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## Abstracts of the 52nd EEMGS & 15th ICAW Meeting

Rovinj, Croatia, 23rd - 27th September 2024

**Supplement 1**
**Vol. 75 / 2024**

# 2024

52nd European Environmental Mutagenesis and Genomics Society (EEMGS)

&

15th International Comet Assay Workshops (ICAW) meeting

Rovinj, Croatia, 23rd – 27th September 2024

**Organised by**

eemgs EUROPEAN ENVIRONMENTAL MUTAGENESIS & GENOMICS SOCIETY

Hrvatskogenetičkodruštvo
CroatianGeneticSociety

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# Opening remarks

Opening remarks
Arh Hig Rada Toksikol 2024;75(Suppl. 1):10-13

Dear Colleagues,

On behalf of the European Environmental Mutagenesis and Genomics Society (EEMGS), it is my great pleasure to welcome you to this year's congress, which is being held together with the biennial congress of our interest group dedicated to the comet assay (International Comet Assay Working Group).

First and foremost, I would like to extend our heartfelt gratitude to The Croatian Genetic Society for their exceptional organisation of this event. Their dedication and attention to every detail have been remarkable, ensuring the success of the congress both scientifically and socially. I speak on behalf of all members of the EEMGS Management Committee when I say that it has been a true pleasure to collaborate with them in the organisation of this event, particularly with Goran Gajski and Marko Gerić, who served as our primary contacts.

The scientific program is full of exciting presentations and discussions. We are expecting to hear new insights from leading experts, as well as fresh ideas from new investigators. This combination of seasoned expertise and innovative perspectives will enrich our understanding and foster vibrant scientific discussion.

I would also like to extend a special welcome to those researchers attending the EEMGS congress for the first time. We hope that your experience here will be so positive that you will want to join us again in the future. Please know that the atmosphere of this congress is both friendly and respectful, as we believe this is the best way to foster scientific progress.

Finally, I wish everyone a fruitful congress both in terms of acquiring new knowledge and forging new collaborations.

Sincerely,

**Amaya Azqueta**
EEMGS president

Dear colleagues, friends, and guests,

On behalf of the Croatian Genetics Society (CGS), it is my great pleasure to welcome you to the 52nd EEMGS and 15th ICAW meetings, jointly organized by the Croatian Genetics Society and the European Society for Mutagenesis and Environmental Genomics, in the beautiful city of Rovinj.

We hope that this congress, like the previous two held in Croatia (the 9th EEMS in Tučepi and the 38th EEMS in Cavtat), will provide valuable insights into the latest scientific achievements and foster new collaborations within our scientific community.

We believe you will enjoy the stimulating program we have prepared, take advantage of the opportunity for fruitful discussions, and explore the charm of Rovinj. For this occasion, we have gathered scientists, experts, and students from across Europe and beyond to share knowledge, exchange ideas, and discuss the latest developments in genetics, mutagenesis, and genomics.

Our venue, Rovinj – with its rich history and stunning coastal scenery – offers the perfect setting for scientific collaboration and unforgettable social experiences.

Once again, welcome and thank you for joining us for this exciting event. We look forward to an inspiring and productive congress.

Sincerely,

**Dušica Vujaklija**
CGS President

Dear colleagues and friends,

On behalf of the International Comet Assay Working Group (ICAWG), we are happy to welcome you to the 15th International Comes Assay Workshop (ICAW) that will take place along with this year's 52nd European Environmental Mutagenesis & Genomics Society (EEMGS) meeting. Following the success of previous ICAW meetings, we are delighted to welcome you to the beautiful town of Rovinj, located on the Istrian peninsula, Croatia, from 25th to 27th September 2024.

Since the ICAWG was founded as a specialist interest group of the EEMGS in 2020, we, as the first executive committee, are glad that from now on we will jointly host ICAW meetings along with the EEMGS, and this year we want to express our deepest thanks to the Center for Marine Research (CIM) of the Ruđer Bošković Institute for hosting the event and allowing us for the first time to extend the hands-on part of our meeting to the field of marine biology and toxicology.

This year, attendees will have the opportunity, besides excellent lectures, to engage in a marine research boat experience and afterwards to catch a glimpse of laboratory work in the facilities of CIM. The programme will cover an introduction to the comet assay along with the latest innovations, including automation, enhancement of the comet-based *in vitro* DNA repair assay, and high-throughput versions using CometChip. Moreover, during the marine boat field trip, participants will be able to spend a few hours on the Adriatic Sea learning about metadata gathering and sampling of aquatic organisms for subsequent laboratory analysis. The lab work will consist of sample analyses that will include filtration for metabarcoding and metatranscriptomics, DNA isolation, microscopy, flow cytometry and preparation of samples for the comet assay.

Besides the rich scientific program, the ICAWG family is devoted to connecting scientists from all around the globe, with special emphasis on networking between our younger colleagues which will hopefully lead not only to future scientific collaborations but also to lifetime friendships.

On behalf of the ICAWG committee, we extend our warmest thanks to all the organisers, lecturers, chairpersons, and participants and especially to our sponsors who have helped to make this conference and ICAW meeting a success.

We wish you all a great time in Rovinj and hope that you catch a view of dolphins at sunset!

**Goran Gajski**
ICAWG Chair

**Andrew Collins**
ICAWG Vice Chair

Dear EEMGS and dear participants of the 52nd EEMGS meeting,

on behalf of the Center of Marine Research (CIM) in Rovinj, I extend our warmest welcome for your stay in Rovinj. It is an honour and privilege to have the 52nd European Environmental Mutagenesis & Genomics Society meeting organized in Rovinj. The much-anticipated scientific discussion of topics related to mutagenesis and genomics in environmentally relevant circumstances is very close to our hearts here in Rovinj. The Center for Marine Research is not only one of the oldest marine research centers around the Mediterranean. It has also a very rich and longstanding history of research in genetics, genomics and mutagenesis. Since its first days, the CIM has focused on research in Biodiversity, bio-geo-chemical and physical Oceanography of the northern Adriatic Sea. However, concomitant to the discovery of DNA as the carrier of inheritable information international teams of researchers started to research the structure, integrity, and characteristics of DNA in marine species and closely collaborated with marine researchers specialized in marine biodiversity and physiology of marine life at the CIM. Since the early days of DNA damage and sequence analysis, these methods were applied at the CIM for physiological studies as well as for phylogenetics and later barcoding. Researchers from the CIM significantly contributed to the design of primers, barcodes, markers, probes for *in situ* hybridization and nowadays bioinformatic downstream analyses and so-called pipelines for environmental genetics and genomics. I am happy to say that today our researchers hold pace and drive the development of experimental design in environmental applications of DNA and RNA-related methods in environmental research in low and high throughput applications, in fundamental research and applied marine environmental management.

