translating research methods to application**
Tim Gant (UK Health Security Agency) UK

07. DNA REPAIR SESSION
Wednesday 17th May: 9:00 - 11:00

Chairs: María Teresa Roldán (Universidad de Córdoba, SPAIN)
Luisa María Sierra (Universidad de Oviedo, SPAIN)

09:00 – 09:45 **Specific killing of BRCA1-deficient cancer cells by depletion of EXO1**
Haico van Attikum (Leiden University Medical Center) THE NETHERLANDS
Invited speaker

09:45 – 10:00 **Influence of orphan base and sequence context on the processing of AP sites by AP lyases in Arabidopsis thaliana**
Marina Jordano Raya (University of Córdoba) SPAIN

10:00 – 10:15 **Role of Phosphatase and Tensin homolog (Pten) in Insulin mediated DNA damage**
Helga Stopper (Institute of Pharmacology and Toxicology, University of Wuerzburg) GERMANY

10:15 – 10:30 **A direct role for HIPK2 in homology-directed DNA repair and the regulation of PARP inhibitor sensitivity**
Thomas G. Hofmann (Institute of Toxicology, Johannes Gutenberg University of Mainz) GERMANY

10:30 – 10:45 **Could DNA damage be a useful biomarker of diagnosis and prognostic in human breast and colorectal cancer?**
Ana Rita Guedes (CECAV– Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro) PORTUGAL

10:45 – 11:00 **Senescence and cell death triggered by the DNA alkylation damage O6-methylguanine**
Bernd Kaina (Institute of Toxicology, University Medical Center, Mainz) GERMANY

08. MACHINE LEARNING SESSION

Wednesday 17th May: 11:30 - 13:00

Chairs: George Johnson (Swansea University, UK)

11:30 – 12:00 **Introduction to Machine Learning in Genetic Toxicology**
Steve Bryce (Litron Labs) USA

12:00 – 12:30 **Deep Neural Networks to Automate Scoring Of The Imaging Flow Cytometry In-Vitro Micronucleus Assay**
Paul Rees (Swansea University) UK

12:30 – 13:00 **Towards a quantitative understanding of the DNA damage response through data-driven dynamical modeling**
Joost Beltman (Leiden University) THE NETHERLANDS

09. NEW CHALLENGES SESSION

Wednesday 17th May: 11:30 - 13:00

Chairs: Ricard Marcos (Universitat Autònoma de Barcelona, SPAIN)
Vanessa Valdiglesias (Universidade da Coruña, SPAIN)

11:30 – 12:15 **Understanding the health risks of exposure to micro- & nano-plastics**
Alba Hernández (Autonomous University of Barcelona) SPAIN
Invited speaker

12:15 – 12:30 **Biological effects of chronic oral exposure to polystyrene nanoparticles in Drosophila melanogaster**
Massimo Aloisi (University of L'Aquila) ITALY

12:30 – 12:45 **Recent achievements in micronuclei characterization**
Jolanta Kwasniewska (University of Silesia Institution in Katowice) POLAND

12:45 – 13:00 **Novel methodology to assess genotoxicity in Food Contact Materials (FCM)**
Maricel Marin-Kuan (Nestlé Research, Vers-chez-les-Blancs) SWITZERLAND

10. ecNGS SESSION
Thursday 18th May: 9:00 - 11:00

Chairs: Vanessa Moraes de Andrade (Universidade do Extremo Sul Catarinense, BRAZIL)
Anthony Lynch (GSK, UK)

09:00 – 09:35	**Overview of ecNGS technologies and applications** Clint Valentine (Twinstrand Bio) USA
09:35 – 10:10	**Cancer driver mutations in the era of pan-cancer genomics** Jiri Zavadil (IARC) FRANCE
10:10 – 10:45	**NGS applications to assess vector mediated genotoxicity in genetic medicine** Patrick van Eijk (Cardiff University) UK
10:45 – 11:00	**ecNGS analysis of induced mutagenesis in the low dose region. Case studies with noteworthy nitrosamines** Anthony Lynch (GSK) UK

11. REGULATORY TOXICOLOGY SESSION

Thursday 18th May: 11:30 - 13:00

Chairs: Birgit Mertens (Sciensano, BELGIUM)
Antonio Guzmán (Alexion Pharmaceuticals, Inc., SPAIN)

11:30 – 12:15 **Modern in vitro screening tools to enhance quantitative chemical risk assessment**
Marc Beal (Health Canada, Ottawa) CANADA
Invited speaker

12:15 – 12:30 **The GENOMARK transcriptomic biomarker demonstrates a high predictivity for genotoxic hazards and utility in potency ranking in human HepaRGTM cells**
Anouck Ingrid Thienpont (Department of *In Vitro* Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel) BELGIUM

12:30 – 12:45 **Assessing DNA damage in Testicular Germ Cells in the Comet Assay**
Ann-Karin Olsen (Norwegian Institute of Public Health, Division of Climate and Environmental Health) NORWAY

12:45 – 13:00 **Assessing the DNA damage potential of cigarette and iQOS emissions in human bronchial epithelial cells**
Michele Davigo (National Institute for Public Health and the Environment (RIVM), Centre for Health Protection) THE NETHERLANDS

12. HUMAN BIOMONITORING SESSION

Thursday 18th May: 11:30 - 13:00

Chairs: Stefano Bonassi (IRCCS San Raffaele Roma, ITALY)
Blanca Laffon (Universidade da Coruña, SPAIN)

11:30 – 12:00 **Understanding blood cell mutational biomarkers for biomonitoring and disease purposes**
Gareth Jenkins (University of Swansea) UK
Invited speaker

12:00 – 12:15 **The importance of gene – environment interactions in Alzheimer disease and the emerging role of epigenetics**
Lucia Migliore (University of Pisa) ITALY

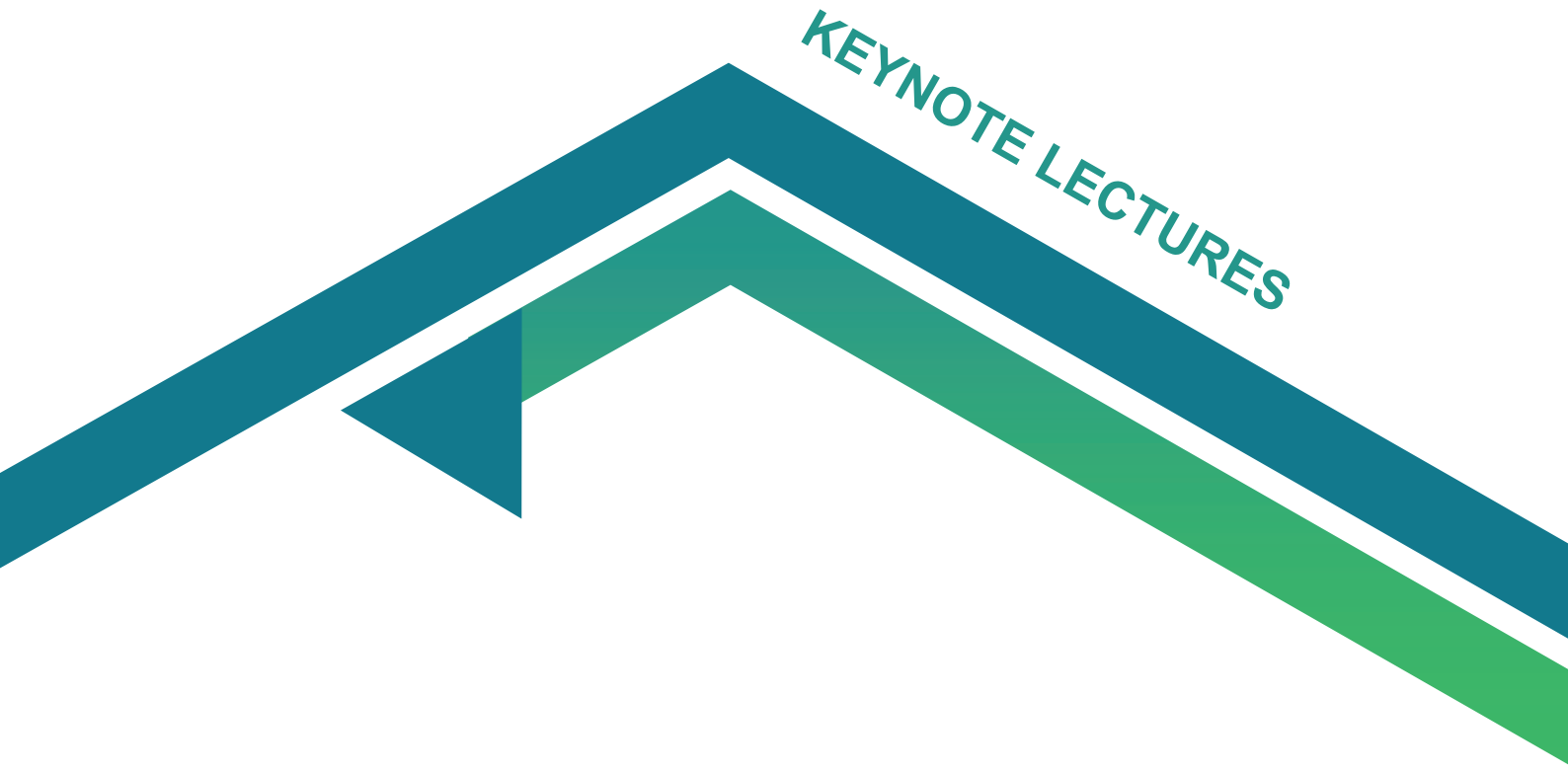
12:15 – 12:30 **Assessment of DNA damage in cumulus cells from infertile women using comet assay**
Vanessa Sousa (University of Porto) PORTUGAL

12:30 – 12:45 **Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes**
Sona Vodenkova (Charles University) CZECH REPUBLIC

12:45 – 13:00 **The use of combined different approaches in assessing the conditions and making prediction models in severely obese BMI ≥ 35 kg m-2 (FFQ, DII, anthropometric, biochemical and DNA damage parameters)**
Mirta Milić (Institute for Medical Research and Occupational Health) CROATIA

HUMN WORKSHOP
Thursday 18th May: 14:00 - 17:30

13:00 – 14:00	**Registration & Luch (provided)** Early afternoon Session 14:00 – 15:40
14:00 – 14:10	**Welcome & Overview of Workshop Goals** *Co-chairs:* Michael Fenech (Genome Health Foundation, AUSTRALIA), Siegfried Knasmueller (Medical University of Vienna, AUSTRIA), Stefano Bonassi (IRCCS San Raffaele Roma, ITALY)
14:10 – 14:40	**The biology of buccal cells and the buccal micronucleus (MN) cytome assay** Claudia Bolognesi (Ospedale Policlinico San Martino, ITALY)
14:40 – 15:10	**Use of buccal cytome assays in the occupational exposure studies** Georg Wultsch (Medical University of Vienna, AUSTRIA)
15:10 – 15:40	**Association of buccal MN cytome assay biomarkers with disease and their relevance for clinical studies** Stefano Bonassi (IRCCS San Raffaele Roma, ITALY)
15:40 – 16:00	**Coffee Break (provided)** Late afternoon session 16:00 – 17:30
16:00 – 16:30	**Impact of nutrition and life style on formation of micronuclei and other nuclear anomalies in buccal cells** Siegfried Knasmueller (Medical University of Vienna, AUSTRIA)
16:30 – 16:45	**Automation of the Buccal Micronucleus Cytome Assay** Michael Fenech (Genome Health Foundation, AUSTRALIA)
16:45 – 17:00	**Artificial Intelligence in Microscope-Based Imaging: Automation of the Buccal Micronucleus Cytome Assay?** Christian Schunck (MetaSystems, GERMANY)
17:00 – 17:30	**Discussion on knowledge gaps regarding the buccal MN cytome assay and a roadmap for its translation into practice** (open to all participants)


KEYNOTE LECTURES

ID 00.1*

Monday 15th May: 16:00 - 17:00

RNA-mediated genome instability and transcription-replication conflicts

Andrés Aguilera*

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Genome instability is a hallmark of cancer cells. As a cell pathology is frequently associated with mutations in factors involved in the DNA damage response (DDR), including DNA replication, repair and recombination. However, different studies have revealed that RNA and transcription can be a natural source of genome instability, both in the absence and upon the action of exogenous genotoxic agents. In most cases this is due to transcription-replication conflicts that are stimulated by co-transcriptional R-loops that if not properly resolved lead to replication fork breakage. We try to define the mechanisms controlling these processes by focusing on two type of factors: RNA metabolic factors and chromatin remodelers and modifiers. We have shown that a number of such factors, including the UAP56/DDX39B, DDX47 and DDX5 RNA helicases, and the SWI/SNF, FACT and SIN3A chromatin remodeler and modifier complexes, are key in preventing or resolving R-loops and their associated transcription-replication conflicts. Interestingly, our recent results indicate that the apparent redundancy of factors controlling the causes of the conflicts and R-loops is in part due to cell cycle phase dependency and a mediator role facilitating the action of DDR factors at stalled replication forks. We will discuss the biological meaning of our new results and their relation with mutagenesis.

Keywords:
Genetic instability; R-loops; Transcription-Replication conflicts; Chromatin; DSB repair, Mutagenesis.

ID 00.2*

Tuesday 16th May: 16:00 - 17:00

Chromosome instability, DNA repair deficiency and cancer: insights from Fanconi anemia

J. Surrallés

Sant Pau Hospital Research Institute, IIB Sant Pau; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) and Universitat Autònoma de Barcelona, Barcelona, Spain
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Fanconi anemia (FA) is chromosome instability disorder characterized at the clinical level by malformations, progressive bone marrow failure and a high predisposition to leukemia and head and neck squamous cell carcinoma (HNSCC) and other tumors. FA cells show chromosome fragility and hypersensitivity to DNA interstrand cross-linking (ICL) mutagens due to deficiency in ICL repair. The only cure of the blood disease is hematopoietic stem cell transplantation and gene therapy appears on the horizon of new treatments thanks to successful clinical trials. Diagnosis and therapy are further complicated by the genetic complexity of the disease. There are at least 22 genes, from FANCA to FANCW, involved in this disease and their products interact in a complex genome stability and tumor suppression network: the FA/BRCA pathway. Notably, 5 out of 22 FA genes (FANCD1/BRCA2, FANCN/PALB2, FANCJ/BRIP1, FANCO/Rad51C and FANCM) are breast/ovarian cancer susceptibility genes in otherwise unaffected mutation carriers. FA HNSCCs are difficult to treat due to extreme toxicity of conventional chemotherapies. Extensive research is underway to genetically and functionally characterize FA tumors in search of therapeutic vulnerabilities. Therapeutic advances based on a deep understanding of the genetics of FA cancers will be shown, including the discovery and repurposing of drugs inducing cancer specific lethality.

Keywords:
Fanconi anemia; cancer; DNA repair; chromosome instability; mutation


SESSIONS
01. New Investigators

ID 01.1
A journey in pursuit of marine genetic toxicology

H.C. Reinardy

Scottish Association for Marine Science, Oban, UK;
The University Centre in Svalbard, Longyearbyen, Norway.
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Understanding organisms responses to contaminants, pollutants, and environment stressors is critical for predicting ecosystem impacts. This is particularly important in light of climate change driving physical changes such as temperature, pH, and transport and spread of contaminants at rates previously not experienced in stable marine environments. Responses can be in the form of detoxification and stress response mechanisms, and species vary in their sensitivity to stress as well as their capacity to respond and adapt. Incorporation of genetic effects analyses into other biological process such as growth, reproduction, and survival can illuminate additional mechanisms of sensitivity and response. These questions have been at the heart of my scientific career for the past fifteen years, couple with the curiosity of understanding lesser-studied species and creatures in the diverse marine habitats. My journey has meandered from temperate regions to warmer subtropical Bermuda shores, up to the cold Arctic of Svalbard and back to familiar British shores, encompassing the diversity of marine life of fish, echinoderms, and zooplankton. Ultimately, this journey has provides overarching insight into how different species can adapt or suffer from a stressful environment, and the wealth of scientific information we can gain for looking into new species, new molecular systems, and new genetic responses. The goal of this story is to inspire aspiring genetic toxicologists to think beyond their immediate shores and to show an example of a international scientific adventure which has delivered rewards in both exciting data and a fun journey.

ID 01.2

Mixtures of genotoxicants: does the principle of additivity applies?

J. Sanders[1,2,*], R. Anthonissen[1], G. Johnson[3], T. Vanhaecke[2] and B. Mertens[1**]**

[1]Scientific Direction of Chemical and Physical Health Risks, Sciensano, Brussels, Belgium
[2]Department of In Vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Brussels, Belgium
[3]Swansea University Medical School, Swansea University, Swansea, UK
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*** equally contributing senior scientists*

Until recently, chemical risk assessment was strongly focused on single compounds. However, in practice, humans are exposed to chemical mixtures instead of just a single compound. Consequently, there is a need to evaluate the combined effects of co-occurring chemicals on human health, including genotoxicity. Different types of combined effects have been described, whereby for non-genotoxic endpoints, the principle of additivity is assumed to generally apply.

The aim of this study was to investigate whether the principle of additivity is also justifiable for genotoxic mixtures. To this extent, two types of binary mixtures were evaluated for their potential to induce chromosome damage *in vitro*. The first consisted of two well-known reference genotoxicants with a similar mode of action, i.e. ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS). The second mixture contained two genotoxic mycotoxins, i.e. deoxynivalenol (DON) and zearalenone (ZEN), known to co-occur in food and feed.

First, *in vitro* micronucleus (MN) data in TK6 cells for the individual compounds were collected in absence of S9 metabolic fraction. Next, benchmark concentrations of the two compounds were compared by applying a specific module in the PROAST software to establish a relative potency factor (RPF), thereby taking into account the optimal parallel fit of the curves. The RPF was then used to express the concentration of one compound as a function of the other and to select a range of binary mixtures expected to induce responses covering different parts of the concentration-response curve. More specifically, binary mixtures with varying concentration ratios of the two compounds of interest (i.e. 1:1, 1:3 and 3:1) were identified and tested in the *in vitro* MN assay. The collected experimental data of the mixtures were then compared to the responses predicted based on the data of the two individual compounds using the PROAST dose-addition model of the BMD approach in R. A first set of experiments was done with EMS-MMS mixtures. The experience gained in these tests was then used for the mixture testing with DON and ZEN. The experimental results of the EMS-MMS mixtures were close to the fitted curve based on the data of the single compounds, indicating that the principle of additivity is applicable to mixtures of EMS and MMS. Analysis of the mixture results with DON and ZEN is currently being finalized.

Keywords:
DNA damage, combined effects, *in vitro* MN assay, BMD approach, dose-addition.

ID 01.3

Estimating the mutation risks conferred by mutational processes

A.K.M. Rosendahl Huber[1]*, F. Muiños[1,2], A. González-Pérez[1,2,3], & N. López Bigas[1,2,3]

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[2]Centro de Investigación Biomédica en Red en Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain
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DNA mutations can disrupt vital cellular functions leading to disase. One of the most striking examples is cancer, which requires mutations disturbing the activity of cancer driver genes. The risk at which these genes are mutated is not equal across mutational processes, as certain positions in the genome have been shown to exhibit considerable variation in mutation rates. This variation in mutation risk is the result of chromatin and sequence differences at multiple scales, the mode of action of the process, and repair of DNA damage. Thus, mutagenic processes may confer varying risks to induce cancer driver mutations. Accurate determination of these mutation risks is important to understand the induction of cancer driver mutations.

We aim to compute the mutation risks for mutational processes across the genome using computational modeling approaches. Currently, we have explored these mutation risks using different modeling strategies, using linear regression and decision tree based machine learning methods such as gradient boosting. As input for modeling, we use mutation probabilities from 33 mutational processes, defined as mutational signatures. Mutations are distributed in genomic bins ranging from 1mb to exonic regions of genes. As determinants for the mutation rates we use the mean scores of epigenetic, replication timing and transcription factor binding site data, which have been known to correlate with absolute mutation counts. Currently, we are able to predict genomic mutation risks accurately for most mutagenic processes (R2 values ranging between 0.6 and 0.8), with tree-based regression performing better at predicting mutational loads. Decomposing the contribution of covariates enables the identification of the determinants behind the variance in mutation rates. The currently generated models provide the basis to calculate relative risks scores for mutagenic processes for specific mutations across the genome. In the future, this will enable the quantification of risk scores of various mutagenic processes to induce cancer driver mutations and neoantigens.

Keywords:
Mutational Signatures; Statistical Learning; Risk Estimation; Carcinogenesis; keywords.

ID 01.4

Negative data is still useful data: investigating and detecting non-genotoxic carcinogens *in vitro*.

Demi Pritchard, Gareth Jenkins

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Swansea University, S. Wales

Approximately, 70-90% of human cancers are induced due to chronic environmental agent exposure. Carcinogens are split into two main groups, genotoxic carcinogens (GC) and non-genotoxic carcinogens (NGC). Where Genotoxic carcinogens act directly on the DNA and non-genotoxic carcinogens use alternative mechanisms to initiate oncogenesis. Currently there are no validated in vitro testing systems for NGCs and due to their complex nature they are largely ignored.

Our *in vitro* testing battery consists of a series of endpoints, trying to unpick the mechanism of action(s) (MOA) utilised by different NGCs. Both the acute and chronic micronucleus (Mn) assays were carried out as well as tests for reactive oxygen species, cell cycle, apoptosis, mitochondrial health and gene expression profiling. Not all NGCs are subject to the current 2 year rodent bioassay, which means some NGCs may slip through the net of testing.

In order to try to understand the complexities of NGCs, a battery of tests investigating MOAs are required. A number of chemicals were subject to this in vitro testing battery however not all of them gave positive results to these assays. The endpoints assessed were traditional acute dosing compared with more human relevant chronic exposures, Micronuclei induction, cytotoxicity, cell cycle perturbations, Reactive oxygen species (ROS) levels, mitochondrial function via a mito stress test and gene expression through a PCR array. The chemicals that gave negative results in these endpoints were: TCDD, rosuvastatin and chloroprene.

Overall, the test battery was successful in unpicking the MOAs used by some NGCs but not all. It was valuable to subject all NGCs to the same tests as it allows for contrasts and comparisons to be made between the differing carcinogens. Future work should have an increased focus on gene expression.

ID 01.5

Modeling human-derived in vitro barriers for environmental toxicology

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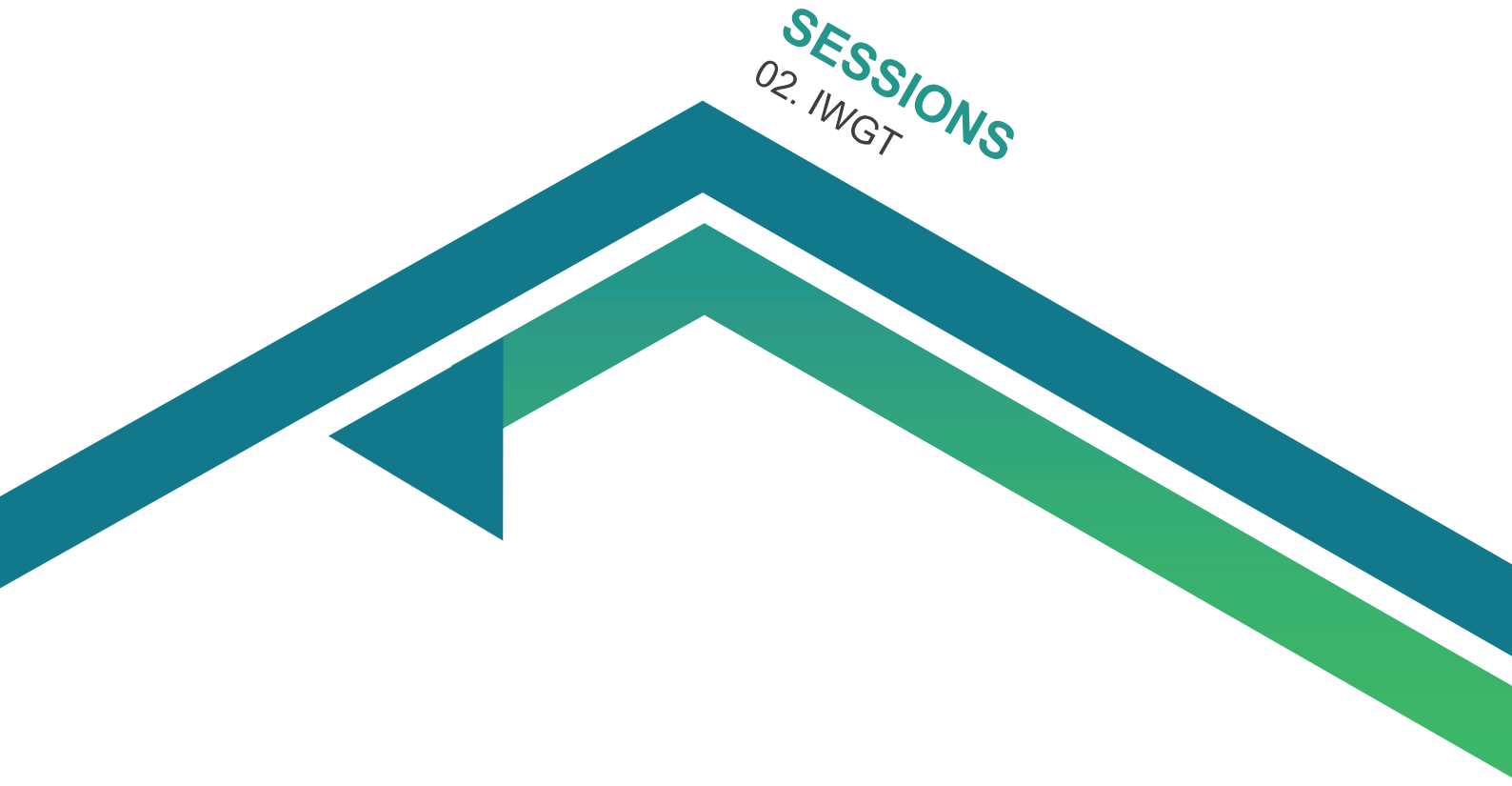
In vitro modeling of cell cultures is widely used in pharmaceutical, medical, food/nutrition and toxicology science. These constructed growth environments support tissue differentiation and mimic tissue-tissue, tissue-liquid, and tissue-air interfaces in a variety of conditions. In toxicology, human-derived in vitro culture models are attracting increasing interest because of the numerous benefits; (1) decreasing the use of in vivo models, (2) rapid and cost-effective experimental methodologies, and (3) the simulation of human physiology and biochemistry in a controlled situation such as (i) the recreation of healthy and disease conditions (e.g., inflammation) and (ii) the coculture with bacterial cells to study host-cell interactions. Therefore, our research group has focused on the modeling of in vitro barrier tissue interfaces for its use in environmental toxicology, concretely, to study the interaction, fate, and effects of engineered nanoparticles (ENPs), and micro- and nano-plastics (MNPLs), recently coined as emergent contaminants. To study the effects of ENPs and MNPLs, three different in vitro systems have been already developed, characterized, and tested: (1) the Caco-2/HT29/Raji-B model that mimics the small intestinal environment, (2) the air liquid interface epithelium of Calu-3 as the bronchial epithelium, (3) and the HUVECs monolayer that closely recreates the endothelium of veins and capillaries. All three models were established in porous transwells® using static conditions. In general, these models have permitted us to visualize and quantify the ENPs (TiO2NPs) and MNPLs (e.g., polystyrene, polyethylene, polylactic acid, etc.) cell internalization, nuclei interaction, and their bio-persistence in tissues, to measure oxidative, genotoxic, and structural damage, and to study the response modulation through batteries of gene expression.

Moreover, successful co-exposures to gut-derived microbiota *(L. rhamnosus)* and ENPs demonstrates the protective role of symbiotic bacteria against contaminants. In conclusion, these models have served us to rapidly test a vast number of actual and potential food and environmental contaminants and provide trustable data to regulators and policymakers. Although some limitations such as the incapacity to perform long-term (>1week) studies were detected, the use of dynamic systems (e.g., microfluidics) could potentially help to the tissue replacement.

Funding:
This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal), the Spanish Ministry of Science and Innovation (PID2020-116789, RB-C43), the Generali Tat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to AH.

Keywords:
Modeling; barriers; nanotoxicology; contaminants; alternatives.


SESSIONS
02. IWGT

ID 02.1

Evaluation of the Standard Battery of In Vitro Genotoxicity Tests for Human Health Risk Assessment through Mathematical Modelling: A Report of the International Workshop on Genotoxicity Testing (IWGT)

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In human health risk assessment of chemical substances, assessment of genetic toxicity for regulatory purposes usually starts with a standard battery of in vitro genetic toxicity tests. This battery, comprising multiple tests, is needed to cover the different genetic toxicity endpoints. The tests that are commonly included in the battery (partly) differ in biology, due to which resulting data may seem contradictory, thereby complicating accurate interpretation of the findings. This could be overcome by using mathematical modelling. To test and discuss the utility of mathematical modelling for evaluating the predictivity of a test battery, a workgroup of the International Workshops for Genotoxicity Testing was convened. We applied mathematical modelling to a large database comprising *in vitro* and in vivo data for genotoxicity, with the aim to evaluate the performance of the *in vitro* test battery to predict in vivo genotoxicity. The results obtained indicate when using a battery of three genotoxicity tests, i.e. a bacterial gene mutation test (Ames), a mammalian cell gene mutation test, and a mammalian in vitro clastogenicity test, combination of two mammalian cell tests showed the highest predictive value for *in vivo* genotoxicity and adding Ames test results has no impact on the prediction of *in vivo* genotoxicity. Further research comparing *in vitro* genotoxicity data with *in vivo* data for the same genotoxicity endpoint will provide additional insights on the predictivity of the standard in vitro genotoxicity battery.

Keywords:
Genetic toxicology, Toxicity prediction, Uncertainty, Hazard assessment, Bayesian modelling, Chemicals.

ID 02.2

In Vivo Genotoxicity Testing Strategies: Report of the International Workshop on Genotoxicity Testing (IWGT)

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The in vivo testing strategies working group (WG) at the International workshop on genotoxicity testing (IWGT) discussed topics related to in vivo testing. Key conclusions from the group will be discussed.

The majority of the WG group agreed that it is unacceptable to reject the conclusions of a negative in vivo erythrocyte MN study solely on the basis of test substance concentrations in blood or plasma falling below the concentration that induced positive results in vitro. Consensus on the evidence required to demonstrate systemic exposure was not reached.

The WG members agreed that the liver MN test is sufficiently validated to develop this test into an OECD Test Guideline, however, the impact of age at the time of dosing warrants further study. The WG agreed that Ki-67 is a reliable marker for mitotic activity, however, the evaluation of longer-lived cell proliferation markers would be valuable. The WG agreed that comparison of comet study results to historical control data (HCD) for determination of biological relevance should not be performed used unless the laboratory can demonstrate stability of their HCD, and that animal (but not study) factors are the predominant source of variance in the HCD. The WG agreed that methodological differences could influence the risk of both false positive and false negative results.

Keywords:
In vivo, Genetic toxicology, Test strategies.

ID 02.3

Assessing the Quality and Making Appropriate Use of Historical Negative Control Data: A Report of the International Workshop on Genotoxicity Testing (IWGT)

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Historical negative control data (HCD) have played an increasingly important role in interpreting the results of genotoxicity tests. Indeed, "Criterion C" can be found in most Organisation for Economic Co-operation and Development (OECD) in vivo genetic toxicology Test Guidelines, and involves comparing responses produced by exposure to test substances with the distribution of HCD. Because of the potential for inconsistency in how HCD are acquired, maintained, described, and used to interpret genotoxicity testing results, a workgroup of the International Workshops for Genotoxicity Testing was convened to provide recommendations on this crucial topic. The Workgroup used example data sets from four in vivo tests, the Pig-a gene mutation assay, the erythrocyte-based micronucleus test, the transgenic rodent gene mutation assay, and the in vivo alkaline comet assay to illustrate how the quality of HCD can be evaluated. In addition, recommendations are offered on appropriate methods for evaluating HCD distributions.

Recommendations of the Workgroup are:

1. When concurrent negative control data fulfill study acceptability criteria, they represent the most important comparator for judging whether a particular test substance induced a genotoxic effect.

2. HCD can provide useful context for interpreting study results, but this requires supporting evidence that i) HCD were generated appropriately, and ii) their quality has been assessed and deemed sufficiently high for this purpose.

3. HCD should be visualized before any study comparisons take place; graph(s) that show the degree to which HCD are stable over time are particularly useful.

4. Qualitative and semi-quantitative assessments of HCD should also be supplemented with quantitative evaluations. Key factors in the assessment of HCD include: i) the stability of HCD over time, and ii) the degree to which inter-study variation explains the total variability observed.

5. When animal-to-animal variation is the predominant source of variability, the relationship between responses in the study and an HCD-derived interval or upper bounds value (i.e., OECD Criterion C) can be used with a strong degree of confidence in contextualizing a particular study's results.

6. When inter-study variation is the major source of variability, comparisons between study data and the HCD bounds are less useful, and consequentially, less emphasis should be placed on using HCD to contextualize a particular study's results.

Keywords:
Historical control data (HCD), Genetic toxicology, Organisation for Economic Co-operation and Development (OECD), Data interpretation, Statistics.

ID 02.4
Summary of 8th IWGT in Ottawa, Canada

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The International Workshops on Genotoxicity Testing (IWGT) are convened approximately every four years to bring together experts in the field of genetic toxicology; they are usually held as satellite meetings to the International Conference on Environmental Mutagens (ICEM). Working Groups (WGs), consisting of experts from industry, regional health authorities and academia are charged with providing consensus recommendations on contemporary issues in genetic toxicology; they reflect the state of the science for each topic.

At the 8th IWGT, held in Ottawa, Canada, the following five WGs were formed:

- Statistical Approaches and Data Interpretation, chaired by Stephen Dertinger, USA.
- Predictivity of In Vitro Genotoxicity Testing, a Mathematical Modelling Approach, chaired by Mirjam Luijten, The Netherlands.
- Transcriptomic Biomarkers, chaired by Roland Froetschl, Germany.
- In Vivo Strategies, chaired by Carol Beevers, UK.
- Dose-response Analysis for Potency Comparisons and Risk Assessment, chaired by Paul White, Canada.

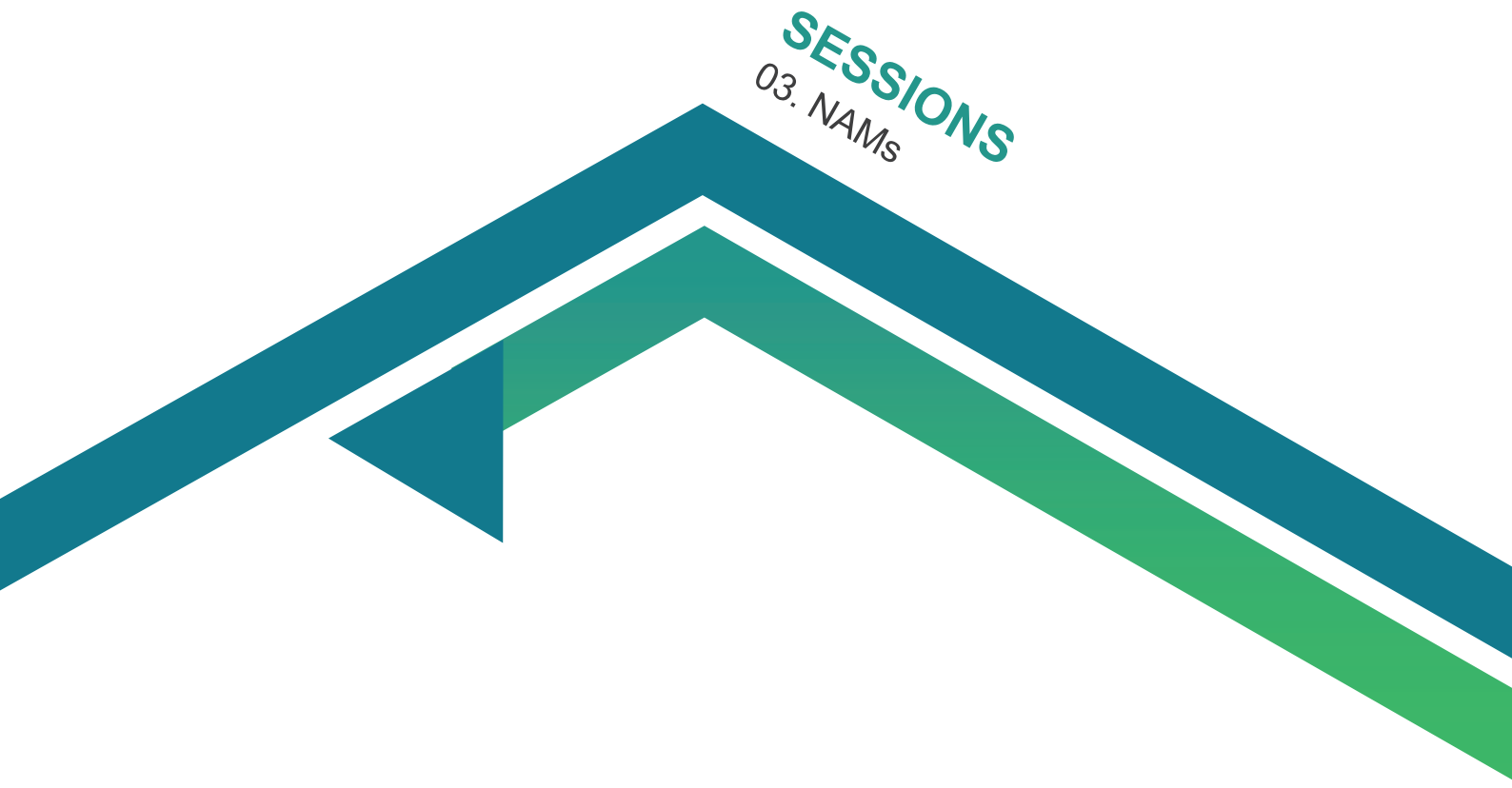
In addition, two plenary sessions critically discussed:

- Epigenotoxicity, chaired by Roger Godschalk, The Netherlands.
- Gene Therapy, chaired by Silvana Libertini, Switzerland.