I am sure you will enjoy the scientific excellence and many fruitful discussions this EEMGS meeting will offer you. You will have the chance to take some glimpses into the marine research at the CIM and the application of modern genomics in marine environmental research. However, I wholeheartedly also wish that you will enjoy the beauty of the northern Adriatic Sea, the scenic views and beautiful corners of the City of Rovinj as well as the culinary experiences the region offers. I thank the organizers of this meeting for having managed and created an excellent program that promises to be a scientific and social highlight. I encourage all participants to use this context and purposefully cross all generational, topical and national borders and make this meeting a networking highlight as well.

On behalf of the Center for Marine Research in Rovinj, I thank the EEMGS for involving the CIM in this meeting and wish you all the best of times in Rovinj.

**Martin A. Pfannkuchen**
RBI CIM Head

**52nd EEMGS and 15th ICAW meeting**
**23rd – 27th September 2024**
**Rovinj, Croatia**

# CONFERENCE PROGRAMME

## Sunday, 22nd September 2024

| | |
|---|---|
| 20:00 – 23:00 | 5th "geNIus" Pub Quiz (*Aperitiv bar Circolo*)<br>- all participants are invited for a casual networking - |

## Monday, 23rd September 2024

9:00 – 11:00 [ToxLearn4EU consortium meeting]

HESI Workshop – Where the rubber meets the road: Transitioning academic research into regulatory requirements
Chairs: C. Chen, M. Vasquez

Part 1. Cross-talk between academic genotoxicity research and regulatory genotoxicity assessment HESI

| Timing | Lecturer | Title |
|---|---|---|
| 13:00 – 13:05 | | Welcome & Introductions |
| 13:05 – 13:35 | Carol Beevers | Reflections on the commonalities and differences between academic and regulatory genotoxicity studies |
| 13:35 – 13:55 | David Lovell | Statistics and experimental design: similarities and differences between regulatory and academic studies |
| 13:55 – 14:15 | Roland Frötschl | What do regulatory agencies look for in studies and how they use academic and regulatory studies |
| 14:15 – 14:35 | Steven Brooks | Genotoxicity in marine organisms, assessing the potential impacts of offshore oil and gas activities on the marine environment |
| 14:35 – 15:00 | | Coffee break |
| Part 2. From academic origins to the regulatory arena: successes and current challenges | | |
| 15:00 – 15:20 | Javed Bhalli | PigA: lifecycle and lessons learned |
| 15:20 – 15:40 | Francesco Marchetti | Duplex sequencing: the roadmap for error-corrected next-generation sequencing |
| 15:40 – 16:00 | Giel Hendriks | From bench to OECD validation: the ToxTracker journey |
| 16:00 – 16:25 | Hans-Jörg Martus | Quantitative-based genotoxicity risk assessment |
| 16:25 – 16:30 | | Closing remarks & conclusions |
| 16:30 – 17:30 | | **The EEMGS General Assembly** |

| | | |
|---|---|---|
| 18:30 – 19:00 | | **Opening ceremony** |
| **Keynote lecture**<br>Chairs: A. Azqueta, G. Gajski | | |
| 19:00 – 20:00 | Rosa Karlić | Exploring the epigenomic context of mutational processes |
| 20:00 – 23:00 | | **Welcome reception (***Restaurant Oleander, Hotel EDEN***)** |

## Tuesday, 24th September 2024

| | | |
|---|---|---|
| **Plenary lecture**<br>Chair: K. Zahradka | | |
| 8:55 – 9:40 | Tomislav Maričić | Neanderthals and genome editing of stem cells: exploring genetic changes that define modern human traits |
| **Session 1a: DNA Structure and Repair**<br>Chairs: I. Ivančić Baće, K. Zahradka | | |
| 9:45 – 10:15 | Marcus S. Cooke | Do nucleic acid modifications have a role in dissecting the health effects of the exposome? |
| 10:15 – 10:45 | Jelena Repar | DNA double-strand break repair and genome stability in the bacterium *Deinococcus radiodurans* |
| 10:45 – 11:00 | Ivana Ivančić Baće | Interplay between DNA repair and CRISPR-Cas adaptation |
| **Session 1b: Aquatic Environments**<br>Chairs: M. Smodlaka Tanković, S. Kolarević | | |
| 9:45 – 10:15 | Sandi Orlić | Microbial diversity as a signal of environmental changes |
| 10:15 – 10:30 | Stoimir Kolarević | Ecogenotoxicology in the Joint Danube Surveys (JDSs) – summary of activities in the past surveys and plans for the upcoming JDS5 |
| 10:30 – 10:45 | Mia Knjaz | First regional reference database of northern Adriatic diatom transcriptomes |
| 10:45 – 11:00 | Josip Madunić | Assessment of domoic acid-induced genotoxicity and oxidative stress in non-target HepG2 liver cells |
| 11:00 – 11:30 | | Coffee break and poster session |
| 11:30 – 13:00 | | **The Frits Sobels Award lecture and the Early Career Award lecture** |
| 13:00 – 14:00 | | Lunch |

| | | |
|---|---|---|
| **Session 2a: Ageing**<br>Chairs: C. Ladeira, D. Vujaklija | | |
| 14:00 – 14:30 | Gordan Lauc | Effects of environmental factors on glycan biomarkers predicting age-related diseases |
| 14:30 – 15:00 | Vlatka Zoldoš | Effects of estrogen on immunoglobulin G glycosylation and biological aging: mapping the downstream signalling pathway |
| 15:00 – 15:15 | Tanima SenGupta | The role of DNA repair in aging and neurodegeneration |
| 15:15 – 15:30 | Sharleen Friese | Trace elements, ageing, and genomic instability in mice |
| **Session 2b: Environmental Toxicants**<br>Chairs: B. Žegura, J. Sanders | | |
| 14:00 – 14:30 | Doris Marko | Data gaps in the risk assessment of mycotoxins |
| 14:30 – 14:45 | Michalis Fragkos | Investigation of the genotoxicity of glyphosate using cell-based assays |
| 14:45 – 15:00 | Henning Hintzche | Genotoxicity of 2-chloroethanol *in vitro* |
| 15:00 – 15:15 | Caroline Quarz | Transcription-coupled nucleotide excision repair protects against the detrimental effects induced by methyleugenol-derived DNA adducts |
| 15:15 – 15:30 | Ariane Schmidt | Threshold concentrations for BPDE-induced cell death are characterised by altered DNA damage signalling and associated with unrepaired double-strand breaks |
| 15:30 – 16:30 | | Poster session |
| 16:00 – 17:30 | | [ToxLearn4EU consortium meeting] |
| 18:00 – 20:00 | | *Rovinj City Guided Tour (from Hotel EDEN)* |
| 20:00 – 22:00 | | Late-night poster viewing |

**Wednesday 25th September 2024**

| | | |
|---|---|---|
| **Session 3a: Cancer**<br>Chairs: S. Bonassi, S. Vodenková | | |
| 9:00 – 9:30 | Duan Chen | Gastric cancer: potential carcinogens, biomarkers, chemoprevention and drug repurposing |
| 9:30 – 9:50 | Chun-Mei Zhao | Proteomics-based system modeling for studying pancreatic cancer |
| 9:50 – 10:10 | Jiří Zavadil | Mutational signature of dietary acrylamide/glycidamide in renal cancer genomes |
| 10:10 – 10:30 | Michael Korenjak | Mutational signatures of tobacco-specific nitrosamines NNN and NNK in cells, animals and humans |
| 10:30 – 10:45 | Julia Stephanie Bruno | Oral tongue cancer infectome in patients with no identified risk factors |
| 10:45 – 11:00 | Natálie Danešová | Changes in mitochondrial DNA in colorectal cancer patients |

| | | |
|---|---|---|
| **Session 3b: New Approach Methodologies**<br>Chairs: R. Frötschl, S. Bryce | | |
| 9:00 – 9:15 | Roland Frötschl | Impact of the ICH S2(R1) guideline on the frequency of irrelevant positive *in vitro* mammalian cell assays |
| 9:15- 9:30 | Steven Bryce | Application of modifying agents to a multiplexed DNA damage assay provides mechanistic information on genotoxicity and molecular targets |
| 9:30 – 9:45 | Nivedita Chatterjee | Role of PMK-1/p38 MAPK in *C. elegans* DNA damage response: A case study with silver nanoparticles |
| 9:45 – 10:00 | Lajos Mátés | Detecting a new class of carcinogens by testing their ability to activate endogenous L1 elements |
| 10:00 – 10:15 | Gladys Mirey | Reference chemicals mode-of-action assessed *in vitro* by HSC micronucleus assay after acute or subacute exposures |
| 10:15 – 10:30 | Evi De Ryck | Assessment of biomarkers in exhaled breath condensate of workers with occupational lung disease |
| 10:30 – 10:45 | Rebekah Beck | High-content *in vitro* micronucleus assay highlights novel links between epigenetic changes and genotoxic outcomes. |
| 10:45 – 11:00 | Danielle Harte | The *in vitro* micronucleus multi-biomarker image stream (ISMN-MB) assay |
| 11:00 – 11:30 | | Coffee break and poster session |
| **New Investigators Session**<br>Chairs: M. M. Nicolai, S. Friese | | |
| 11:30 – 12:00 | Marina Tenório Botelho | Reviewing comet assay as a tool in marine ecotoxicology |
| 12:00 – 12:15 | Bérénice Chavanel | Mutagenic effects of ethanol and acetaldehyde in oral cancer: an experimental modelling approach |
| 12:15 – 12:30 | Julie Sanders | Quantitative genotoxicity assessment of mycotoxin mixtures |
| 12:30 – 12:45 | Anne Lene Nordengen | Effect of a personalized intensive dietary intervention on DNA damage and repair in colorectal cancer patients |
| 12:45 – 13:00 | Lieselot Hemeryck | DNA adduct formation associated with specific environmental, dietary, and lifestyle habits among kidney transplant patients |
| 13:00 – 14:00 | | Lunch |
| 14:00 – 14:15 | | Zeiss sponsored talk |
| 14:15 – 14:30 | | Inel sponsored talk |
| **Plenary lecture**<br>Chair: D. Vujaklija | | |
| 14:30 – 15:15 | Nenad Ban | Revealing the machinery for the production of proteins in human cells |
| **15th International Comet Assay Workshop Part 1**<br>Chairs: G. Gajski, A. Haverić | | |
| 15:30 – 16:00 | Andrew Collins | The comet assay in middle age |
| 16:00 – 16:30 | Stefano Bonassi | Biomarkers of effect: a journey from exposure monitoring to predictors of adverse health outcome |
| 16:30 – 17:00 | Siegried Knasmüller | Search for the most reliable genotoxicity test |
| 17:00 – 17:30 | Sabine A.S. Langie | Revolutionizing DNA repair analyses: latest enhancements to the comet-based *in vitro* DNA repair assay |

**European Centre for Ecotoxicology and Toxicology of Chemical Session**
Chair: B. van Ravenzwaay, K. Meurer

| | | |
|---|---|---|
| 15:30 – 15:50 | Bennard van Ravenzwaay | ECETOC's transformational program A framework to incorporate NAMs in regulatory toxicology |
| 15:50 – 16:10 | Sylvia Escher | Toxicological effects in 28-day studies compared to 90-day studies – what do we miss after short term exposure? |
| 16:10 – 16:40 | Sergio Perez | Examples of the ECETOC framework incorporating NAMs in risk assessment |
| 16:40 – 17:00 | Krista Meurer | Smart *in vivo* studies: using new technologies in the 28-day studies |
| 17:00 – 17:15 | | Discussion |
| 17:30 – 18:30 | | Poster viewing |
| 20:00 – 00:00 | | **Gala dinner (***Primi terreni, Hotel GRAND PARK***)** |

## Thursday, 26th September 2024

**Next-generation Sequencing Session Part 1**
Chair: A. Lynch

| | | |
|---|---|---|
| 9:00 – 9:15 | Anthony Lynch | Introduction |
| 9:15 – 9:45 | Francesco Marchetti | Advancing quantitative genetic toxicology and genomic technologies to reduce and replace conventional rodent mutagenicity tests |
| 9:45 – 10:15 | Anne Ashford | Extended analysis of NDMA mutagenicity using Duplex Sequencing on an *in vivo* Muta™Mouse mutation assay |
| 10:15 – 10:30 | Paula Štancl | Exogenous and endogenous carcinogens driving different mutational signatures in uveal and skin melanoma |