The products of each of the WG discussions, which are preceded by extensive analysis of available information, are one or several publications built around the consensus statements on each of the topics. These consensus statements focus on regulatory applications of the technology or topic(s) under discussion; thus, direct interpretation in a regulatory context is facilitated. For the podium discussions, reviews will be published to describe various aspects of the field and the state of the science.

In the current session, presentations will be given on three of the WG topics (i.e., Statistical Approaches, Predictivity of In Vitro Testing, and In vivo Strategies). Summaries of the discussion outcomes and consensus statements of the other activities will also be presented.

Keywords:
IWGT, genotoxicity, guidelines, new technologies, regulatory testing.


SESSIONS
03. NAMs

ID 03.1
3Rs: from complexity to predictivity

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The application of the 3Rs principle, Reduce, Refine, Replace (Directive 2010/63 EU), asks for scientific innovation with a continuous development of methodologies. Non Animal Methods (NAMs), from cell cultures to tissue-based assays, from in vitro epithelial barriers for oral, respiratory and topical exposure (i.e. alveolar, dermal, intestinal barrier) to 3D cultures and organoids and/or spheroids, are useful predictive tools, in order to answer to the requirements of biological complexity. Moreover it is clear that the new approach is looking to a strategy, integrated or tiered, through combined tests, instead of stand alone methods, that are a few and with limited application, like in skin irritation (OECD 439) or corrosion (OECD 431).

Considering systemic toxicity like carcinogenicity, genotoxicity, reproductive toxicity or the multiple mechanisms of Endocrine Distuptor Chemicals (EDCs), new alternative methods are necessary to solve complex endpoints.

If the new vision implies Integrated Testing Strategies (ITS), Integrated Approaches for Testing and Assessment (IATA) or Defined Approaches for Testing and Assessment (DA), on the other hand research is looking to advanced technologies like Microfluidic Perfusion Systems (MPS), which could expand their applicability from toxicity testing to biomedical research.

A predictive 3Rs interdisciplinary approach, addressed to investigate different aspects of a multilevel science, considering humans, animals and environment through a One health vision, is the future goal.

Keywords:
3Rs; Non Animal Methods; Predictivity; Complexity.

ID 03.2

The human HepaRG cell line: a brief history of its use in genetic toxicology: advantages, limits and future directions

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The genotoxicity assessment of chemicals is becoming increasingly dependent upon the use of in vitro models. Therefore, to take into account the impact of human metabolism in the genotoxicity response of chemicals, human liver cell lines have been studied and used in genotoxicity testing strategies. In addition to being a physiologically relevant model, HepaRG cells have been found to be an excellent model of human hepatic metabolism and toxicity, having nuclear receptor functionality, and expression of phase I and II enzymes, drug transporters, similar to primary human hepatocytes. Based on these characteristics, many researchers have focused their efforts to determine the capacity of the HepaRG cell model to detect human genotoxicants by comet assays and micronucleus tests. Their work found that this model is very sensitive and specific in genetic toxicology, and could be suitable to integrate into the current in vitro testing strategy. In addition, other biomarkers of genotoxicity have been investigated in HepaRG cells, such as γ-H2AX, expression of targeted genes (TGX-DDI, GENOMARKS), cometchip and high throughput multiparametric assays. Recently, with the development of new approach methologies (NAMs), 3D HepaRG models have been tested with the Cometchip technology and high throughput micronucleus assays, and have given promising results. Altogether these data suggest that HepaRG cells could provide a major contribution to in vitro genotoxicity assessment in in vitro genotoxicity assessement. Moreover, in the case of next-generation risk assessment of chemicals, the combination of more predictive approaches such as 3D co-culture models using HepaRG cells combined with OMICS, POD, and IVIVE approaches need to be explored.

ID 03.3

In vitro cell transforming capacity of different types of nano-plastics

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Nano-plastics are environmental pollutants that have received increasing interest during the last years. They are manufactured as such for specific industrial purposes or generated as secondary products resulting from the fragmentation of larger plastics. The broad distribution through the different environmental compartments makes humans susceptible to being unavoidably and continuously exposed to nano-plastics via different exposure routes. In addition, due to their persistent nature, they may bioaccumulate in different organs and tissues, raising concerns on their potential effects on human health, including the induction of carcinogenic processes.

Traditionally, short-term genotoxicity tests have been performed to assess genotoxic carcinogens, as DNA damage and mutations are key initiating events of carcinogenicity. However, carcinogenesis is a multi-stage process that involves not only initiation, but also promotion, and progression events. The latter ones cannot be detected by the genotoxicity assays. On the other hand, in vitro cell transformation assays, as the validated Bhas-42 cell transformation assay, allow the in vitro simulation of the in vivo initiation and promotion stages of carcinogenesis, thus, the detection of a broader range of carcinogenic agents.

In the present study, polystyrene (PS), polyethylene terephthalate (PET), and polylactic acid (PLA) nano-plastics were assessed using the Bhas-42 cell transformation assay. These plastic types are among the most represented in our daily life as food packages, building insulation, plastic films, etc. Preliminary results indicate the lack of PS' carcinogenic potential, in agreement with the reported literature. On the other hand, PET induces a significant promoting activity, but not initiating one, at the highest tested dose (200 µg/mL). Experiments with PLA are currently ongoing.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 965196 (PlasticHeal).

Keywords:
nano-plastics, cell transformation, carcinogenicity, Bhas-42 cells.

ID 03.4

In silico modelling of crosstalk between DNA damage and oxidative stress for prediction of cellular adversity

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Drug Induced Liver Injury (DILI) is a major problem for the drug development industry. Therefore, we urgently need methods that predict drug adversity with high fidelity and early in the drug development process. Computational approaches are thought to contribute to this challenge because they can make predictions and increase mechanistic understanding in a time and cost efficient way. Exposure of cells to toxic compounds activates various stress pathways and activity within these pathways is known to contribute to DILI. Therefore, computational descriptions of stress pathway activity based on experimental measurements could be a crucial step in the improvement of adversity predictions.

Genotoxic chemicals mainly cause activation of the DNA damage response (DDR), whereas other xenobiotics give rise to the oxidative stress response (OSR). Activation of these pathways can also occur via pathway crosstalk. Currently available computational models describe these individual stress pathways well with ordinary differential equation models, yet do not include crosstalk. Therefore, we here investigate the effect of connecting OSR and DDR models via multiple previously reported modes of crosstalk. Specifically, with our models we investigate the stimulating effect of NRF2 on MDM2 and NQO1 on P53, and the inhibitory effect of phosphorylated P53 on SRXN1 and of SRXN1 on BTG2. We compare the behaviour of the coupled model with previously published data of HepG2 GFP-reporter cells exposed for 60 hours to various concentrations of Diethyl Maleate and Etoposide, respectively OSR and DDR inducers. Model simulations show that the mentioned interactions can indeed qualitatively explain the characteristic dynamics of OSR and DDR proteins, and we are investigating which interactions are required to explain the dynamics also quantitatively. In the future, we aim to couple the model further to models describing the pharmacokinetics and cellular adversity, thereby rendering a novel approach for DILI prediction.

Keywords:
DNA damage response; Oxidative Stress response; computational modelling; Drug Induced Liver Injury;

ID 03.5

Perspectives for DNA adductomics in large-scale exposomics: upscaling sample preparation and preprocessing data

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Study of DNA adduct formation can be highly informative since DNA adducts are markers of exposure to chemical stressors as well as markers of effect, indicating increased risk. Complimentary to targeted DNA adduct analysis, untargeted DNA adductomics can be used for the detection of both known and unknown DNA adducts. DNA adductomics is a relatively young field of research however and methodological improvements can still be made. Therefore, the first objective of the current work was to evaluate thermal acidic vs. enzymatic DNA hydrolysis, followed by DNA adduct purification and enrichment using solid-phase extraction (SPE) or fraction collection; and hydrophilic interaction (HILIC) vs. reversed phase liquid chromatography (RPLC) coupled to high resolution mass spectrometry (HRMS) for DNA adductome mapping and modelling (i.e. assessed using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)). At the time, we also lack a clear view on how to preprocess untargeted data. Preprocessing is in fact seldomly applied, apart from signal correction for DNA concentration per sample and the use of a limited number of internal standards. Nevertheless, to obtain high-quality data and improve biological interpretability, proper data preprocessing is an absolute requirement. The second objective of this work was thus to investigate the use of QC (quality control) compared to iQC (internal QC) and QC-based robust locally estimated scatterplot smoothing (LOESS) signal correction in the data preprocessing workflow (based on i.a. superior clustering of QCs, and percentage of explained variance by principal components 1 and 2 in Principal Component Analysis (PCA) plots).

OPLS-DA demonstrated that HILIC compared to RPLC allowed better modeling of the tentative DNA adductome, particularly in combination with thermal acidic hydrolysis and SPE; resulting in more valid models, with an average cumulative Q2(Y) and cumulative R2(Y) of 0.930 and 0.998, respectively. Important to note is that thermal hydrolysis excels in simplicity, cost and time efficiency compared to enzymatic hydrolysis, and can accomodate high-throughput DNA adductomics.

Thermal acidic hydrolysis-SPE-HILIC-HRMS therefore qualifies as the most promising starting point for DNA adductome mapping and modelling in large-scale exposomics. Regarding data preprocessing, QC normalization outperformed iQC and LOESS, and may therefore be put forward as the default data normalization strategy.

Keywords:
Sample extraction; Liquid chromatography; DNA adductome mapping; Data normalization; Upscaling.

ID 03.6

Oxidative Stress Disrupt Differentiation of Human Induced Pluripotent Stem Cells

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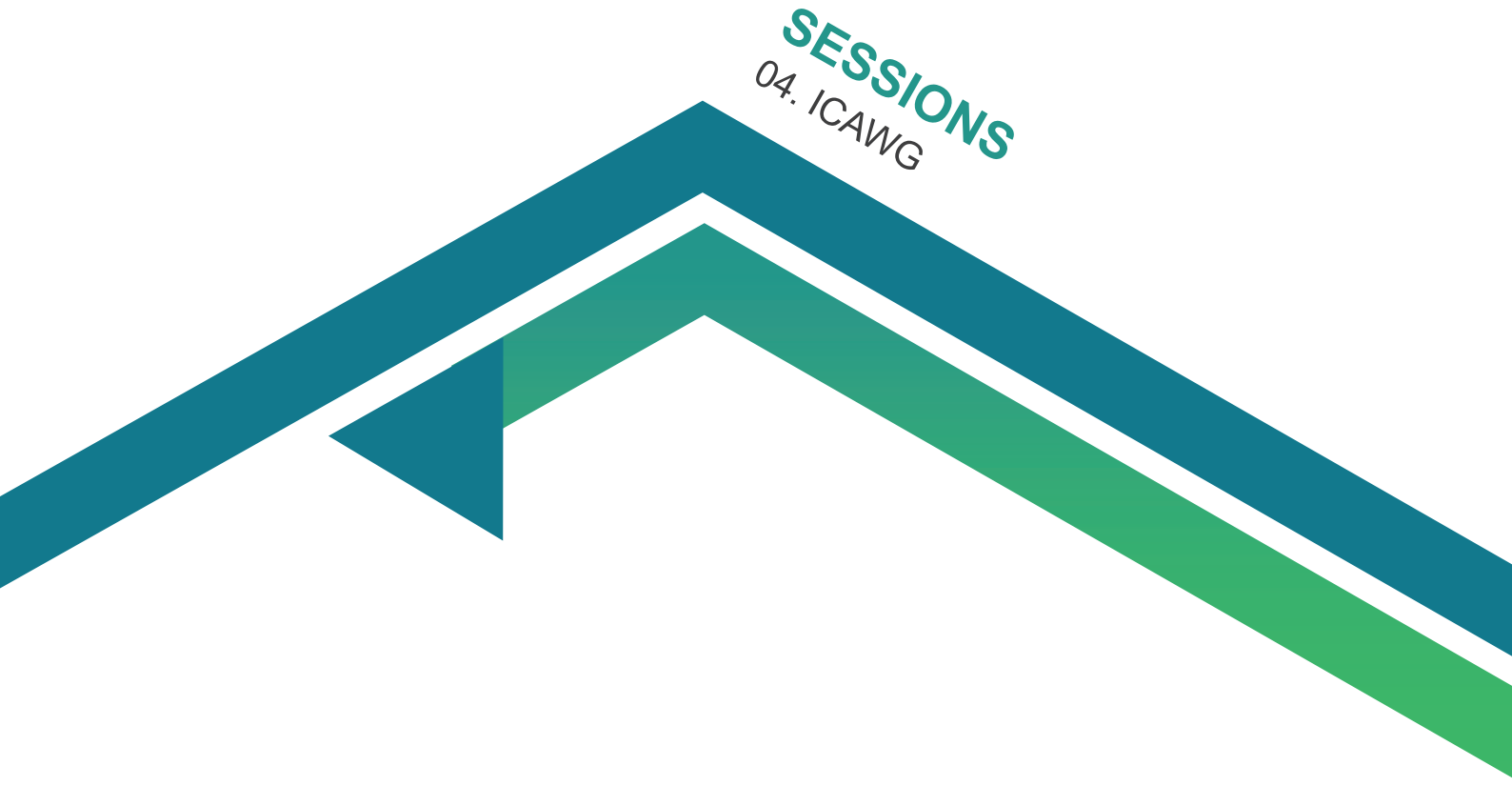
An array of substances mediate induction of reactive oxygen species (ROS) and it is thus important to understand its potential implications. One unexplored area is the process of early embryonal development.

We thus embarked on addressing this issue by exposing human induced pluripotent stem cells (hIPSC) during differentiation into the three germ cell layer specific cell types, cardiomyocytes, hepatocytes and neural rosettes in the Reprotracker assay. Model compounds inducing oxidative stress such as Potassium Bromate (KBrO3) and radiation (X-rays) were investigated along with benzo(a)pyrenediolepoxide (BPDE), and the cell cultures were exposed continuously (KBrO3 and BPDE) or intermittent (X-rays) to the compounds during the differentiation. Well-established human teratogen (thalidomide/retinoic acid) and non-teratogen (saccharin) were included as assay controls.

Cell viability was first broadly assessed to instruct selection of exposure concentrations/ doses of the agents. The status of differentiation of hiPSCs into the three germ layer specific cell types was assessed by detection of morphological abnormalities and decline in contraction of cardiomyocytes combined with expression patterns of biomarker genes using qRT-PCR. Extended gene expression analyses of genes involved in differentiation to all three germ cell layers, DNA damage response, DNA repair and epigenetic regulation is being conducted. Preliminary data will be presented.

The preliminary data suggest that ROS-producing compounds lead to disruption of differentiation into neural rosettes indicating developmental toxicity. The finding was supported by the gene expression patterns of the biomarker genes PAX6 and NESTIN.

Keywords:
Human induced pluripotent stem cells; reactive oxygen species; DNA damage; differentiation, germ cell layer.


SESSIONS
04. ICAWG

ID 04.1

Advanced 3D cell models and zebrafish embryos: A bridge between in vitro and in vivo

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The number of animals used in research has increased tremendously with the advancement of research and the development of pharmaceutical, medical and industrial fields. Under current EU legislation, toxicity testing is mandatory for all new chemicals and products before they are placed on the market. International regulations and guidelines for safety testing require animal testing as a follow-up when positive results are obtained in bacterial and mammalian in vitro models. Because currently used in vitro test systems are highly inaccurate and not sufficiently reliable, as indicator cells do not reflect the metabolism of chemicals in the human body, many chemicals are prematurely and often unnecessarily tested in vivo, which is ethically questionable and not in line with EU legislation on the welfare and protection of laboratory animals (3R strategy: reduce, refine, replace). Therefore, researchers are developing alternative approaches that are more reliable and relevant for human exposure to replace or at least reduce the number of animal experiments. One of the alternative approaches in toxicology research is advanced three-dimensional (3D) cell models, which more closely resemble the in vivo microenvironment than the traditional two-dimensional (2D) in vitro cell models currently used. Another model that is gaining popularity in toxicology research and is considered as a replacement for animal testing, is the zebrafish (Danio rerio) embryo model. The European Commission Directive 2010/63/EU currently allows experiments on fish embryos at the earliest life stages without regulating them as animal experiments. The presentation will discuss advanced 3D cell models in vitro developed from a human hepatocellular carcinoma (HepG2) cell line and zebrafish embryos as alternatives to animal testing in toxicology research.

Keywords:
Alternative to animal testing, 3D cell model, zebrafish embryo model.

ID 04.2

Utility of 3D HepaRG spheroid model for testing genotoxicity using high-throughput CometChip platform

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Three-dimensional (3D) culture systems are becoming more popular than 2D cultures for genotoxicity evaluation as several studies have demonstrated improved cell-to-cell interactions and tissue-like structures in 3D that are limited or lacking in 2D cultures. In the current study, HepaRG spheroids were formed using metabolically competent human HepaRG cells. 3D spheroids formed in 96- or 384-well ultra-low attachment plates were then exposed to various concentrations of 34 test articles that encompassed 8 direct-acting and 11 indirect-acting genotoxicants/carcinogens as well as 15 compounds that show different genotoxic responses in vitro and in vivo. High-throughput CometChip assay was employed to evaluate DNA damage along with concurrent cytotoxicity assessment by the ATP assay in both 2D and 3D cultures. 3D HepaRG spheroids compared to 2D cultures appeared to maintain a stable phenotype for up to 30 days with higher levels of albumin secretion, cytochrome P450 gene expression, and enzyme activities. 3D spheroids also demonstrated a higher sensitivity than 2D cultures for detecting both direct- and indirect-acting genotoxicants/carcinogens, indicating a better reporter of in vivo genotoxicity. DNA damage dose-response data when quantified using PROAST software, 3D spheroids showed generally lower or similar benchmark dose values compared to 2D HepaRG cells, but they were more comparable to primary human hepatocytes. These results demonstrate that 3D models can be adapted to the CometChip technology for high-throughput genotoxicity testing and that 3D HepaRG spheroids may be a reliable and pragmatic in vitro approach for the hazard identification and risk assessment of potential human genotoxic carcinogens.

ID 04.3

Use of the CometChip® for the *in vitro, in vivo* and Fpg-modifed assay

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The high throughput 96 macrowell system with a spatially encoded array of microwells patterned in agarose, has gained in popularity in recent years. The commercial version (CometChip®) has only been used when applying the standard *in vitro* version of the assay, and comparisons with the more commonly used protocols, such as the 2 gels/slide, have not been published. In this work, new protocols to allow the adoption of the enzyme-modified comet assay as well as the analysis of *in vivo* samples were developed. Results obtained using the CometChip® were compared with those obtained with the classic 2 gels/slide version.

TK6 cells were treated with different concentrations of methyl methanesulfonate (MMS) or hydrogen peroxide (H2O2), for the standard version of the assay, or potassium bromate (KBrO3) in the case of the enzyme-modified version. Appropriate solvents were used as negative controls. For the *in vivo* comet assay, snap frozen samples of liver, kidney and duodenum were obtained from Wistar rats orally dosed with 200 mg/kg MMS and sacrificed after 3 hours.

Adapting CometChip® protocol for the use of the enzyme formamidopyrimidine DNA glycosylase (Fpg) requires modifications in washing steps before the enzyme incubation, together with changes in overlay agarose and enzyme concentrations. In the case of the analysis of *in vivo* samples, adjustments in the temperature of certain steps were needed. After adapting the CometChip® protocol, results obtained in the in vitro standard and Fpg-modified comet assay and in the *in vivo* assay are comparable to the ones obtained when using the 2 gels/slide protocol. It is important to note that different systems need different protocols to obtain comparable results.

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Keywords:
Comet assay, CometChip, Fpg, *in vivo, in vitro.*

ID 04.4

Evaluation of antioxidative, antigenotoxic, and anticancer activities of commercial medical mushrooms products: Agaricus blazei, Cordyceps Sinensis and Immune Assist

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Mushrooms have been evaluated for their nutritional and medicinal properties for centuries. We evaluated the biological activities of commercial products of Cordyceps Sinensis (CS), Agaricus blazei (AB), and Immune Assist (IA) (all produced by Aloha Medicinals). Immune Assist (IA) is made from extract of six species of medical mushrooms: Agaricus blazei - Cordycepssinensis - Grifola frondosa - Ganoderma lucidum - Coriolus versicolor - Lentinula edodes.

The antioxidant evaluation showed that CS had strong OH scavenging properties and moderate reducing power, while its DPPH scavenging ability was weak. AB displayed remarkable ·OH scavenging properties, moderate reducing power, and modest DPPH scavenging activity, similar to IA. Also, CS, AB and IA displayed significant antigenotoxic effects in human peripheral blood cells against H2O2-induced DNA damage.

Further, the cytotoxicity of CS, AB, and IA to HS-5 (bone marrow stroma cells), MCF-7 (human breast epithelial cell line), and MDA-MB-231 (human breast carcinoma cell line) were assessed using MTT assay. AB and IA could inhibit the proliferative action of mentioned cancer cell lines after 72h in a dose-dependent manner, while there were no effects on HS-5 cell lines proliferation, while CS did not show anti-proliferative/ cytotoxic activity on cancer cell lines.

Current findings remain a significant challenge for the usage of medicinal mushrooms in the field of cancer prevention/treatment, and thus an active area of future research.

Keywords:
Medical mushrooms; antioxidative properties; antigenotoxicity; cytotoxicity.

ID 04.5

Maternal exercise during pregnancy modulates genetic and biochemical damage caused by high consumption of fructose in offspring

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The consumption of fructose increased exponentially over the past decades. Especially during pregnancy a poor diet, such as high fructose consumption, can have adverse health effect via fetal programming. Thus, preventive measures to minimize the effects of poor diet in this period are necessary. Therefore, the objective of this study was to evaluate whether the practice of voluntary physical exercise (VPE) can reduce the adverse effects of chronic consumption of fructose, from the beginning of life and/or until the gestational period, on the metabolism and genome of pregnant females and their offspring. For this, 70 Swiss female mice with 21 days of life received fructose (FRU; 20%/L) in the hydration bottle and/or practiced VPE for 8 weeks (pre-pregnancy). Females were divided into 4 treatment groups: G1-Water; G2-Water+VPE; G3-FRU; G4-FRU+VPE. After the lactation period, the offspring of the 4 experimental groups were separated by sex. The mothers were euthanized after lactation. The offspring (males and females) continued on the respective treatments and were euthanized at 60 days of age for genetic and biochemical evaluations. It was observed that the consumption of fructose increased the serum fructose concentration in the mothers and offspring, and that the VPE decreases these parameters. In addition, fructose was genotoxic (assessed via comet assay) and mutagenic (micronucleus assay) in the mothers' peripheral tissues and VPE had a preventive effect on these parameters. An increase in the adiposity index was observed in male offspring in the FRU group and a decrease in the FRU+VPE group. Furthermore, fructose lead to hepatic steatosis in the offspring and VPE was able to decrease the area of steatosis. Also, fructose led to genotoxicity in the offspring and VPE was able to modulate this effect, reducing damages.

The practice of physical exercise in the control group did not significantly affect the mother nor the offspring. In conclusion, we observed that intervention with VPE had genetic and biochemical benefits in blood and liver of the mothers and their offspring, counteracting the adverse effects of fructose.

Keywords:
Fetal programming; Fructose; Physical exercise; Genotoxicity; Mutagenicity.


SESSIONS
05. Ecotoxicology

ID 05.1

The challenge of assessing contaminants of emerging concern and microplastics

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The list of contaminants of emerging concern (CECs) has steadily increased during the last decades including a broad spectrum of organic and inorganic compounds showing different physicochemical and toxicological properties. Some of the more prominent groups include persistent and mobile organic compounds (PMOCs), polar pesticides, pharmaceuticals, flame retardants, a significant number of chemical groups employed as plastic additives, personal care products, anthropogenic particles including nanomaterials and micro-and nano plastics, among others. For the analysis of organic chemicals liquid chromatography separations coupled with mass spectrometry analysers (LC-MS), have been the techniques of choice for environmental analysis. Nowadays, thanks to its unique ability to measure analytes based on accurate mass, full-spectrum high-resolution mass spectrometry (HRMS) can simultaneously gain qualitative and quantitative information on a virtually unlimited number of analytes.

In this presentation, several examples of the analysis of CECs using targeted, non-targeted and suspected screening approaches will be presented, together with recent assessment of their potential negative impacts in the environment and human health.

Keywords:
CECs, PFASs, nanomaterials, natural toxins, micro-nano plastics

ID 05.2
Abstract for EMGS~ Malaga~ March 2023

Assessing the Impact of Persistent and Emerging Contaminants on Aquatic Organisms

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The aquatic environment is often the ultimate recipient of increasing amount and range of contaminants, in all probable combinations. This presents a major challenge to protect the quality and sustainability of natural resources. The continued developments of integrated and multidisciplinary approaches incorporating analytical, biological (including 'omics'), statistical and computational technologies are realising the importance of natural organisms, which could serve as sentinel or surrogate to correlate human and ecosystem health in order to improve environmental risk assessment (ERA). In this context, we have attempted to develop and validate a range of sub-lethal biological or biomarkers responses in ecologically important species such as bivalve mollusks. The broader aims have been to determine the relative sensitivity of the biomarkers and the species following exposure to a range of well known (e.g. metals, PAHs) and emerging contaminants (e.g. engineered nanoparticles or ENPs) in combinations with other pollutants (e.g. PAHs). Linking 'toxicokinetics' with 'toxicodynamics' processes and using appropriate analytical, statistical and computational modelling tools, our studies compliment the observed biological responses with bioavailability and tissue-specific body burden of the contaminants. The synthesized information from our studies offer information pertaining to potential detrimental impact of contaminants on the health of the organisms. The adopted approach could be translated in other organisms and environmental conditions for the protection of human and ecosystem health. We however need to evaluate inherent limitations and challenges of various available tools, while aiming to readily integrate them into regulatory frameworks.

ID 05.3

Hydrogen peroxide-induced oxidative damage in sea urchin (Paracentrotus lividus) DNA

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Hydrogen peroxide (H2O2) is regularly used in Scottish salmon aquaculture as an antiparasitic treatment for sea lice infestation. After application, H2O2 is released into the environment and reactive oxygen species produced during the breakdown process which can induce oxidative damage in DNA of non-target marine organisms, such as sea urchins. The objective of this study was to assess oxidative DNA damage in adult sea urchins and embryos derived from gametes that were exposed to environmentally realistic H2O2 concentrations. Exposure concentrations of H2O2 were based on those obtained from previous models of aquaculture farm treatments. Damage to DNA was determined by the modified fast micromethod (Liu et al. 2022), which was adapted from the fast micromethod and is similar to the modified comet assay [used formamidopyrimidine-DNA glycosylase (FPG) and endonuclease-III (Endo III) for additional quantification of oxidised purine and pyrimidines]. Acute 1-h exposure of adult sea urchins to a bath treatment of 500µM H2O2 resulted in significantly increased oxidative DNA damage in coelomocytes of 0.05±0.2 (mean±SEM, n=5, $p<0.05$, ANOVA) in strand scission factor (SSF) that returned to control levels after 3h and suggested DNA repair. Embryos cross fertilised with H2O2-exposed sperm (with confirmed DNA damage after 1mM H2O2 exposure for 10min) and unexposed eggs showed elevated oxidative DNA damage of up to 0.1±0.01 SSF (mean±SEM, n=3, $p<0.05$, ANOVA) at 3 and 24h (blastula) post fertilisation. No evidence of DNA repair was indicated up to 24h, but average levels of SSF returned to control levels at 48h (gastrula) possibly due to DNA repair. This study used low concentrations of H2O2 in short-term exposures and demonstrated DNA damage in both adult benthic sea urchins and pelagic spawned gametes with evidence of DNA repair in both cases at 3h in coelomocytes and 48h in embryos.

Keywords:
Genotoxicity; DNA damage; oxidation; methylation; modified fast micromethod, FPG, Endo III, McrBC.

ID 05.4

Plant comet assay in biomonitoring of air pollution in Sarajevo, Bosnia and Herzegovina

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The World Health Organization recognizes air pollution as a significant health and environmental concern. Threatening air pollutants are a common predicament of developing countries. Located in a valley and surrounded by high mountains, Sarajevo, the capital of Bosnia and Herzegovina (B&H), suffers temperature inversions during autumn/winter which trap harmful particles thus exacerbating their effects. Local geomorphology, vehicle emissions, coal combustion from households, and the lack of adequate environmental policies profoundly influence the air quality. The detrimental effects of air pollution are well-known through various human biomonitoring studies. However, biomonitoring studies are inevitably hindered by human lifestyle and individual metabolic variance. Plants, on the other hand, are continuously exposed to a variety of environmental factors, thereby presenting compelling models for periodic air pollution biomonitoring. Ligustrum vulgare L. (wild privet) is a semi-evergreen hedge plant from Oleaceae family, widespread across Europe, East Asia and North America. It is tolerant towards a wide array of soils and environmental conditions. We applied plant comet assay in order to compare DNA damage in L. vulgare leaves sampled at two urban and one rural site, with respect to season, leaf position and the stage of development. Tail intensity (TI) values showed significant differences between urban and rural sites ($P < 0.001$), but also between adult and young leaves. At urban sites, outer adult leaves revealed higher TI compared to inner adult, and both outer/inner young leaves. At rural sites, those parameters did not significantly differ. In regards to COVID-19 pandemic, TI at urban sites was lower after the lockdown compared to pre COVID-19 period. Given its ubiquitous distribution and genome size, our results demonstrate that L. vulgare is a reliable model for plant comet assay and air pollution biomonitoring. Nevertheless, ongoing monitoring and periodic sampling should provide more information concerning air data correlation, plant physiology and air pollution effects.

Keywords:
DNA damage; Ligustrum vulgare; plant comet assay; tail intensity; air pollution.


SESSIONS
06. ECETOC

ID 06.1

An Introduction to ECETOC's workshops on Point of Departure and IVIVE modelling

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'Omics technologies have been part of the research toolkit since the late 1990s. Right from the start there were predictions about how these technologies would revolutionize toxicology allowing greater insight into pathological changes, understanding of mechanisms, and in deriving points of departure. There were, however, several challenges identified including recording of metadata, processing of data, and finally interpretation. ECETOC was amongst the first to systematically explore the application of 'omics methods, now incorporated into the group of methods known as New Approach Methodologies (NAMs), to regulatory toxicology. Outputs have led to projects included in the workplan of the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST) group of the OECD and to the drafting of OECD Guidance Documents for omics data reporting. The workshop on the derivation of a Point of Departure (POD) from 'omics data, jointly with the one on PBPK models for in vitro-in vivo extrapolation provide a framework for the use of NAM data for quantitative risk assessment. Workshop presentations demonstrated that 'omics data developed within robust frameworks for both scientific data generation and analysis can be used to derive a POD. The issue of noise in the data was discussed as an important consideration for identifying robust omics changes and derive a POD. As such variability or "noise" can comprise technical or biological variation within a dataset but should clearly be distinguished from homeostatic responses. Adverse Outcome pathways (AOPs) were considered to be a useful framework on which to assemble omics methods, and several case examples were presented in illustration of this point. What is apparent is that high dimension data will always be subject to varying processing pipelines and hence interpretation, depending on the context they are used in. Yet, they can provide valuable input for regulatory toxicology, with the pre-condition being robust methods for the collection and processing of data together with a comprehensive description how the data was interpreted, and conclusions reached.

Keywords:
Point of Departure, IVIVE, 'omics.

ID 06.2

Physiologically Based Kinetic Modelling applications in Chemical Risk Assessment

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Physiologically based kinetic (PBK) modelling, which mathematically represents the body of a given species using chemical-specific and physiological information, is a fast-growing tool in toxicology. With this presentation, a perspective on the evolution of this tool, together with learning principles, will be given. The first part will introduce elements and a step-by-step process to develop a PBK model using the Open System Pharmacology Suite (OSPS)- PK-Sim platform. Briefly, an introduction to the building blocks and the equations representing the tissues in the body will be presented along with relevant exposure routes. The second part of the talk will be on the application of the tool and the extrapolations that can inform chemical risk assessment, especially with insight into the quantitative in vitro to in vivo extrapolations (QIVIVE). This approach is defined as the extrapolation of in vitro effect concentrations to in vivo bioequivalent exposures; the approach is considered a new approach methodology (NAM) and can be applied in different industrial sectors. The presentation will end with the recent recommendations from the ECETOC IVIVE workshop (Najjar et al. 2022; https://doi.org/10.1007/s00204-022-03356-5), to improve regulatory adoption to gain confidence in PBK modelling for QIVIVE in regulatory use.

Keywords:
PBPK model, QIVIVE, Human and Environmental Health.

ID 06.3

Determination of point of departures based on high throughput *in vitro* metabolomics

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Metabolomics *in vitro* poses a high potential for a biologically driven risk assessment approach. We recently developed a platform for high throughput in vitro testing based on LC-MS targeted metabolomics in HepG2 cells. This screening platform was optimized for various parameters such as cell seeding density, passage number, sample preparation, analytical method, etc. and could be used to identify different liver toxicity mode of actions. To determine biologically relevant dose concentrations useful for *in vitro* to *in vivo* extrapolation, it is required to determine a point-of-departure (PoD). We present here an approach to determine PoD from a dose response curve determined from multivariate metabolomics data. This approach establishes an important connection for translating results from *in vitro* systems to *in vivo* and thus human relevance. Taken together, our metabolomics *in vitro* system demonstrates applicability for mode of action identification in vitro as well as POD determination.

Keywords:
Metabolomics; metabolomics in vitro; point of departure; PoD.

ID 06.4

Summary of the ECETOC workshop from IVIVE to PODS – translating research methods to application

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'Omic technologies have been in use by the scientific community for a quarter of a century and over that time have matured, particularly for use in research or investigative toxicology. Initial applications in the Toxicological space for the 3Rs and pathological refinement has been achieved at the research level. Translation to application in routine regulatory hazard assessment has been though more challenging. There are several reasons for this with the pre-eminent being technical and data complexity, transformation, reporting and interpretation. Technical issues have been largely addressed and data transformation and reporting are addressed with reporting frameworks from the OECD which ECETOC instigated with workshops in 2015 and 2016 and subsequent adoption by the EAGMST committee of OECD. Issues with interpretation are still to be adequately addressed for regulatory purposes. For this reason this aspect has been recently adopted onto the ECETOC work plan and in due course will be taken to OECD. In this workshop will examine dose setting using QIVIVE, a critical issue in ensuring that conclusions from complex data are meaningful and not just a reflection of cellular stress resulting from exceedance of the Maximum Tolerated Dose. Then in two omics session will examine meaningful methods to extract Benchmark Dose or point of departure data (POD) from both metabolomics and transcriptomics to inform on hazard and derive Health Based Guidance Values. These presentations will provide insight into the progress now being made in the translation of these research methods from the bench to routine application in toxicological hazard assessment for regulatory purposes and provide a useful summary of the current state of the art and future direction.

Keywords:
Transcriptomics, Metabolomics, POD, IVIE.

ID 06.5

Applying transcriptomic benchmark modelling for toxicological decision making

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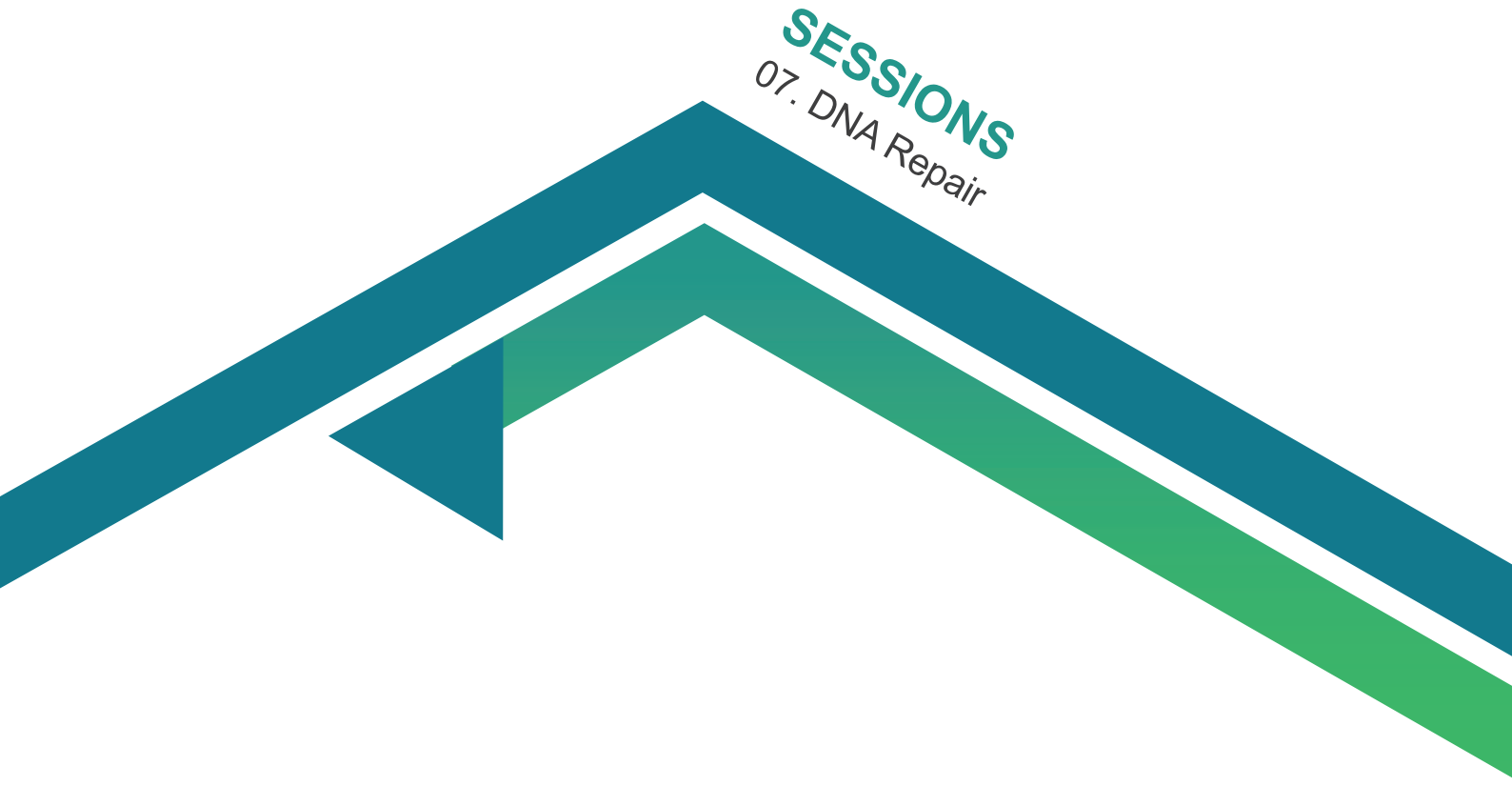
Risks from chemical exposure are managed using reference doses extrapolated from a Point of Departure (PoD) that is derived from the no observed effect concentrations, or by calculating the benchmark dose (BMD) of pathological observations seen in standardised animal studies. This approach is founded on the premise that humans and ecological species will be sufficiently protected by ensuring chemical exposure is below the dose at which a defined observable endpoint is expected. Pathological observations in animal studies have been used, since at least the 1940s, to determine these PoDs. However, using traditional animal studies is low throughput and resource intensive, resulting in only a limited number of known chemicals having been evaluated.