**15th International Comet Assay Workshop Part 2**
Chairs: S.A.S. Langie, M. Milić

| | | |
|---|---|---|
| 9:00 – 9:30 | Marcus S. Cooke | Is automation the next innovation for the comet assay? |
| 9:30 – 9:45 | Ann-Karin Hardie Olsen | The *in vivo* comet assay: uncovering DNA damage in testicular germ cells |
| 9:45 – 10:00 | A. Nurşen Başaran | DNA damage in patients with end-stage renal disease |
| 10:00 – 10:15 | Miroslav Mišík | Impact of different electromagnetic fields on DNA stability *in vitro* |
| 10:15 – 10:30 | Victoria Claudino Bastos | Enzyme-modified CometChip: detection of enzyme-specific DNA damage with high-throughput comet assay |
| 10:30 – 11:00 | | Coffee break |

| | | |
|---|---|---|
| **Next-generation Sequencing Session Part 2**<br>Chair: A. Lynch, A.K. Hardie Olsen | | |
| 11:00 – 11:30 | Martin Pfannkuchen | Genomics in coastal oceanography |
| 11:30 – 12:00 | Giel Hendriks | MutaTracker, a novel approach method to measure gene mutations using error corrected NGS to gain understanding of the genotoxic mode of action. |
| 12:00 – 12:30 | Ann-Karin Hardie Olsen | Embarking on decoding stem cells: ecNGS of hIPSCs exposed to environmental mutagens during trilineage differentiation using the ReproTracker assay |
| **15th International Comet Assay Workshop Part 3**<br>Chairs: V. Moraes de Andrade, M. Gerić | | |
| 11:00 – 11:15 | Vanesa Moraes de Andrade | Antigenotoxic effects of melatonin in obese mice |
| 11:15 – 11:30 | John Einset | An important limitation of the comet assay |
| 11:30 – 11:45 | Agnes Draxler | Age-related DNA damage in middle-aged hospitalized COVID-19 patients |
| 11:45 – 12:00 | Vidya Balakrishnan PV | Evaluation of nanoparticles induced genotoxicity in human peripheral blood lymphocyte using CBMN assay and comet assay: An *in vitro* study |
| 12:00 – 12:15 | Elisa Sáenz-Martínez | Evaluation of potassium bromate as a positive control in the *in vivo* fpg-modified comet assay for the detection of oxidized bases |
| 12:15 – 12:30 | Camille Guyon | Study of genotoxic effects on exocrine pancreas after chronic dietary exposure to cocktail of pesticides |
| 12:30 – 13:00 | | Light lunch |
| **Partnership for the Assessment of Risks from Chemicals SYNnet 2nd Forum**<br>Chairs: S. Namorado, N. Alygizakis | | |
| 13:05 – 13:35 | Philip Marx-Stölting | Hazard assessment in PARC - first outcomes and synergies |
| 13:35 – 13:50 | Maria João Silva | Closing regulatory data gaps on the genotoxicity and non-genotoxic carcinogenesis of natural toxins and bisphenols |
| 13:50 – 14:05 | Marc Audebert | New approach methodologies to facilitate and improve the hazard assessment of non-genotoxic carcinogens |
| 14:05 – 14:20 | Emmanuel Demuynck | Development of an AOP-based IATA for Genotoxicity: Building an AOP network for genotoxic events leading to permanent DNA damage |
| 14:20 – 14:35 | Levi M.T. Winkelman | Meta-analysis for quantifying a genotoxicity AOP linking DNA alkylation to increase in mutations and chromosomal aberrations |
| 14:35 – 14:50 | Ana Tavares | Genotoxicity biomarkers in occupational biomonitoring studies: linking exposure to preclinical effect |
| 14:50 – 15:05 | Cynthia Recoules | Set up of a human 3D multi-cellular liver model for non-genotoxic chemical carcinogen detection |
| 15:05 – 15:30 | | Discussion |
| 15:30 – 16:00 | | **EEMGS Closing and Award Ceremony** |

**Friday, 27th September 2024**

| | |
|---|---|
| 15th International Comet Assay Workshop hands-on | |
| Morning | Marine Research Boat session |
| | Lunch break |
| Afternoon | Centre for Marine Research Lab session |

# Abstracts of the 52nd EEMGS meeting

eemgs EUROPEAN ENVIRONMENTAL MUTAGENESIS & GENOMICS SOCIETY

*Keynote lecture*

# K-1
# Exploring the epigenomic context of mutational processes

Rosa Karlić
University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Bioinformatics Group, Zagreb, Croatia
*rosa@bioinfo.hr*

The cell-of-origin (COO) of cancer affects the treatment options, prognosis, and outcomes for individual cancer patients. Despite technological advances, correct identification of the cell-of-origin still poses a challenge for around 2–5% of cancer patients diagnosed with cancer of unknown primary, for whom the primary site cannot be correctly inferred using currently used diagnostic methods. In earlier research, we demonstrated that the landscape of somatic mutations in cancer correlates with the chromatin marks of the cell-of-origin. We developed an innovative machine learning-based approach that can accurately identify the cancer's primary site by leveraging various epigenetic features of the cell-of-origin and the somatic mutations present in the cancer genome. Additionally, we expanded our analysis to show that this principle applies to common cancer types and subtypes, and allows us to precisely identify the exact cell type from which the cancer originated. Beyond identifying the COO, models that relate the epigenetic features of the cell-of-origin to cancer somatic mutations can be utilised to study the mechanisms of mutation accumulation across various genomic regions and identify potential driver genes linked to cancer-related pathways. Model accuracy can be further enhanced by incorporating information on mutational signatures – distinct mutational patterns caused by different processes. This supports previous findings which indicated that the relationship between chromatin and the mutational landscape also depends on the specific process that produced the mutations. Overall, these insights underscore the critical role of the specific cell type of origin and the mutational process in shaping the mutational landscapes of different cancer types.