The application of omics methods to the risk assessments of industrial chemicals and agrochemicals has been proposed by the scientific community for two decades. However, although research has demonstrated that it may be possible to use omics data to derive a PoD, in practice it has not been used. To aid this uptake process, recently Johnson et al (Toxicol. Sci. 190(2), 2022, 127) proposed a logical framework that outlined 4 key principles that must be accepted to give confidence in using omics data to set a PoD: (1) transcriptomics is a reliable tool to detect altered gene expression caused by a chemical treatment; (2) altered gene expression is an indicator of adverse or adaptive biological responses to a stressor; (3) a benchmark dose based PoD can be set using a concerted molecular change (CMC) in transcriptomics data measured in a short term in vivo studies; and (4) these transcriptomic PoD supports a human health protective risk assessment.

The first two principles are well supported by published literature. Recently, the latest in a series of ECETOC workshops on use of omics data, explored the third principle. It explored a number of case studies and concluded that 'omics data, when developed within the extant robust frameworks for data generation and analysis, can be used to derive a PoD. Here I will show that BMD analyses of CMCs in transcriptomics data from multiple tissues in a 14-day rat dietary toxicity studies supports human health protective risk assessments for several of our recent crop protection development candidates. Therefore, we can conclude that transcriptomics data can be used to set endpoints for human health risk assessments of repeat dose systemic toxicity.

Keywords:
toxicogenomics; benchmark dose modelling; point of departure; crop protection


SESSIONS
07. DNA Repair

ID 07.1

Specific killing of BRCA1-deficient cancer cells by depletion of EXO1

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BRCA1 and BRCA2 are essential genome maintenance factors that function in the repair of DNA Double-Strand Breaks (DSBs) by homologous recombination (HR). Cancer patients that carry tumors with loss-of-function mutations in BRCA1 or BRCA2 often benefit from treatment with PARP inhibitor therapy, which specifically kills HR-deficient tumor cells. However, clinical responses are rarely long-lasting due to resistance to PARP inhibitor treatment. We therefore sought to identify novel therapeutic opportunities to treat HR-deficient tumors. Our studies revealed that genetic inactivation of the exonuclease EXO1 is severely toxic to BRCA1-deficient cells, but not to BRCA1-proficient cells. Mechanistically, our data suggest show that loss of EXO1 results in DSB formation, potentially due to a defect in the maturation of Okazaki fragments. BRCA1/EXO1 double-deficient cells are severely compromised in their capacity to repair these DSBs, resulting in genomic instability and cell death. Taken together, we have uncovered EXO1 as a novel synthetic lethal target with therapeutic potential to treat patients carrying BRCA1-deficient tumors.

ID 07.2

Influence of orphan base and sequence context on the processing of AP sites by AP lyases in Arabidopsis thaliana

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Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions arising by spontaneous loss of a nitrogenous base due to the susceptibility of the N-glycosidic bond to undergo hydrolysis reactions. Moreover, AP sites are also enzymatically generated as intermediates during the Base Excision Repair (BER) pathway, in which DNA glycosylases catalyze the excision of modified bases from DNA. AP sites may be processed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that the sequence flanking the AP site and the orphan base opposite the lesion may determine the enzyme responsible for its processing and the repair efficiency.

We analyzed the activity of the major Arabidopsis AP lyase (FPG) on DNA substrates containing an abasic site opposite C or G in different sequence contexts. AP sites opposite G are common intermediates during the repair of deaminated cytosines, whereas AP sites opposite C frequently arise from oxidized guanines. We found that FPG shows a preference for AP sites opposite C, but such specificity is modulated by the DNA sequence context surrounding the lesion.

To identify possible mechanisms responsible for the opposite base preference displayed by FPG we performed DNA binding assays with different DNA substrates, as well as with reaction products. We found that FPG binds DNA with high affinity regardless of the presence of and AP site, suggesting that the enzyme binds DNA non-specifically and slides randomly in search for its target. We have also observed that FPG binds DNA substrates containing AP sites with the same affinity independently of the base opposite the lesion. However, FPG remains bound with higher affinity to its incised reaction product when G is the orphan base. This result suggests that the higher activity of FPG for AP sites opposite G is not due to increased affinity for the substrate, but to decreased product dissociation. Our results shed light on the role of AP lyases in the processing of a ubiquitous DNA lesion.

Keywords:
Base Excision Repair, AP sites, AP lyase, FPG.

ID 07.3

Role of Phosphatase and Tensin homolog (Pten) in Insulin mediated DNA damage

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Endogenous substances such as hormones may cause DNA damage when present at pathophysiological levels. For example, hyperinsulinemia is a characteristic of early type 2 diabetes mellitus, resulting from the oversecretion of insulin. The association between elevated insulin levels and cancer is well-known, however, the precise role of insulin in cancer development is far from clear. In this study, our focus was the role of Phosphate and Tensin homolog (Pten), a tumor suppressor dual phosphatase that negatively regulates PI3K/AKT signaling downstream after insulin activation. For further elucidation Phosphatase and Tensin homolog (Pten), a tumor suppressor phosphatase that plays a role in insulin signaling by negative regulation of PI3K/AKT and its downstream targets, was investigated here.

We used dihydroethidium staining to measure reactive oxygen species (ROS) formation and analyzed DNA damage using comet assay, micronucleus test, and an antibody staining against phosphorylated H2Ax. Our findings showed an increase in insulin-mediated ROS formation and as a result, elevated DNA damage by using a pharmacological Pten inhibitor in a liver cell line. Furthermore, the knockdown of Pten in a mouse model increased oxidative stress and yielded increased DNA double-strand breaks in the liver tissue.

We conclude that Pten is involved in oxidative stress and genomic damage induction in vitro and that this may also explain the in vivo observations. This further supports the hypothesis that the PI3K/AKT pathway is responsible for the damaging effects of high levels of insulin.

Keywords:
Insulin, DNA damage, oxidative stress, Pten, insulin signalling.

ID 07.4

A direct role for HIPK2 in homology-directed DNA repair and the regulation of PARP inhibitor sensitivity

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Our cells are under continuous attack by exogenously and endogenously evoked DNA damage resulting in different types of DNA lesions. DNA double-strand breaks (DSBs) are the most deleterious type of genome damage and are in principle repaired by two pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR). Homology-directed repair of DSBs efficiently counteracts genome instability and cancer formation. Moreover, cancer cells frequently show defects in DNA repair and particularly HR-deficiency of cancer cells can be therapeutically exploited since such cells are sensitized to poly(ADP-ribose) polymerase inhibitor (PARPi) treatment.

Work from us and others has established the tumour suppressor Homeodomain Interacting Protein Kinase 2 (HIPK2) as a central player in DNA damage-induced cell fate control. Upon DSB induction by ionizing radiation (IR), HIPK2 is activated by a mechanism involving checkpoint kinase ATM and site-specific HIPK2 autophosphorylation, and triggers cell death through the p53 pathway. Although the cell death regulatory function of HIPK2 in response to irreparable DNA damage is well-established, its function upon repairable DNA damage remains largely obscure.

Intriguingly, our unpublished data identified a direct role for HIPK2 in homology-directed DSB repair. We found that HIPK2 forms a protein complex with DSB repair factors BRCA1 and 53BP1, and physically accumulates at IR-induced DSBs in an ATM-regulated manner. DSB reporter assays and Sister chromatid exchange analysis revealed an essential role for HIPK2 in DSB repair by HR. Mechanistically, we found that HIPK2 binds and site-specifically phosphorylates BRCA1, thereby regulating BRCA1 protein stability. Accordingly, HIPK2 depletion or pharmacological inhibition of HIPK2 results in declined BRCA1 levels and potentiates IR sensitivity and PARPi toxicity in BRCA1-proficient cells. Together, our results identify a direct role of HIPK2 in HR repair, and suggest HIPK2 inhibition as novel strategy to sensitize BRCA1-proficient cancer cells to PARPi.

Keywords:
DNA repair; homologous recombination; PARP inhibitor; HIPK2

ID 07.5

Could DNA damage be a useful biomarker of diagnosis and prognostic in human breast and colorectal cancer?

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Breast and colorectal cancers are the two most common and deadly cancers worldwide. Mechanisms underlying the increasing number of sporadic cases of these cancers are not fully understood, but environmental exposure and lifestyle have been implicated. Among causes of cancer development, genomic instability plays a major role due to constant endogenous and exogenous aggressions to cells that cause mutations when the damage is not repaired. Recently, understanding the mechanisms that drive DNA instability in cancer cells has become an increasingly important area of research, as it has the potential to contribute to the development of new diagnostic tools for cancer. Although controversial, DNA damage is more commonly associated with carcinogenesis, and it has been considered by some authors as a potential biomarker for cancer diagnosis and prognosis. To clarify this issue, we assessed basal levels of DNA damage, in peripheral blood lymphocytes of 58 breast and 27 colorectal cancer patients and a control group of 11 healthy individuals, using the simple comet assay. This work was approved by the local ethic committee and each patient gave a written consent. Most of the studied cancer cases (95.7%) are not hereditary and may be associated with sporadic cases. Results showed that DNA strand breaks levels of all cancer patients are higher when compared to healthy individuals (t-test for independent samples, P=0.001), and are independent of age, gender, and lifestyle, such as smoking and drinking habits, suggesting that high levels of DNA damage are associated with cancer. Particularly, we observed that breast cancer patients in stage III of the disease have a significantly increased level of DNA damage than individuals in stages I and II of the disease (one-way ANOVA, P=0.02), revealing that there was a positive linkage between DNA damage level and the disease stage. In turn, results from colorectal cancer patients did not allow the same conclusions to be drawn, since most patients were stage II. Additionally, results suggest that colorectal cancer patients with a worse prognosis tend to have higher levels of basal DNA damage at diagnosis.

Altogether, these findings support the potential value of DNA damage assessment as a useful biomarker for diagnosis and prognosis in these cancer patients. However, DNA damage is not sufficient for a cancer diagnosis since many factors can contribute to DNA damage and further tests are required for a definitive diagnosis.

Keywords:
Cancer, breast, colorectal, DNA damage, biomarker.

ID 07.6

Senescence and cell death triggered by the DNA alkylation damage O6-methylguanine

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Alkylating agents are potent mutagens and carcinogens. They are also cytotoxic, which is harnessed in cancer chemotherapy. In glioblastoma therapy, the 1st line drug is temozolomide (TMZ), which induces various DNA lesions including O6-methylguanine (O6MeG). If not repaired, O6MeG gives rise to DSBs due to futile mismatch repair (MMR) cycles, which finally trigger ATR/ATM activation and the DNA damage response (DDR). This leads to activation of apoptotic and the senescence pathways. We have shown that apoptosis and senescence follow the same kinetics, but different dose-responses. Thus, 8 d after treatment senescence reached a 3-fold higher level than apoptosis, indicating senescence is a main trait. Analysis of TMZ-induced senescent cells show high levels of trimethylated H3K9 and H3K27, both marker for senescence, high amounts of DNA double-strand breaks, which were located outside of telomers, and a sustained activation of the DDR. Although TMZ-induced DSBs persist in senescent cells, radiation-induced DSBs are still repaired. To further elucidate the role of O6MeG in apoptosis and senescence induction following TMZ treatment, we used a tet-on system to induce MGMT at defined stages after TMZ treatment and in senescent cells. Upregulation of MGMT immediately after TMZ treatment caused complete abrogation of apoptosis and senescence, while MGMT upregulation >3d following TMZ treatment had no impact on apoptosis and senescence induction. Upregulation of MGMT in senescent cells showed neither reduction of senescence nor induction of apoptosis in the senescent population. Senescent cells were also found in post-treatment cancer specimens. Overall, the data show that O6MeG-triggered senescence is a main response following TMZ treatment of glioblastoma cells and that O6MeG is required for induction, but not for maintenance of the senescent state. The amount of O6MeG required for triggering apoptosis and CSEN was determined. A search for senolytic drugs revealed some natural compounds of promising activity. The role of senolytics in cancer therapy will be discussed. Supported by DFG KA724/31-1.


SESSIONS
08. Machine Learning

ID 08.1
Introduction to Machine Learning in Genetic Toxicology

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Any modern application of technology in use today likely has some component of machine learning (ML) or artificial intelligence (AI) associated with its development or execution. ML/AI has become such a ubiquitous part of our lives that we often take it for granted and rarely acknowledge what it is doing for us or conversely, we overly exaggerate its influence and consider it a potential threat to our workforce and society. Regardless of these perceptions, there is no denying the impact of AI/ML on our lives, and more specifically on science. Perhaps one of the most well-recognized roles of machine learning in science is in analyzing datasets that were previously inaccessible. “Big data” has frankly become an understatement compared to the reality in which we find ourselves with our ability to generate large volumes of data in a very short period of time. Examples of general applications of AI/ML in science will be reviewed.

When we move to genetic toxicology specifically, we continue to see the utility of AI/ML. As New Approach Methodologies development more efficient ways of generating high content output, the need for analytical schemes that can easily deal with large or complex data structures is critical. From in silico work studying structure activity relationships to in vitro studies using pattern recognition for multiplexed biomarker assessments and large meta analyses of in vivo data, there are applications and opportunities for ML to contribute greatly for improvements in our workflow. This presentation will highlight several applications of ML in the analysis and interpretation of data from current genetic toxicology methods.

D ID 08.2

eep Neural Networks to Automate Scoring Of The Imaging Flow Cytometry In-Vitro Micronucleus Assay

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The *in vitro* micronucleus assay is a globally used technique for the quantification of DNA damage required for regulatory compound safety testing in addition to inter-individual monitoring of environmental, lifestyle and occupational factors. However, it relies on time-consuming and user-subjective manual scoring. This presentation will discuss progress towards fully automating the scoring of the assay to provide a robust and reliable technique which can be applied at any laboratory without any requirement for parameter tuning.

We focus on using imaging flow cytometry to provide single-cell, brightfield and nuclear stained images in a high-throughput manner. Images were captured for the cytokinesis-block micronucleus method using methyl methane sulphonate and carbendazim exposures to TK6 cells at three different laboratories. The images were scored manually into mono, bi, tri and tetra -nucleated categories alongside the same phenotypes exhibiting micronuclei. Apoptotic cells, metaphase spreads and cellular debris events were also scored. This human-curated data provided a large training set enabling assessment of wide-ranging automated scoring algorithms.

The large number of single-cell images acquired using imaging flow cytometry (> 10,000 / replicate) makes this type of data ideal for the application of deep learning neural networks. We will discuss the use of several different types of deep learning algorithms and the optimisation of the analysis pipelines to increase the accuracy of detection. We will also discuss the generalisation of these methods to traditional microscopy images.

ID 08.3

Towards a quantitative understanding of the DNA damage response through data-driven dynamical modeling

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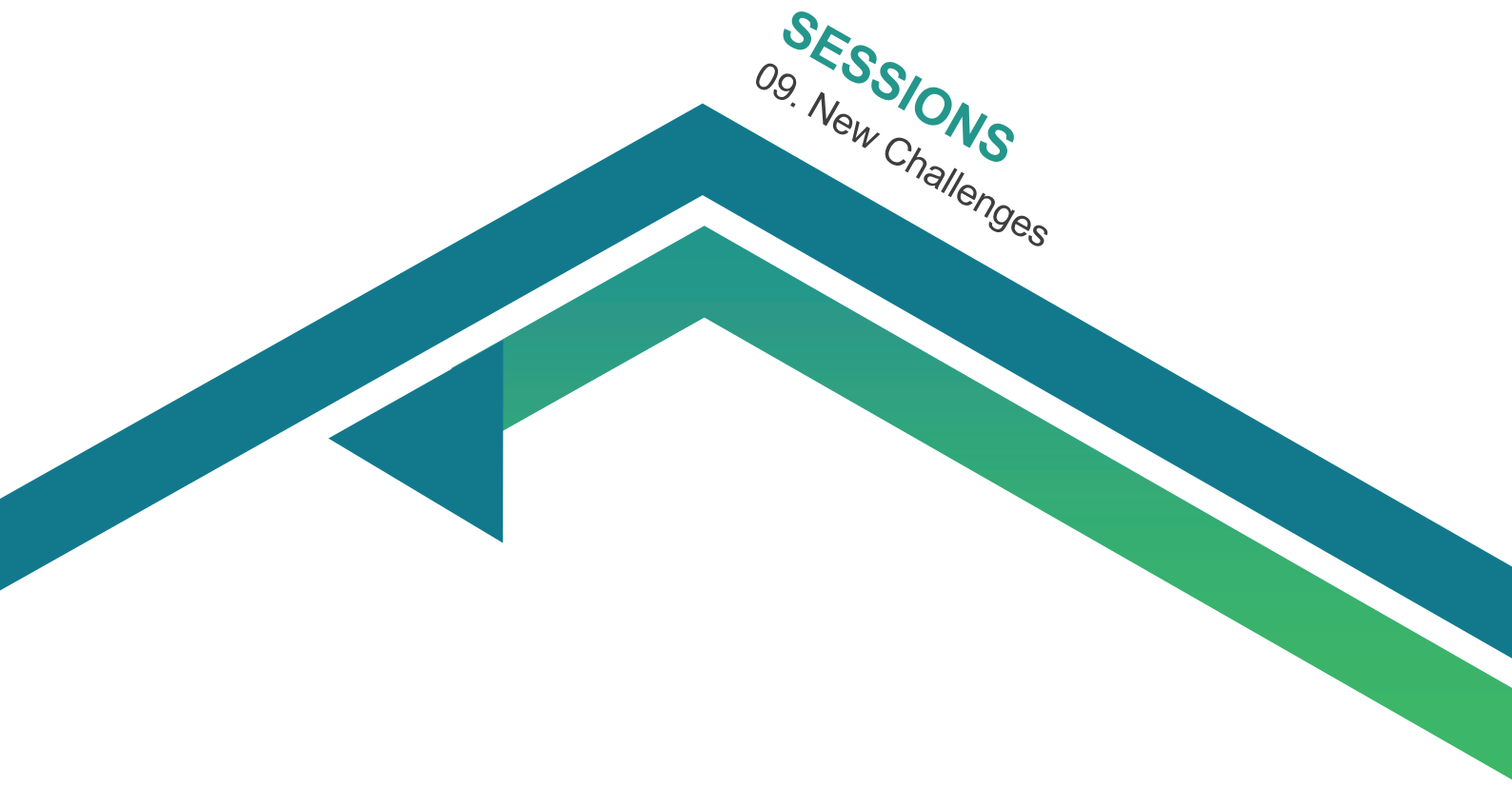
Among the most important proteins regulating homeostasis is the transcription factor p53, primarily known for its function to maintain genomic stability, regulate transient and permanent cell cycle arrest and apoptosis. Activated p53 transcriptionally regulates the expression of many proteins, among which are MDM2, p21 and BTG2. MDM2 functions as a direct inhibitor of p53 by targeting it for ubiquitination, whereas the proteins p21 and BTG2 are known for their regulatory function in cell cycle arrest. Although the general wiring of the molecular interactions in the DNA damage response (DDR) is well known, this is not the case for its detailed quantitative dynamics. An aspect that is not fully understood includes the relative importance of p21 and BTG2 in determining cell cycle arrest. Moreover, it is unclear whether DDR dynamics differs between cell lines compared to primary cells, which may affect cancer susceptibility.

Therefore, we developed a computational dynamical model that describes the dynamics of DDR regulator p53 and targets MDM2, p21 and BTG2, and applied it to time-lapse imaging data of HepG2 reporter cells of these components. With this model, we first generated simulations of virtual primary human hepatocytes (PHHs) and compared the results to transcriptomics data for PHH donor samples. Our analysis showed that model-based extrapolation from HepG2 to PHH can be done for some DDR elements, yet our analysis also reveals that such extrapolation is inaccurate for the regulator MDM2. Second, we studied the quantitative relation between protein expression and cell cycle arrest, by extending our DDR computational model with the cell cycle. We calibrated model parameters to cell cycle data acquired with HepG2-FUCCI reporter cells, thus determining the importance of p21 and BTG2 in their stimulation of G1 and G2 cell cycle arrest. The protein dynamics could predict the G2 cell cycle arrest in HepG2 cells treated with cisplatin.

In conclusion, our work illustrates the relevance of studying pathway dynamics in addition to gene expression comparisons, which allows a quantitative understanding of pathway dynamics, and supports translation of cellular responses from cell lines to primary cells.

Keywords:
DNA damage response; cell cycle; computational modelling; HepG2 cells; primary human hepatocytes.


SESSIONS
09. New Challenges

ID 09.1
Understanding the health risks of exposure to micro- & nano-plastics

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Micro- & nano-plastics (MNPLs) are considered emergent pollutants widely spread over all environmental compartments. They are mostly generated form the degradation of the huge amounts of plastic waste that can be found in the environment. There is evidence that humans can internalize these MNPLs through inhalation and ingestion and that the small size of the plastic particles may allow for absorption and systemic biodistribution. This exposure scenario is aggravated by the fact that MNPLs are thought to have the potential to act as vectors for other well-known harmful contaminants. Nevertheless, and despite of the fact that MNPLs research is a very fast-moving field, information on the biological impacts of MNPLs in mammalian models remain unclear, and the limitations in current methodologies do not allow for accurate MNPLs exposure and hazard evaluation. More solid evidence is therefore needed to enable science-based risk assessment of MNPLs that can be utilized to protect human health and ecosystems from its derived adverse effects.

In this context, the present talk will focus in presenting the current science on MNPLs research, the key knowledge gaps in this area, and the approaches developed and results obtained in the frame of the large-scale EU Project PlasticHeal (www.plasticheal.eu/en), aimed at developing front-end tools to study the impact and mode of action of MNPLs, to benefit the identification and management of safety issues arising from MNPLs human exposure. These include advances in the areas of MNPs sampling and monitoring, human exposure and fate, MNPs-induced effects, and MNPs risk evaluation. Special attention will be given to complex in vitro systems, new approach methodologies, MNPLs-induced long-term effects, and genotoxicity.

Funding:
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Keywords:
Microplastics, nanoplastics, health, risk, exposure, effects, knowledge gaps.

ID 09.2

Biological effects of chronic oral exposure to polystyrene nanoparticles in Drosophila melanogaster

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One of the most significant environmental issues of our time is plastic pollution. Only a small part of plastic is recycled and, as result of chemical and physical processes, the plastic released into the environment breaks down into tiny particles that can cross biological tissue barriers. These fragments contaminate everything, from fresh and salty water to the food we consume and the air we breathe. Furthermore, due to the lipophilic nature of the nanoplastics, they accumulate in fatty tissues, biomagnifying the dose to which organisms are exposed. Given the high levels of exposure, it is crucial to assess the impact of nanoplastics on human metabolism and related health risks.

Drosophila melanogaster is a suitable animal model for toxicity and genotoxicity research. The aim of our study is to characterize the biological effects of oral exposure to polystyrene nanoparticles (PSNPs) on fruit flies. PSNPs are commonly used in food packaging and are one of the most widespread plastic pollutants. In order to characterise the PSNPs we used fluorescein-conjugated PSNPs and carried out a SEM analysis on the PSNPs themselves, the food additioned with PSNPs and the feces of wilde-type flies reared on the contaminated food. Fluorescence microscopy analysis revealed that PSNPs tend to accumulate in intestinal and fat body cells of flies fed with the additioned food, indicating that PSNPs cross the intestinal epithelium and are released into the circulatory flow.

Trypan blue staining on the intestine showed tissue damage following the ingestion of nanoparticles. Measurable physiological and behavioural endpoints (developmental timing, male fertility, adult body weight and larval crawling) were analyzed. In both pupal formation and adult hatching, a significant extension of development was observed along with a significantly reduced ability of adult flies to react to additional stress, such as starvation. Moreover, we tested the sensitivity of DNA damage repair (DDR) mutants to PSNPs as an indirect indication of genotoxicity. The analysis was conducted on the ligIV mutant, known to be hypersensitive to DNA damage induced by ionising radiation (IR) in early developmental stages and with a shortened lifespan under conditions of nutritional stress. Preliminary results suggest an effect on chromosome integrity and thus a genotoxic effect of PSNPs. We are currently focusing on this question to better define its health-related aspects.

Keywords:
Drosophila, Genotoxicity; Nanotoxicology; Nanoplastics;

ID 09.3
Recent achievements in micronuclei characterization

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Among numerous genotoxicity assays, the micronucleus (MN) test is especially recommended to evaluate the genotoxic effects of chemical and physical agents. MN test is less time-consuming and easier to perform compared to the chromosomal aberrations (CA) assays. An analysis of the MN frequency is the basis for genotoxicity assessment.

Although the knowledge on different aspects of the origin, structure, genetic activity of micronuclei in plants has been explored in recent years, there is still much less that is known in humans and animals. Over the past years, distinct approaches have allowed an understanding the mechanisms of formation of the micronuclei. Especially modern cytogenetics techniques have revolutionized knowledge on the chromatin that is involved in micronuclei. From the data that are available for animals and humans, micronuclei can be lost from the cells and incorporated into the nucleus. Thus the issues of the fate of micronuclei will be considered.

The latest methodological developments in the field of molecular cytogenetics to describe the fate of micronuclei, with particular attention to plants will be present. We provide a comprehensive overview of the current knowledge on MN characteristics in plants focuses on critical scientific problems: Is the distribution of DNA damage that led to micronuclei formation random? What is the origin of plant micronuclei? Are epigenetic processes involved in micronuclei formation? How could there be a role of the genetic activity of chromatin in the formation of micronuclei? The technical innovations that have been developed and used for human research is shown.

Keywords:
cytogenetics; DNA damage; micronuclei; mutagenesis.

ID 09.4

Novel methodology to assess genotoxicity in Food Contact Materials (FCM)

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According to the Food Contact Materials Regulation (EC1935/2004), materials and articles in contact with food should not release constituents into the food at levels harmful to human health. Packaging migrating substances may include intentionally added substances (IAS) and non-intentionally added substances (NIAS). The IAS refers to known substances used in the manufacture of the FCM while NIAS are often unknown substances found in the FCM. As reported, NIAS can represent more than half of the substances found in a migration mixture and the evaluation of their safety is difficult to carry out, requiring the identification of the toxicologically most relevant unknown chemicals in the materials.

According to regulatory recommendations, exclusion of mutagenicity in FCM is required. Consequently, the identification of genotoxic/mutagenic chemicals in FCM extracts and migrates is needed. Standard tests such as the Ames assay are recommended for the detection of mutagenicity in pure chemicals but not suitable for this type of application due to inadequate limit of detection (LOD). In this context, a novel procedure combining high-performance thin-layer chromatography (HPTLC) with genotoxicity bioassays (SOS Umu-C assay) has been successfully developed. Moreover, such an approach is likely to facilitate the identification of active substances.

This HPTLC-Umu-C assay was applied to assess the genotoxic potential of paper-based packaging materials as case study. Paper-based samples, spiked or not with a reference genotoxic compound, were tested using this methodology. The bioactive bands were both analyzed with LC-HRMS for chemical identification and evaluated with the AMES assay to detect DNA-damaging properties.

The compounds responsible for the genotoxic activity were identified and their mutagenic effect assessed using the Ames-MPF assay. These results demonstrate the power of the approach integrating HPTLC-Umu-C, Ames assay and LC-HRMS to address the genotoxic potential of FCMs. This approach will likely become a significant contributor not only for packaging safety but also for other food related as well as environmental and cosmetics fields.

Keywords:
HPTLC, genotoxicity, bioassays, mutagenicity, LC-HRMS.


SESSIONS
10. ecNGS

ID 10.1
Genomic Safety Assessment with ecNGS: The Next Generation

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Error-corrected next-generation sequencing (ecNGS) is an effective tool for not only detecting the induction of mutations from a carcinogenic exposure, but in carefully quantifying individual mutations too. The current genetic toxicology test battery includes several in vitro and in vivo mutation assays but their applicability to different genomic loci, tissues, organs, and species is limited. ecNGS methods promise disruption to the field of genomic safety with richer data, broader applicability, greater accessibility, and universal translation given that the analyte of the assay is simply DNA.

NGS technologies, in general, output nucleotide resolution data at incredible speed and throughput which, when coupled with the increase in accuracy from an error-corrected approach, enable ultra-sensitive mutation detection. At this scale, rare mutational data can be used for advanced bioinformatics analysis such as mutation spectra deconvolution, pattern matching, and inferring the mode of action of a mutagenic exposure.

Among ecNGS techniques exists duplex sequencing which improves sequencing accuracy over standard NGS by 100,000-fold and permits the detection of rare de novo mutations at frequencies of 1×10-8 through a process of independently tracking both strands of individual DNA molecules and then comparing the strands to eliminate technical errors.

Recent advances in using ecNGS for genomic safety assessment have proved innovative, and multiple ecNGS approaches now exist for generating a mutagenesis or carcinogenesis readout. This talk will summarize the history of ecNGS as a tool for genomic safety, the current landscape of ecNGS options for mutagenesis and carcinogenesis readouts, and reveal how ecNGS should transform the future of not only drug and chemical safety assessment but also of optimizing gene editing therapies, assessing the life-integrated mutagenic exposures of living individuals, linking contamination to cancer clusters, and biomonitoring those at risk to occupational carcinogens such as firefighters and astronauts.

Keywords:
ecNGS; mutagenesis; genetic toxicology; genomics; duplex sequencing.

ID 10.2
NGS and toxicogenomic signatures of human carcinogens

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The International Agency for Research on Cancer (IARC) aims to identify preventable cancer causes; to this end, its existing research programs employ innovative NGS-based approaches to investigate the mutagenic effects of known or suspected human carcinogens. The selection of the tested compounds is primarily informed by the priorities set for carcinogen evaluation and classification by IARC. The effects of the candidate substances are studied in unique collections of human and rodent tumours or in vitro experimental exposure models, by using NGS conducted at the genome-scale. At the core of the NGS analysis is the identification of mutational signatures, the specific mathematical readouts that reveal the external and endogenous mutagenic mechanisms operative in cells, and their impact on cancer driver genes.

Mutational signatures can be ascertained by well-established NGS applications (e.g. WES, WGS), and recent reports indicate that they can also be obtained from error-corrected/ecNGS-generated data. Despite their superior accuracy and sensitivity in true mutation detection, the presently used ecNGS applications typically produce rather low-count mutation spectra and might not be ready for prime-time use in robust signature analysis relying on a large number of somatic/acquired events. Further developments of the ecNGS approaches for reliable mutational signature identification are thus warranted.

One rather unexploited area of applying NGS to studying the mutagenic fingerprints of carcinogens is the genome-scale analysis of mutational signatures and cancer driver events in archived FFPE or EFPE biospecimens. Successful examples of such investigations will be presented, including the discovery of novel mutational signatures in archived tumours associated with exposures to dietary contaminants, iatrogenic agents, and industrial chemicals.

Overall, this presentation will illustrate the power of the NGS approach in improving our understanding of extrinsic cancer causes, and its value in supporting disease prevention efforts aimed at the reduction of modifiable cancer-causing exposures.

Keywords:
Mutational signature; cancer driver; genome-scale NGS; carcinogen; cancer prevention.

ID 10.3

NGS applications to assess vector mediated genotoxicity in genetic medicine

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Currently, the development of gene editing (GE)-based cell and gene therapies involves the use a variety of NGS-based methods for detecting off-target editing. However, none of these has achieved gold standard status since all have certain limitations.

INDUCE-seq was specifically developed to overcome these. As a result, this has greatly improved the efficiency and precision of detecting off-target break sites in the genome following GE. At present all methods, including INDUCE-seq, generate hierarchical lists of potential off-target editing sites based on frequency of GE-induced break formation, coupled with breaks located at sites with homology to the guide sequence. These combined potential off-target lists are subsequently analysed to determine the mutation frequency found at each off-target site. Subsequently, these are compared to the mutation frequencies found in unedited (control) cells. This information is used to aid safe and efficacious guide selection to manufacture the cell and gene therapy product.

Here, we describe the use INDUCE-seq to report the detection of off-target editing for a range of different guides employed during CRISPR-Cas9 gene editing. Furthermore, we report the measurement of the corresponding mutation frequencies at these locations using error-corrected NGS by Duplex-seq. We reveal the relationship between the transient formation of editing-induced DNA breaks versus fixed mutational endpoints.

At present off-target break nomination still relies upon relatively simple frequency-based calling of off-targets, with arbitrary thresholds applied to generate hierarchical break lists. We extend our analysis of the genomic break data generated by INDUCE-seq to demonstrate that it is replete with additional genomic information. We show that the adaptation of AI algorithms and the development of additional bioinformatics pipelines make it possible to extract latent information from INDUCE-seq datasets that will advance the safe and efficacious design of future cell and gene therapies.

Keywords:
Gene editing; CRISPR-Cas; Off-targets; INDUCE-seq; AI.

ID 10.4

ecNGS analysis of induced mutagenesis; case studies with noteworthy nitrosamines

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The N-nitrosamines, NDMA and NDEA, are environmental mutagens that have been identified as contamination impurities in some commonly used drugs, resulting in several product recalls. These nitrosamines were evaluated in transgenic gene mutation assays (Muta™mouse or Big Blue® rats, respectively). NDMA mutation was determined at the transgenic lacZ-locus in liver and the endogenous Pig-a gene in peripheral blood, following 28-day dosing. Acute treatments were included to investigate the accumulation and/or additivity of individual dose effects on NDMA mutation induction. NDEA mutation was determined at the transgenic cII-locus. Liver was selected because it is the most sensitive organ for tumour and mutation induction in rodents. There were dose-dependent increases in liver lacZ (NDMA) and cII (NDEA) mean mutation frequencies following 28-day repeat dosing, or after a single dose of NDMA (10 mg/kg). The No Observed Genotoxic Effect Levels (NOGEL) were determined for both nitrosamines. NDEA liver tissue samples were subsequently analysed for DNA mutations using DS (Duplex Sequencing, Twinstrand-Bio) and genome-representative panels of loci to determine mutation frequency directly. The results showed broad agreement with the cII mutation frequency data, with DS revealing slightly higher sensitivity. NDMA samples are currently being analysed. NDMA and NDEA mutagenesis will be discussed in terms of historically defined mutation signatures for both compounds obtained in traditional mutation assays and tri-nucleotide signatures that have been identified for alkylating agents in the catalogue of somatic mutations in cancer (COSMIC). As DS does not rely on phenotypic selection, it will be interesting to compare results and ascertain whether any selection bias exists in the historical data.

Keywords: NDMA, NDEA, Mutation, Duplex-Sequencing, Transgenic gene mutation assays.


SESSIONS
11. Regulatory Toxicology

ID 11.1

Modern in vitro screening tools to enhance quantitative chemical risk assessment

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Chemical risk assessments are undergoing a renaissance as the international regulatory community moves away from animal toxicity tests in favour of robust non-animal alternative tests. These alternatives to animals are often referred to as new approach methodologies (NAMs), which are broadly defined as any novel method and/or approach that can support chemical risk assessment without using animals (i.e., in vitro or in silico methods). Advancements in NAMs are enabling higher-throughput and/or higher-content genotoxicity assessment. Furthermore, toxicokinetic models can be employed to model chemical disposition and support interpretation of NAM data in an in vivo context. Recent work has established that application of in vitro to in vivo extrapolation (IVIVE) models to NAM data tends to provide surrogate points-of-departure (PoDs) that are protective of human health relative to in vivo animal PoDs. Additional efforts are exploring the utility of computational models that estimate freely dissolved concentrations of test chemicals by accounting for in vitro assay conditions and physicochemical properties. These models are capable of providing a better estimate of the cellular exposure conditions compared to the nominal concentration in aqueous solution. Finally, the emergence of three-dimensional cell cultures and complex organ-on-a-chip technologies, that recapitulate aspects of human physiology, are further bridging the gap between NAMs and in vivo toxicity assessment. For instance, liver-on-a-chip technologies can use human-derived cell lines to provide a more robust assessment of genotoxicants that require metabolic activation and can be coupled with other organotypic tissues (i.e., gut-on-a-chip) to mimic complex physiological processes. The presentation will highlight NAM developments and provide examples where NAM testing strategies are being employed to screen chemicals with a potential for concern.

Keywords:
new approach methodologies; in vitro to in vivo extrapolation; organ-on-a-chip; risk assessment.