*Supported by HRZZ IP-2019-04-9308 "A statistical modelling approach to predict the cell-of-origin and investigate mechanisms of cancer development".*

*Plenary lecture*

## PL-1
## Neanderthals and genome editing of stem cells: exploring genetic changes that define modern human traits

Tomislav Maričić
Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany
*tomislav_maricic@eva.mpg.de*

As a species, humans are strikingly different from our closest living relatives, bonobos and chimpanzees, and much less so from our closest extinct relatives, Neanderthals and Denisovans. Underlying these differences in traits are genetic differences. By comparing sequenced Neanderthal genomes with a large dataset of modern human genomes and with genomes of great apes, we have identified genetic changes that distinguish us not only from living apes but also from extinct Neandertals and Denisovans. One could call these changes a genetic recipe for what makes us modern humans. The critical challenge, however, is to determine which of these genetic changes have functional significance in shaping our modern human traits. In this talk, I will discuss examples that demonstrate the power of genome editing and pluripotent stem cells to unravel the functional relevance of genetic changes relevant in the evolution of modern humans. This research fuels our ongoing quest to understand the fundamental genetic basis of what distinguishes us as modern humans.

*Supported by the Max Planck Society.*

*Plenary lecture*

## PL-2
## Revealing the machinery for the production of proteins in human cells

Nenad Ban
ETH Zurich, Zurich, Switzerland
*ban@mol.biol.ethz.ch*

Our group is interested in understanding the process of expression of genetic information that leads to the production of functional proteins. This process requires an intricate interplay between the protein synthesis machinery and an ever-growing list of cellular components that control protein synthesis and participate in protein biogenesis. Building on our studies that provided some of the first blueprints for understanding the eukaryotic protein synthesis machinery including the cytosolic and mitochondrial ribosomes, we are now investigating protein synthesis in human cells using a combination of structural, biochemical, and biophysical experimental approaches. We are particularly interested in understanding the regulation of protein synthesis and the biogenesis of cytosolic and membrane proteins. I will present examples of recent results that contribute to our understanding of the network and the coordination of cellular factors that interact with translating ribosomes in human cells to control protein synthesis and ensure accurate protein production.

[1] Gamerdinger et al. 2023. https://doi.org/10.1126/science.adg3297
[2] Jaskolowski et al. 2023. https://doi.org/10.1038/s41594-023-00990-0
[3] Jomaa et al., 2022. https://doi.org/10.1126/science.abl6459
[4] Kobayashi et al. 2018. https://doi.org/10.1126/science.aar7924

# DNA Structure and Repair

*Invited lecture*

# IL-1
# Do nucleic acid modifications have a role in dissecting the health effects of the exposome?

Marcus S. Cooke[1], Mu-Rong Chao[2,3], Yuan-Jhe Chang[2,3], and Chiung-Wen Hu[4,5]
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The concept of the exposome encompasses all the environmental exposures, both exogenous and endogenous, across the life course. Many, if not all, of these exposures can result in the generation of reactive species, and/or the modulation of cellular processes, that can lead to a breadth of modifications of DNA, the nature of which may be used to infer their origin. Because of their role in cell function, such modifications have been associated with various major human diseases, including cancer, and so their assessment is crucial. Historically, most methods have been able to only measure one or a few DNA modifications at a time, limiting the information available. With the development of DNA adductomics, which aims to determine the totality of DNA modifications, a far more comprehensive picture of the DNA adduct burden can be gained. Importantly, DNA adductomics can facilitate a "top-down" investigative approach whereby patterns of adducts may be used to trace and identify the originating exposure source. However, it is increasingly clear that nucleic acids, more broadly, are important targets for exposome-derived reactive species and that more comprehensive analysis approaches are needed to incorporate these. We recently described nucleic acid adductomics, which, in addition to DNA and RNA monoadducts, can detect a variety of combinations of DNA-, RNA- and protein crosslinks in nucleic acids and even human urine [1]. We propose that nucleic acid adductomics, together with other 'omic approaches, represents a major tool for unravelling the complexities of the exposome and hence a better understanding of the environmental origins of disease.

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[1] Hu et al. 2024. https://pubs.acs.org/doi/10.1021/acs.est.3c04674

*Invited lecture*

# IL-2
# DNA double-strand break repair and genome stability in the bacterium *Deinococcus radiodurans*

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Bacterium *Deinococcus radiodurans* can repair hundreds of simultaneous DNA double-strand breaks (DSBs) caused by γ-radiation. This extreme DSB repair is based on the presence of multiple genome copies broken in random places; homology between the genome fragments guides genome reassembly. The DSB repair in *D. radiodurans* is accurate and efficient, prompting us to explore its potential limitations. We have shown that extreme levels of DNA assault, as well as inactivation of the *recA* gene, introduce inaccuracies in the DSB repair in this bacterium. The absence of RecA, the main bacterial recombinase, compromises genome stability and causes gross genome rearrangements in *D. radiodurans*. Still, even in the absence of RecA, *D. radiodurans'* radiation resistance is comparable to that of *E. coli* showcasing an important role for RecA-independent DSB repair in *D. radiodurans*. We have characterized this RecA-independent repair through PacBio sequencing of *recA* isolates with rearranged genomes. The detected rearrangements consisted of large deletions in chromosome II. The mechanism behind these deletions utilized short (4–11 bp) repeats, suggesting that large genome deletions in *D. radiodurans recA* mutants occur during DSB repair via the alternative end-joining (A-EJ) mechanism. We have also found footprints of the A-EJ mechanism during the divergence of *D. radiodurans* wild-type strains in different laboratories, demonstrating that A-EJ is an important component of the *D. radiodurans'* DSB repair toolkit.

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# O-1
# Interplay between DNA repair and CRISPR-Cas adaptation

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To protect themselves from invasive mobile genetic elements (MGEs), prokaryotes have developed numerous defence strategies. The defence strategy of the CRISPR-Cas adaptive immune system against MGEs is to remember them by integrating a small piece of foreign DNA (protospacer) into the CRISPR locus as a new spacer. This process of spacer integration is called naïve CRISPR adaptation and is mediated in *Escherichia coli* by the Cas1-Cas2 complex. The spacers are later transcribed into long pre-crRNA and processed by Cas proteins into short crRNAs (CRISPR RNA) that guide the effector complex (Cascade) to recognise the target DNA. After recognition, the DNA is degraded by the nuclease/helicase Cas3. Naïve adaptation in *E. coli* is supported by the host DNA repair protein RecBCD, which is involved in the preparation of suitable short single-stranded (ssDNA) substrates for integration. These ssDNA substrates are generated during the processing of double-stranded DNA (dsDNA) breaks that occur at broken replication forks. In our previous work, we showed that RecBCD helicase activity is essential for this step and that recombination inhibits adaptation. In this work, we have further investigated other DNA repair proteins and their potential role in naïve adaptation in *E. coli*. We will present our current results and progress in understanding the interplay between DNA repair and naïve adaptation.