ID 11.2

The GENOMARK transcriptomic biomarker demonstrates a high predictivity for genotoxic hazards and utility in potency ranking in human HepaRGTM cells

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New Approach Methodologies (NAMs) and improved testing strategies are needed to modernize genotoxicity assessment and reduce reliance on experimental animals. We previously developed GENOMARK, a transcriptomic biomarker that consists of 84 genes to identify genotoxic substances in metabolically competent human HepaRG™ cells. Initial work demonstrated a high prediction performance of GENOMARK in classifying compounds as genotoxic or non-genotoxic based on qPCR gene expression data collected at a low cytotoxic concentration, the IC10. A qualitative classification of chemicals as genotoxic or non-genotoxic is generally insufficient for risk assessment. Therefore, there is growing global interest in developing quantitative methods for the analysis and interpretation of genotoxicity dose-response data. In this study, we demonstrate how GENOMARK gene expression data can be used quantitatively, i.e. for potency ranking of genotoxic chemicals. First, we evaluated the applicability of GENOMARK to higher-throughput platforms, such as RNA-Seq and TempO-Seq. These sequencing platforms generate a larger amount of gene expression data in a shorter timeframe compared to qPCR to increase the throughput and facilitate the combination of GENOMARK with other transcriptomics-based biomarkers. The work confirms that GENOMARK shows a high predictivity using these high-throughput technologies. In addition, we applied benchmark dose (BMD) analysis to demonstrate that GENOMARK can be used in ranking genotoxicants based on their potency. Finally, we compared the performance of GENOMARK and another biomarker of DNA damage, i.e. TGx-DDI, for genotoxicity hazard calls and potency ranking. Overall, the results suggest that transcriptomic biomarkers for genotoxicity such as GENOMARK can rapidly and effectively identify genotoxic hazards while simultaneously providing additional information on potency that is useful for modern risk assessment.

Keywords:
genotoxicology, quantitative assessment, benchmark dose, high-throughput toxicogenomics, new approach methodologies.

ID 11.3

Assessing DNA damage in Testicular Germ Cells in the Comet Assay

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The in vivo comet assay is a widely used genotoxicity assay, however currently the OECD test guideline 489 (TG489) does not recommend obtaining testicular germ cell data since testicular cell suspensions contain a mixture of germ cells and somatic cells. In absence of the scarcely available in vivo germ cell mutagenicity data, demonstration of interaction with the germ cell genome is warranted for classification as Muta 1B instead of Muta 2 when combined with positive findings of somatic mutagenicity in vivo. An approach to specifically assess testicular germ cells within TG 489 is thus highly demanded. We here provide a proof-of-concept to selectively analyse round spermatids and primary spermatocytes. We utilize semi-automated comet assay recordings of both DNA damage (% tail intensity) and DNA content (total fluorescence intensity) of individual comets, combined with visual comet identification, to distinguish testicular comet populations based on their different DNA content/ploidy and physical appearance. Comet populations are identified through 1) visual discrimination of DNA content distributions, 2) setting DNA content thresholds, and 3) fitting a normal three mixture distribution function. Primary spermatocyte comets can be identified during scoring based on their particularly large physical size combined with high DNA content. We harvested biological materials from an extensive rat experiment testing five classical agents to facilitate inter-laboratory validation. Preliminary data will be presented. Valuable information regarding genotoxic potential as well as distribution of substances to gonads can be gathered. Both somatic and germ cell data can be obtained from the same animals in accordance with the 3R-principle. Our adaptation adds a versatile, sensitive, rapid and resource-effective assay to the currently limited toolbox for regulatory germ cell mutagenicity assessment. Considering the increasing global production of and exposure to potentially hazardous chemicals, new and easily implementable methods are urgently needed. The framework proposed herein may facilitate improved assessment of male germ cell mutagenicity.

Keywords:
Germ cell; OECD TG 489; Mutagenicity; Round spermatid; primary spermatocyte.

ID 11.4

Assessing the DNA damage potential of cigarette and iQOS emissions in human bronchial epithelial cells.

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It is widely recognized that use of combustible cigarettes (CCs) is associated with an increased risk of developing different types of cancer. Nowadays, heated tobacco products (HTPs) such as iQOS are gaining popularity worldwide, mainly because they are considered less harmful than CCs due to the lower levels of toxicants in their emissions. However, it is unknown whether the impact of iQOS emissions on human health is indeed significantly lower compared to cigarette smoke. Specifically, the potential of iQOS emissions to damage DNA in comparison with cigarette smoke is unexplored.

Therefore, in this study, the bronchial epithelial cell line BEAS-2B was exposed to different (v/v) % of extracts of cigarette or iQOS emissions for 1h and (oxidative) DNA damage was assesses by the alkaline comet assay. In addition, to determine the oxidative potential of both extracts, we quantified the radicals and the reactive oxygen species by means of electron spin resonance (ESR).

Strikingly, HTP extract (HTPE) was found to contain significantly higher levels of (carbon-centred) radicals and reactive oxygen species compared with cigarette smoke extract (CSE), suggesting higher oxidative potential and presumably higher genotoxic impact. Moreover, all the assessed CSE concentrations (1%, 3% and 5%) induced significantly more oxidative DNA damage compared to vehicle-exposed cells. We are currently assessing DNA damage in response to different HTPE concentrations. Furthermore, we are also assessing mRNA abundancy of several DNA repair genes in BEAS-2B cells exposed to CSE and HTPE. In addition, we performed RNA sequencing of human primary bronchial epithelial cells (PBECs) exposed to sub-lethal CSE and HTPE concentrations, and we are investigating whether the exposure significantly affected pathways involved in DNA damage and/or DNA repair.

Keywords:
Heated Tobacco Products; DNA damage; oxidative potential; DNA repair genes; RNA sequencing.


SESSIONS
12. Human Biomonitoring

ID 12.1

Understanding blood cell mutational biomarkers for biomonitoring and disease purposes.

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Micronuclei (Mn) form from mitotic errors during the telophase stage of mitosis. Mn are easily measured in isolated human lymphocytes stimulated to grow ex vivo by using microscopy-based approaches. Mn can be caused either by systemic genotoxic exposure or by underlying disease states in vivo. Another easily measurable blood cell mutation employs the PigA mutation locus. Erythrocytes with mutated PigA can rapidly be identified by flow cytometry coupled to fluorescent antibodies for GPI anchored proteins (e.g. CD55, CD59), with mutated cells losing fluorescence.

We have been assessing the combined use of Mn and PigA as endpoints to comprehensively study human mutation. We have collected blood from over 500 patients and volunteers and assessed lymphocyte Mn and erythrocyte PigA. We have observed that both mutational endpoints are elevated in older participants and have shown associations with modifiable lifestyle factors such as BMI, smoking status, diet, medication and other factors. We have also noted increasing levels of both Mn and PigA mutations in the blood cells of patients with oesophageal cancer.

In an effort to better understand the origin of these blood cell mutations, we have explored underlying causative genotoxic factors. We have shown that lymphocyte Mn from cancer patients are more likely to contain whole chromosomes and that lymphocytes from cancer patients are more sensitive to aneugen exposure (vinblastine) in a “challenge assay”. We have shown that patient lymphocytes show an adaptive response to oxidative stress, with those bearing high Mn levels being resistant to ROS induced Mn in a ex vivo challenge assay. We also show that patient plasma samples show markers of oxidative stress (GSH), activation of the cGAS-STING pathway (IFN-b) and that human plasma can ultimately induce Mn in TK6 cells in an ROS dependent manner in some patients. Further efforts are needed to better understand the underlying cause of these blood cell mutational events in populations.

In conclusion, this kind of multi-mutation approach may be useful in both biomonitoring studies and in understanding the role of internal secondary mutations induced by various diseases including cancer.

ID 12.2

The importance of gene – environment interactions in Alzheimer disease and the emerging role of epigenetics

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Although a minority of Alzheimer's disease (AD) forms are single-gene disorders, showing an autosomal dominant inheritance pattern, most AD cases appear to be a complex disorder that is likely to involve multiple susceptibility genes and environmental factors. Much work has been done to identify susceptibility genes in AD, however the contribution of individual genes to the increase or decrease in risk appears generally low, as evidenced by the odd ratio values found.

Twin studies represent traditional methods to reveal the importance of environmental and genetic influences in complex traits/diseases. A plethora of studies on monozygotic or dizygotic twins discordant for numerous complex diseases, including AD, was performed, many of which came to the same conclusions: in twins discordant for a complex-age-related disease what differs is not the genome, but the epigenome.

Epigenetic modifications are common in complex diseases, including neurodegenerative diseases. For AD the most robust disease biomarkers resulting from candidate gene approach and epigenome-wide association studies (EGWAS) in post-mortem cortical samples and peripheral blood cells, will be reported.

Moreover epigenetics has been explaining us since the beginning of this century, how environmental factors such as diet, lifestyle, alcohol, smoking, pollutants, can interact with our genome. In recent years, more and more evidence has been accumulated confirming that experiences and the environment leave epigenetic marks on genes. These epigenetic modifications are similar to those found in pathological tissue samples, and in some cases a specific correspondence between environmental exposures and epigenomic alterations was found. Environmental factors, mainly nutrients but also life style factors, are key effectors towards epigenetic modifications.

Emerging evidence indicating that epigenetic changes are important cellular and molecular correlates of neurodegenerative diseases resulting from chronic neurotoxic chemical exposure will be reported, as well as specific environmental exposures linked to epigenetic modifications of AD-related genes.

Finally the periods most vulnerable for our epigenome to the effects of environmental factors as well as the importance of the reversibility of epigenetic modifications will be highlighted, in view of therapeutic strategies but also of potential primary prevention strategies.

Keywords:
Genetic factors; environmental factors; epigenetic biomarkers; Alzheimer disease.

ID 12.3

Assessment of DNA damage in cumulus cells from infertile women using comet assay

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Infertility affects approximately 15% of reproductive-aged couples, which brings an increasing demand to prevent and explore biomarkers for this reproductive and health burden. The comet assay is being used to assess sperm DNA damage, demonstrating that a high level of DNA damage affects reproductive ability. To the best of our knowledge, in women no correlation was obtained mainly due to the scarcity of studies and difficulty in obtaining those tissues. Cumulus cells surround the oocyte, establishing mutual interdependence, fundamental for oocyte development and fertility processes.

This work aims at optimizing and implementing the comet assay protocol to assess DNA damage in cumulus cells (CC) to be applied on samples belonging to infertile women.

The alkaline comet assay was optimized in whole blood and CC samples, using as a positive control cells treated with MMS (0.5 mM). Comet assay was performed in cryopreserved blood and CC of six samples from infertile women, with mean age 33.7 ± 3.4 (range 28-37 years). Data was analyzed using SigmaPlot version 14.0 (Systat Software®Inc.,Chicago, IL, United States). T-test and Mann-Whitney U test were used for continuous comparisons.

The optimization of the assay showed that the concentration of low melting point agarose and the density of cells per gel were critical steps for establishing optimal conditions. In addition, human CC is a very difficult tissue to obtain, conditions cannot be repeated, hence the assay optimization is of paramount importance. Our findings showed that the DNA damage measured by the comet assay was significantly increased in CC compared to the levels found in blood (systemic DNA damage).
These results seem to indicate that the DNA damage found in CC may be related to infertility, as evidenced in male infertility. However, further studies with a higher number of participants are needed to confirm these results.

This study allowed us to successfully implement the comet assay in CC and blood samples from infertile women. Furthermore, positive findings were observed in CC compared to a systemic tissue. Despite the need to increase the number of samples tested, this study highlights the importance of using these two tissues to compare results of DNA damage and supports the use of CC to target the oocyte status as a biomarker with clinical impact in female infertility treatments.

Funding:
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Keywords:
Comet assay, DNA damage, women infertility, cumulus cells.

ID 12.4

Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes

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The dysfunction of mitochondria is one of the cancer hallmarks. Mitochondria evince a limited DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN in cancer have reported ambiguous results; most were based on a case-control design and were inconsistent for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing chromosomal instability, while telomere length (TL) maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. Similar to mtDNA-CN, the reports on TL as a biomarker for cancer risk, patient therapy response and/or survival are contradictory. MtDNA-CN and TL are highly variable across cell types but maintained within a constant range according to the specific tissue type. It has been demonstrated that mitochondrial biogenesis and energy production were decreased in telomerase-deficient mice with severe telomere dysfunction. It thus has been hypothesized that telomere alteration affects not only oxidative defence mechanisms but also mitochondrial functions. The deregulation of the telomere-mitochondria axis, as caused by ageing or other physiological factors, triggers carcinogenesis. We, therefore, investigated mitochondria and telomere changes in colorectal cancer (CRC), one of the leading causes of cancer-related deaths. Our study particularly aimed to look closely at mtDNA-CN, mtDNA damage, TL, and the expression of mitochondrial transcription factor A and telomerase reverse transcriptase in association with CRC patient outcomes. Our cohort included deep-frozen tumour tissue, adjacent non-tumour tissue, and blood from 163 untreated sporadic CRC patients. We isolated DNA and RNA from these samples and measured particular molecular biomarkers using a quantitative-polymerase chain reaction assay. Currently, the experiments are running and after collecting the experimental data, comprehensive statistical analysis using patient clinical and follow-up data will be performed.

The results will be presented during the conference and we believe that they may aid improvements in the current understanding of CRC, by identifying the role of mtDNA-CN and TL in CRC pathogenesis. This study was financially supported by the Ministry of Health of the Czech Republic (NU22J-03-00033); and by the Grant Agency of the Czech Republic (21-04607X).

Keywords:
Mitochondrial DNA; telomere length; DNA damage; colorectal cancer; patient outcomes.

ID 12.5

The use of combined different approaches in assessing the conditions and making prediction models in severely obese BMI ≥ 35 kg m-2 (FFQ, DII, anthropometric, biochemical a nd DNA damage parameters)

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The data on genome stability in the obese/severely obese are scarce, although obesity and its comorbidities are linked with higher cancer risk. This is the first study where parameters from different approaches without correlation were used for the severe obese conditions estimation (n=53). Results were analysed for: 1) daily intake of food groups, nutrient intake and dietary inflammatory index (DII), 2) anthropometric, 3) biochemical and 4) parameters of three DNA damage assessment assays (Fpg-modified-, alkaline comet- and micronucleus (MN)cytome- assay). DNA damage, BMI and basal metabolic rate (BMR) correlated with cell proliferation changes; DII with oxidative DNA damage; and groups with higher DNA damage than expected (tail intensity >9% and >12.4%, MN >13), consumed daily, weekly, and monthly more often some type of food groups. Results demonstrated that some type of damage can start earlier in the obese individual lifespan, such as nuclear buds and nucleoplasmic bridges, then comes decrease in cell proliferation and then elevated MN frequencies; and that obesity can have an impact on changes in blood cell counts and division. Assays were able to demonstrate groups of sensitive individuals that should be further monitored for genomic instability and cancer prevention. DNA damage, biochemical and anthropometric parameters should be combined for further obese monitoring, better insight into biological changes in the severely obese, and a more individual approach in therapy and treatment. Patients should also get proper education about the foodstuff with pro- and anti-inflammatory effects.

Keywords:
DII; FFQ Norfolk food questionnaire; alkaline comet assay; micronucleus cytome assay; obesit.

POSTER COMMUNICATIONS

P01

High exposure to anesthetic is associated with DNA damage and apoptosis in veterinarians

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Inhalational anesthetics are routinely used worldwide for general anesthesia in both humans and animals. Therefore, health care professionals who work in operating rooms have occupational exposure to waste anesthetic gases, which may be associated with toxic effects. Considering the lack of studies in veterinary professionals, this is the first study to monitor trace concentrations of anesthetic and evaluate genetic instability and apoptosis in veterinarians. After approval from the ethical committee, questionnaires were applied and written informed consent was obtained from veterinarians exposed to the anesthetic isoflurane (exposed group) and from volunteers without occupational exposure (control group). Biological samples were collected and blinded analyzed for cytokinesis-block micronucleus (MN) assay for evaluation of DNA damage; viability and apoptosis in lymphocytes (CD3+) were detected by flow cytometry; and urine samples were analyzed by gas chromatography-mass spectrometry for isoflurane trace concentrations as internal marker of exposure (biological monitoring). In addition, air samples from veterinary operating rooms were measured by infrared analyzer to detect isoflurane trace concentrations (environmental monitoring). Both groups were similar regarding demographic and anthropometric data ($p > 0.05$). Regarding the urinary concentration of isoflurane in the exposed group, an average of 26 ± 24 µg/l urine was found; as expected, no isoflurane was detected in urine samples from the control group. Environmental monitoring showed 10 ± 8 ppm of isoflurane in veterinary operating rooms (considered above the international limit). The exposed group had a higher frequency of MN ($p = 0.04$) and apoptosis (annexin+/7-AAD-; $p = 0.044$) than control group whereas the control group had a higher frequency of viable cells ($p = 0.017$) than exposed professionals. In conclusion, high levels of trace concentrations of isoflurane in urine and workplace are associated with DNA damage and apoptosis in lymphocytes. Therefore, the findings highlight the need to mitigate anesthetic pollution in the work environment to reduce occupational exposure in professionals who work in veterinary operating rooms to minimize the impact of anesthetic toxicity.

Funding:
FAPESP and CNPq

Keywords:
Occupational exposure; isoflurane; veterinarians; micronucleus; cytotoxicity

P02

Oxidative and inflammatory effects in normal tissues of mice exposed whole body to combined treatments with conventional radiotherapy or protontherapy and a PARP inhibitor

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This work belongs to the ToxIP3 program which aims to assess toxicity to healthy tissues of protontherapy versus photontherapy and their combination with a PARP inhibitor: olaparib. PARP inhibitors have a recognized radio-sensitizing effect by causing an increase in unrepaired DNA breaks after irradiation. However, their toxicity in association with irradiation has not yet been adequately studied *in vivo.*

For this purpose, C57Bl6 mice were whole-body irradiated with photon or proton beams +/- olaparib. Survival was stronger decreased after protons than after photons. Olaparib did not modify the survival or the weight of unirradiated mice but strongly decreased them when associated to irradiation. Blood and various organs were collected after the onset of acute toxicities. Skin, brain, lung, heart, small intestine and liver were cryomilled and biomarkers of genotoxicity, oxidative stress and inflammation were measured. Genotoxicity, measured by 8-oxodG level in lymphocytes, was significantly increased only after photon irradiation. PARP1 activity was also increased in all organs only after photons and the addition of olaparib decreased it. Moreover, olaparib caused oxidative damage to lipids (malonedialdehyde level) and proteins (carbonyls level) varying according to the tissues with an inverse effect when combined with photons or protons. The level of plasma inflammatory cytokines was increased after photons or protons. Olaparib decreased TNF-α, IFN-γ, but also IL-10, and increased IL-6 and IL-12p70.

In conclusion, protons leaded to: an increase in acute toxicity compared to photons (mouse survival and weight, PARP1 activity) but a decrease in oxidative damage to lipids, proteins and DNA in the majority of tissues. The combination with olaparib leaded to an increase in acute toxicity (survival, weight, PARP1 activity, pro- and anti-inflammatory cytokines) but also to a decrease in other pro-inflammatory cytokines and in oxidative damage to lipids (after photons only). This study will enable the clinical use of olaparib associated with photon or proton beam radiotherapy.

Keywords:
Irradiation; normal tissues; PARPi; genotoxicity; inflammation.

P03

Evaluation of air pollution effects on human population: a Zagreb case

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In terms of exposome, air pollution is a life-long and constant source of exposure affecting human organs, tissues, cells, and molecules. It is considered responsible for more than 3 million premature deaths annually. Currently ongoing HUMNap project investigates possible associations between air pollutants and biomarkers of exposure and early biological effects. The evaluation of results is divided in two parts. In part 1 we examined historical data (2011-15) of cytogenetic biomarkers in Zagreb population (N=130) and corresponding air quality data. Measured air pollution parameters were largely below regulatory limits, except for B[a]P. There were no significant positive associations indicating contribution of air pollutants to increased genome damage for designated period.

In part 2, in 2021-22 we recruited a new cohort (N=60) and included biomarkers of exposure (benzene, toluene, ethylbenzene, o-, m- and p-xylene) and effect (blood comet assay, buccal and blood micronucleus assay). For air quality data, the results were in agreement with results from part 1. Similarly, tested parameters did not impact biomarkers of exposure nor the genotoxicity biomarkers. The focus of the project will now turn to other cities with different air pollution burden, and will expand the number of evaluated biomarkers in order to find possible links between air pollution and the biomarkers of effect, improve prediction models, and to serve in better risk assessment of general public. Supported by the Croatian Science Foundation (HUMNap project 1192 and the work of doctoral student K. Matković).

Keywords:
Air pollution; genotoxicity; human population.

P04

Blood molecular profile to predict genotoxicity from exposure to antineoplastic drugs – a comparison with cytokinesis-block micronucleus assay results

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Gentoxicity is an important information that should be included in human biomonitoring programmes design. Cytogenetic methods are usually laborious and time-consuming, therefore new molecular methods development is an added value. The aim of this study was to evaluate if the molecular profile of previously frozen whole blood as acquired by Fourier Transform Infrared (FTIR) spectroscopy, allow to assess genotoxicity in occupational exposure to antineoplastic drugs in hospital professionals, as obtained by the lymphocyte cytokinesis-block micronucleus (CBMN) assay. It was considered peripheral blood from hospital professionals occupationally exposed to antineoplastic drugs (n = 46) and from a non-exposed group (n = 46). It was first evaluated the metabolome from defrosted whole blood by methanol precipitation of macromolecules as haemoglobin followed by centrifugation. The metabolome molecular profile resulted in 3 ratios of spectral bands significantly different between the exposed and non-exposed group ($p<0.01$) and a spectral principal component-linear discriminant analysis (PCA-LDA) model enabling to predict exposure with 73% accuracy. After optimization of the dilution conditions of defrosted whole blood, it was also possible to obtain a higher number of significant ratios of spectral bands, i.e. 10 ratios significantly different at $p<0.001$, pointing the method high sensitivity and specificity. Indeed, the PCA-LDA model based on the molecular profile of whole blood enabled to predict the exposure at an accuracy, sensitivity and specificity of 92%, 93% and 91%, respectively.

All this was achieved based on 1mL of defrosted blood, in a high-throughput mode, i.e., based on the simultaneous analysis of 92 blood samples, in a simple and economic mode. The method presents therefore very promising potential for high-dimension screening of genotoxic effects from exposure to genotoxic substances.

Keywords:
Molecular profile, FTIR-spectroscopy, Genotoxicity, Cytokinesis-blocked micronucleus assay, blood.

P05

Cellular effects of cannabinoids from Cannabis sativa in vitro

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Products containing Cannabis-derived cannabinoids such as cannabigerol, cannabidiol, and cannabinol can be bought worldwide without restrictions. Often these products are advertised as highly beneficial for human health; however, the research community is divided. Some studies have shown that Cannabis phytoproducts have antioxidant, anti-inflammatory, and anticarcinogenic properties. Still, others claim that the bioactivity of these phytoproducts is not adequately characterized, and their accurate benefits and disadvantages are unclear. Therefore, this study aimed to investigate the cellular effects of pure cannabigerol, cannabidiol, and cannabinol on the human lymphoblastoid cell line, TK6, and the human hepatoma cell line, HepG2, using the cytokinesis–block micronucleus assay. Cannabidiol significantly increased micronucleus formation in human lymphoblastoid but not in hepatoma cells. Furthermore, cannabidiol caused a significant reduction of cytokinesis–block proliferation index in both cell lines. However, the lack of increment in micronucleus formation in cannabidiol-treated hepatoma cells differs from published data and needs further investigation regarding the reasons for this difference. The preliminary data for cannabigerol and cannabinol showed that they could possibly induce micronuclei formation in both cell lines at concentrations ≥ 10 µM. At the same time, the effects on cytokinesis–block proliferation index are still unclear. Therefore, these cannabinoids require further experiments to elucidate their effects in vitro.

Keywords:
Cannabis sativa; cannabinoids; genotoxicity; proliferation.

P06

Metabolism and membrane transporters influence the genomic damage induced by pyrrolizidine alkaloids in a co-culture model system

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Pyrrolizidine alkaloids (PAs) are natural phytotoxins distributed extensively in thousands of plants species. PAs require metabolic activation in the liver to instigate toxicity. Humans are exposed with PAs via cross-contamination in food products, spices and herbal medicines. PA induced hepatic sinusoidal obstruction syndrome (HSOS) is mainly characterized as hepatic sinusoidal endothelial cell (HSECs) damage which later leads to hepatotoxicity and carcinogenicity. However, the mechanism is not yet fully known because HSECs in the liver lack metabolic enzymes.

To mimic the in vivo situation to some extend in vitro, we co-cultured HepG2 liver cells with metabolically inactive fluorescence labelled HeLa cells (HeLa H2B-GFP) and analyzed micronucleus formation in the HeLa cells after treatment with PAs. The genomic damage induced by the PAs europine, riddelline and lasiocarpine was investigated and increased micronucleus formation was observed in HeLa H2B-GFP cells after treatment of the co-culture with PAs. The CYP450 inhibitor ketoconazole, and the outwards membrane transporter inhibitors verapamil (MDR1 inhibitor) and benzbromarone (MRP2 inhibitor) reduced the micronucleus formation. Mitotic disturbances as a possible mechanism of micronucleus formation were also observed in HeLa cells after treatment of the co-culture.

Thus, within the applied co-culture model system, PAs were activated by HepG2 liver cells and the metabolites were taken up by HeLa cells in which they induced genomic damage.

Keywords:
Include; pyrrolizidine alkaloids; micronuclei; HepG2 cells; HeLa H2B-GFP cells.

P07

Viability assay and DNA double strand break induction in nervous system cells exposed to cerium dioxide nanoparticles

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Cerium dioxide nanoparticles (CeO2 NP) show antioxidant enzyme mimetic properties and free radical scavenging activity. These properties make them a promising material for biomedical applications, but their potential adverse effects are not totally understood yet. Our objective was to assess the biological behaviour of CeO2 NP in human neuronal and glial cells. After carrying out the physical-chemical characterization of the CeO2 NP and analysing their ability to be taken up by neuronal and glial cells, the possible alterations in cell viability and induction of DNA double strand breaks were determined by means of MTT assay and γH2AX assay, respectively. The possible existence of interference of the NP with the assay methodologies was previously addressed and corrected when necessary. The results obtained showed that, even though there was a significant dose- and time-dependent internalization of the NP by both cell lines, the CeO2 NP generally presented scarce cyto- or genotoxicity, essentially depending on the NP exposure time and being restricted to higher doses. These results provide a better understanding of the interaction of CeO2 NP with cellular systems and their possible adverse effects, specifically at nervous system level.

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Keywords:
Cerium dioxide nanoparticles; MTT assay; γH2AX assay; glial cells; neuronal cells.

P08

Analysis of cytotoxicity and genotoxicity of differently charged gold nanoparticles in human SH-SY5Y neuronal cells

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Gold nanoparticles (AuNP) have aroused great interest in the last years due to their potential for biomedical applications. Due to their small size, these NP can cross the blood-brain barrier, which makes them good candidates for the treatment of diseases related to the central nervous system. For all these applications, they must be introduced in the body, so it is essential to discard any potential harmful effects. Our objective was to evaluate the influence of surface charge on biological behaviour of AuNP by assessing the cytotoxic and genotoxic effects induced in neuronal cells exposed to AuNP with different charge, i.e. cationic, anionic and neutral. SH-SY5Y cells were treated with each type of nanoparticle for 3 and 24h. Cytotoxic effects were analysed by changes in cell viability, whereas genotoxic effects were assessed by γH2AX assay. Also, cellular uptake was evaluated by flow cytometry. The results obtained showed different toxicological behaviour depending on the surface charge: cationic NP showed cytotoxic effects, but not anionic and neutral NP. Furthermore, cationic and neutral AuNP showed a low genotoxic potential, while anionic NP did not induce DNA double-strand breaks. Cell uptake analysis did not provide conclusive results likely because the extremely small size of the NP hinders their detection by the flow cytometer.

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Keywords:
Gold nanoparticles; MTT assay; γH2AX assay; neuronal cells.

P09

Assessment of sea pollution in the Boka Kotorska Bay, Montenegro with relocated gilthead sea bream (Sparus aurata) as an indicator of genotoxicity

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The Boka Kotorska Bay, located in the southeastern Adriatic Sea, is subjected to anthropogenic pressure due to population growth and the increased number of vessels in its waters. In the past decade, the focus of our research group has been the assessment of the reliability of biomarkers in aquatic organisms in the field of eco/geno-toxicology.

As a useful strategy for biomonitoring marine pollution we used the active approach (comet assay and micronucleus test in conjunction with relocation and cage exposure). Among the various assays in this field, the comet assay stands out due to its significant potential for discriminating DNA damage between the groups of aquatic organisms of the same species collected or exposed at different sites. In the case of the Boka Kotorska Bay, marine fish species Sparus aurata (gilthead sea bream) is of peculiar interest because it is commercially farmed in the Bay.

Having in mind the aforementioned, we relocated the S. aurata from an aquaculture farm to more impacted sites within the Bay. After two weeks of exposure, blood from the fish heart was taken, and afterwards muscle tissues were prepared for trace element determination. Genotoxicity was measured by comet assay and micronucleus test in blood cells. The level of accumulation of metals and metalloids in the muscle was also measured and based on the obtained data, the level of tissue burden with these elements was calculated using the Metal Pollution index. Finally, the results were combined by Integrated Biological Responses analysis to present all biomarkers of interest with a single, general "stress index".

The results indicated differences in the investigated sites based on the "stress index". As expected, the level of DNA damage (measured by both comet assay and micronucleus test) was significantly higher in animals exposed at the sites under stronger anthropogenic impact.

Keywords:
Genotoxicity; Sparus aurata; Boka Kotorska Bay; comet assay; micronucleus test.

P10

Buccal micronucleus cytome assay in Armenian diabetes patients

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Diabetes mellitus (DM) is associated with a risk of serious health complications. An increased frequency of micronuclei (MN) was reported in DM patients (both in lymphocytes and exfoliated buccal cells).

It would be of interest to study MN and other nuclear anomalies in buccal cells of Armenian DM patients because of extremely high homogeneity of population (98.1%). It is notable that the prevalence of DM (both types in population of Armenia is between 5.6% and 8.5%

In our pilot study buccal cells of DM patients (type 1 and type 2; n=25/group) as well as healthy persons (control) were evaluated for MN and other nuclear anomalies. Several patients with latent autoimmune diabetes in adults (LADA) also were included in MN evaluation (we could not find publications on this topic in literature). The buccal MN cytome assay was applied for analysis. Several blood parameters were also monitored including the most important, HbA1c levels.

We found significantly increased frequencies of MN in DM type 1 and 2 patients as well as in LADA patients compared with controls. The mean ratio (MN in patients/ MN in controls) was 3.8, 2.5 and 2.3 respectively. The frequencies of all other nuclear anomalies also were significantly higher in all patients compared with the controls. Hence, our preliminary data show increased levels of MN and other nuclear anomalies in DM and LADA patients. Gender and age did not influence levels of MN and other nuclear anomalies in buccal cells.

Keywords:
Micronucleus; Nuclear anomalies; Buccal mucosa; Diabetes mellitus.

P11

Development of a micronucleus test using the EpiAirway™ organotypic human airway model

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The use of organotypic human tissue models in genotoxicity has increased as an alternative to animal testing. Genotoxicity is generally examined using a battery of in vitro assays such as Ames and micronucleus (MN) tests that cover gene mutations and structural and numerical chromosome aberrations. At the 7th International Workshop on Genotoxicity Testing, working group members agreed that the skin models have reached an advanced stage of maturity, while further efforts in liver and airway models are needed [Pfuhler et al., Mutat. Res. 850-851 (2020) 503135]. Organotypic human airway model is composed of fully differentiated and functional respiratory epithelium. However, because cell proliferation in organotypic airway models is thought to be less active, assessing their MN-inducing potential is an issue, even in the cytokinesis-blocking approach using cytochalasin B (CB) [Wang et al., Environ. Mol. Mutagen. 62 (2021) 306-318]. Here, we developed a MN test using EpiAirway™ in which epidermal growth factor (EGF) was included as a stimulant of cell division.

By incubating EpiAirway™ tissue with medium containing various concentrations of CB, we found that the percentage of binucleated cells (%BNCs) almost plateaued at 3 µg/mL CB for 72 h incubation. Additionally, we confirmed that EGF stimulation with CB incubation produced an additional increase in %BNCs with a peak at 5 ng/mL EGF. Transepithelial electrical resistance measurement and tissue histology revealed that CB incubation caused the reduced barrier integrity and cyst formation in EpiAirway™. Adenylate kinase assay confirmed that the cytotoxicity increased with each day of culture in the CB incubation period with EGF stimulation. These results indicated that chemical treatment should be conducted prior to CB incubation. Under these experimental conditions, it was confirmed that the frequency of micronucleated cells was dose-dependently increased by apical applications of two clastogens, mitomycin C and methyl methanesulfonate, and an aneugen, colchicine, at the subcytotoxic concentrations assessed in %BNCs.

Well-studied genotoxicants demonstrated capability in an organotypic human airway model as a MN test system. For further utilization, investigations of aerosol exposure, repeating exposure protocol, and metabolic activation are required.

Keywords:
Micronucleus test; organotypic human airway model; EGF; clastogens; aneugen.

P12

Internalization, toxicity, and genotoxicity of ultra-small non-magnetic iron oxide (III) nanoparticles in cultured cell lines and Drosophila larvae in vivo

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Iron oxide nanoparticles have been used in the last years as therapeutic agents in biomedicine. Among the wide variety of these particles, the ultra-small (< 10 nm), non-magnetic ones, composed by a ferrihydrite core covered with tartaric and adipic acids (FeAT-NPs) are showing promising properties. In published data, these nanoparticles have shown an efficient absorption rate by the enterocytes, conserving their nanoparticulated form, and a good solubilization into free iron inside the cells but not in the intestinal lumen. All these characteristics allow FeAT-NPs to be considered as a potential anemia treatment. However, the solubilization of these nanoparticles inside the cytosol might produce reactive oxygen species (ROS) that could trigger protein, lipid, and DNA oxidative damage. Thus, a systematic investigation of their activity, including biological effects and chemical behavior, should be performed.

In this work, we evaluated FeAT-NPs uptake, as well as their solubilization within the cell cytosol of different human cell lines, using inductively coupled plasma mass spectrometry (ICP-MS), alone or in combination with high performance liquid chromatography (HPLC). Cell viability, cell proliferation and ROS induction were analyzed in these cell lines after exposure with different FeAT-NPs concentrations, and the possible induced genotoxicity was evaluated with the alkaline Comet and the micronucleus (MN) assays. In addition, in vivo toxicity and genotoxicity of these nanoparticles were evaluated using Drosophila melanogaster as the model organism, with the eye SMART assay that detects somatic mutation and mitotic recombination.

Results revealed that FeAT-NPs are taken up efficiently, in a cell type-dependent manner, with a minimum dissolution. These results correlated with no effects on cell proliferation and minor effects on cell viability and ROS induction for all the studied cell lines. With respect to genotoxicity, comet assay results revealed significant induced DNA damage only in nucleotide excision repair deficient GM04312 cells, whereas MN data show no clastogenic activity in Hep-G2 cells.

Additionally, the Drosophila results showed that FeAT-NPs were genotoxic in vivo only with the two highest tested concentrations (2 and 5 mmol·L-1 of Fe) in surface treatments. These data altogether seem to show that FeAT-NPs represent a safe alternative for anemia treatment, with high uptake level and controlled iron release.

Keywords:
Iron oxide nanoparticles; uptake and solubilization; cytotoxicity; genotoxicity: SMART, comet and micronucleus assays.

P13

Genotoxicity of selected hydrogels loaded with iron oxide nanoparticles for potential application in regenerative medicine

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Current advanced methods in regenerative medicine are trying to develop suitable materials that can restore and reproduce the favorable and natural environment needed for skin regeneration. The development of advanced multifunctional materials for wound treatment with the ability to provide multiple functions at once is crucial for clinical application. The utilization of nanohydrogels in regenerative medicine provides an innovative way to treat skin injuries.

As it is important to evaluate the biosafety of nanohydrogels as a degradable biomaterial for use in the biomedical field, the aim of this study was to determine the genotoxic effects of newly prepared nanocomposites. The model system represents different types of skin cells, keratinocytes (HaCaT), and fibroblasts (HFF-1). The experiments were focused on determining the genotoxic effect of nanocomposites and their individual components in in vitro conditions. Three hydrogels (Alginate, Pluronic F127, and GelMA) with different chemical compositions and iron oxide nanoparticles were used for nanohydrogel build-up. For genotoxicity determination, we used three different methods: comet assay, fpg-modified comet assay, and micronucleus test to determine aneugenic, clastogenic, and DNA damage.

Initial results after 24 h nanohydrogel exposure, measured by comet assay showed a significant increase in DNA damage in the case of GelMA nanohydrogel. We did not observe any DNA damage in the two other nanohydrogels. Subsequently, we used an fpg-modified comet assay to determine if this DNA damage is caused by base oxidation. However, we did not observe any differences between samples with and without fpg enzyme treatment. We assume that DNA damage is a result of single or double-strand breaks incurred as the attempted repair of UV radiation-induced base damage in DNA. From the results of the micronucleus test, we noticed a higher amount of apoptotic and necrotic cells after GelMA exposure, also the presence of micronuclei was significantly higher.

Keywords:
wound healing, regenerative medicine, nanoparticles, nanohydrogels. This work was supported by ENM III/2019/861/TENTACLES; project VISION No. 857381 and VEGA No. 2/0121/21.

P14

Determination of proliferation and genotoxic effect of thymol and acetyl thymol on in vitro intestinal model

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Thymol has a proven bioactive effect on colorectal cancer cells. However, its properties such as low solubility and cell penetration prevent its wider application. Therefore, a new hydrophilic derivative - acetylthymol - was synthesized. In our study, we treated colorectal cancer tumor cell lines (HT-29 and HCT-116) with thymol or acetylthymol on a concentration scale for 24 hours. Proliferation was determined using time-lapse microscopy with an Incucyte® Zoom device. The genotoxic effect of substances was analyzed by the comet assay method.