# Aquatic Environments

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*Invited lecture*

# IL-3
# Microbial diversity as a signal of environmental changes

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Aquatic ecosystem functioning is influenced by different types of organisms. The microbial community is sensitive to changes in its living environment, reflecting the structure and function of the aquatic ecosystems. Microbes play a crucial role in regulating and transforming major bioactive elements, recycling organic matter to benthic food webs, as well as in the degradation of organic pollutants. Environmental perturbations usually contribute to the reduction of biodiversity, shifts in community composition, the removal of sensitive species and the selection of the more tolerant ones. With the advent of novel molecular tools, a more depth understanding of these changes has been detected. However, microbes live in diverse communities that interact with other organisms and the environment, making their impact difficult to predict. Also, the relationships between microbes, climate change, and human well-being are in constant need of more advanced studies and collaboration across disciplines to address these complex interactions.

*Supported by Croatian Science Foundation (HRZZ IP-2020-02-9021).*

## O-2
# Ecogenotoxicology in the Joint Danube Surveys (JDSs) – summary of activities in the past surveys and plans for the upcoming JDS5

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The Joint Danube Surveys (JDSs) are expeditions organised by the International Commission for the Protection of the Danube River each 6 years starting from 2001. The key purpose of JDSs is to gather vital data on carefully selected elements of water quality across the entire length of the Danube River and its major tributaries. These expeditions harmonise water monitoring practices across the Danube countries, following the EU Water Framework Directive (WFD) committing Member States to achieve good water quality. Additionally, surveys provide an opportunity as a platform for testing new and advanced methodologies and approaches. Our group participated in JDS3 and JDS4 with an ecogenotoxicological program customised for each survey. In JDS3 (2013), we applied a passive monitoring program using comet assay in the haemolymph of freshwater mussels (*Unio* sp. and *Sinonodonta woodiana*) and the blood of freshwater fish (*Alburnus alburnus*) as sentinel organisms. We have processed 34 sites along the river and the results indicated the presence of genotoxic potential in the stretch of the river affected by untreated wastewater. In JDS4 (2019), we tested the efficacy of genotoxicological endpoints as one of the lines of evidence (LoEs) in the *in situ* assessment of pollution effects in freshwater ecosystems using *A. alburnus* as a bioindicator species. Additional LoEs used in the study were: component-based methods relying on water quality data, effect-based methods employing *in vitro* genotoxicological analyses of water extracts and field-derived species inventories to assess and indicate ecological status/potential based on national and JDS4 data. For the JDS5, scheduled for summer 2024, we have developed a program combining active and passive biomonitoring approaches to provide additional insight into the efficiency of the methodologies used.

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## O-3
## First regional reference database of northern Adriatic diatom transcriptomes

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Marine microbial communities form the basis for the functioning of marine ecosystems and the conservation of biodiversity. With the application of metagenomics and metatranscriptomics in marine environmental studies, significant progress has been made in analysing the functioning of microbial communities as a whole. These molecular techniques are highly dependent on reliable, well-characterised, comprehensive and taxonomically diverse sequenced reference transcriptomes of microbial organisms. To address this need, a set of 12 individual transcriptome assemblies derived from 6 representative diatom species from the northern Adriatic Sea grown under 2 environmentally relevant growth conditions (eutrophic *vs.* phosphate-deprived) was created. After filtering the reads and assembly, an average number of 64,932 transcripts per assembly was obtained, of which an average of 8,856 were assigned to functionally known proteins. Of all assigned transcripts, an average of 6,483 proteins were taxonomically assigned to diatoms (*Bacillariophyta*). A higher number of assigned proteins was detected in the transcriptome assemblies of diatoms grown under the F/2 condition and approximately 50 % of the mapped proteins were shared between the two growth conditions. The resulting diatom reference database for the northern Adriatic, focussing on the response to nutrient limitation as characteristic for the region and predicted for the future world oceans, provides a valuable resource for analysing environmental metatranscriptome and metagenome data. At the same time, the established diatom reference database for the northern Adriatic is a reference and supporting tool for transcriptomics of further diatom and diatom-containing communities.

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## O-4
## Assessment of domoic acid-induced genotoxicity and oxidative stress in non-target HepG2 liver cells

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Domoic acid (DA), a potent marine neurotoxin produced by the diatom *Pseudo-nitzschia*, is primarily recognized for its toxic effects on marine mammals and fish. However, its impact on human non-target cells remains inadequately understood. In this study, we investigated the cytotoxic and genotoxic effects of DA on human hepatocellular carcinoma (HepG2) cells. HepG2 cells were exposed to graded concentrations of DA (0.001–10 μg/mL) for different periods (4, 24, and 72 h) and the effects on cell viability, proliferation and induction of DNA damage were investigated. Our findings indicate that DA exposure at the concentrations tested did not significantly alter the viability, proliferation, or cell cycle dynamics of HepG2 cells. DA did not induce DNA double-strand breaks as assessed by the γ-H2AX assay, however, it caused a significant dose- and time-dependent increase in DNA damage, manifested as DNA single-strand breaks or alkali-labile sites, as evaluated by the alkaline comet assay. Additionally, elevated malondialdehyde levels after DA treatment were indicative of oxidative damage to lipids. Overall, our results suggest that the toxic effects of DA on non-target HepG2 cells are minimal and caused by oxidative stress. However, the exact action of DA is unknown and thus further studies are warranted to comprehensively elucidate the mechanisms underlying DA toxicity, in particular concerning chronic exposure scenarios, and its potential implications for non-target human cells. This is especially important in light of today's climate change, which favours harmful algal blooms and the growth of DA producers.

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# The Frits Sobels Award Lecture

# Innovative approaches in nanotoxicology: the future of risk assessment

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Nanotoxicology has been around for two decades, yet research into the safety of nanomaterials remains challenging. It depends on an intimate knowledge of the physicochemical properties of nanomaterials, and of the interactions with their microenvironment. Nano-specific features, including properties in exposure medium and complex matrices, state of agglomeration, and cellular uptake should be considered. Existing OECD Test Guidelines (TGs) need to be adapted for application to nanomaterials, and new methods developed, standardised and pre-validated for future TGs. Fast and efficient hazard assessment and an understanding of the complexity of potential nanomaterial risks require a shift from traditional risk assessment to more complex and holistic approaches. The need for high-quality FAIR data (findable, accessible, interoperable and reuseable) and for large publicly available databases has been recognised. To support the harmonisation of data reporting, over 75 data entry templates (including for genotoxicity tests) have been developed for reporting experimental data. The harmonised templates improve the reliability of interlaboratory comparisons, data reuse, and meta-analyses and can facilitate the safety evaluation and regulation process for nanomaterials. The field of nanotoxicology is advancing with the development of New Approach Methodologies (NAMs) such as advanced lung, liver and other more complex organ- and tissue-specific models and high-throughput methods that better reflect and simulate the relevant physiological processes in the human body. They represent a paradigm shift in toxicology and genotoxicity assessment, offering efficient, cost-effective, and ethical alternatives to traditional animal testing methods. NAMs will play an important role in the next generation of risk assessment, revolutionising risk assessment across various regulatory domains, and providing innovative ways to evaluate the potential hazards associated with chemicals including advanced materials, nanomaterials, nano- and micro-plastics.

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**Mária Dušinská**
Fritz Sobels Award winner 2024

**Award committee**

*In a close-run competition, Maria was voted the 2024 Frits Sobels Award winner. With her extensive publication record and hugely successful acheivements within the EU FP7 and Horizon funding frameworks, Maria thoroughly deserves this presitigous award.*

# The Early Career Award Lecture

# *C. elegans* as a model for understanding genomic integrity

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*Caenorhabditis elegans* (*C. elegans*) is a well-established multicellular model organism in DNA repair research owing that most DNA repair pathways found in bacteria, yeast, mammals, and humans are highly conserved in the nematode and genetic manipulations are easily conducted in the worm. However, methods for specifically detecting DNA damage are scarce. Classical genotoxicity testing still relies mainly on expensive and time-consuming animal experiments or cell culture systems with limited transferability, while meaningful multicellular model organisms in the niche between *in vitro* and *in vivo* are not yet routinely used. By developing and utilizing novel methods for assessing DNA damage (alkaline unwinding assay, 8-oxo-guanine quantification) in *C. elegans* we provide reliable endpoints for investigating the genomic integrity in a multicellular organism. In combination with investigations of the DNA damage response (quantification of poly(ADP)ribosylation) and DNA repair (gene expression studies, sensitivity assessment of DNA repair "deletion mutants/knock-downs"), as well as endpoints of possible underlying mechanisms for genotoxicity (*e.g.* oxidative stress), we can assemble a complete model system for genotoxicity testing from (oxidative) stress endpoints, to activation of the DNA damage response and DNA repair to measuring the DNA damage itself, thus creating a modern approach for genotoxicity testing. The methods have been tested for efficiency, reliability, and sensitivity with known and well-established positive controls. Current investigations focus on applying these methods for substance testing – from metals to plastics, to understand their impact on genomic integrity, neurodegeneration, and ageing.

**Merle M. Nicolai**
Early Career Award winner 2024

**Award committee**

*Merle is an outstanding scientist, and the committee was unanimous in their decision to award the 2024 Early Career Award to her. She made a significant contribution to this "wormy business", has had post doctorial positions at numerous leading laboratories within our field, received the GUM Young Scientist Award, and has contributed greatly to the New Investigators, EEMGS society, as well as to GUM.*

# Ageing

*Invited lecture*

# IL-4
# Effects of environmental factors on glycan biomarkers predicting age-related diseases

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Glycans are the ultimate layer of molecular complexity that modifies proteins with complex chemical structures integrating genetic, epigenetic, and environmental information. Alternative glycosylation (attaching different glycans to the same glycosylation site on a protein) modulates protein function and in this way actively participates in the transition from health to disease. Hundreds of genes are involved in the complex pathway of glycan biosynthesis and glycome composition is significantly heritable, but also strongly affected by current and past environment. By analysing over 200,000 individuals, we demonstrated that glycans have significant biomarker potential in predicting the risk of different age-related diseases, including cardiovascular diseases and diabetes. However, since glycans biomarkers are under significant environmental influence, they also change in the response to different pharmacological and lifestyle interventions aimed at decreasing the disease risk. Therefore, glycans have a great potential for the development of biomarkers for the personalisation of preventive healthcare and the first such biomarkers, like the GlycanAge biomarker of biological age, are already commercially available.

*Invited lecture*

# IL-5
# Effects of estrogen on immunoglobulin G glycosylation and biological ageing: mapping the downstream signalling pathway

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The immunologic potency of immunoglobulin G (IgG) is modulated by glycosylation, but mechanisms regulating this process are still insufficiently understood. Several studies suggested the role of sex hormones in differences in IgG glycans between women and men, most prominently with respect to galactose. In addition, alternative glycosylation of IgG participates in biological ageing. In women, the sharp and most notable change coincides with the perimenopausal period. We investigated the effect of estrogen on IgG glycosylation by analysing IgG and total serum glycomes in 36 healthy premenopausal women enrolled in a randomized controlled trial of the gonadotropin-releasing hormone analogue ($GnRH_{AG}$) leuprolide acetate to lower gonadal steroids to postmenopausal levels and then randomized to transdermal placebo or estradiol ($E_2$) patch. While the induced suppression of gonadal hormones resulted in significant changes in the IgG glycome composition, most notably galactosylation and sialylation, supplementation with Estradiol ($E_2$) was sufficient to prevent or reverse these changes. Depletion of $E_2$ primarily affected B cell glycosylation, but not liver glycosylation. In order to unravel downstream molecular pathways linking estrogen and IgG glycosylation we conducted a series of experiments *in vitro* using the CRISPR/dCas9 molecular tools. Previously identified GWAS hits for IgG galactosylation and sialylation, *RUNX1*, *RUNX3*, *SPINK4*, and *ELL2,* were functionally validated in the FreeStyle 293-F cell-based transient system expressing IgG antibodies with stably integrated dCas9-VPR and dCas9-KRAB cassettes for gene up- and down-regulation. Following gene manipulations, secreted IgG was analysed for glycosylation using HILIC-UPLC and MS. The results established estrogen as an *in vivo* modulator of IgG galactosylation in women.