For a comprehensive assessment of the effect of thymol and the newly synthesized derivative - acetylthymol, the proliferative and genotoxic effect was also determined in 3D culture on colorectal cancer tumor cells. 3D cell culture ensures greater stability, while better representing real cell aggregation, morphology, and mutual cell interaction. As a result, the creation of a more complex microenvironment was ensured, which to a greater extent corresponds to the real conditions in vivo. Spheroids were formed after 5 days using ULA (ultra-low attachment) microplates. Subsequently, the proliferation and genotoxic effects of thymol and acetylthymol were analyzed and compared using the methods mentioned above.

Our results demonstrated that a newly synthesized hydrophilic derivative of thymol with targeted chemical structure modification acts more effectively on both colorectal cancer cell cultures in 3D at much lower concentrations than thymol alone. Comet assays have shown a significant increase in DNA damage for the newly synthesized derivative even at non-cytotoxic concentrations. The HCT-116 cell line showed higher DNA damage values than HT-29. Incucyte Zoom noted the effect of thymol and acetylthymol on the proliferation of both tumor cell lines. The results confirmed our assumption that the newly synthesized hydrophilic derivative can act more effectively than thymol. In the future, we would like to focus on determining the expression of selected proteins using the Western blot method.

This work was supported by a European Union's Horizon 2020, No 857381, project VISION.

Keywords:
Thymol, acethyltymol, spheroids, proliferation, comet assay.

P15

Development of automated image-based gH2AX and micronucleus assays for efficient genotoxicity and mutagenesis screening in the advanced 3D human hepatocytes HepoidR model

The liver is essential in the elimination of environmental and food contaminants. Given the significant inadequate data resulting from interspecies differences between rodents and humans in xenobiotic metabolism, the development of novel relevant in vitro human models is crucial to investigate the genotoxicity and mutagenesis of compounds that undergo metabolic activation. The in vitro gold standard model primary human hepatocytes suffer from a limited lifespan and lack of proliferation while the differentiation of the hepatic HepaRG cell line requires high concentration of dimethyl sulfoxide which restricts its usefulness for drug-metabolism studies.

We have developed a DMSO-free advanced 3D model of human hepatocytes named HepoidR, that allows concomitant proliferation and differentiation of human hepatocytes (primary and HepaRG cells) cultured in collagen matrix. Cells rapidly organize into characteristic polarized hollow spheroids of differentiated hepatocytes exhibiting high levels of liver-specific functions and xenobiotic metabolism enzymes expression and activities after a few days of culture and for at least 4 weeks. Traditional genotoxicity assays are labour intensive and time consuming. Here, we have applying machine learning models from the open access software Fiji and develop computational approaches to automate the quantification of gH2Ax and micronucleus from the 3D HepoidR model, thus reducing analysis time and minimizing human bias. We have studied the effects of well-known DNA reactive carcinogenic (MMS, MMC, colchicine, vinblastine, DMH, AFB1) and negative compounds (ethionamide, DEHP, methylcarbamate) to validate the methods and show that they can be used to efficiently discriminate the genotoxic effects of various class of molecules.

Taken together, our results show that the highly differentiated HepoidR model associated with automized analysis is an adapted tool for acute and long-term genotoxic in vitro assays and environmental chemicals risk assessment.

P16

The Role of UV exposure and novel compounds on the immunogenicity and genotoxicity of skin cancer

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In the UK, melanoma is the 5th most common cancer. The treatment of melanoma is a challenge for clinicians because of its aggressive behaviour and metastatic status. In the last 20 years, Retinoid therapy has produced remarkable results in the treatment of malignant melanoma. The published data suggested that several gene signalling pathways are involved in the mechanism of action of Retinoic acid as an anti-cancer drug.

Retinoids have been used to treat lots of different diseases including breast cancer, colorectal cancer and melanoma. Meanwhile, as retinoids act as an anti-cancer agent for different cancers. So, the aim of the study is to investigate Retinoic acid (RA), as a potential novel agent for the treatment of melanoma. The objective is to investigate the effects of RA on the lymphocytes of healthy individuals and melanoma patients' lymphocytes and to the detect anti-cancer activity of RA on two melanoma cell lines FM55 and CHL-1 as compared to untreated cells using the Comet assay. The H2O2 and ultraviolet A+B (PUVA light) (315-350nm) were used to cause oxidative stress. A concentration of RA 20µg/ml and 30µg/ml were used to treat the lymphocytes in the comet assays.

The lymphocytes from melanoma patients showed increased DNA damage as compared to healthy individuals (*$p<0.05$). There was no damage observed in healthy lymphocytes, but it produced significant (***$p<0.001$) reduction in the DNA damage of Melanoma lymphocytes in the Comet assay. Moreover, The RA 20µg/ml significantly decreased the oxidative stress caused by hydrogen peroxide and UVA+B rays. Hence, RA is effective in both groups using the Comet assay. Immunocytochemistry was used to visualize the expression of P53, Ki-67 and S100 proteins. Immunocytochemistry results demonstrated that the expression of protein P53 is nuclear in CHL-1 cells and cytoplasmic in FM55 cells. It also suggested that the expression of protein ki67 in CHL-1 and FM55 is nuclear and the expression of protein S100 is absent in both cell lines.

Keywords:
Melanoma, Retinoic Acid, Comet Assay, Immunocytochemistry, UVA.

P17

Epigenotoxic effects of Bisphenol-A mediated by its metabolite reducing DNA methylation

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Bisphenol-A (BPA) is commonly used in the manufacture of polycarbonate plastics and epoxy resins and is known to possess a weak estrogenic activity. BPA is also an environmental contaminant with adverse health effects suspected to be mediated through epigenetic mechanisms. We have reported that the FLO1-dependent flocculation of transgenic yeast transformed with human DNA methyltransferase genes (DNMT yeast) is a useful tool in epigenotoxicology studies. In this study, we have examined the effects of BPA in the presence of metabolic activation (S-9 mix) on the transcription level of the FLO1 gene in the DNMT yeast. In the presence of S-9 mix, BPA reduced the intensity of reporter green fluorescence protein (GFP) driven by the FLO1 promoter. The metabolite of BPA 4-methyl-2,4-bis(p-hydroxyphenyl)- pent-1-ene (MBP) also exhibited similar effect on this promoter activity. Moreover, BPA in the presence of S-9 mix showed only a weak while MBP had no inhibitory activity on the expression of GFP reporter gene controlled by a modified FLO1 promoter lacking some CpG sites. FLO1 mRNA expression level was also decreased by both S-9 mix activated BPA and MBP. Furthermore, the global DNA methylation level in the human HEK293 cells was reduced by MBP as well. These results indicate that BPA metabolites, including MBP, have inhibitory effect on DNA methylation. Our DNMT yeast assay provides a novel in vitro method for screening for chemicals that can alter the epigenome also by a mechanism dependent on their metabolic activation.

Keywords:
Bisphenol-A; metabolite; DNA methylation.

P18

Epigenetic changes in recreational runners from a clean and an air polluted locality

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Air pollution has an unfavourable impact on the environment as well as on human health. It has been proven that exposure to polluted air, mainly in large cities or industrial zones, increases the risk of diseases such as cancer and cardiovascular disorders. Small non-coding RNA molecules (microRNAs; single-strand, approximately 22 nucleotide long RNA molecules) play a crucial role in the epigenetic regulation of gene expression. In their mature form, they complementary bind to mRNA, which causes its silencing. These molecules are affected by environmental pollutants, including airborne contaminants and have been implicated in human cancers.

Physical activity (including running) is a well-established factor positively affecting human health. Among other physiological roles, it improves respiratory and cardiovascular systems, supports immunity, as well as neurological and psychological functions and in turn, it contributes to the delayed onset of age-related diseases, thus potentially increasing lifespan. Although the area of the Czech Republic (Central Europe) is relatively small (78 781 km2), the level of air pollution highly differs across the country. In this study, RNA was isolated from collected blood, microRNA libraries were prepared and sequenced. MicroRNA expression in 393 volunteers was compared between and within two localities with different levels of air pollution or between runners and non-runners. Overall, from fourteen comparisons, most deregulated microRNAs (42) were detected when female runners were compared with non-runners in the clean locality. On the contrary, the highest deregulation rate (log2FC) of a single microRNA was observed in comparison of runners from the polluted and the clean locality. Due to deregulation of these microRNAs, various biological, including cancer-related, pathways may be altered which might lead to numerous diseases progression.

This research project was funded by the Ministry of Education, Youth and Sports of the Czech Republic: project Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798, which is co-financed by the European Union (European Structural and Investment funds; Operation Programme Research, Development and Education).

Keywords:
Air pollution; Czech Republic; running; microRNA expression.

P19

Impact of air pollution to oxidative stress markers in mothers and newborns (Summary of the long-term research in the Czechia)

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In 2014, while studying the impact of air pollution on oxidative DNA damage [measured via 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)] and lipid peroxidation [measured via 15-F2t-isoprostane (15-F2t-IsoP)] in mothers and their newborns from Karvina (a polluted region) and Ceske Budejovice (CB) (a control locality), 8-oxodG and 15-F2t-IsoP levels were expected to increase with increasing concentration of air pollutants. While in winter 2014 in newborns from Karvina the 8-oxodG levels were significantly increased ($P<0.001$) compared to CB, in mothers from Karvina oxidative DNA damage levels were significantly decreased in the same period compared to mothers from the control locality ($P<0.05$). This may be explained by adaptation of the adult organism to adverse environmental conditions and development of protective mechanisms.

The 15-F2t-IsoP levels generally followed the same trend as 8-oxodG levels. The exception was observed for lipid peroxidation in samples from newborns collected in summer 2013, when 15-F2t-IsoP levels were significantly higher in the control group ($P<0.001$). This could be a result of the effect of other independent factors (e.g. type of delivery or anesthesia applied during delivery). Multivariate regression analysis of the effect of air pollution on oxidative stress in newborns from Karvina showed PM2.5 concentrations to be a significant predictor for 8-oxodG levels. Exposure to PM2.5 and B[a]P significantly affected lipid peroxidation.

The project "Healthy Aging in Industrial Environment" (HAIE) was carried out in 2018-2022 to verify these results. The partial aim of the study was again to analyze the same oxidative stress markers in urine and plasma from non-smoking mothers and newborns from the above-mentioned localities. Sampling of biological material was performed during the whole year including two time periods with different levels of air pollution: in summer (low pollutant levels) and in winter (high pollutant levels). Metabolites of polycyclic hydrocarbons (PAHs) were determined in urine.

Concentrations of pollutants (PAHs, PM2.5) in the air of Karvina and CB were obtained from web pages of The Czech Hydrometeorological Institute. Number of newborns included in the HAIE (N=250/locality) was higher compared to the previous study (N=100/locality). As the previous study, the morbidity of children will be monitored after 2 years. Comprehensive results, including a comparison of these projects, will be presented at the conference.

Supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Keywords:
Air pollution; Newborns; Oxidative DNA damage; Lipid peroxidation; effect of other independent factors.

P20

Background Level of Unstable Chromosome Aberrations in the Kazakhstan Population: a Human Biomonitoring Study

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Kazakhstan is known as a country with a complex radioecological situation resulting from different sources of exposure to radiation such as a natural radiation background, extensive activities of the industrial system of the former Soviet Union and a well-known testing of nuclear power weapons occurred in the Semipalatinsk Test Site (STS) area during the period 1949–1989. Moreover, approximately 41% of the world's reserves of uranium are concentrated in Kazakhstan, especially in the North and West regions with their uranium ore and thorium-containing provinces where the most significant deposits of uranium and thorium and their daughter decay products are located
.
For all these reasons, Kazakhstan's current situation in terms of radiological safety provides a unique opportunity for a wide range of studies including biomonitoring studies and retrospective dosimetry investigations. Several studies concerning radiation exposure rely heavily on the quantification of the radiation-induced chromosome aberrations, such as dicentric chromosomes (Dic) and acentric fragments (Ace) in the peripheral blood lymphocytes (PBLs) of exposed and potentially exposed subjects.

The present study focuses on the assessment of the background of dicentric chromosomes in Kazakhstan's population, which is the starting point in the retrospective dose assessment of irradiated people, since the baseline level of spontaneous dicentrics can vary significantly in different populations.

Dicentric chromosomes, are specific radiation-induced aberrations occurring at a very low level in unirradiated persons and increasing in a linear or linear quadratic manner after exposure to high- or low-LET radiation, respectively. This means that, in the context of the biological dosimetry, estimates of an absorbed whole-body dose can be determined by the dicentric frequency observed in PBLs.

In this context, aiming to determine the background frequency of chromosome aberrations in the population of Kazakhstan, considering the heterogeneity of natural radiation background levels of its large territory, a selection of 40 control subjects living in four cities of North, South, West and East Kazakhstan has been investigated.

Keywords:
Human biomonitoring study, Kazazhstan population, radiation exposure, biological dosimery.

P21
Maternal diet quality and health status of newborns

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Persistent organic substances (POPs) are compounds of mainly anthropogenic origin that persist in the environment for a long time, become part of food chains, and then accumulate in living organisms. Because of that, POPs levels are monitored and some of them has already been banned or at least significantly restricted.

In our study, we analyzed the diet of 53 pregnant women from the Czech Republic. The women simultaneously recorded in detail all the food they ate for one week during the last month of pregnancy and collected a quarter of all portions in food boxes. From the dietary records, we obtained information about the quantity and quality of the diet and determined the concentrations of 67 different persistent organic pollutants in the collected samples. These pollutants belong to five different groups – polychlorinated biphenyls, organochlorine pesticides, brominated flame retardants, perfluorinated compounds and polyaromatic hydrocarbons. Furthermore, we determined the levels of 8-isoprostane in cord blood plasma samples in order to determine the degree of oxidative damage in newborns. Subsequently, we evaluated possible associations between maternal diet quality and POPs intake with birth weight and neonatal oxidative damage.

Dichlorodiphenyldichloroethylene (DDE) was the only substance present in all 352 daily food samples. DDE is a metabolite of the pesticide DDT. The use of DDT has been prohibited in the Czech Republic since 1974.

The concentrations of most POPs in the diet did not reach any established limits. The exception was perfluorinated compounds, whose permissible cumulative weekly intake was exceeded in four women. Concentrations of polychlorinated biphenyls and organochlorine pesticides were higher in samples with higher amounts of fat, concentrations of DDT were higher in samples with higher amounts of dairy products, and concentrations of polyaromatic hydrocarbons were higher in samples with higher amounts of cereals.

We did not find an association between birth weight and dietary intake of persistent organic substances. Birth weight was within the normal range for all monitored newborns. 22 % of the birth weight variability was explained by a positive association between maternal protein intake and the mother's weight before pregnancy. 48 % of the neonatal oxidative damage variability was explained by positive association with DDT intake and a negative association with protein intake.

Keywords:
8-isoprostane; birth weight; DDT; maternal diet quality; maternal protein intake; oxidative stress; persistent organic pollutants.

P22

Extending ToxTracker with duplex sequencing to further understand the mode of action of genotoxic substances and their mutagenic potential.

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The standard genotoxicity testing strategy typically investigates induction of gene mutations, chromosomal aberrations, and numerical chromosome changes. ToxTracker® is an in vitro mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways to identify direct DNA damage induction as well as indirect genotoxicity caused by oxidative stress and protein damage. The assay provides insight into the genotoxic mode of action (MOA) and can discriminate between clastogenic and aneugenic compounds. ToxTracker was shown to predict in vivo genotoxicity of compounds with a >90% sensitivity and specificity.

The TwinStrand DuplexSeq™ mutagenesis assays use a highly accurate sequencing technique that tracks both strands of the sequenced DNA molecules to limit sequencing errors. The DuplexSeq mutagenesis assays can detect and characterize mutations induced upon chemical exposure and are supported with an easy-to-use bioinformatics pipeline.

To combine the MOA information and accurate detection of gene mutations, we applied the TwinStrand DuplexSeq Mouse Mutagenesis Assay in the ToxTracker reporter cells to further unravel the MOA of genotoxic substances and determine their mutagenic potential. Providing a mutational fingerprint of compounds helps to further explore the MOA of genotoxic substances, thereby improving the in vitro genotoxicity prediction. In a pilot study, we tested the genotoxic substances N-ethyl-N-nitrosourea, benzo[a] pyrene, and potassium bromate in ToxTracker and determined their mutational fingerprint using the DuplexSeq Mouse Mutagenesis Assay.

Keywords:
Genotoxicity; mutagenesis; mode-of-action; duplex sequencing.

P23

Effect of the oxidation status of reduced graphene oxide on the genotoxicity of human bronchial cells

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Graphene-based materials (GBM) are a broad family of novel carbon-based nanomaterials suitable for many nanotechnology applications. However, the increasing market of these materials raises concerns on their possible impact on human health, especially via the inhalation route in occupational settings. Several studies have focused on the assessment of the influence of different physico-chemical properties on the toxicity of GBM. However, the broad variability of materials and cellular systems used preclude the identification of the key physico-chemical parameters. Here, we evaluated the in vitro cytotoxicity and genotoxicity towards human bronchial cells of four reduced graphene oxide (rGO) materials that only differ on the carbon-to-oxygen (C/O) ratio.

The physicochemical characterization of the selected rGOs was conducted by means of oxygen content, microscopy and spectroscopy analyses. The effect on viability of human bronchial epithelial (16HBE14o-) cells was evaluated by the WST-8 assay, whereas the effects on reactive oxygen species (ROS) production by the fluorescent DCFDA probe. Then, the in vitro genotoxicity of the four materials, was evaluated towards human bronchial epithelial (16HBE14o-) cells by the micronucleus (MN) test (assessing chromosome damage) and the comet assay (assessing DNA damage).

Each material presented a different oxygen content, ranging from 1 to 12 %. According with the WST-8 assay, all the materials significantly reduced cell viability after 3 and 24 h exposure (EC50 values ranging from 4.6 to 30.8 µg/mL), with slightly different potencies not dependent on the C/O ratio content. A similar observation was evidenced by the analysis of ROS production but highlighted the capability of all the materials to significantly increase ROS level after 3 and 24 h exposure. The results obtained by the in vitro comet assays indicate that none of the materials induced DNA damage after exposing 16HBE14o- cells to 1.5-50 µg/mL, for 3 and 24 h. Regarding the MN test, two rGOs showed an increase in MN frequency after exposing cells to 1.5-50 µg/mL, for 24 h. However, the genotoxic response did not correlate with the content of oxygen of the materials.

This research was funded by the Finnish Work Environment Fund (GrapHazard, project number 200338), which was supported by the SAF€RA programme.

Keywords:
Reduced graphene oxide; oxidation status; comet; micronuclei.

P24

An interlaboratory validation trial of the ToxTracker Assay for Genotoxic Mode of Action Assessment according to OECD guidelines.

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ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon chemical exposure. ToxTracker contains six different GFP-tagged reporter cell lines that together allow the accurate identification of genotoxic substances and discrimination between induction of DNA damage, oxidative stress and/or protein damage in a single test. More recently, the assay was extended to allow the discrimination between clastogenic and aneugenic compounds.
The ToxTracker assay is currently being evaluated in a large international inter-laboratory validation study, approved by the OECD. The goal of this prospective validation study is to explore the applicability of ToxTracker for regulatory applications, establish the transferability and reproducibility of the assay and to explore how it can be applied to improve the in vitro genotoxicity testing strategies. The validation has been conducted strictly following OECD guidance document 34.

ToxTracker was transferred to seven laboratories. The validation labs were trained to perform the assay and tested a training set of compounds to show their proficiency to run ToxTracker. Next, the labs evaluated a selection of 64 coded, well-established genotoxic and non-genotoxic chemicals with each compound being tested in three labs independently. All the experimental work has been completed and data have been analyzed. The accuracy to predict genotoxicity, as well as the intra- and inter-laboratory reproducibility were determined. In this poster, we will give an overview how the ToxTracker validation was performed and the most important results from this interlaboratory validation.

P25

Evaluation of the impact of lifestyle parameters on global DNA methylation and hydroxymethylation in a French cohort

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Cancer development and progression are intimately linked with epigenetic alterations. Of these, DNA methylation on cytosine, a repressive mark inhibiting transcription, have been widely studied. Cytosine hydroxymethylation has received less attention, although perturbations of this mark have also been associated with cancer. Most studies investigate DNA methylation alterations at the level of individual loci, thought to reflect potential alterations in gene expression. Another view upon DNA methylation can be obtained while looking at global methylation levels genome wide, thereby reflecting potential alterations of the mechanisms driving DNA methylation.

In this study, we use an UHPLC-MS/MS method for the quantification of methylated and hydroxymethylated cytosine. The method was validated using the bioanalytical method guideline of European medicines agency. Quality control samples were periodically injected to check the robustness of the results. Global DNA was obtained from neutrophils in a population of 551 individuals affiliated to the French agricultural social protection, previously included in the EPIBIO97 study and biobank. Enrolment for this cohort began in 1997 and included 42.2% of women. Their age ranges from 17 to 76 years old with a mean of 44.4. Their BMI ranges from 18.1 to 44.1 with a mean of 25.4. 33.7% of theme had already smoked at inclusion while 66.3% never did.

The blood samples were accompanied with data from questionnaires regarding individual health parameters, life habits and agricultural work exposures. Using statistical analysis, we linked epigenetic marker levels with several of these life habit parameters to assess their effects on epigenetic biomarkers. Using this method, we highlighted an increase of global methylation in women. We observed a decrease of methylation and hydroxymethylation correlated with higher BMI. Older people were associated with a decrease of hydroxymethylation level.

Finally, smokers were associated with lower hydroxymethylation level. These results were already described in region specific analysis of DNA. This study brings a more global approach of these parameters with a number of samples rarely studied in the literature. As a follow-up of the present results, DNA hydroxymethylation and methylation will be analysed in line of agricultural exposures of the studied population; to further explore the link between agricultural occupational exposures and risk of cancer.

Keywords:
Methylation; Hydroxymethylation; Global DNA; UHPLC-MS/MS; Agricultural cohort.

P26

In vitro cell responses and cell-cell interactions upon xenobiotic exposure of renal and hepatic cells

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Traditionally, *in vitro* test systems are based on two-dimensional (2D) cell cultures, which are associated with inherent limitations. Lately, 3D models (spheroids) have also proven to be a very useful and promising tool in environmental toxicology, including long-term repeat dose studies enabling the exposure to lower concentrations of pollutants that are relevant also for real human exposure.

Two human cell lines, hepatocarcinoma cells (HepG2) and renal proximal tubule epithelial cells (TH1) were used to evaluate the biological activity of two chosen xenobiotics, aflatoxin B1 (AFB1) - a potent genotoxic hepatocarcinogen and ifosfamide (IFO) - a synthetic analog of cyclophosphamide that has a nephrotoxic effect, in various *in vitro* test systems (2D – monolayer, 3D – spheroids, co-cultures).

The objective of this study was to evaluate the cytotoxic and genotoxic effects after 2h and 24h exposure to AFB1 and IFO, the changes in ROS production, and the expression of enzymes involved in the metabolism of xenobiotics, especially the P450 cytochrome complex.

The evaluation has shown that in 2D models, short-term as well as long-term exposure to IFO decreased the cell viability of both TH1 and HepG2 cells, while the cytotoxic effect of AFB1 was detected only after long-term exposure in both cell lines. The genotoxic effect was determined by comet assay and micronucleus test. AFB1 and also IFO were able significantly to increase the level of DNA strand breaks in both in vitro systems, while the most damaging was 24h IFO exposure. Chosen xenobiotics significantly increased the percentage of micronuclei in both cell lines after 2h and 24h treatment.

In 2D after 24h cell exposure, we have also measured the low significant increase in ROS production. The effect of both chemicals has also caused changes in CYPs expression (CYP1A2, CYP2B6, CYP3A4) in both cell systems which was determined by western blotting.

As expected, our results showed different cell responses upon AFB1 and IFO treatment, confirming the differences between cell lines along with the culture conditions. This study was supported by VEGA grant 2/0121/21, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, and APVV 20-0494.

Keywords:
Toxicity, xenobiotics, 3D cultures, kidneys, liver

P27

Antimicrobial activities of biocompatible nanocapsules loaded with essential oils and their cyto/genotoxicity in human keratinocyte cell line

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Essential oils (EOs) of Thymus capitatus (Th) and Origanum vulgare (Or) were encapsulated in biocompatible poly(ε-caprolactone) nanocapsules (NCs).

These nanosystems exhibited antifungal, antibacterial and antibiofilm activities against Candida albicans, Staphylococcus aureus and Escherichia coli. Th-NCs and Or-NCs were more effective against all tested strains than pure EOs and at the same time were not cytotoxic on human keratinocyte cell line (HaCaT). The genotoxic effects of EO-NCs and EOs on HaCaT were evaluated using a comet assay for the first time, revealing that Th-NCs and Or-NCs did not induce DNA damage compared with untreated control HaCaT cells in vitro after 24 h. The cells morphological changes were assessed by label-free live cell Raman imaging.

This study demonstrate the ability of poly(ε-caprolactone) nanocapsules loaded with thyme and oregano EOs to reduce microbial and biofilm growth and could be an ecological alternative in the development of new antimicrobial strategies.

This work was supported by the VEGA Grant Agency of the Slovak Republic (grant No. 2/0055/20) and COST Action (CA17140), supported by COST (European Cooperation in Science and Technology).

Keywords:
Essential oils, cyto/genotoxicity, antimicrobial activities.

P28

Role of oncometabolites succinate and fumarate in the response to induced DNA damage

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Genetic mutations in genes coding Krebs cycle enzymes, like succinate dehydrogenase (SDH) and fumarate hydratase (FH), cause a buildup of succinate and fumarate. These increases disrupt energy metabolism and lead to tumor development; moreover, they seem to affect chromatin structure by inhibiting histone and DNA demethylases, what, joined with their inhibition of some DNA repair proteins, contribute to DNA repair impairment that, in turn, might influence cancer therapy. To check the effects of oncometabolite accumulation on the response to different DNA-induced damages, three cell lines: PC12 (rat adrenal medulla pheochromocytoma), A2780 (ovarian carcinoma), and GM04312 (human fibroblast deficient of the nucleotide excision repair system), were treated with hydrogen peroxide or cisplatin. The DNA damage response (DDR) analysis included apoptosis, cell cycle progression, viability, clonogenic activity and genomic instability assays, and the determination of cisplatin induced DNA adducts using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The presence of SDHB and FH proteins was determined by immunofluorescence. PC12 cells, used to check the effects of metabolites on the response to induced DNA oxidative damage, revealed firstly that the metabolites were not toxic and did not affect cell cycle progression, although they showed a slight increase in DNA damage, likely due to impaired repair of spontaneous damage. In addition, they altered apoptosis and the induced DNA damage, showing differences between the analysed oncometabolites.

A2780 and GM04312 cells were used to study the role of oncometabolites in the response to induced DNA cross-links. Results of GM04312 cells reveal an increase in DNA-adducted Pt, but only in co-treatments with cisplatin and oncometabolites. Results of A2780 cells show that oncometabolites were not toxic, but increased DNA damage, both spontaneous and induced ones, with a statistically significant positive correlation between the amount of DNA-adducted Pt and the DNA damage detected with the comet assay, also in cotreatments. This work suggests that the impact of oncometabolites on the repair of DNA strand breaks, may be extended to other types of DNA damage, like cross-links, probably related to their effect on homologous recombination repair (HRR).

Keywords:
Oncometabolites: succinate and fumarate; DNA damage response; cisplatin; hydrogen peroxide.

P29

Comparative analysis of miniaturized Ames assay variations for substances with ambiguous testing outcomes

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The Ames assay is based on the concept of bacterial reverse gene mutation, and it is proven to be the most widely applied test in mutagenicity assessment. Increasing emphasis is placed on the development of miniaturized versions of the traditional Ames test with an accentuated goal to reduce the necessary amount of test chemicals, reagents, and liver microsomal S9 fraction resulting in a reduction of test animals. The miniaturized assays, especially Ames MPF, facilitate higher throughput by allowing parallel testing of a large number of samples in the compound screening phase. However, these miniaturized assay variations suffer from the lack of regulatory acceptance, which is (at least in part) due to the ambiguity of the results gained with the miniaturized Ames assays versus the traditional method.

Our goal is to provide an insightful picture on the performance of the miniaturized Ames assay variations in the context of testing a selection of chemicals with known equivocal assay outcomes conferred to the traditional Ames test. Herein we present a comparative analysis of the Ames MPF system, the MicroAmes6 assay, and results from the NTP database.

Previous publications showed a good correlation between the agar-plating assays and the Ames MPF system, which we now further corroborate with selected compounds. An important motivation behind our efforts is to show that scaling down is possible without significantly altering the accuracy and sensitivity of the assay. Therefore, we compare in this study the sensitivity of different miniaturized assays with compounds tested false negative elsewhere (in-press to date).

Our experimental results further strengthen the applicability of miniaturized Ames assays in various use cases, for example, early phases of drug development, impurity testing, and additional research and development scenarios. Taken together, our findings indicate that the miniaturized Ames tests provide highly reliable and cost-effective alternatives to the traditional Ames assay for the assessment of mutagenic chemicals.

Keywords:
Ames Test; miniaturized; MPF; MicroAmes; MiniAmes.

P30

In Vitro Cytotoxicity and Genotoxicity Assessment of Novel Cellulose Nanomaterials using intestinal cells

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Cellulose nanomaterials (CNMs) have been investigated for several applications, including in food and food packaging (e.g. as candidates for zero-calorie filler/thickener/stabilizers; as substitutes of petroleum-based food packaging materials). The widening of these applications will lead to human exposure via oral route, and potentially, to adverse health outcomes. To contribute to the CNMs safety evaluation, the aim of this study was to analyse the in vitro cytotoxicity and genotoxicity of two new micro/nanofibrillated celluloses (CMF/CNFs), using the HT29-MTX-E12 human intestinal cell model. CNMs were synthetized from industrial Eucalyptus globulus kraft and their physicochemical properties were characterized. Upon cells exposure to 3.1 - 200 µg/mL of CNMs during 24 h, the cytotoxicity was evaluated by the MTT and clonogenic assays, and the genotoxicity by the cytokinesis block micronucleus (CBMN) and comet assays.

None of the CNMs was cytotoxic in the concentration-range tested. Concerning genotoxicity assessment, CMF induced a significant level of DNA damage (comet assay) in cells exposed for 3h to 25, 50 and 100 µg/mL and for 24h, to 50 µg/mL, compared with controls. No increases were observed with the FPG-modified comet assay compared with negative control. Cells treatment with the CNF for 3h significantly increased DNA damage at 14.3, 25, 50 µg/mL while a 24h treatment produced significant damage at 50 µg/mL, compared with control. For the latter concentration, induction of oxidative DNA damage was observed for both time points. In contrast, no increase in chromosomal damage was observed using the CBMN assay upon 52h of exposure. To our knowledge, this is the first study in which CNMs were evaluated for their genotoxic effects using the HT29MTX-E12 cell model, relevant for their potential ingestion. Our findings show that cytotoxicity, the endpoint generally used to assess their biocompatibility, is not sufficient to assess their safety to humans. Ongoing studies including the in vitro simulation of human digestion will allow a more comprehensive assessment of CNMs safety. This should be done at an early stage of their development, to ensure their sustainable and innovative application in food technology.

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Keywords:
Cellulose nanofibres; gastrointestinal effects; genotoxicity, nanotoxicology.

P31

Effect of cell treatment procedure on *in vitro* genotoxicity assessment

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Chronic exposure to low-dose contaminants is a major public health issue, so it is important to determine their potential hazard and minimize the risk. Up to now, the majority of in vitro toxicological experiments are conducted after an acute 24h treatment that did not represented the human exposure. Recently, new in vitro approaches have been proposed to study chemical toxicological effect over several days in order to be more predictive of a realistic exposure scenario. In this study, we investigated the genotoxic potential of chemicals with the γH2AX and pH3 biomarkers, with different cell treatment procedure. We tested reference compounds (direct or bioactived clastogen, aneugen and apoptotic inducer) in the human liver-derived HepaRP cell line and used different cell treatment duration, with or without a release period, before genotoxicity analysis. Data were analysed with the Benchmark dose approach. We demonstrated that the detection of clastogenic compounds (notably DNA damaging agent) was more sensitive after three days of repeated treatment compared to one or three treatment over 24h. On the opposite, we observed that aneugenic chemicals were more easily detected as genotoxic after a 24h exposure compared to a 3-day repeated treatment. A 3-day release period after the last treatment, decrease substantially the genotoxicity measurement, whatever the chemical tested. In conclusion, in the cell line used, there are some important difference between a one day acute and a three-day repeated treatment protocol, indicating that different cell treatment procedure may permit to differentiate the chemical genotoxic mode of action.

Keywords:
Genotoxic compounds; repeated exposure; release; γH2AX; pH3.

P32

Utilizing the multicellular model organism C. elegans for investigations of genomic integrity

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Caenorhabditis elegans (C. elegans) is a well-established multicellular model organism in DNA repair research as most DNA repair pathways found in bacteria, yeast, mammals, and humans are highly conserved in the nematode and next to many other advantages, genetic manipulations are fairly easy to conduct in the worm. In contrast to this, methods for specifically detecting DNA damage are scarce. Classical genotoxicity testing still relies mainly on expensive and time-consuming animal experiments or less transferrable cell culture systems, while meaningful multicellular model organisms in the niche between in vitro and *in vivo* are not yet routinely used.

Transcriptome analysis can be a powerful tool for initial pathway identification for (geno)toxicity. By developing and utilizing novel methods for assessing DNA damage (alkaline unwinding assay, 8oxo-guanine quantification) in C. elegans we provide reliable endpoints for investigating specifically the genomic integrity in a multicellular organism. In combination with investigations of the DNA damage response (poly(ADP) ribosylation quantification), DNA repair (gene expression studies, sensitivity of DNA repair deletion mutants), and endpoints of possible underlying mechanisms for genotoxicity (oxidative stress), we are able to assemble a complete model system for genotoxicity testing from (oxidative) stress, activation of the DNA damage response/ DNA repair to measuring the DNA damage itself - thus creating a modern approach for genotoxicity testing.

Keywords:
C. elegans, 3R, alkaline unwinding, PARylation, DNA repair.

P33

New Findings from the Study "Healthy Ageing in an Industrial Environment

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The molecular epidemiological study as part of the Project of Excellent Research "Healthy Aging in Industrial Environment" addressed the effect of selected environmental factors on the health and aging of the population.

The Moravian-Silesian region of the Czech Republic was identified as the study site based on the hot spot of air pollution in Central Europe. The South Bohemia region, which is being used as control locality, is considered as the region with the lowest air pollution level in the Czech Republic. The study cohorts included 500 mothers who long-lived in the regions and their newborns; 125 city policemen who worked mostly in streets with higher levels of pollution; and 400 recreational runners who may be negatively impacted by running in polluted air.

Biological material (venous blood, umbilical cord blood, plasma, urine, maternal dietary samples and breast milk after delivery) was collected from the volunteers. A series of biochemical and epigenetic tests were performed in all cohorts (oxidative damage, micronucleus test, DNA methylation level, miRNA expression profiles, contaminants in diets, etc). The study also hypothesized the adaptation to environment in city policemen. In city policemen from industrial region, biomarkers of early effects (=CpG sites) participating in regulation of genes involved in neurodegeneration, diabetes and respiratory diseases were found by performed DNA methylation analysis. In newborns born in polluted region frequent occurrence of demethylated sites in autoimmune diseased such as asthma, allergy and general disorder of immune functions.

By analysing the diet of mothers before child birth, insufficient intake of vegetables, dairy products and fibers was found. Concentration of PAHs in food is higher in air-polluted locality. Birth weight was higher with higher protein intake and maternal body weight. Oxidative damage in newborns was lower with protein intake in the mothers' diet. In recreational runners, the highest number of deregulated miRNAs was detected in female runners from a low pollution area compared to non-runners.

Analysis of micronuclei proved their significantly lower frequency in policemen from polluted area which confirms the earlier analysed trends that's suggested hypothesis on adaptation of population chronically exposed to high concentrations of the air pollutants. This study was supported by the European Regional Development Fund under Grant HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).

Keywords:
Air pollution; Epigenetics, Molecular epidemiology, Public Health,

P34

Multi-omic and phenotypic reprogramming of oral cells following individual or combined exposures to arsenic and smokeless tobacco

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Ground water contamination with arsenic is a global public health problem, as chronic arsenic exposure can cause serious health damage including cancer. There is also a growing concern regarding the enhancement of arsenic toxicity by lifestyle habit co-exposures, such as smokeless tobacco (SLT) products extensively consumed in Asian countries. Here we studied the in vitro (epi)genomic and phenotypic effects of sodium arsenite (SA), SLT (sadagura) extract, and of SA+SLT combined, to gain new mechanistic insights relevant to oral carcinogenesis. Upon acute exposure, cell viability and genotoxicity were measured in normal oral keratinocyte cultures. Flow cytometry and live-cell imaging analyses were employed to study cell cycle dynamics and apoptosis induction. Temporal transcriptomic analyses by RNAseq addressed the acute exposure effects, while the epigenetic changes following chronic exposure were assessed by DNA methylome analysis.

A dose-dependent decrease in metabolic activity was observed upon individual SA/SLT exposures, an effect exacerbated by co-exposure. Increased γ-H2AX immunofluorescence was observed across exposure conditions and comet assay demonstrated significantly increased DNA tail formation following 24-hr SA+SLT co-exposure compared to the individual treatments. Cell-cycle analyses revealed an increase in the sub-G1 cell population in the SA and SA+SLT exposure groups, suggesting possible cell death, a result manifesting more rapidly upon co-exposure. Annexin V staining and Incucyte analysis combined with caspase inhibition indeed revealed increased apoptotic cell death. Gene expression analysis revealed general upregulation of genes implicated in DNA repair and ROS formation, while we observed upregulation of genes involved in key processes such as apoptosis, ubiquitin-dependent proteolysis, and chromatin organization especially upon SA treatment. Following chronic exposure (4-5 weeks), we observed enhanced DNA hypomethylation, primarily upon the SA and SA+SLT exposures. Associated genes were involved in processes including cell differentiation, apoptosis and ROS response.

The results of our integrated multi-omic and phenotypic analyses offer new mechanistic insights into the action of arsenic and smokeless tobacco, with potential relevance for human populations at risk of oral cancer due to the co-exposure.

Keywords:
Multi-Omics, Mechanisms, Arsenic, Smokeless tobacco.

P35

Identification of epigenetic biomarkers in COPD and lung cancer using minimally invasive samples

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Lung cancer (LuCa) is the leading cause of cancer deaths worldwide, partially because is an asymptomatic disease in early stages. It also shares many symptoms with chronic obstructive pulmonary disease (COPD), which is considered a risk factor for development of lung cancer, as is tobacco use. In addition, lung cancer and COPD are difficult to diagnose due to sampling complexity and the absence of biomarkers.

5-methylcytosine (5-meC) is an epigenetic mark that causes gene silencing. Altered DNA methylation patterns are common in a growing number of human diseases, such as cancer. Tumour cells display local hypermethylation of tumour-suppressor gene promoters and global hypomethylation of gene-poor regions and repetitive sequences.

In this work, we aimed to develop a highly sensitive methodology for detection of epigenetic biomarkers for early diagnosis of lung cancer and COPD using minimally invasive samples (exhaled breath condensate and blood plasma)

.For this purpose, samples were classified into four groups: 1) control group without risk factor (healthy); 2) smokers risk factor group (Smokers); 3) chronic obstructive pulmonary disease risk factor group (COPD) and 4) lung cancer group (LuCa). DNA was extracted, bisulfite-modified, and quantitative methylation specific PCR (qMSP) perfomed to determine methylation status for a group of genes selected previously associated with COPD or LuCa (p16, Rassf1, Shox2, pTGER4 and Line1).

To date, blood plasma samples have been analysed and preliminary data show Rassf1 methylation in 38% LuCa, 10% COPD, 12.5% Smokers and 12.5% healthy subjects. Furthermore in Shox2, methylation was observed in 22% of patients in LuCa group and no methylation was observed in the rest of selected genes in any patient. These preliminary data suggest that methylation of Rassf1 and Shox2 could be useful epigenetic biomarkers for diagnosis of lung cancer using minimal invasive samples.

Keywords:
Lung cancer, COPD, epigenetics, DNA methylation, early diagnosis, minimally invasive samples.

P36

Variations of Dietary Trace Element Supply and its Consequences on Genomic Stability in the Murine Cerebellum

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Trace elements are essential micronutrients involved in various physiological pathways. Therefore, dysregulation in trace element homeostasis can result in a number of diseases. Trace element levels can be adapted to physiological need and nutritional supply, but only to a certain extent. Shifts in their homeostases can affect the redox status of the cell, thereby interfering with several cellular processes, among them the maintenance of genomic stability. Instead of investigating effects of isolated trace element deprivations, this work focuses on the more relevant approach to study modulated supply conditions with multiple trace elements in parallel and their consequences on DNA damage levels in the murine cerebellum. Here, the trace elements of interest are manganese, iron, copper, zinc, and selenium.

Adult male and female mice received suboptimal or adequate supply of copper, zinc, or selenium for eight weeks. Additionally, aiming to investigate trace element interactions in further detail, also combinations of dietary depletion of those three elements were fed. Trace element levels in the cerebellum were analysed via inductively coupled plasma-tandem mass spectrometry. In order to assess DNA damage levels, DNA strand breaks and alkali-labile sites were determined by alkaline comet assay.

It was confirmed that the established diet was indeed suitable to reduce concentrations of copper and selenium in the murine cerebellum whereas zinc contents remained unaffected by dietary supply. Even though dietary iron was not modulated, cerebellar iron levels were influenced by copper supply. However, none of these variations showed consequences on the levels of DNA strand breaks and alkali-labile sites in the cerebellum.

This emphasizes the tight regulation of trace element homeostasis and high priority of genomic stability maintenance, especially in sensitive brain tissue.

Keywords:
Tace elements, comet assay, cerebellum.

P37

Evaluation of Opuntia Ficus Indica (OFI) extract as a potential natural radioprotector

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Many natural substances and extracts have been tested for the capacity to mitigate ionizing radiation-induced damage in normal cells, due to their efficacy and lower side effects and toxicity compared to synthetic radioprotectors. Opuntia Ficus Indica (OFI) is an edible plant growing in arid and semiarid climates with a wide distribution over the world. Besides its importance as a dietary source due to the nutritional value of both OFI fruits and cladodes (rich in minerals, vitamins and antioxidants), the extracts obtained from different parts of the plant (mainly flowers, fruits and seeds) have been gaining increasing attention due to their promising health-promoting properties. In this context, also extracts obtained from OFI cladodes have already been proven to exert cytoprotective, antioxidant, antigenotoxic, antitumoral and anti-inflammatory activities, opening to the possibility to exploit significant amounts of OFI waste products for health protection applications.

The aim of the present study was to evaluate the possible radioprotective effect of an extract obtained from OFI cladode in human peripheral blood lymphocytes (PBLs) undergoing exposure to 60Co γ-rays. Such extract has been prepared by cutting the cladodes in cubic pieces and macerating in distilled water for 24 hours in the dark. Subsequently the macerated material was separated by different filtration stages and characterized by HPLC.

Whole blood samples from healthy volunteers were pre-treated with different non-cytotoxic concentrations of OFI extract for 24 hours; after OFI incubation, the PBLs were exposed to 60Co γ-rays (0.5 and 1 Gy). Radiation-induced chromosome damage was evaluated by the Cytokinesis-Block Micronucleus (CBMN) assay.

The analysis of the results is currently ongoing, in order to evaluate the possible protective effect of OFI extract against radiation-induced increase of micronuclei that might be used in further studies to demonstrate the health-related applications of OFI waste products as powerful radioprotective agent.

Keywords:
Opuntia Ficus Indica (OFI); cladode extract; natural radioprotector; ionizing radiation; micronucleus assay.

P38

Prospective study of cytotoxic and genotoxic effects of Combretastatin A4

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Combretastatins are a class of natural phenols found in the bark of Combretum caffrum, commonly known as South African Bush Willow. Despite having a similar name, combretastatins are unrelated to statins, a family of cholesterol-lowering drugs.

Combretastatin A4 have been shown to be one of the most potent tubulin-depolymerizing agent. Microtubules control chromosomal segregation and cytokinesis during mitosis in both cancer and stromal cells and contribute to overall tumor growth. Consequently, microtubule inhibitors interfere with cell cycle progression and induce apoptosis in cancer cells *in vitro.*

The aim of this study was to investigate the potential gentoxic effect of Comretastatin A4 (CA4) in isolated peripheral blood mononuclear cells (PBMC) in Comet assay in order to establish is there any DNA damage in healty non-dividing cells. The aim also was to explore potential cytotoxic activity of CA4 against human cervical carcinoma (HeLa) cell line.

Genotoxicity of CA4 was evaluated on PBMC in a range of 9 concentrations (from 1 nM to 200μM). Non of the tested concentrations showed genotoxiceffect. The same range of different concentrations of CA4 (from 1 nM to 200μM) were applied to evaluate potential cytotoxicity in a monolayer culture of HeLa cells using the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. After 24h incubation with CA4, there was a significant reduction in cell viability in all concentrations above 250 nm, while IC50 (half maximal inhibitory concentration) was 123 ± 0.06396 μM.

We concluded that CA4 does not have gentoxic effect on PBMC, and that it reduce cell viability of cancer HeLa cell lines. These results are especialy importanat because they showed that CA4 does not damage the DNA molecule in healthy human cells, but achieves its cytotoxic effect on malignant cells in the same range of concentrations.

Keywords:
DNA damage, genotoxicity, cytotoxicity, Comet assay, MTT assay.

P39

Sensitivity to temozolomide in glioblastoma: role of epigenetically regulated DNA repair genes

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Resistance to antitumor therapies is a major problem in the treatment of cancer patients. The resistance to therapy has been correlated, among other causes, with the action of DNA repair mechanisms that remove the damage caused by an antitumor agent. Glioblastoma (GBM) is an aggressive form of brain tumour with a low survival rate, due in large part to resistance to temozolomide (TMZ), a DNA alkylating agent used in combination with radiotherapy as first-line treatment after surgery. A role in TMZ resistance has been proposed for high expression of DNA repair proteins such as MGMT (O6-meG DNA methyltransferase), or MPG (N-Methylpurine DNA Glycosylase). However, increased levels of MGMT or MPG only explain a small percentage of TMZ-resistant tumours. In recent years there has been increasing interest in studying the role that epigenetic silencing of DNA repair genes plays in tumour generation and in antitumor therapy. Indeed, a widely used biomarker to predict response to TMZ in GBM is the MGMT promoter methylation status. A still open question is whether events involving epigenetic silencing in other DNA repair genes, besides MGMT, can be correlated with sensitivity or resistance to TMZ in tumour cells. Here, we have characterized a panel of GBM cell lines by analysing their sensitivity to TMZ. Furthermore, we have examined their capacity of repair TMZ-induced DNA damage, as well as the expression and DNA methylation levels of different DNA repair genes. Our ultimate goal is to contribute to achieve a better knowledge of GMB and facilitate the identification of novel predictive genetic biomarkers and/or therapeutical targets for treatment of this very aggressive tumour.

Keywords:
Glioblastoma, temozolomide sensitivity, BER, epigenetics.

P40

Genotoxic effects of Taxifolin on lymphocytes and MDA-MB-231 breast cancer cell line

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Breast cancer is aggressive cancer in women, and according to recent statistics, it is the most common cancer among women worldwide. DNA damage and repair mechanisms directly affect the mechanisms of cancer; therefore, improving DNA damage repair represents a logical treatment and prevention strategy. Studies have shown that taxifolin (dihydroquercetin) belongs to the subclass flavanonols in the flavonoids, a well-known antioxidant with DNA repair properties in cancers, with reduced metastases and mortality.

In the present study, the genotoxic effects of taxifolin were evaluated on peripheral lymphocytes from breast cancer patients compared to healthy donors using the Comet assay. Furthermore, the cytotoxicity effect of taxifolin on the MDA-MB-231 breast cancer cell line was assessed by using Comet assay.

Also, the cell viability effects of taxifolin were measured on the MDA-MB-231 breast cancer cell line using the cell counting kit-8 (CCK-8).

The comet assay was performed on the healthy blood samples which were treated with different doses of taxifolin to evaluate the genotoxic effect of taxifolin on the healthy blood samples. The results showed that the highest reduction in the genotoxicity on the treated healthy blood samples was 20 µl of 60 µM concentration of taxifolin. Our comet assay results indicated that taxifolin as an antioxidant could have considerable efficacy and potential in repairing the DNA of lymphocytes in patients with breast cancers and increasing DNA damage in the MDA-MB-231 breast cancer cell line. The best dose of taxifolin was observed as 20µl of 60µM concentration with the least and highest genotoxicity in treated whole blood lymphocytes of breast cancer samples and in treated MDA-MB-231 breast cancer cell line, respectively.

The cell proliferation and cytotoxicity assay results in 30 minutes, 2, and 12 hours as 60 µl of 60 µM concentration shows the least cell viability in comparison with the non-treated samples (****, $p<0.0001$); while at 24 hours treatment of 40 µl of 60 µM concentration of taxifolin shows least viability compared to the non-treated samples (****, $p<0.0001$). Although, the IC50 value was calculated to be 40µl of 60 µM concentration, which indicates the amount of taxifolin is needed to inhibit MDA-MB-231 breast cancer cell line proliferation by half. The results from (CCK-8) indicate that taxifolin as an antioxidant could have a considerable cytotoxicity effect on the MDA-MB-231 breast cancer cell line.

This study's results revealed that taxifolin could be used as anti-breast cancer in terms of its cytotoxic effect and promoting apoptosis in the cancer cell and no harm to healthy cells. More investigation to detect the mechanism of action and targeted genes are demanded to understand this flavonoid 's function at the molecular and cellular level.

Keywords:
Breast cancer; Genotoxicity; Taxifolin; Comet assay; MDA-MB-231 cell line.

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DNA replication stress and an ATR-HIPK2 signaling branch mediate genotoxicity of the mycotoxin Aflatoxin B1

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Mycotoxins, including Aflatoxin B1 (AFB1), pose major medical problems since they can contaminate human and pet food. Mycotoxin incorporation is linked to acute and chronic toxicity by causing acute liver damage and inflammation, while chronic exposure is linked to liver carcinogenesis. Although it is well established that AFB1 causes bulky, mutagenic DNA base adducts, the detailed mechanisms and DNA damage signalling events underlying acute AFB1 cytotoxicity are still incompletely defined. The DNA damage-activated kinase HIPK2 is a central regulator of cell death in response to genotoxic stress. The role of HIPK2 in mycotoxin toxicity remains currently unknown.

Here we analysed the molecular events and DNA signalling pathways underlying acute mycotoxin genotoxicity. Our results revealed a critical role for HIPK2 in AFB1-mediated genotoxicity. Acute exposure of human liver cells to AFB1 results in site-specific HIPK2 autophosphorylation and activation. In consequence, active HIPK2 phosphorylates p53 at Ser46 and triggers cell death through both ferroptosis and apoptosis induction. Pharmacological HIPK2 inhibition inhibits p53 Ser46 phosphorylation. Furthermore, we found that AFB1 exposure triggers DNA replication stress involving checkpoint kinase ATR activation, RPA phosphorylation and stalling of replication forks, reflected by DNA fibre assays. In addition, by making use of select kinase inhibitors we demonstrate that AFB1-induced HIPK2 activation is mediated by checkpoint kinases ATR thereby linking HIPK2 activation to DNA replication stress. Confocal microscopy revealed that upon AFB1 exposure active HIPK2 accumulates at DNA breaks in an ATR-dependent manner, suggesting HIPK2 activation takes place at DNA breaks. Pharmacological inhibition of ATR and the down-stream activated checkpoint kinase ATM inhibits AFB1 cytotoxicity in liver cells, suggesting DNA damage checkpoint kinase inhibition as potential intervention option to mitigate acute AFB1 hepatotoxicity. Collectively, our results reveal DNA replication stress as a critical mechanism of AFB1 hepatotoxicity through triggering a detrimental ATR-HIPK2-p53 signalling axis. Finally, our data suggest select DNA damage checkpoint kinase inhibitors as potential antidots to treat acute mycotoxin toxicity.

Keywords:
mycotoxins; Aflatoxin B1; DNA replication stress; ATR; HIPK2.

P42

A framework for interpreting in vitro genotoxicity data: Using mechanistic data to interpret positive results

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Non-animal methods are increasingly being used for regulatory decision making by agencies worldwide because of their potential to reliably and efficiently produce information that is fit for purpose while reducing animal use. The prediction of genotoxic hazard to humans usually follows a stepwise approach, beginning with an *in vitro* battery consisting of a gene mutation test in bacteria, an *in vitro* test for chromosomal damage and/or gene mutation in cultured mammalian cells. Depending on the in vitro test results and regulatory requirements, the *in vitro* battery may be followed in some cases by *in vivo* testing.

When the standard battery of two or three in vitro genotoxicity tests was retrospectively analysed it was found that a high percentage of in vivo non-genotoxic or rodent non-carcinogenic compounds gave positive results in at least one in vitro test. The *in vitro* chromosome damage assays gave a high percentage of misleading positive results, often related to choice of cell line and confounded by differing methods of estimating cytotoxicity. To better interpret in vitro genotoxicity results, it has been suggested that all available information including in silico and *in vitro* data should be considered in a holistic weight of evidence approach. Clarification of the mechanism of action (MoA) of the test chemicals proves particularly valuable for decision-making.

This poster presents illustrative case studies showing how MoA assessment using non-animal methods such as the ToxTracker system, as well as other non-animal methods, can provide information on the potential genotoxic mode of action. Examples are presented where results from in vitro genotoxicity tests can be rationalised with a holistic data driven approach, potentially reducing the need for in vivo follow-up testing. Mode of action information allows for a more accurate and concrete assessment of genotoxicity. Investigation of MoA using targeted methods without animal testing has great potential to clarify positive *in vitro* genotoxicity test results, thereby avoiding *in vivo* follow-up testing.

Keywords:
In vitro positives, Mode of action, ToxTracker, Reduce *in vivo* follow-up.

P43

Mutagenic and genotoxic potential produced by a mixture of the cyanotoxins Anatoxin-a and Cylindrospermopsin

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The simultaneous occurrence of various cyanobacterial toxins can potentially induce toxic effects different than those observed for single cyanotoxins, as interactions may occur. This is a more common scenario in nature, where multiple toxins are frequently found in cyanobacterial blooms. Furthermore, the need for further studies based on the toxicity of cyanotoxins mixtures has been stated by the European Food Safety Authority (EFSA). Nevertheless, toxicological information on the topic is still scarce.

The aim of this study was to assess the mutagenic and genotoxic potential of mixtures of two of the most relevant cyanotoxins, Anatoxin-a (ATX-a) and Cylindrospermopsin (CYN), using a basic battery of in vitro test consisting of the bacterial reverse mutation test (Ames test, OECD 471) and the micronucleus (MN) assay (OCDE 487). Mixtures of 1:1 ATX-a/CYN were used to perform both assays. The Ames test were tested in five Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, TA1537) in a concentration range from 0.125 to 2 µg/mL of toxins in presence and absence of S9 fraction from rat livers as metabolic activation system. The MN assays were performed on L5178YTk± cells in absence (0.084-1.35 µg/mL ATX-a/CYN) and in presence (0.125-2 µg/mL ATX-a/CYN) of S9 fraction. The exposure periods ranged between 4 and 72 h depending on the assay. There were no mutagenic effects after bacteria exposure to ATX-a/CYN mixture with or without metabolic activation in the concentrations range assayed. Significant changes were observed in the MN test of the mixture with or without S9 metabolic fraction, whereas previous studies only showed toxic effects in the presence of the S9 fraction after individual exposure to CYN. These results highlight the need for a specific evaluation of the genotoxicity of cyanotoxin mixtures, as their effects cannot be extrapolated from those of the individual cyanotoxins.

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Keywords:
Anatoxin-a, cylindrospermopsin, genotoxicity, mutagenicity, mixture.

P44

Mutagenic and genotoxic assessment of pure anatoxin-a

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In recent years, the proliferation of cyanobacterial blooms has significantly increased due to water eutrophication and climate change. The capacity of certain cyanobacterial strains to produce cyanotoxins is of public concern due to its toxic effects on humans, animals, and the environment. One of the most important cyanotoxins is anatoxin-a (ATX-a), a globally distributed freshwater neurotoxin that has been linked to human and animal poisonings. The main route of exposure of this cyanotoxin is oral, through the consumption of contaminated water and food. However, in spite of its importance, the toxicological database of ATX-a is limited.

For this reason, the aim of this study was to investigate the potential mutagenicity and genotoxicity of pure ATX-a, as they play a key role in the risk assessment of any food contaminant. Two different in vitro tests recommended by the European Food Safety Authority (EFSA) have been used. The mutagenicity of ATX-a was assessed by the bacterial reverse-mutation assay using the Salmonella typhimurium TA1537, TA98, TA100, TA102, TA1535 strains (Ames test, OECD 471) and its genotoxicity was investigated by the in vitro micronucleus (MN) assay (OCDE 487) in L5178Y Tk+/− cell line, both in absence and presence of metabolic fraction S9. The ranges of concentrations of ATX-a tested were from 0.125 to 20 µg/mL for 72 h in the Ames test and from 1.25 to 20 µg/mL for 4 h in the MN assay. The results showed no mutagenic effect in any of the tested strains in the range of concentrations tested. By contrast, in the MN assay, a significant increase of percentage of binucleated cells with micronuclei (BNMN) was observed after exposure to 10 µg/mL ATX-a in absence of the metabolic fraction S9. To our knowledge, these are the first results showing a potential genotoxicity of ATX-a in the in vitro MN assay. Nevertheless, further studies are needed to elucidate the toxicity profile of ATX-a.

Acknowledgements: This research was funded by the Spanish Ministerio de Ciencia e Innovación (PID2019-104890RB-I00/AEI/10.13039/501100011033). C. Plata-Calzado is grateful for the financial support of the Junta de Andalucía (PREDOC_00447 contract). Leticia Diez-Quijada Jiménez thanks to the Junta de Andalucía for the award of a postdoctoral grant as Doctoral Researcher Junta de Andalucia, grant number POSTDOC_21_00130.

Keywords:
Anatoxin-a, genotoxicity, mutagenicity, Ames test, micronucleus assay.

P45

Stem cells as unique models to study long-term effects triggered by micro- and nanoplastic exposure: a focus on cell transformation and differentiation.

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The wide diversity of human exposures across individuals and throughout their lifetime calls for new approaches and models to implement risk assessment strategies. In this context, stem cells offer a unique possibility to study specific functionalities of immature cells, cell fate, and cell transformation. Due to their long lifespans, stem cells can be particularly susceptible to long-term exposures and the accumulation of abnormalities could lead to a differential impact compared to short-lived cells, including the emergence of cancer stem cells. Therefore, we propose stem cells as a relevant model for the evaluation of the potential impact of micro- and nanoplastic (MNPLs) on human health. The population is continuously exposed to these small plastic particles that can translocate through physiological barriers and cause mild but relevant effects related to cytotoxicity, ROS generation, DNA damage, and pro-inflammatory response alterations. The information regarding the bioaccumulation of MNPLs is still limited but the extended exposure could be expected to induce accumulative adverse effects such as mutagenesis and carcinogenesis, aspects insufficiently explored until now.

Our work aims to develop stem cell-based models useful to provide information on the potential long-term effects of MNPLs by evaluating endpoints related to transformation onset and stemness imbalance. On the one hand, we have developed an in vitro exposure approach in which we continuously exposed a model of breast stem cells (MCF10A) to polystyrene (PS) and polyethylene terephthalate (PET) nanoplastics for 5 months. Although we have not identified significant changes in the transformed status of the cells at a functional level, we have observed an increasing tendency in their anchorage-independent growth and migration capacities that points to MNPLs as inducers of a permissive context for cell transformation. Ongoing work is focused on identifying potential underlying molecular effects by transcriptomic and kinase activity profiling analyses. In parallel, we are looking for new stem cell models relevant to evaluate the potential cell fate-deregulating effect of MNPLs exposure that may require further in-depth studies.

This work could contribute with relevant data regarding the impact of MNPLs on cell fate deregulation and stem cell transformation, and further highlight the interest in incorporating stem cell models in risk assessment strategies.

Keywords:
Stem cells; micro- and nanoplastics; long-term exposure; in vitro transformation; stemness imbalance.

P46

Evaluating micronucleus frequency of circulating lymphocytes as a potential biomarker for oesophageal adenocarcinoma

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The late presentation of symptoms leads to poor prognosis in patients with Oesophageal adenocarcinoma (OAC). Patients with pre-malignant Barrett's oesophagus (BO) stay under surveillance to monitor level of cell dysplasia. Our group is interested in investigating lymphocyte micronucleus frequency (L-MN%) as a potential blood-based biomarker to prioritise patients for endoscopy. Previous work within our group involving blood samples obtained from healthy volunteers (n=34), patients with gastro-oesophageal reflux disease (GORD) (n=63), Barrett's oesophagus (BO) (n=34) and OAC (N=43) showed that OAC patients had significantly elevated lymphocyte micronucleus frequencies (L-MN%) than non-cancer controls ($p<0.001$) It is our current aim to investigate the mechanism behind the increased lymphocyte MN%(L-MN%) in patients with OAC.

To assess individual susceptibility to DNA damage, lymphocytes from healthy volunteers (n=15), patients with GORD(n=9), BO (N=9), and OAC(n=9) were stimulated with phytohemagglutinin and treated with hydrogen peroxide (H2O2), sodium deoxycholate (DCA) and vinblastine. L-MN% was assessed using the cytokinesis-block micronucleus assay.For H2O2 and DCA treatments there is no difference in L-MN% between patient groups, however there is a negative correlation (P value = 0.0005 and 0.0046 respectively) between MN% and MN% fold change. Plasma levels of reduced glutathione (GSH) were increased in patients with a higher L-MN% (P-value =0.0091). This suggests that the lymphocytes of some individuals exposed to higher levels of oxidative stress in-vivo have adapted to reduce further DNA damage. Following kinetochore staining of patient lymphocytes, it was observed that OAC patients have higher levels of centromere positive MNi, indicating an aneugenic mechanism behind the increase. This is further supported as the L-MN% fold change following treatment with vinblastine appears higher in cancer patients, compared to non-cancer controls.

Innate immune receptor cyclicAMP-AMP synthase (cGAS) recognises dsDNA from micronuclei, activating the Stimulator of Interferon Genes (STING) via cGAMP and leading to the upregulation of type 1 interferons e.g., IFN-B. OAC patients have significantly higher levels of IFN-B in their plasma, activation of this pathway with be evaluated following measurement of cGAMP in plasma. Whilst further work is required, the results are promising in the validation of L-MN% as a potential biomarker for risk of cancer progression.

Keywords:
Micronuclei, Lymphocytes, Biomarker, Oesophageal Cancer.

P47

Long-term exposure to nanoplastics alters molecular and functional traits related to the carcinogenic process

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Micro/nanoplastics (MNPLs) are considered emergent pollutants widely spread over all environmental compartments. Although their potential biological effects are being intensively evaluated, many doubts remain about their potential health effects in humans. One of the most underdeveloped fields is the determination of the potential tumorigenic risk of MNPLs exposure. To shed light on this topic, we have designed a wide battery of different hallmarks of cancer applied to prone-to-transformed progress MEF cells exposed to polystyrene nanoplastics (PSNPLs) in the long term (6 months). Interestingly, most of the evaluated hallmarks of cancer are exacerbated after exposure, independently if they are associated with an early tumoral phenotype (changes in stress-related genes, or microRNA deregulation), advanced tumoral phenotype (growing independently of anchorage ability, and migration capacity), or an aggressive tumoral phenotype (invasion potential, changes in pluripotency markers, and ability to grow to form tumorspheres). This set of obtained data constitutes a relevant warning on the potential carcinogenic risk associated with long-term exposures to MNPLs, specifically that induced by the PSNPLs evaluated in this study.

Keywords:
Carcinogenesis; Long-term exposure; Oncogenic phenotype; Polystyrene nanoplastic.

P48

In vivo standard and Fpg-modified comet assay: study of lysis conditions

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Cell lysis is one of the steps in the comet assay. It is a popular belief that it should endure for at least 1 hour, and it is common practice to extend it, conveniently pausing the experiment, sometimes until next day (i.e. giving an overnight or a 24-hour lysis). It has also been published that it could be fully removed in the standard in vitro comet assay or reduced to 5 minutes regarding the enzyme-modified version. However, the influence of lysis length regarding the in vivo version of the assay is not well established.

For that purpose, a single oral dose of 200 mg/kg methyl methanesulfonate (MMS) to induce strand breaks, or 5 mg/kg MMS or 400 mg/kg potassium bromate (KBrO3) to induce Fpg-sensitive sites, was administered to male Wistar rats (n=3 rats/group). Negative control rats were dosed with saline solution.

After 3 hours, the animals were sacrificed. Liver, kidney and duodenum were removed from animals administered with 200 mg/kg MMS, while liver and duodenum were taken when the lower dose of MMS was used. In the case of the animals treated with 400 mg/Kg KBrO3 only the kidney was extracted. These tissues were processed using the standard alkaline or the Fpg-modified comet assay, as appropriate, and lysed for 5 minutes, 1 hour, overnight, or given no lysis. The influence of lysis pH was also studied in the Fpg-modified comet assay, using neutral (pH 7) and alkaline (pH 10) conditions for the 1 hour lysis timepoint.

Regarding the standard comet assay, no significant differences were found among different lysis lengths, including the absence of lysis. However, as expected, the lysis step is necessary to measure Fpg-sensitive sites. Concerning MMS, the levels of Fpg-sensitive sites detected increased along with lysis duration. In contrast, this effect was not found in rats treated with KBrO3. Moreover, decreasing lysis solution pH seems to have an impact on results obtained with MMS, reducing the Fpg-sensitive sites obtained, which is not the case for KBrO3-induced lesions. This could be due to the different nature of the Fpg-sensitive sites detected in animals administered with MMS or with KBrO3.

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Keywords:
Comet assay, *in vivo*, Fpg, lysis duration.

P49

Investigation of base excision repair and oxidative stress in preeclampsia-complicated placentas – a pilot study

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Although the molecular pathways underlying pre-eclampsia (PE) are not entirely elucidated yet, the pathogenesis of PE involves elevated levels of oxidative stress in the placenta. Oxidative stress leads to DNA damage, including 8-oxoguanine (8-oxoG), which can interfere with normal placental development. Fortunately, oxidative stress induced DNA damage is repaired by base excision repair (BER), and a decreased ability to repair DNA may be involved in PE.

Therefore, the current pilot study investigated the correlation between PE and BER activity of the placenta. Additionally, we investigated the relationship between BER and antioxidant activity. We observed a significantly lower BER incision activity in PE-complicated placentas when compared to healthy controls by means of a comet-assay based assessment of DNA repair related incision activity. Furthermore, gene expression of various genes that are involved in the initial steps of BER (including OGG1, APEX1 and NEIL1) was significantly lower in PE (n=9) than in healthy control placentas (n=11). The expression of APEX1 correlated with BER incision activity (r= 0.72, P= 0.0027). Additionally, we observed a positive correlation between mitochondrial copy number and BER incision levels (r= 0.67, P= 0.0192). Higher antioxidant capacity was observed in PE, which was negatively correlated with BER incision capacity.

In conclusion, these data show that antioxidant and DNA repair defenses interact in the response to oxidative stress in the placenta and that decreased BER may play a role in the development of PE.

Keywords:
preeclampsia; oxidative stress; BER; antioxidants; mitochondria;

P50

Heritability of baseline frequency of micronuclei in buccal cells: the SMP-twin study.

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The analysis of the spontaneous frequency of micronuclei in buccal cells is applied in human biomonitoring studies to quantify genomic damage associated with environmental and occupational exposures, dietary, lifestyle habits as well as clinical outcomes (1-3). It is important to identify the critical factors affecting interindividual variability to evaluate with confidence whether the variations observed among individuals are due to environmental factors or the individual's genetic make-up.

One of the most robust methods for identifying sources of variation in humans is to analyse a specific trait in twins. The comparison of the similarity between pairs of genetically identical twins (monozygotic, MZ) and fraternal twins (dizygotic, DZ) allows to measure the relative contribution of genetics, as opposed to environment, to a given trait.

The design of the present study is aimed at investigating the heritability of baseline frequency of micronuclei in buccal cells from a population of 150 pairs of healthy MZ and DZ twins, enrolled from the Italian Twin Registry, aged from 18 to 80 years. Information on health status, smoking habits and mental well-being status were obtained through questionnaires administered to the enrolled subjects. Biological samples, including buccal cells, serum, plasma and blood mononuclear cells, were appropriately stored in a biobank.

A secondary aim of this study is to determine if the mental well-being status may affect the frequency of micronuclei in buccal cells. A recent study by Reimann et al. (2020) highlighted a possible contribution of depression on DNA damage in buccal cells and suggested the need of further studies to determine whether this analysis could be predictive of mental disorders. In the present study, the analysis of DNA damage will be focused on a subgroup of MZ twins discordant for mental well-being status to better investigate this association with the powerful tool provided by the twin model.

In addition, the measure of micronucleus frequency will be integrated with the analysis of individual levels of biomarkers of oxidative stress and antioxidant capacity to investigate the influence of oxidative stress on this marker of DNA damage.

P51

Applying a test strategy to investigate toxic effects using Schyzosacchromyces pombe

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The yeast Schizosaccharomyces pombe is a good alternative model organism for the study of substance toxicity and, more specifically, for the investigation of toxic mechanisms. The objective of this study was the validation of a test strategy to investigate the main effects of various toxic compounds using several strains.

A series of chemical compounds whose mechanism of action is perfectly known and that act as inducers of different toxicity mechanisms was used: carbendazime, which causes interference in microtubules, hydrogen peroxide and potassium chloride which cause cellular stress, and hydroxyurea, which induces DNA damage.

The growth in liquid media of different strains of S. pombe deficient in the genes of interest exposed to the compounds for 16, 18 and 20 hours have been compared. Thus, in cases where particular sensitivity was detected in the MPH1Δ strain, interference in microtubules was considered; for Sty1Δ, Sty1Δpmk1Δ and Pap1Δ, oxidative stress was stablished; DNA damage was correlated with the Rad3Δ strain; global sensitivity was obtained for a strain deficient in several MDR proteins; and the defence mediated by specific efflux pumps was identified with PMD1, BFR1, MFS1 and CAF5 deficient strains.

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Keywords:
Toxicity; genotoxicity; yeast; Schizosaccharomyces pombe.

P52

Cytotoxic and Genotoxic Effects of Patient-derived Plasma on Healthy and Cancer-derived Cell Lines

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Genome instability and mutation drive carcinogenesis. Many everyday factors can have adverse effects on the genome. Monitoring the mutational effects of plasma and serum derived from patients diagnosed with: GORD, Barrett's oesophagus, and upper GI cancer can reveal and ultimately predict future cancer risk. For this study, plasma was isolated from healthy volunteers and non-cancer patients attending the local endoscopy department and cancer patients attending the oncology department. Micronucleus frequency (MN%) was measured in two cell lines: healthy lymphocyte-derived cell line (TK6) and oesophageal cancer-derived cell line (OE33). Additionally, other effects were also determined using several end-point measurements, including confocal microscopy imaging, cell cycle, invasion, and migration of cells upon plasma/serum treatment.

PIG-A is the catalytic subunit of the Phosphatidylinositol Acetylglucosaminyl transferase enzyme, encoded by the PIGA gene, which encodes an X-inked enzyme that is involved in GPI anchor synthesis. Measuring mutations in the PIGA gene allows for identifying individuals more prone to developing cancer due to exposure to harmful reagents. This study measures the transformation in the PIGA gene in healthy volunteers and non-cancer patients referred to an endoscopy department and cancer patients. This is an attractive tool to study the level of exposure, such as those associated with diet and exercise.

P53

A novel form of gallic acid to improve the compound bioavailability and cancer

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Gallic acid (GA), a natural phenolic compound, has numerous biological properties such as antimalarial, antibacterial (against Bacillus subtilis, Staphylococcus aureus and Escherichia coli), antiviral, anti-inflammatory and anticancer activities. In this poster we presented the methodology that allows for the synthesis of a range of sulphonamide analogous to gallic acid (GA), 3,4,5-trimethoxybenzenesulfonamide TMBS and other derivatives of GA and their antioxidant properties were investigated. CCK-8 assay was used to investigate the inhibition of cell proliferation and apoptosis cell death of GA and its sulphonamide analogue on the A549 lung cancer cell line. Different concentrations of GA (20, 30, and 40 μM) and 3,4,5-trimethoxybenzenesulfonamide (TMBS), (30, 40 and 60 μM) were used to treat A549 cancer cell line for 30 minutes, 12, 24 and 48 hours. The apoptosis rate of GA and TMBS was higher than without treatment and positive control. Furthermore, TMBS showed a higher inhibition rate than GA.

The genotoxic effects of these compounds were assessed on a model for non-small cell lung cancer (A549) and lymphocytes from lung cancer patients and healthy controls by using the Comet assay. The Comet assay was carried out on 40 blood samples, 20 from patients with lung cancer and 20 from healthy volunteers. The most promising compound synthesised showed approximately a 2-fold more potent effect with a p-value of 0.001 compared to negative control and 1.4-fold reduced DNA damage when compared to gallic acid. The results of the Comet assay on the cell line showed that both compounds are not as genotoxic. However, adding an oxidising agent such as hydrogen peroxide increased their genotoxicity by up to 3-fold. These results clearly demonstrated that this compound is an effective compound to repair DNA damage compared to gallic acid and can pave the way for developing an effective drug. Intriguingly, the new derivatives suggest a higher level of antioxidant effect compared to GA.

Keywords:
Gallic acid; 3,4,5-trimethoxybenzenesulfonamide (TMBS); antioxidant; genotoxic; Comet assay.

P54

Evaluation of Cytotoxicity and Genotoxicity of Nano-sized Plastic Particles

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Polystyrene nanoparticles (PS-NPs) are one of the most represented plastic NPs in the environment and one of the main concerns of PS-NPs exposure is their genotoxic potential [1]. In this study, we continued the investigation of PS-NPs genotoxicity and cytotoxicity. For in vitro studies, lymphocytes of healthy donors were treated with 80 nm size PS-NPs. Cytotoxicity was evaluated using an acridine orange/ethidium bromide stain mix, and primary DNA lesions were investigated by comet assay after short-term (3 h) and long-term (24 h) incubation with PS-NPs. Chromosomal damage was assessed by micronucleus assay and chromosome aberration assay. For in vivo studies, Somatic Mutation and Recombination Test (SMART) in Drosophila Melanogaster was applied.

Tested PS-NPs concentrations were not cytotoxic in human peripheral blood lymphocytes. However, a negative correlation between the nuclear division index and NPs concentrations was observed, suggesting that PS-NPs could have a cytostatic effect. The comet assay results revealed that after long-term incubation, all tested PS-NPs concentrations induced a statistically significant increase in %TDNA (% Tail DNA). Also, the majority of selected PS-NPs concentrations induced a statistically significant increase in the frequency of micronuclei, besides that it was dose-related. However, the chromosome aberration test and SMART results showed that none of the tested PS-NPs concentrations induced a significant increase in chromosome aberration number in vitro or somatic mutations in vivo. Overall, results suggest the genotoxic potential of polystyrene nanoparticles, yet highlight the need for additional studies to assess the bio-accumulation and genotoxicity of these NPs in different cell lines and organisms.

Keywords:
Nanoplastic, Comet Assay, Micronucleus Assay, Somatic Mutation and Recombination Test, Chromosome Aberrations.

P55

Activities of the Iberoamerican Network of Toxicology and Chemical Safety with the Hispanic Organization of Toxicology

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This poster shows the close collaboration between the Hispanic Organization of Toxicologists of the Society of Toxicogy (HOT-SOT), with the Ibero-American Network of Toxicology and Chemical Safety (RITSQ). This network was created by the joint initiative of the University of São Paulo, member of IUTOX (Dr. Barros) and the Higher Council for Scientific Research, and the Spanish Association of Toxicology, then its president (Dr. de la Peña); We started activities in 2006 and since then we have held 3 RITSQ meetings, in Montreal, Canada (2007), in Barcelona Spain (2010) and in Mérida, Mexico (2016). In the sixteen years of the existence of the aforementioned RITSQ, we use the web (http://ritsq.org), and where we have presented: news, congresses, courses, news from the festival, and to date we have presented 134 posters , on the website are published by year, all the posters presented in congresses until.

2022; The objective of the RITSQ is to publish the information provided to us by e-mail, information on congresses and courses of toxicological interest and chemical safety, publishing all the information on the aforementioned website. In the past year we have communicated the activities held with the Hispanic toxicology organization with which we collaborate and materialize it in this poster number 134. The attached table shows a summary of the published data: news, congresses, courses, network report RITSQ and the number of pages visited, data that has been shown annually since 2018, for this reason we ask potential organizers to kindly provide us with the relevant information about the event and publish it in advance on the web.

Keywords:
Users, sessions and number of pages visited.

P56

Genomic instability in blood cells from bovine with spontaneous tumours: an OneHealth perspective

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Genomic instability is a hallmark of carcinogenesis, that acts in the conversion of a normal into a premalignant cell. The aim of this preliminary study, is evaluate DNA damage in different spontaneous tumours, as well as establish a correlation between malignity and the DNA damage itself. OneHealth is a recent approach that links animal with human medicine which is nowadays more relevant. In order to perform this study, the alkaline comet assay was performed on blood samples from 5 animals with neoplastic disease: 2 cutaneous melanomas, 1 cutaneous papilloma, 1 vesical hemangiosarcoma and 1 undifferentiated tumour. The results obtained from DNA damage in blood lymphocytes (in arbitrary units 0-400) were 20±5.34 in the cutaneous papilloma; 22±8.50 and 34±10.12 in the two cutaneous melanomas; 89±19.12 in the vesical hemangiosarcoma and 168±5.5 in the undifferentiated tumour. All animals presented values below 170 A.U., compared to the other cases, the undifferentiated tumour and vesical hemagiosarcoma (which have the higher values) reflect a greater DNA damage. Interestingly, these tumours present histological features suggestive of high biological aggressiveness and distant metastasis. The papilloma, a benign tumour, presented the lowest value. So far results indicate that the damage is higher in aggressive tumours compared to non-invasive tumours. This data belongs to a far more extensive study that will be carried out with the establishment of a control group, as well as obtaining more samples and applying inquests; in order to connect the animals, owners and environmental aspects in an one health perspective of understanding cancer progression.

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Keywords:
DNA damage, comet assay, bovine tumours, blood cells

P57

Analysis of Polycyclic Aromatic Hydrocarbons in Size Fractionized Atmospheric Aerosol Obtained by Personal Monitoring

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Personal monitoring represents a useful tool to obtain more detailed information on exposure histories, microenvironment concentrations and air pollution sources in studied areas. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. Long-term inhalation exposure to PAHs has been associated with different types of cancer, cardiovascular, respiratory and other diseases. Airborne particles (PM) of different sizes can bound various amounts and types of PAHs depending on their volatility and molecular structure. In our study, we employed SKC Leland Legacy Pump with Sioutas Cascade Impactor to analyse 20 US EPA and EU 15+1 priority PAHs in different size fractions of PM (<0.25, 0.25–2.5, and >2.5μm). A total set of 129 volunteers from two localities of the Czech Republic: industrial area of the city of Ostrava (n=65) and a control area of České Budějovice city (n=64), participated in 24h personal air sampling taking place from August 2019 to August 2021. Personal monitoring data were accompanied by a detailed questionnaire and PM2.5 measurements from the nearest monitoring stations. Extraction of the target PAHs was carried out by organic solvent extraction in an ultrasonic bath. The analytical method for PAH determination was developed using gas chromatography coupled to tandem mass spectrometry in electron ionization. The total amount of 20 PAHs ranged from 0.1 to 42.2 ng/m3. Concentration of Benzo(a)pyrene, an air quality standard, ranged from 0.01 to 3.27 ng/m3. The EU daily averaged concentration limit of 1.0 ng/m3 was exceeded in 20 out of 129 volunteers, especially those exposed in the industrial locality and the winter season. The particle size distribution showed 87% of the total amount of PAHs to be bound to PM0.25 (PM<0.25μm) and only 1% to the fraction larger than 2.5μm. Comparing the industrial and control areas, Ostrava air contained twice as high mean concentrations of PM-bound PAHs compared to České Budějovice. The highest concentrations were observed in January and February, indicating the significant contribution of local heating. The results of the personal monitoring contribute to the identification of local and seasonal sources of PAHs and specific activities with high PAH exposure.

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Keywords:
PAHs; personal monitoring; air pollution; particulate matter; size franctions.

P58

Potential effects of in vitro digestion on the physicochemical and biological characteristics of polystyrene nanoplastics

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In humans, the main exposure route to micro/nano plastics MNPLs is via ingestion.To effectively determine the toxicological profile of MNPLs, the role of digestion must be considered. Accordingly, this study aims to determine the influence of the in vitro digestive process on the toxicity of polystyrene nanoplastics (PSNPLs) in three different human leukocytic cell lines. Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) we have determined that all particles are spherically shaped, with similar appearance and sizes but, the digested particles show a relevant tendency to agglomerate. The hydrodynamic radius shows that digested particles have a larger hydrodynamic size, and the polydispersity index (PdI) indicates that the non-digested particles are more monodisperse. These results agree with the results of the Z-potential showing that the digested particles have less Z-potential. Also, the particles were analyzed by Nano tracking analysis (NTA) to measure the hydrodynamic radius supporting the previously obtained data. Cell uptake was evaluated with the fluorescent polystyrene counterparts (fPSNPLs and dfPSNPLs) by flow cytometry and confocal microscopy. Results show that the three selected cell lines internalize more dfPSNPs than fPSNPLs. When cell viability was assessed, only moderate effects were observed at the highest concentration of dPSNPLs in TK6 at exposures lasting for 24/48 h. No intracellular ROS production was observed in any of the cell lines at 24/48 h and, finally, genotoxic damage induction was detected only at 24 h exposure in THP1 cells, and at the highest concentrations. No oxidative DNA damage was detected at any time and in any cell line. Finally, the visualization and characterization of proteins on the surface of PS particles was done by Raman spectroscopy showing the association of proteins on the particle surface and information about the secondary protein structure on particle surface.

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Keywords:
Nanoplastics, Polystyrene, digestion, cell uptake, cytotoxicity.

P59

A new source of representative secondary PET nanoplastics. Obtention, characterization, and hazard evaluation

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Micro and nanoplastics (MNPLs) are emergent environmental pollutants requiring urgent information on their potential risks to human health. One of the problems associated with the evaluation of their undesirable effects is the lack of real samples, matching with those resulting from the environmental degradation of plastic wastes. To such end, we propose an easy method to obtain polyethylene terephthalate nanoplastics from water plastic bottles (PET-NPLs) but, in principle, applicable to any other plastic goods sources. An extensive characterization indicates that the proposed process produces uniform samples of PET-NPLs of around 100 nm, as determined by using a multi-angle and dynamic light scattering methodology. An important point to be highlighted is that to avoid the metal contamination resulting from methods using metal blades/burrs for milling, trituration, or sanding, we propose to use diamond burrs to produce metal-free samples. To visualize the toxicological profile of the produced PET-NPLs we have evaluated their ability to be internalized by cells, their cytotoxicity, their ability to induce oxidative stress, and to induce DNA damage. In this preliminary approach, we have detected their cellular uptake, but without the induction of significant biological effects. Thus, no relevant increases in toxicity, reactive oxygen species (ROS) induction, or DNA damage -as detected with the comet assay- have been observed. The use of real samples, as produced in this study, will generate relevant data in the discussion about the potential health risks associated with MNPLs exposures.

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Keywords:
Nanoplastics, polyethylene terephthalate, PET, cell uptake, cytotoxicity.

P60

Cognitive impairment due to biological ageing process related to DNA damage assessed by the comet assay

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Cognitive impairment is costly and invalidating. While age is the strongest known risk factor for declining cognitive function, other risk factors include environmental exposure to pollutants, unhealthy diets, or toxic habits, among others. Since these risk factors have been related to DNA damage, the promising potential of comet assay to explore the association between DNA damage and cognitive dysfunction due to biological ageing was examined. We reviewed and summarized recent studies exploring the relationship between DNA damage evaluated by means of the comet assay and cognitive function both in animal models and in humans. A general overview of studies determining cognitive dysfunction related to DNA damage due to the biological ageing process is provided. The review confirmed the potential of the comet assay to further explore the link between DNA damage, as indicative of genomic instability, and cognitive impairment in different research and clinical areas. Studies analysed support a considerable relationship between DNA damage and cognitive impairment, mainly affecting executive functions, working memory and attention. These cognitive domains are crucial to daily functioning and occupational performance, with important clinical implications.

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Keywords:
ageing; cognitive impairment comet assay; DNA damage.

P61

Hazard assessment of different-sized polystyrene nanoplastics in a human B-lymphoblastoid cell line

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The environmental presence of micro/nanoplastics (MNPLs) is an environmental and human health concern. Such MNPLs can result from the physicochemical/biological degradation of plastic goods (secondary MNPLs) or can result from industrial production at that size, for different commercial purposes (primary MNPLs). Independently of their origin, the toxicological profile of MNPLs can be modulated by their size, as well as by the ability of cells/organisms to internalize them.

To get more information on these topics we have determined the ability of three different sizes of polystyrene MNPLs (50, 200, and 500 nm) to produce different biological effects in human B-lymphoblastoid cells (Raji-B).

The results show that none of the three sizes was able to induce toxicity (growth ability) in the tested cell type; Although transmission electron microscopy and confocal images showed cell internalization and their quantification by flow cytometry demonstrated an important uptake by Raji-B cells. This uptake was negatively associated with the size. Interestingly, when the loss of mitochondrial membrane potential was determined, dose-related effects were observed. These effects were observed for the three different sizes. Finally, when oxidative stress induction was evaluated, no clear effects were observed for the different tested combinations.

Our conclusion is that size and biological endpoints, are aspects modulating the toxicological profile of MNPLs.

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Keywords: polystyrene nanoplastic size, Raji-B cells, uptake, oxidative stress, mitochondrial membrane potential.

P62

Studying the effects of nanoplastics derived from teabags in an in vitro model of the intestinal epithelium

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The increasing presence of micro- and nano-sized plastics in the environment, and in the food chain, is of growing concern. Plastics from consumer goods can break down into micro and nanoplastics (MNPLs), migrate into the food, and facilitate their human ingestion. A good example is the teabags, which has become a new source of MNPLs since the traditional paper bags were substituted with “biodegradable” plastic bags. Among the different studies on the potential hazard effects of MNPLs, those related to the MNPLs released from teabags are practically inexistent. To cover this gap, we have isolated MNPLs from teabags and used a model of in vitro intestinal barrier to perform toxicological studies. The model consists of the co-culture of human gut-derived cells: enterocytes-like cells (Caco-2 and goblets-like cells (HT-29). The results from SEM-EDX and FTIR confirmed that the particles derived from teabags were polylactic acid (PLA). Moreover, using the Nano Z-sizer we could detect a hydrodynamic size of approximately 120 nm. Finally, TEM and SEM images showed the spheric shape and confirmed the size from both PLA in suspension and in the teabag tissue. Following, we aimed to study the interaction between PLA and the cells in monocultures and in differentiated co-cultures (Caco-2/HT29). Using confocal microscopy, we confirmed that PLA-NPLs could internalize into all the cells after 24 h. Moreover, NPLs were seen reaching the cell nucleus compartment. Using flow cytometry, we could quantify that PLA-NPLs had a 100% of internalization when exposing HT-29, but only 60% of internalization when exposing Caco-2 cells, suggesting that NPLs internalization may vary depending on the cell type or in vitro model. However, no significant cytotoxic effects were observed when analyzing the intracellular ROS, and the barrier integrity by TEER and LY. Therefore, this study opens new insights regarding the bio-persistence effects of MNPLs, reinforces the urgent need for multidisciplinary studies that further investigate the MNPLs as emergent contaminants, and could serve as an example for regulatory agencies to pay close attention to the regulation of MNPLs production and management.

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Keywords:
Polylactic acid; nanoplastics; food contaminants; gastrointestinal tract; *in vitro.*

P63

Establishment of an in vivo micronucleus assay using flow cytometry

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The micronucleus test is an essential part in the characterization of the genotoxic potential of a test compound. This assay is used for the detection of chromosomal damage by evaluating the formed micronuclei. Micronuclei emerge from chromosome fragments or whole chromosomes, which have failed to be incorporated correctly into one of the daughter nuclei during cell division. As genotoxic substances are known to be potentially mutagenic, the micronucleus test is an inherent part in the safety evaluation of pharmaceuticals.

In this study an in vivo micronucleus test using flow cytometry on peripheral blood was established. Experiments were performed with the alkylating compounds cyclophosphamide (CP) and ethyl methanesulfonate (EMS). Measurements were restricted to the youngest reticulocytes, displaying the transferrin receptor (CD71). This ensured that only recent DNA lesions were observed. To avoid the splenic elimination of micronucleated reticulocytes (MN-RETs) reported in rats, CD-1 mice were used through the study. Peripheral blood from the vena facialis was collected 24 hours after treatment for two consecutive days. The frequency of micronucleated reticulocytes was determined by measuring 20 000 reticulocytes using flow cytometry.

In contrast to previously published data, both EMS concentrations with 200 and 225 mg/kg bodyweight displayed only moderate effects with an overall 1.7-fold increase in MN-RETs. CP at a dosage of 20 mg/kg bodyweight resulted in a 3.8-fold inductions of MN-RETs and may serve as a validated positive control in further studies. It could be demonstrated that flow cytometry is excellent for the evaluation of the micronucleus test, as it is a reliable, time-saving method for the measurement of micronuclei in the peripheral blood of mice.

Keywords:
Genetic Toxicology, Micronucleus Assay, Flow Cytometry.

P64

Deciphering the effects induced by nanoplastics in peripheral blood immune cells

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Humans can internalize nanoplastics (NPLs) through inhalation and ingestion due to the small size, allowing for their absorption, reaching the bloodstream, permitting their systemic biodistribution. Indeed, NPLs have been detected in human whole blood samples representative of the general population. Although several studies in invertebrates have linked NPLs exposure to disruption of the immune system, and some have assessed the in vivo immunotoxicity of NPLs in small mammals, information on the effects of NPLs on the human immune system is still lacking. In the present study, we exposed ex vivo human peripheral blood from healthy donors to several representative NPLs, not only to the most used (polystyrene. PS) but also to polyethylene terephthalate (PET) and to polylactic acid (PLA). To explore the influence of the physicochemical characteristics of NPLs on the analyzed effects, different sizes were chosen: 50 nm PS with different surface characteristics (pristine PS, carboxyl (-COOH), and amino (-NH2)), 150 nm PET, and 250 nm PLA. After 24 h of blood exposure to NPLs, white blood cells (WBC) were isolated and processed for flow cytometry analysis and single-cell RNA sequencing (scRNA seq). Our results show that NPLs internalize in human WBC, suggesting that the internalization dynamics of the NPLs depend on the WBC sub-population, and that it is modulated according to the type and functionalization of the particle.

The analysis of the scRNA seq data has permitted us to identify differentially expressed genes between each WBC population for the different NPL exposures. Remarkably, relevant proinflammatory markers are enhanced by NPLs exposure, suggesting the influence of NPLs in the production of an inflammatory environment in the body, thus altering human health and conferring an immune system more prone to be altered. Overall, these findings show us for the first time the NPLs effects on the human immune blood cells and emphasize that may be fundamental to control the NPLs exposure.

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Keywords:
Nanoplastics; exposure; scRNA seq; immune system; inflammation.

P65

Title Mutagenic evaluation of a rodenticide in the in vivo micronucleus assay

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Numerous investigations related to rodent control have been carried out in our country, developing technology for the production of new rodenticides under tropical conditions. Biorat is a biological rodenticide whose active principle is the bacterium Salmonella enteritidis var. Danysz. This product was produced by the LABIOFAM Company and due to its actions as a biological control it has a wide potential on rodents.

The development of these products necessarily has to carry in parallel an evaluation system that makes it possible to know with certainty the impact that such biotechnological products may cause on human and animal health and the environment. For this reason, it is essential to carry out a biological evaluation of these biopesticides, which includes studies on their possible toxic and genotoxic activity.

Among the genetic assays with mammals, the Mouse Bone Marrow Micronucleus Assay has been widely used as an indicator of in vivo genotoxicity, which is why our work has as its main objective to determine the genotoxic potential of Biorat using this assay.

The results obtained indicate that the evaluated dose of the product does not induce cytotoxic or genotoxic effects on polychromatic erythrocytes (PCE) of the mouse bone marrow; therefore this product does not cause chromosomal damage in vivo under our experimental conditions.

Keywords:
Micronucleus Assay, Genotoxicity, Mutagenesis.

P66

Effect of pH and Duration of Electrophoresis on Comet Tail

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The comet assay is widely used for measuring DNA damage and DNA repair. After embedding the cells in agarose, cells are lysed to yield supercoiled nucleoids. In case of DNA strand breaks, the supercoiling of DNA is relaxed and after electrophoresis, moved towards to anode and forms a comet tail. Together with increasing DNA strand breaks, we observe an increase in the percentage of DNA in the tail. However, a question has remained unanswered whether the comet tail contains DNA fragments or loops. We have now revisited this question.

To develop an understanding of what might be in the comet tail, we have conducted alkaline and neutral comet assay by using human lymphoblastoid cell line TK6. The TK6 cells were either treated with the alkylating agent methyl methanesulfonate (MMS, 100µM for 4h) to induce at this low concentration presumably single strand breaks leading to loop formation or with the unspecific protein kinase C inhibitor staurosporine (1 µM for 4 h) to induce apoptotic cells yielding DNA fragments and ghost cells. After treatment, we conducted alkaline as well as neutral comet assay with the same samples under different electrophoresis durations (0, 10, 20, 30, 40, and 60 min). We observed an increasing percentage of DNA in the tail as well as tail length over time in electrophoresis with both substance treatments. There was no saturation of this increase, which may be an indication of the presence of DNA fragments in the tail of comets after treatment with both substances. In addition, we have observed a faster migration of apoptotic small DNA fragments, which leads to a reduced number of visible comets and loss from the comet tail area in staurosporine-treated samples. There seem to be subtypes of cells after staurosporine treatment, possibly dependent on the cell cycle phase of treatment, with some cells dissolving or disappearing into very small non detectable DNA fragments and others harboring visible comets. In addition, we could show that the results of the neutral comet assay didn`t differ from the alkaline comet assay, which indicates that both endpoints are able to detect single and double-strand breaks.
Altogether, the tail of comets might contain both loops and fragments after treatment with the genotoxin methyl methanesulfonate, whereas staurosporine yields apoptotic cells with DNA fragments.

Keywords:
Comet assay, DNA fragments, DNA loops, alkaline comet assay and neutral comet assay.

P67

HPF1 stimulates PARP1 and PARP2 to autoPARylation and histone modification

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Poly-ADP-ribosylation (PARylation) is an essential post-translational modification of biomolecules. Poly(ADP-ribose)polymerase 1 and 2 (PARP1/2) are the main enzymes, that synthesize PAR in a nucleus. These enzymes and PARylation catalyzed by them are involved in many biological processes, including DNA damage response. The PARP1/2 proteins serve as sensors for detecting DNA lesions and the signal performed with these enzymes is necessary to repair DNA damages [1]. A new histone PARylation factor (HPF1) which modulates PARP1/2 activity was discovered recently. By completing the active site of PARP1/2, HPF1 switches the PARylation specificity to serine residues. Thereby, HPF1 functions in the complex with PARP1/2 involved in the PARylation of histones [2]. It was shown that HPF1 leads to the shortening of synthesized PAR, and enhances NAD+-hydrolysis catalyzed by PARP1. However, the general picture of HPF1 interaction with PARP only begins to clear up. In the presented study, we demonstrate for the first time that HPF1 can stimulate the autoPARylation of PARP1/2 and the heteroPARylation of histones in the context of nucleosomes. Interestingly, stimulation is promoted by the incomplete serine-specificity switch. We have shown that only a large excess of HPF1 over PARPs can switch the activities to NAD+ hydrolysis. We provide evidence that HPF1 can promote opposite effects on different stages of the PARP1/2-catalyzed reaction: it stimulates early stages and inhibits elongation by shielding of amino acid residues important for PAR chain elongation [3]. Moreover, we show that HPF1 more efficiently stimulates PARP2, compared with PARP1 and the effect of stimulation is dependent on the structure of DNA damage. The HPF1-dependent histone PARylation catalyzed by PARP2 is specifically stimulated by a 5'-dRP containing BER DNA intermediate [4]. We suggest a specific role of PARP2 in the ADP-ribosylation-dependent modulation of chromatin structure in the DNA-damage response. The work was supported by grants from RSF 21-64-00017

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Keywords:
PARP1, PARP2, HPF1, nucleosome, DNA repair.

P68

Long-term effects of PS and PET nanoplastics in lung cell lines

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The exponential production of plastics, along with an inefficient recycling system, have resulted in an alarming accumulation of plastic waste. Degradability of these polymers in the environment results in the so-called micro and nanoplastics (MNPLs). In humans, besides ingestion which has already been pointed as an important route of exposure to MNPLs, attention is now paid to inhalation, considered also as a major source of exposure. Although MNPLs have recently been identified in the lungs of living people, very few is known about their chronic effects on health. This study tries to identify the long-term adverse effects of two of the most common plastic wastes: polystyrene (PS) and polyethylene terephthalate (PET) nanoplastics, (NPLs). For that, we have used two cell lines representing the proximal and distal lung epithelia: Beas-2B (bronchial cell line) and A549 (alveolar cell line) exposed to long-term treatments (up to 15 weeks). By performing a battery of in vitro assays, testing the ability of PS and PET to reach cell cytoplasm, their genotoxic potential, and some cell transformation hallmarks (proliferation rate, anchorage independent growth, migration potential, invasion ability or tumorsphere generation), we have observed the high ability of PS and PET NPLs to penetrate the cell, although no genotoxic nor carcinogenic potential have been anticipated. Transcriptomic data for both cell lines exposed to PS and PET under 24 h exposure and chronic exposure (15 weeks) was analysed to distinguish altered genes and pathways. Briefly, from these results we can highlight that A549 cell line reveals an upregulation of immune response and TNFA pathways after chronic exposure to both PS and PET, and a downregulation of mitotic spindle pathway in all the conditions. In Beas-2B cells, although the list of deregulated genes is higher, there is no clear pattern regarding specific altered pathways.

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Keywords:
Nanoplastics, Chronic exposure, Cytotoxicity, Transcriptomics.

P69

Drosophila melanogaster as bioindicator of microbiome dysfunction

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The gastrointestinal (GI) microbiota is tightly linked to intestinal homeostasis and crucial for the overall host health. Several factors such as bird mode, genetics, diet, stress, and pollution have been reported to highly shape microbial communities. When the microbiota is impaired or altered, common inflammatory, metabolic disorders, as well as oncologic and neurologic diseases are more likely to be developed. In general, GI disorders in the human population have exponentially increased during the last decade and are linked to anthropogenic pollution and contamination. Therefore, there is an urgent need to develop new approaches methodologies to rapidly study the impact of emergent contaminants on the microbial GI. Because of their unique properties (e.g., time and cost-effective handling, large offspring, and genetic manipulation is fast and inexpensive) we propose the use of the fruit fly, Drosophila melanogaster, to study microbial dysbiosis. In this study, we aimed to develop and optimize an innovative protocol describing (1) the best bacterial isolation method from the host, (2) the finest bacterial DNA isolation kit, and (3) the optimal concentration of bacterial DNA needed for sequencing, as well as the impact of these factors to the microbial gamma and alpha diversity. This will be assessed by using a MinION sequencer (Oxford Nanopore Technologies). The results indicate that separating the gut from the larvae body ameliorates the bacterial DNA (~ 2.5 ng/µL) discrimination from the host DNA (~ 40 ng/µL). Using the larvae phase, instead of adult flies, makes possible the gut separation from the rest of the body. On the other hand, we have seen that a wide number of bacteria is lost when filtering the lysates with a 10 µm-mesh filter to isolate bacteria from undigested tissues. The kit that yielded more bacterial gDNA per sample was the QIAamp PowerFecal Pro DNA Kit. We finally found that in 50 larvae guts there were an average of 2 ng/µL of bacterial DNA. At least 40 ng are needed to proceed with the 16s rRNA gene amplification PCR > barcoding PCR > End-prep > and MINIon sequencing. Future experiments will conclude whether the above-mentioned conditions alter the alpha and beta diversity. Further experiments are planned to work with (i) 150 or 300 larvae guts and with (ii) adult flies to study the overall adult microbiome.

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Keywords:
Microbiota; gastrointestinal; Drosophila; experimental models; contaminants.

P70

Polystyrene nanoplastics: Surface- and size-dependent effects on human primary endothelial cells

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Although plastic wastes are considered long-lasting and stable in the environment, they undergo fragmentation to micro- and nanometer-level particles, named micro- and nanoplastics (MNPLs), under the influence of different physical and chemical factors. During this weathering process, plastics suffer from changes in their physicochemical surface properties that can influence their toxicological profile. Nowadays, there is increasing evidence suggesting that environmental MNPLs can reach the human body through different pathways: ingestion, inhalation, and dermal contact. Indeed, MNPLs have been detected in whole blood samples representative of the general population. However, the potential effects of MNPLs on the health of exposed individuals are still unknown and require further research. In the present study, polystyrene nanoplastics (PSNPLs) and human umbilical vein endothelial cells (HUVECs) were used to better understand what are the toxicokinetic and toxicodynamic interactions of MNPLs with the vascular system. Representative PSNPLs of different sizes (PS-COOH 30 nm, 50 nm, and 100 nm) and surface characteristics (pristine PS, carboxyl (-COOH) and amino (-NH2)) were included in the study. Our results suggest that although all PSNPLs are internalized by HUVECs, the internalization dynamic is modulated based on the functionalization and the size of the particle. Interestingly, our flow cytometry data shows that both PS-COOH 50 nm and PS-COOH 100 nm can modify the morphology of the cell and increase its inner complexity/granularity. When analyzing possible toxic effects by treating the cells with a concentration of 100 µg/mL we observe that only PS-NH2 50 nm can reduce cell viability (- 40% vs control; 12 h treatment). Finally, our study of intracellular ROS generation when treating the cells with 100 µg/mL of the different PSNPLs for 24 h shows an increase in ROS production with carboxylated PS at different time points. Overall, our results indicate a surface- and size- dependent effect of PSNPLs on HUVEC cells.

Funding:
This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal), the Spanish Ministry of Science and Innovation (PID2020-116789, RB-C43), the Generalitat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to AH.

Keywords:
Nanotoxicology, nanoplastics, primary cells, HUVEC.

P71

Proficiency Establishment of the OECD 488 Big Blue® Transgenic Rodent Somatic and Germ Cell Mutation Assay

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Assessing the mutagenic potential and risk to human health and the environment of new drugs and chemicals is a global regulatory requirement. OECD 488 transgenic rodent (TGR) mutation assays are key in determining the in vivo mutagenicity risk of substances that have positive in vitro mutagenicity data. The Big Blue® rat is a suitable TGR model that allows investigation of in vivo mutation at the cII locus within the genome integrated lambda bacteriophage shuttle vector.

Gentronix conducted an initial proficiency assessment for the detection of cII locus mutation frequencies in duodenum, liver, and male seminiferous tubule derived germ cells from Big Blue® rats, and compared data with the historical laboratory, with extension of the exercise to then include glandular stomach, bone marrow and kidney. The exercise used frozen tissues banked during previously conducted Big Blue® rat studies, where a 28 day exposure period was followed by either a 3 or 28 day (somatic tissues) or 28 day (germ cells) fixation period for untreated/vehicle and N-ethyl-N-nitrosourea (ENU; positive control) treated animals. Genomic DNA was extracted from tissues using Agilent DNA RecoverEase methods, and packaged using Agilent Transpack reagents to create infectious phage particles that were then expressed using the G1250 E. coli strain plated on agar. Plates were incubated at 2 temperatures: 37°C (both wildtype and mutated phage enter the lytic cycle [packaging efficiency]) or 24°C (only cII mutated phage enter the lytic cycle), and the resulting phage plaques scored. Packaging e-ciency was demonstrated across all tissues with the mean number of phage screened from each DNA sample >200,000 in both untreated/vehicle and ENU treated animals, in accordance with OECD 488 (2022). ENU treatment significantly increased mutant frequencies over controls ($p<0.001$) for all tissues tested (mean mutation frequency fold increases of: 16, glandular stomach; 20, duodenum; 5, liver; 16, bone marrow; 8 kidney; 7, male germ cells). Absolute mutant frequencies for all tissues in the untreated/vehicle control groups were consistent with the 95% control limits of previously generated historical laboratory data. These data illustrate that Gentronix is proficient in the methods of transgene recovery from genomic DNA and in reproducing expected mutant frequencies for positive and negative controls in the Big Blue® TGR assay across a range of somatic tissues and in germ cells.

Keywords:
Transgenic rodent; mutagenicity; OECD 488.

P72

Toxicological assessment of ZnO nanoforms to substantiate grouping

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Various zinc oxide nanoforms were grouped to fulfil information requirements according to the European Chemicals Regulation, REACh. The grouping was to be substantiated by new experimental data on selected nanoforms within this group [1]. The toxicological endpoint was a comet assay using the inhalation route of exposure.

28 Nanoforms of ZnO were characterized: (i) size distribution and aspect ratio (by TEM, according to the NanoDefine Method), (ii) dustiness and aerodynamic diameter (by the small rotating drum method, EN 17199:4), (iii) dissolution rate in lysosomal simulant (by continuous flow system; according to the 2, 3]). Two nanoforms with identical size and shape, but different surface coating (uncoated and hydrophobic) were selected for inhalation testing. Soluble zinc sulfate monohydrate and micron-sized ZnO particles were tested as reference substances at equimolar Zn concentrations. Rats were exposed to up to 8 mg/m³ ZnO for 14 days according to OECD test guideline (TG) no. 489. The target tissues analyzed in the comet assay were the nasal epithelium and the lung (site of contact) as well as the liver and the bone marrow.

Zinc oxide nanoparticles caused local toxicity at the respiratory tract. In animals exposed to 8 mg/m³ coated nano ZnO increased neutrophil counts in blood was observed. Other systemic effects were not observed in any of the tested substances. The COMET assay did not show genotoxic effects in any of the examined tissues. Nor were there any changes after exposure of high concentration of ZnO or ZnSO4 monohydrate. Both ZnO nanoforms, micron-sized ZnO particles and Zn-ions in solution did not cause genotoxicity. The nanoforms of ZnO were comparable and maybe grouped together. No pronounced difference was found between nanoforms and pigment grade ZnO. This grouping approach helps to minimize the number of animal studies to be performed.

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Keywords:
zinc oxide; nanoforms; COMET assay; grouping.

P73

Biomonitoring and predictive modelling of genomic instability in childhood obesity

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Epidemiological evidence indicates obesity in childhood and adolescence to be an independent risk factor for cancer and premature mortality in adulthood. Pathological implications from excess adiposity may begin early in life. Obesity in childhood and adolescence is concurrent with a state of chronic, low-grade inflammation, a well-known aetiological factor for DNA damage. In addition, obesity in childhood and adolescence has been associated with micro-nutritional deficiencies. Vitamin D has attracted attention for its anti-inflammatory properties and role in genomic integrity and stability.

We have conducted research aimed at devising a novel approach for predicting genomic instability in childhood obesity via the combined, non-invasive assessment of adiposity, DNA damage, systemic inflammation, and vitamin D status. We carried out a cross-sectional study with participants, aged 10–18, recruited from schools and paediatric obesity clinics in London. Our results support the hypothesis that childhood obesity is associated with increased genomic instability. Importantly, we have found that obesity, vitamin D and oxidative DNA damage can together predict genomic instability.

Non-invasive biomonitoring and predictive modelling of genomic instability in young patients with obesity may contribute to the prioritisation and severity of clinical intervention measures.

Keywords:
Childhood obesity; genomic instability; Vitamin D; DNA damage; inflammation.

P74

Comet Assay validation of male germ cells isolated from seminiferous tubules

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The *in vivo* alkaline comet assay is a well-established method for detection of DNA damage in somatic cells, either as a follow-up testing of positive or equivocal in vitro test results or to evaluate local genotoxicity. Although it is widely used on somatic cells, there are only few studies available demonstrating its capability to detect mutagenicity in germ cells, which is more often required for an optimal risk assessment. Moreover, other alternative methods, assessing the induction of germ cell mutations, require very large numbers of animals and often lack practicality and efficiency. Therefore, an internal validation study was performed to verify the performance of the alkaline comet assay with male germ cells isolated from the seminiferous tubules.

For calculation of inter-analyst variability, three analysts independently prepared single cell suspensions of germ cells isolated from the seminiferous tubules, which were collected from male gonads. As freezing of the gonads led to cell damage and resulted in non-evaluable results, the cell suspensions were directly used to prepare comet slides. As the comet slides can be stored for a period of up to 3 months at room temperature under dry conditions and protected from light, they can be analyzed later without initiating a new animal study, depending on the results in somatic cells.

Within the validation study, experimental competency was successfully demonstrated, proving the ability to obtain single cell suspensions of sufficient quality for the performance of the alkaline comet assay. As required by the OECD TG 489, a positive response with three increasing concentrations of ethyl methane sulfonate (EMS) was noted, indicating that the positive control had reached the gonads and the observed DNA damage is concentration-related. Moreover, repeatability and inter-analyst variability were calculated and showed small standard deviations both within the dose group and between analysts.

In summary, the integration of analysis of germ cells isolated from the seminiferous tubules into the standard OECD TG 489 test protocol for somatic mutation offers an effective option for germ cell mutation testing and optimizes and reduces the use of animals while requiring less time and resources.

Keywords:
comet assay, male germ cells, Wistar rat, seminiferous tubules, OECD 489.

p75

Development of an AOP-based IATA for genotoxicity

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Genotoxicity assessment of chemicals currently involves a tiered approach starting with an in vitro testing battery covering bacterial and/or mammalian cell gene mutations, and structural and numerical chromosome aberrations followed by in vivo testing in case of a positive result. However, this strategy has several limitations including the high number of misleading positive results triggering unnecessary animal testing, the limited mechanistic information provided, and the insufficient integration of new approach methodologies (NAMs).

One strategy to include NAMs in chemical risk assessment involves the development of 'integrated approaches to testing and assessment (IATAs)'. IATAs integrate existing information and newly generated data from NAMs or traditional toxicity tests to address a specific regulatory question. Genotoxicity is a particularly interesting area for developing IATAs as many innovative non-animal methods have become available over the last years. The selection of NAMs to be included in an IATA should be structured and science-driven, a process in which adverse outcome pathways (AOPs) could play an important role.

This project, performed within the framework of the Horizon Europe Partnership for Assessment of Risks from Chemicals (PARC) and involving many different partners, aims to deliver an AOP-based IATA for genotoxicity to support the transition to animal-free genotoxicity assessment. As a first step, inventories of AOPs and NAMs linked to genotoxicity have been compiled. Nineteen AOPs related to DNA damage were found in the AOP-wiki. These AOPs are in different stages of development and ten of them are already in the OECD work plan. Similarly to Huliganga et al. [1], an AOP network has been drafted based on the four most advanced AOPs. This network will be used as a starting point to design a more extended AOP network with "increase in gene mutations" and "increase in chromosomal aberrations' as adverse outcomes. In addition, in the AOP network, each NAM of the inventory was linked to the key event it can measure.

Based on the AOP network, a first version of the IATA will be drafted. Where needed, knowledge gaps within the network will be filled, including quantification of key event relationships. Finally, the newly developed IATA's applicability will be evaluated in case studies.

Keywords:
Genotoxicity, Adverse Outcome Pathway (AOP), Integrated Approaches to Testing and Assessment, Chemical risk assessment, New Approach Methodologies (NAMs).

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P76

Lung barrier establishment using Calu-3 cell line as an *in vitro* model to study the pulmonary effects of micro and nanoplastics

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Micro and nanoplastics (MNPLs) are contaminants of emerging concern (CEC) generated from the degradation of plastic waste and with the potential to affect the human health. Ingestion of this particles has been identified as the first route of human exposure. However, recent studies have pointed out the presence of MNPLs in the air also as an important source of human exposure by inhalation. Therefore, huge numbers of these contaminants of different sizes, chemical compositions and shapes have been identified in the air of indoor and outdoor scenarios. In addition, as a direct prove of exposure, in a recent study MNPLs were found in the different parts of the lung of the majority of tested volunteers. Despite the potential effects that these particles could cause after a prolonged exposure in the respiratory system and in the whole organism, the studies deciphering the possible effects are scarce. In this study, we propose an in vitro lung barrier using Calu-3 cell line as a pulmonary model to study the effects of polystyrene (PS), polyethylene terephthalate (PET), and polylactic acid (PLA) nanoparticles after long and repeated exposures. The formation of the barrier after 16 days of culture gives a more realistic model to study the toxicological effects of MNPLs by representing a pseudostratified epithelium, secreting mucus and generating microvilli. Preliminary results by using this model, showed no effects on the stability of the barrier after 1 week of repeated exposure. However, an increase in the permeability and internalization of particles were observed from the first 24 hours of treatment. Further experiments will be conducted in order to confirm the preliminary results and to assess other parameters such as the passage of the particles through the barrier.

Keywords:
Lung barrier; in vitro model, nanoplastic toxicity.

P77

Molecular dosimetry of alkylating agents: Quantification of O6-methylguanine and correlation with DNA double-strand breaks, apoptosis and senescence

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Alkylating agents are potent environmental mutagens that are also used in cancer chemotherapy. One of the methylating drugs applied 1st line in brain cancer therapy is temozolomide (TMZ). Similar to other SN1 alkylating agents it induces N- and O-base adducts, including O6-methylguanine (O6MeG). Although being a minor lesion, O6MeG is responsible for almost all genotoxic, cytotoxic and cytostatic effects caused by the so-called O6-methylating agents. How many O6MeG adducts are required to induce specific cell responses was not precisely known. Therefore, we determined the dose-dependent formation of O6MeG in glioblastoma cells by mass spectroscopy and set it in relation to DSBs, p53Ser15 phosphorylation, apoptosis/necrosis and cellular senescence. Our results indicate a linear increase of O6MeG, along with DSBs and p53Ser15 phosphorylation. Apoptosis/necrosis and senescence also increased linearly, with senescence being the main response induced. A possible threshold for the induction of apoptosis in A172 was indicated by the Hockey-stick model at a concentration of 2.5 µM TMZ, while no threshold was detected in LN229 cells. In all cell lines no threshold was detected for the endpoint senescence. In A172 cells, treatment with 20 µM TMZ induced 14.000 O6MeG adducts, which gave rise to 32 DSBs (measured by yH2AX and 53BP1 foci), 12 % cell death and 35 % senescence. In LN229 cells, 20 µM of TMZ induced 20.600 O6MeG adducts, 66 DSBs, 24 % cell death and 52 % senescence.

Since this dose of TMZ approximates the serum concentration that can be achieved in cancer therapy, the data can be translated to the therapeutic situation, indicating that the intra-tumoral concentrations of TMZ trigger a significant amount of cytotoxic and cytostatic responses such as apoptosis and senescence.

P78

Is the genetic toxicology assessment of oligonucleotide therapies warranted?

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The European Federation of Pharmaceutical Industries and Associations (EFPIA)–Preclinical Development Expert Group (PDEG) established an Oligonucleotide Working Group (OWG) to review industry experience of developing oligonucleotide therapeutics (ONTs). As synthetic drugs, the nonclinical safety evaluation follows ICH guidelines for small molecules which requires the full battery of genotoxicity tests. However, ICHS2(R1) guidance does not specifically refer to ONTs, and these compounds are not expected to interact chemically (e.g., by covalent binding) with DNA or other cellular targets, such as mitotic microtubules, that could result in genotoxicity.

The current literature was reviewed for evidence of various hypothetical mechanisms considered potential hazards for ONT genotoxicity, including effects on deoxyribonucleotide triphosphate pools, integration into DNA, triplex formation, effects on DNA repair and cell cycle regulation. The EFPIA-OWG also identified additional factors for consideration, i.e., 1) ONT class/modality; 2) conjugated targeting ligand/delivery platforms; 3) ONT sequence; and 4) ONT specific chemical modifications. To address these and other factors, an industry survey was undertaken (covering biotech and pharma across US, Europe, and Japan) regarding ONT testing under ICHS2(R1). Preliminary analysis indicates genotoxic effects are not observed with a variety of different ONT chemistries and modalities, confirming previous observations (PMID 26978711, 30139307, 28295562) and expanding on the knowledgebase of well precedented modifications. Initial survey analysis will be presented and placed into context with ONT-specific considerations and testing precedence. The EFPIA-OWG will continue to investigate the survey results and recent literature to build a weight of evidence approach for recommendations regarding future genotoxicity testing requirements.

Keywords:
Oligonucleotide therapeutics, genetic toxicology, EFPIA, PDEG .

P79
CARCINOGENIC TRAITS OF ALUMINIUM

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Aluminium is the most abundant metal and, after oxygen and silicon, the third most abundant of all elements in the Earth's crust. Highly reactive, aluminium is rarely found in nature in its free state. This could be one reason why this element is not part of any known physiological process and therefore not essential for life.

Because of its abundance, chemical versatility, and low cost, aluminium is extracted from its natural sediments and used in numerous industrial products and procedures, including cosmetics, food additives and drinking water purification procedures. As a result of chronic exposure, aluminium accumulates in certain organs such as the liver, bone, or mammary gland. Aluminium is recognized as a neurotoxin when it reaches very high concentrations in the body (e.g., in chronic renal insufficiency). Apart from this specific situation, it is considered essentially harmless. With respect to cancer, epidemiological studies investigating potential associations between aluminium exposure and cancer incidence are scarce and provided conflicting results.

Published work from our research group shows that at concentrations in the range of those measured in human organs, aluminium, in the form of AlCl3, enters mammalian cells within one hour and induces chromosome abnormalities – mainly DNA double strand breaks – upon 24 hours exposure in a dose-dependent manner. Part of these data were obtained with V79 cells, frequently used in human regulatory toxicology for the assessment of chemical carcinogens. Prolonged exposure to the same concentrations of aluminium consistently makes normal mammary epithelial cell models capable of forming aggressive tumors in immunodeficient and immunocompetent mouse models. Our results unveil a carcinogenic potential of aluminium and warrant a reappraisal of its postulated innocuity.

Keywords:
Aluminium; breast cancer; chromosomal instability; chemical carcinogenesis.

P80

Transcriptome profiling of human hepatocyte cell line HHL-16 in response to aflatoxin B1.

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Dietary exposure to aflatoxin (AFB1) can cause acute aflatoxicosis and liver cancer, and is associated with immune suppression and growth impairment. A non-neoplastic human hepatocyte cell line 16 (HHL-16) was used to understand the effects of AFB1 on the transcriptome and identify molecular pathways underlying toxicity and health effects.

HHL-16 cells were treated with different concentrations of AFB1 and the MTT assay was used to assess the cytotoxicity of AFB1. RNA samples were extracted for RNA-Sequencing and bioinformatic analysis (RNA-Seq) (Novogene Co. Ltd (Cambridge, UK)). RT-qPCR was used to validate gene expression of several differentially expressed genes.

A dose-dependent pattern of AFB1 toxicity was observed. RNA sequencing revealed 280 significantly up-regulated and 296 significantly down-regulated genes in HHL-16 cells after 20 µg/ml AFB1 treatment for 24 hours. In GO-term analysis of differentially expressed genes (DEGs), three main categories were identified: biological process, cellular component, and molecular function. KEGG pathway enrichment analysis indicated that DEGs were significantly enriched in the following pathways: cytokine-cytokine receptor interaction, NF-kappa B signalling pathway, TNF signalling pathway, IL-17 signalling pathway, Amoebiasis, MAPK signalling pathway, and lipid and atherosclerosis. In the most significant DEGs associated KEGG pathway, cytokine-cytokine receptor interaction pathway, 15 genes (IL24, IL11, IL6, LIF, CXCL8, BMP2, CXCL2, TNFSF15, NGFR, CXCL3, IL1RN, IL10RA, IL1RL1, TNFRSF9, CSF2) were up-regulated, and 4 genes (INHBB, IL17RE, TNFSF18, TNFSF4) were downregulated. Further, in the validation of the DEGs by RT-qPCR, dose-dependent increases of IL6, CCL20 and BMP2, and dose-dependent decrease of NDP gene expression were found in HHL-16 cells after 5, 10, and 20 µg/ml AFB1 treatments for 24 hours.

Conclusions: AFB1 modulates the expression of genes related to the pathways that play important roles in inflammatory response, growth, and cancers.

P81

Hazard assessment of polystyrene nanoplastics in primary human nasal epithelial cells, focusing on the autophagic effects

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The human health risks posed by micro/nanoplastics (MNPLs), as emerging pollutants of environmental/health concern, need to be urgently addressed as part of a needed hazard assessment. The routes of MNPL exposure in humans could mainly come from oral, inhalation, or dermal means. Among them, inhalation exposure to MNPLs is the least studied area, even though their widespread presence in the air is dramatically increasing. In this context, this study focused on the potential hazard of polystyrene nanoplastics (PSNPLs with sizes 50 and 500 nm) in human primary nasal epithelial cells (HNEpCs), which constitute the first line of cells acting as a physical and immune barrier in the respiratory system. Primarily, cellular internalization was evaluated by utilizing laboratory-labeled fluorescence PSNPLs with iDye, a commercial, textile pink-colored dye, using confocal microscopy, and found PSNPLs to be significantly internalized by HNEpCs. After, various cellular effects, such as the induction of intracellular reactive oxygen species (iROS), the loss of mitochondrial membrane potential (MMP), and the modulation of the autophagy pathway in the form of the accumulation of autophagosomes (LC3-II) and p62 markers (a ubiquitin involved in the clearance of cell debris), were evaluated after cell exposure. The data demonstrated significant increases in iROS, a decrease in MMP, as well as a greater accumulation of LC3-II and p62 in the presence of PSNPLs. Notably, the autophagic effects did indicate the implications of PSNPLs in defective or insufficient autophagy. This is the first study showing the autophagy pathway as a possible target for PSNPL-induced adverse effects in HNEpCs. When taken together, this study proved the cellular effects of PSNPLs in HNEpCs and adds value to the existing studies as a part of the respiratory risk assessment of MNPLs.

Funding:
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Keywords:
Polystyrene nanoplastics; HNEpCs; oxidative stress; mitochondrial membrane potential; autophagy.

P82

Effect of trypsin-EDTA on expression of DNA damage repair enzyme OGG1 in human limbal- and conjunctival- epithelial cells.

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Enzymatic cell dissociation may provide cells for experimental cultures and tissue engineering. We hereby examine corneo-scleral ring-associated limbal- and conjunctival- epithelial cell morphology, as well as expression of DNA base-excision repair (BER) enzyme OGG1 during incubation in trypsin-EDTA.

All experiments were conducted in accordance with the Declaration of Helsinki and Local Committees for Medical Research Ethics. Post-mortem samples of organ cultured human corneo-limbal rings were incubated in 0.05% trypsin-EDTA for 0 (control) or 3 hours, fixed, embedded, sectioned and stained with H&E and processed for immunohistochemistry (IHC) for protein expression of OGG1.

In sections stained with H&E, limbal and conjunctival epithelium in control samples showed regular bi- or multilayered morphology. Incubation in trypsin-EDTA for 3 hours induced detachment of the superficial, as well as the basal limbal and conjunctival cells. Complete detachment of epithelial cells from the basement membrane was also observed. A low-level expression of OGG1 was detected by IHC in control samples, and any noticeable alteration in expression could not be observed during incubation in trypsin-EDTA.

Damage to DNA may, when unrepaired, interfere with cell function, differentiation, proliferation and viability. Dissociation of cells by trypsin-EDTA may induce molecular stress and damage. In vivo, oxidized DNA bases are normally repaired by BER enzymes including OGG1. Noticeable changes in the protein levels of this particular repair enzyme could not be observed during incubation in trypsin-EDTA in the present study.

Keywords:
DNA damage, repair enzyme, trypsin-EDTA, limbal epithelial cells, conjunctival epithelial cells.

P83

A modified alkaline comet assay to measure base excision repair in mitochondria

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Base excision repair (BER) is the major repair pathway for oxidative DNA damage removal, taking place in nuclei and mitochondria. Both in nuclear and mitochondrial DNA (mtDNA), the BER process is initiated by DNA glycosylases, such as 8-oxoguanine DNA glycosylase 1 (OGG1), that recognize and incise N-glycosidic bonds between the damaged base and deoxyribose. MtDNA is exposed to reactive oxygen species more than nuclear DNA due to its proximity to the electron transport chain. Functional BER, keeping mtDNA intact, is necessary for the proper cell energetic metabolism and for preventing mtDNA mutations leading to various aging-related diseases. The comet-based in vitro DNA repair assay is generally used to monitor BER activity in whole cell lysates. However, comet assay protocols for assessing BER activity only in mitochondria were until now missing. Our modified comet-based BER assay does not use a whole cell lysate as in the conventional protocol but a mitochondrial protein extract enabling assessment of the BER activity exclusively in mitochondria.

BER activity was assessed using crude mitochondria pellets isolated, using Percoll density gradient ultracentrifugation, from homogenized liver tissues of Zucker fatty and spontaneously hypertensive (ZSF1) rats; including obese and lean hypertensive rats investigated at ages 8-9, 22-23, and 34-35 weeks old (n=8-10/group). BER activity was compared to healthy control Wistar rats (n=8); 22-23 weeks old. The purity of isolated mitochondria was checked and confirmed on a Western blot. Pilot data showed that BER activity in mitochondria from obese (n=3) is about 1.3-fold lower than lean (n=4) ZSF1 rats (P=0.03; BER activity = 25.2% ± 4.8 versus 34.4% ± 2.9), while average mitochondrial BER activity of Wistar control rats was similar to the lean ZSF1 rats (33.0% ± 8.0). Analysis of all other comet assay images is ongoing and a more complete data set will be presented at the EEMGS meeting.

Currently, we are verifying our approach on mitochondrial proteins and whole cell lysates from wild-type and Ogg1-deficient (Ogg1-/-) mouse embryonic fibroblasts (MEFs). The study is funded by the Programme EXCELES (LX22NPO5102).

Keywords:
Base excision repair; alkaline comet assay; mitochondria; oxidative mtDNA damage.

HESI Genetic Toxicology Technical Committee

HESI WORKSHOP

Quantitative Interpretation of Genetic Toxicity Dose-response Data for Risk Assessment and Regulatory Decision-making – State of the Science, Applications, and Persistent Challenges

Monday 15 May 2023
09:00 – 15:00

Objectives / Goals
Genetic toxicology is moving from dichotomous hazard identification to quantitative dose-response analyses for potency ranking, risk assessment, and regulatory decision-making. Advancements in quantitative methods is driven by several factors including the availability of tools that facilitate dose-response analyses (e.g., Benchmark Dose software), as well as advances in the application of risk assessment concepts to interpretation of genetic toxicity test results. The workshop will provide an overview of recent developments in quantitative interpretation of genetic toxicity dose-response data; first by outlining basic concepts and best practices for PoD (point of departure) determination, then by outlining approaches to extrapolate below the PoD for risk assessment and regulatory decision-making. The latter half of the workshop will provide examples of applications related to impurities in pharmaceutical products. More specifically, examples that demonstrate how an approach based on quantitative dose-response analyses can be used to determine human exposure limits to potent genotoxicants.

The morning session will provide recommendations regarding suitable benchmark response (BMR)/critical effect size (CES) values for genetic toxicity endpoints; additionally, for the uncertainty factors (UFs) required to extrapolate below the BMD. The latter are required for determination of human exposure limits, sometimes referred to as HBGVs (Health-based Guidance Values), below which the likelihood of adverse health effect can be deemed negligible. Presentations will outline recommendations for UFs to account for interspecies variability, variability across individuals (i.e., intraspecies), and the effect of treatment duration. Presentations will also address the critical need to develop and deploy methods for quantitative interpretation of in vitro dose-response data generated using new approach methodologies (NAMs). Ongoing efforts have demonstrated that analyses of NAMs dose-response data, coupled with in vitro to in vivo extrapolation, can be used for potency ranking, chemical screening and prioritization, and risk assessment.

The afternoon session will focus on the application of quantitative methods to genotoxic impurities that cause deleterious changes in genetic material via a variety of mechanisms. These genotoxic impurities are emerging as a common problem in drug development, as well as in certain marketed pharmaceuticals. Although the proactive reduction of these impurities through advanced chemistry and suitable safety assessment is excellent, there are emerging cases where a safety assessment based on quantitative dose-response analyses is needed for regulatory decision making. Speakers will provide an overview of the problem of genotoxic impurities, with a focus on the results of quantitative dose-response assessments based on analyses of genetic toxicity and cancer bioassay data. The application of duplex sequencing, in combination with standard genotoxicity testing strategies, for determination of PoDs and mechanism, will also be discussed. The workshop will close with a critical discussion of regulatory considerations related to quantitative interpretation of genetic toxicity dose-response data.

ID HESI.01

Quantitative Interpretation of In Vivo Mutagenicity Dose Response Data for Risk Assessment and Regulatory Decision-Making

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Interpretations of in vivo genotoxicity test results have traditionally involved dichotomous hazard identification. However, recent works have established a paradigm involving quantitative dose response analyses for determination of PoD (point of departure) values, followed by extrapolation below the PoD for chemical prioritization, risk assessment and regulatory decision-making. Standardized methods to analyze dose-response data have been established; the most robust approach determines the BMD (Benchmark Dose). The CES (critical effect size) is a key parameter for BMD determination; for in vivo genotoxicity endpoints, the emerging consensus value is 50%. The use of BMDs to determine health-based guidance values (HBGVs) commonly requires the application of assessment factors (AFs) to account for interspecies differences, variability in individual sensitivity, less-than-chronic treatment, and possibly effect severity. Interspecies adjustment commonly uses animal-to-human body-size scaling. AFs to adjust for variability in human sensitivity are the subject of considerable controversy. Analyses of published genotoxicity dose-response data scrutinising the effects of compensatory pathway deficiency indicate that a default AF of 10 for sensitivity differences is likely appropriate. Published dose-response data can also be used to evaluate the utility of a default AF to adjust for less-than-chronic treatment durations. An initial comparison of chronic and acute genotoxicity datasets suggests that a default AF of 10 may not be sufficient. The need of an additional AF for effect severity is also the subject of continued debate. Although the aforementioned AFs are commonly multiplied to provide a composite value, the approach can result in values that are unnecessarily conservative, particularly for substances such as aneugens. An alternative approach involves the use of the MOE (Margin of Exposure) concept that simply examines the ratio of the PoD to the estimated level of human exposure. The minimum acceptable MOE is often set at 10,000, with values below 10,000 indicating the need for intervention. Another alternative involves the use of informatic tools such as APROBA to conduct approximate probabilistic analyses, i.e., analyses that consider inherent uncertainties within the AF values used to determine HBGVs. The approach permits critical examinations of the uncertainties associated with HBGVs such as the RfD (Reference Dose).

ID HESI.02

The interpretation of in vitro dose-response data for risk assessment and regulatory decision-making

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Chemical risk assessments are routinely performed using data derived from rodent toxicity tests; however, there is increasing awareness that non-animal alternatives are more ethical and potentially more relevant for evaluating human risk. Indeed, global efforts are establishing new guidelines to reduce or replace the use of animals in toxicity testing. Given these international pressures, there is an urgent need to build scientific confidence in the effective use of non-animal alternatives for quantitative risk assessments. An Expert Working Group (EWG) of the 8th International Workshop on Genotoxicity Testing (IWGT) was convened to initiate discussions towards the standardization of testing strategies and data interpretation for quantitative in vitro genotoxicity concentration-response data for risk assessment. The EWG first reviewed the in vitro mammalian cell assays used currently for genotoxicity assessment. The variability and maximal response of in vitro tests were examined to estimate biologically relevant critical effect sizes for use in point-of-departure (POD) determination. Next, the EWG reviewed the results of computational models employed to determine human-relevant PODs from in vitro concentration-response data. Lastly, the EWG evaluated risk assessment applications for which in vitro data are ready for routine use, and applications where further validation efforts are required. The EWG concluded that in vitro genotoxicity concentration-response data can be interpreted in a risk assessment context; however, additional research will be required to address remaining uncertainties and limitations before broadly applying an in-vitro-only strategy in regulatory decision making.

Keywords:
new approach methodologies, risk assessment, genotoxicity.

ID HESI.03

Nitrosamine impurity issues and potential resolutions

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Johnson et al., (2021) was a publication from the Health and Environmental Sciences Institute Genetic Toxicology Technical Committee (HESI GTTC), where in vivo mutagenicity dose response data was compared to in vivo cancer bioassay dose response data to calculate benchmark dose confidence intervals and permitted daily exposures (PDE). Certain benefits of the approach were presented, along with some aspects where additional data and refinement were considered on the use of the in vivo mutation data that was available at the time. These include the critical effect size, the uncertainty factors used, as well as justification for using the PDE approach. Nitrosamines have become an important class of impurities within pharmaceuticals, and there are continuing efforts and advancements to ensure patient safety. It is also very important that there is not unjustified withdrawal of marketing authorisation, and precision in the risk assessment approach is paramount to this. During the presentation and discussion, we will focus on the current projects on nitrosamine risk assessment and best practise.

Johnson, G.E., Dobo, K., Gollapudi, B., Harvey, J., Kenny, J., Kenyon, M., Lynch, A., Minocherhomji, S., Nicolette, J., Thybaud, V., Wheeldon, R., and A. Zeller 2021. Permitted daily exposure limits for noteworthy N-nitrosamines. Environmental and Molecular Mutagenesis, 62(5), pp.293-305.

ID HESI.04

In vivo genetic toxicity assessments for nitrosamines

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Some N-nitrosamines may be considered potent carcinogens in animals because metabolically activated N-nitrosamines may form stable DNA adducts that lead to mutations and initiation of cancer in animals. However, approximately 18% N-nitrosamines that have been tested in previous animal carcinogenicity studies show no indication of carcinogenic potential. Furthermore, the carcinogenic potencies for N-nitrosamines span about 4 orders of magnitude with TD50s overlapping non-nitrosamines that are not in the cohort of concern. The lifetime cancer bioassays in rodents are time- intensive and associated with high experimental costs. Assessment of in vivo genotoxicity in rodents to determine a robust point of departure is a practical surrogate for assessment of nitrosamines. Here we use NDEA as a case study to compare the sensitivities of three in vivo methodologies including the transgenic rodent assay (TGR), Duplex Sequencing (DS) and Comet assay in both Big Blue mice and rats. NDEA was administered to Big Blue® or wild type rodents at a wide range of doses to enable a robust dose-response analysis. Both comet and TGR assays detected significant increases in the genotoxicity of NDEA at doses of 1 and 3 mg/kg/day in the liver of rodents and the BMD ranges calculated from both assays are largely overlapping. The DS appears to be slightly more sensitive than TGR assay in detecting a statistically increase in MF in rat liver exposed to NDEA at dose of 0.1 mg/kg/day. However, none of the three in vivo assays detected a genotoxic effect caused by exposures to NDEA equal to or lower than 0.01 mg/kg/day, suggesting a no observed genotoxic effect level (NOGEL) could be observed for NDEA. Overall, this work shows that the results of both Comet and DS assays have a good agreement with the gold standard TGR assay in dose-response assessment of a well-studied N-nitrosamine and could be an excellent alternative for the assessment of nitrosamines *in vivo.*

ID HESI.05

Defining a NOGEL for mutation induction in Muta™Mouse following exposure to N-Nitrosodimethylamine (NDMA)

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The N-nitrosamine, NDMA, is an environmental mutagen and has been identified as a contamination impurity in some commonly used drugs, resulting in several product recalls. NDMA was evaluated in an OECD compliant Muta™Mouse assay (28-day oral dosing) across 7 doses (0.02-4 mg/kg/day) using an integrated design that assessed mutation at the transgenic lacZ locus in various tissues and the endogenous Pig-a gene, along with micronucleus frequencies in peripheral blood. Liver pathology was determined together with NDMA disposition. Acute treatments were included to investigate the accumulation and/or additivity of individual dose effects on mutation induction. Liver was included since it is the most sensitive organ for tumour and mutation induction and bone marrow for reasons of comparison to the micronucleus endpoint (used to assess clastogenic potential). NDMA was negative for mutation induction in bone marrow (lacZ) and peripheral blood (Pig-a mutation or micronucleus induction) when tested up to 4 mg/kg/day. There were dose-dependent increases in lacZ mean mutation frequency in liver, lung and kidney following 28-day repeat dosing or in liver after a single dose (10 mg/kg). The No Observed Genotoxic Effect Level (NOGEL) was determined for these tissues and the dose response data were analysed using bench-mark dose modelling. NDMA mutagenicity in liver was not stochastic in terms of mutation additivity at the lacZ locus with evidence of an overall reduction in mutation frequency following repeat dosing compared with acute dosing for the same total dose. Liver toxicity was observed (≥1.1mg/kg/day) and these data will be discussed in terms of NDMA exposure, hepatic toxicity, and mutagenicity, together with bench-mark dose modelling. The results will be integrated using an adverse outcome framework and the implications for human risk assessment presented.

Keywords:
NDMA, Mutamouse, mutation, LacZ and Pig-a

ID HESI.06

Regulatory considerations related to mutagenic impurities in pharmaceuticals

Roland Froetschl

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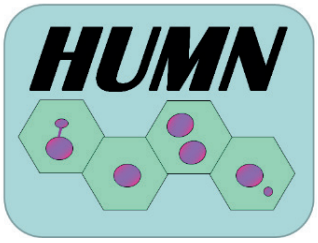
Genetic Toxicology data are only used for quantitative risk assessment of genotoxic compounds with an indirect (not-DNA-reactive) mechanism of action. For such compounds a threshold can be determined below which no genotoxic effect is expected. Directly-DNA-reactive compounds are considered potentially mutagenic at any dose. Theoretically, even one molecule may reach and react with DNA and cause a mutation. Therefore, standard battery genetic toxicology data are primarily used to qualitatively characterize a molecule as mutagenic or not. For mutagenic molecules further assessment concentrates on the mode of action of genotoxicity to identify DNA-reactive molecules considered of high concern for human health and exempted from using quantitative genetic toxicity data in risk assessment. Such molecules are then generally regulated according to ICH M7(R1) deriving a substance specific AI based on carcinogenicity data or applying a generic TTC of 1.5 µg/d in case of lack of adequate carcinogenicity data.

Research of recent years challenges this paradigm promoting the future use of quantitative evaluation of (especially) in vivo genetic toxicology data for risk assessment of all mutagens. Few examples for using quantitative assessment of mutations to derive exposure limits of compounds have been published, e.g. EMS, benzo(a)pyrene or NDMA, using extensive in vivo mutation and mechanistic datasets. Examples with corresponding cancer data to quantitatively correlate risk for mutations and risk for cancer as the major apical disease are exceptions and have limitations. Still fundamental questions of the quantitative relationship of mutations and cancer need to be answered. A harmonized framework for evaluating the quantitative correlation of mutation risk and cancer risk is needed to use quantitative mutation data for defining health protective exposure levels for humans. A database of reference compounds to validate the quantitative relationship of mutation and cancer is recommended to support the use of quantitative genetic toxicity data for risk assessment. Definition of modifying factors needed for extrapolation of mutational risk from animal to human is considered crucial to protect humans from mutations and genetically determined health risks. For this, understanding of the crucial molecular and cellular parameters and their variability in humans is needed.

Keywords:
mutation; cancer; risk assessment; quantitative.



HUMN WORKSHOP

HUMN Workshop on the buccal micronucleus cytome assay – New horizons for its implementation in human biomonitoring and clinical studies

Thursday 18 May 2023
14:00 – 17:30

Objectives / Goals
Micronuclei are expressed in cells that have structural chromosome aberrations and/or defects in the mitotic apparatus that leads to failed segregation of chromosome fragments or whole chromosomes during mitosis. The lagging chromosome fragments or whole chromosomes are excluded from the two main nuclei at anaphase/telophase and are ultimately surrounded by membrane to form micronuclei. Measurement of micronuclei in eukaryotic cells, including human cells, has become one of the most widely used methods to measure chromosome instability and the DNA damaging effects of environmental and endogenous genotoxins. The best validated of these methods in humans is the lymphocyte cytokinesis-block micronucleus assay in which micronuclei, and other related nuclear anomalies, such as nucleoplasmic bridges and nuclear buds, are scored exclusively in cells that have completed one nuclear division which are identified as binucleated cells after blocking cytokinesis with cytochalasin-B.

Another method to measure micronuclei in humans is to use buccal cells which are post-mitotic epithelial cells that can be collected in a minimally invasive manner from the inside of the mouth. In this method micronuclei and other nuclear anomalies such as nuclear buds can be observed and scored without the need of ex vivo culture of cells. Because of the relative ease of collecting, preparing, fixing and storing buccal cells there is growing interest in further developing and validating this assay for biomonitoring studies of human populations. Recent reviews have shown that the association of buccal micronucleus frequency with exposure to genotoxins, and a wide range of age-related degenerative diseases such as cancer and cardiovascular disease, is similar to that observed with lymphocytes. However, there are some important knowledge and technological gaps regarding the buccal micronucleus assay. The knowledge limitations include (i) lack of prospective studies showing that an elevated MN frequency in buccal cells predicts an increased risk of developmental and degenerative diseases and (ii) lack of knowledge on whether mitotic rate in the basal layer of the buccal epithelium substantially affects MN frequency. The technological gaps include (i) lack of automated systems to score MN frequency in buccal cells which is critical given the lower incidence of MN in buccal cells relative to lymphocytes, (ii) lack of algorithms that can distinguish MN from other nuclear anomalies such as nuclear buds or distinguish between different types of cell death such as cells that have nuclei with condensed chromatin, karyorrhexis or pyknosis.

The workshop is designed to discuss the current status of the buccal MN cytome assay and determine the most important near term and long term goals to further validate the assay and enable its more practical application in human studies. Presentations will be given by six experts on the use of the buccal MN cytome assay including the mechanisms and biology of MN formation in buccal cells, application of the assay in occupational exposures to genotoxins, the association with disease, nutrition and lifestyle and the potential and challenges for automation. The detailed agenda is shown below.

ID HUMN.01

The biology of buccal cells and the buccal micronucleus (MN) cytome assay

Bolognesi

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The buccal mucosa forms the primary barrier for the inhalation or ingestion route representing a preferred target site for early events induced by genotoxic agents entering the body. The oral epithelium, composed by multiple layers of cells, maintains itself by continuous cell renewal whereby new cells produced in the basal layer by mitosis migrate to the surface replacing those that are shed. Basal cells impacted by genotoxic agents express the genetic damage, as chromosome breakage or loss, during the cell division.The daughter cells differentiate then exfoliate into the buccal cavity and can be easily collected and analysed. The micronucleus assay applied in exfoliated cells represents a minimally invasive approach to evaluate genomic damage in biomonitoring studies.

The micronucleus(MN) assay in buccal cells was established in 1980 to evaluate the genotoxic effects induced by chewing tobacco at the site of exposure. The buccal MN assay has been largerly applied in the last 40 years in biomonitoring human populations exposed by inhalation to a variety of genotoxic and carcinogenic agents or by oral continuous . The MN test was also used to evaluate the effects of anti-cancer chemopreventive agents, the impact of nutrition and lifestyle factors. A large number of studies appeared more recently on the application of the buccal MN assay in the follow-up of cancerous and precancerous oral lesions and as a biomarker of chromosomal instability in patients with cancer or with different chronic diseases. Based on the data available, the association of MN in buccal cells with some diseases appears to be as robust as MN in lymphocytes.

The MN assay was successfully applied to evaluate the MN frequency as a marker of chromosome damage. More recently the MN assay evolved in the “buccal MN cytome” including the scoring of the different cell types and nuclear anomalies providing a comprehensive evaluation of the biomarkers of DNA damage, biomarkers of cell death, biomarkers of cytokinetic defects or arrest. The data collected in biomonitoring occupational or environmental exposure and in clinical studies suggest an added value for the evaluation of the cytome biomarker profile.

Keywords:
Buccal mucosa, exfoliated cells, micronucleus, biomonitoring, clinical studies.

ID HUMN.02

Use of buccal cytome assays in the occupational exposure studies

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So far about 200 studies have been published which concern the formation of micronuclei (MN) in buccal cells of different groups of workers. The first investigation with iron exposed workers was published already 30 years ago. Most studies (55) concern the impact of exposure to agricultural chemicals followed by workers that are exposed to petroleum and its derivatives (24). A similar number of investigations was conducted with medical staffs (exposed to anesthetic gases, cytostatics and radiation) (22). Further studies were conducted with medical students and anatomy laboratory staff which are exposed to formaldehyde (14). Less frequently studied groups are miners, electroplaters, welders, painters and carpenters.

We conducted in the last decade studies with the latter groups and found a clear positive result in individuals which work in the furniture production but not in electroplaters. Also with workers that are exposed to chicken manure (used for energy production) negative results were obtained. It is notable that in all these studies the number of nuclear anomalies which reflect acute cytotoxicity was significantly higher in exposed subjects. A clear increase of MN was found in cotton weavers (in Pakistan) that are exposed to cotton dust. Our latest study concerned the induction of DNA damage in street markers that are exposed to silica crystals and various chemicals. A clear increase of genotoxic effect with a duration of work was detected. This is the first study which demonstrated increased genetic damage in this occupational group.

In several investigations MN rates were comparatively studied in buccal and nasal cells and similar effects were detected in both cell types. Furthermore, in some occupational studies (in total 49) the MN frequencies were monitored in parallel in lymphocytes and buccal cells and significant correlations of the results were found.

The currently available data indicate that MN studies with buccal cells are a cost-effective, rapid and simple approach to find out if workers are exposed to genotoxic carcinogens. This method could complement the chemical exposure measurements which are currently used to control the safety of workers.

Keywords:
micronuclei, nuclear anomalies, occupational exposure, health risks.

ID HUMN.03

Association of buccal MN cytome assay biomarkers with disease and their relevance for clinical studies

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Micronucleus (MN) test in exfoliated buccal cells is widely applied with different purposes, mostly to assess the genotoxic impact of environmental and occupational exposure to genotoxic agents. In the last years the investigations on a potential clinical application of the assay, mainly in patients with oral cancer and oral premalignant lesions, substantially increased. Given the limited extent of clinical data concerning MN frequency in buccal cells, results on buccal cells were compared with MN frequency in lymphocytes in cancer and non-cancer diseases, and will be discussed extensively. In all diseases examined, MN in lymphocytes and exfoliated cells were higher than in controls, with the exception of prostate cancer. The ratio of MN frequency in subjects with disease vs controls in lymphocytes (2.3 and 2.0 for non-cancer diseases and cancer, respectively) was significantly lower than the corresponding estimates observed in exfoliated cells (3.6 and 6.1). The best association was found for those cases in which MN were measured in cells from the same tissue in which cancer was diagnosed (i.e., oral cancer). How to validate and translate the application of MN assays into clinical practice will be discussed, and a possible roadmap driving this process will be illustrated. Critical steps are the following: a) differentiate disease patients from unaffected individuals and identify important variables that can modify the MN biomarker in healthy and disease subjects; b) drive the transition from the use of MN assays at group level to the individual level; and 3) run prospective cohort studies and randomised controlled trials to verify that MN assays are predictive of disease and that MN frequency modification alters disease outcomes. Pragmatic trials will also be required before inclusion in routine clinical practice, to provide the decisive evidence to support their adoption by the medical and public health community.

ID HUMN.04

Impact of nutrition and life style on formation of micronuclei and other nuclear anomalies in buccal cells

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Only few dietary studies have been realized in which the impact of vitamins (vitamin and provitamin A, tocopherol, folate) on buccal micronuclei (MN) were studied and in most of them (>90%) evidence for beneficial effects were found.

The technique was also frequently used to study the consequences of consumption of various drugs. Consistently positive effects were observed in tobacco chewers (> 20 studies) and in heavy smokers (> 15). Interestingly, we observed an inverse correlation between the nicotine contents of cigarettes and MN formation while a positive correlation with the tar contents was observed. In a well-designed older trial evidence for a synergistic effect between alcohol consumption and smoking was observed. Alcohol intake per se caused no clear effects in a number of investigations.

Several studies showed that betel and areca nuts chewing (with and without tobacco) and consumption of khat leaves lead to increased MN frequencies. This observation may explain the high incidence of oral cancer in areas where these chewing habits are prevalent. It is also notable that synthetic derivatives of ephedrine as well as synthetic and natural cannabinoids led to increased MN frequencies in in vitro experiments with cells from respiratory/oral tract. On the contrary, no evidence of MN induction was seen in a study which we realized in South America (Peru) with coca leave chewers (i.e. in this case even a decrease of the MN frequencies was observed).

A substantial number of studies (in total 17) concern the effects of mobile phone specific electromagnetic fields. High quality studies (n=4) yielded consistently negative results. Also in our investigation with highly controlled exposure via head phones (Knasmueller et al., unpublished) no evidence for positive results was found.

Taken together, the available data show that MN assays reflect health risks as a consequence of exposure to certain drugs; the results of dietary studies are scare and no firm conclusion can be drawn.

Keywords:
micronuclei, life style, cancer, drugs, mobile phone radiation.

ID HUMN.05

Automation of the Buccal Micronucleus Cytome Assay

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Originally, the buccal micronucleus technique was a simple assay in which only micronuclei (MNi) are scored. It eventually evolved into a complex two-stage cytome assay in which cells are first classified into seven types (Basal, Differentiated, Binucleated, Condensed chromatin, Karyorrhexis, Pyknotic, Karyolytic cells) and secondly MNi and nuclear buds (NBUD) are scored in differentiated cells only. Both the relative frequency of the various cell types and the number of differentiated cells with MNi and/or NBUD have diagnostic value with regards to toxic environmental exposures, poor lifestyle, malnutrition and a wide range of diseases. Scoring this complex profile of biomarkers is laborious and limits the possibility of doing genetic toxicology studies efficiently using a relevant epithelial cell type that can be obtained in a minimally invasive manner.

Therefore, there is a legitimate need to automate some of the best validated biomarkers of the Buccal Micronucleus Cytome assay and ultimately achieve a fully automated system that scores all the biomarkers. In my presentation I shall discuss which of the buccal biomarkers may be easier to measure automatically by image analysis and present some preliminary data with DAPI stained slides using the Metafer system. A key question is which slide preparation and staining system is most suitable for both visual and automated scoring of buccal cell biomarkers?

Keywords:
Buccal, micronucleus, cytome, scoring, automation.

ID HUMN.06

Artificial Intelligence in Microscope-Based Imaging: Automation of the Buccal Micronucleus Cytome Assay?

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Artificial intelligence (AI) has become a key factor for automated microscope-based image analysis. The power of artificial neural networks in the evaluation of digital image content opens unimagined possibilities for automating even complex assays.

MetaSystems uses Deep Neural Networks (DNN) in its scanning software Metafer to classify objects based on criteria determined by the algorithm. These networks are trained with large amounts of pre-classified image data (supervised learning).

The Buccal Micronucleus Cytome Assay is increasingly used in epidemiological studies to investigate the influences of nutrition, lifestyle, and exposure to genotoxins. The assay looks at many different cell classes and markers, and evaluation of a very large number of cells is required for a significant result. Automation of the assay would therefore be highly desirable.

We would like to take advantage of the HUMN workshop to discuss with the participants the different possible approaches for automation of the Buccal Micronucleus Cytome Assay using DNN.

Keywords:
Artificial Intelligence; Buccal Micronucleus Cytome Assay; Automation; DNN.


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