# O-5
# The role of DNA repair in ageing and neurodegeneration

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Genomic instability and mitochondrial dysfunction are the major risk factors for ageing and neurodegeneration. However, the underlying mechanisms are poorly understood. Here, we show that the Base Excision Repair pathway (BER) causes genomic stress, which promotes age-dependent neurodegeneration. A physiological level of BER DNA glycosylase causes mitochondrial and nuclear genomic instability, which promotes the degeneration of dopaminergic neurones upon ageing. BER deficiency prevents *a*-synuclein-induced neurotoxicity and maintains neuronal function with age. This apparent paradox is caused by the modulation of mitochondrial transcription which activates mitohormesis. The dependence of neuroprotection on mitochondrial transcription emphasises the interplay of BER and transcription regulation during physiological ageing. Finally, whole-exome sequencing of genomic DNA from idiopathic PD patients (Parkinson's disease) suggests that BER might influence susceptibility to PD in humans. Base excision repair causes age-dependent accumulation of single-stranded DNA breaks that contribute to Parkinson's disease pathology.

[1] SenGupta et al. 2021. https://doi.org/10.1016/j.celrep.2021.109668

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## O-6
## Trace elements, ageing, and genomic instability in mice

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As essential micronutrients, trace elements are involved in various cellular processes. One of these is the maintenance of genomic stability, which is hypothesised to be impacted by trace element disbalances as well as during ageing. In this project, multiple assays were established for the evaluation of different parameters of genomic stability in mice. These include the determination of DNA strand breaks and oxidative base lesions, incision activity, characterising the initial step of base excision repair, as well as DNA ligation efficiency, presenting the final step of almost every DNA repair pathway. Animal experiments with mice of different age groups fed various degrees of suboptimal trace element supply have been carried out. The above-mentioned parameters of genomic stability and other endpoints have been studied in different murine tissues, including the liver, cortex, and cerebellum. Those results shed light on the impact of trace elements and ageing on genomic instability.

# Environmental Toxicants

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*Invited lecture*

# IL-6
# Data gaps in the risk assessment of mycotoxins

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Species of the genus *Alternaria* are known to produce a broad spectrum of structurally diverse secondary metabolites with toxic properties. Occurrence data focus so far on Alternaria toxins for which reference compounds are commercially available, like alternariol (AOH), its monomethyl ether (AME), tenuazonic acid (TeA) and tentoxin (TEN). Little is known so far on the occurrence of perylene quinones. Several members of this toxin class have been already identified as highly genotoxic, but reliable occurrence data are still missing. *Alternaria* toxins are often found in mixtures. The presence of TeA is a clear indication of infestion but does not allow us to conclude on the presence of toxic perylene quinones. Cultivation of an *Alternaria alternata* on rice resulted in a toxin mixture with immunosuppressive and antiestrogenic properties. Toxicity-guided fraction was applied to identify potential contributing factors. The assessment of the immunomodulatory effects, performed by applying the NF-kB reporter gene assay in THP-1 Lucia monocytes, revealed the limited contribution of AOH to the effects exerted by the complex mixture. TeA showed no effect on the NF-kB pathway up to 250 μM, whereas perylene quinones were identified as suppressing the pathway activation at concentrations ≥1 μM. The evaluation of the antiestrogenic effects, performed in Ishikawa cells by applying the alkaline phosphatase assay, revealed the ability of selected perylene quinones to suppress the enzyme activity in the low micromolar range. Given the potential risk of detrimental impacts stemming from alterations in endocrine and systemic immune responses by the investigated mycotoxins, data on the occurrence of these perylene quinones in food and feed are urgently needed. Further studies are needed to elucidate their underlying mechanisms of action and comprehensively evaluate the health risks posed by these toxins.

# O-7
# Investigation of the genotoxicity of glyphosate using cell-based assays

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Our genome is constantly challenged by intracellular and environmental factors that can damage DNA and cause cancer. Glyphosate is the main component of a widely used herbicide called Round Up and acts as a potent inhibitor of protein synthesis. Even though glyphosate has been widely used for the last forty years, it has been classified as a potential carcinogen by the IARC and its use in agriculture has been associated with the development of non-Hodgkin lymphoma. To this end, we set out to investigate the genetic toxicity of this chemical using *in vitro* cell-based assays. Human osteosarcoma (U2OS) cells were cultured and treated with different concentrations of glyphosate. They were then analysed by immunofluorescence using an antibody against γH2AX, an established marker for DNA damage. A significant proportion of the treated cells showed a large number of foci for γH2AX, indicating a mild genotoxic effect of glyphosate at concentrations larger than 1500 μM. The compound was shown not to be cytotoxic at all concentrations tested. To further investigate the effect of glyphosate on U2OS cells, a micronucleus test was performed. This assay showed an effect of glyphosate on the induction of genomic instability, as the number of micronucleated cells in glyphosate treated samples was significantly higher than that in the control samples. Moreover, glyphosate-treated cells showed a significant number of unstable anaphases, indicating an unknown mechanism of action that affects genomic stability and needs to be further investigated. Taken together, our data reveal the genotoxic activity of glyphosate using cell-based assays, which may require a reconsideration of the use of this chemical in agriculture.

# O-8
# Genotoxicity of 2-chloroethanol *in vitro*

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A number of food items were recently withdrawn from the market in the European Union due to the presence of ethylene oxide residues in sesame seeds, spices, or food additives. In some non-European countries, ethylene oxide is used as a fumigant to sterilise food for protection against microbial contamination. Ethylene oxide degrades rapidly into a 2-chloroethanol metabolite, which is mainly found in contaminated food. As ethylene oxide is classified as a carcinogen without a threshold value, there is no intake level without a health risk. The maximum residue levels, established by regulatory authorities, refer to the sum of ethylene oxide and 2-chloroethanol. As the data on 2-chloroethanol is unclear and contradictory, regulatory risk assessment authorities assume that 2-chloroethanol should be treated in the same way as ethylene oxide, since it may pose a comparable health risk. The aim of this study is to fill the data gap with regard to the genotoxicity of 2-chloroethanol. To this end, the human cell lines HeLa and TK-6 were exposed to 2-chloroethanol and, for direct comparison, to ethylene oxide. In order to derive relevant concentrations for subsequent genotoxicity experiments, the cytotoxicity of the two substances was investigated after 4 and 24 hours of treatment using the MTT assay and counting the number of cells. Genotoxicity was assessed using the alkaline comet assay and the cytokinesis-block micronucleus test. 2-chloroethanol showed cytotoxic effects at very high concentrations (≥80 mM). Genotoxic effects, as indicated by a concentration-dependent increase in the incidence of micronuclei and DNA strand breaks, only occurred at cytotoxic concentrations after 24 hours of treatment. No genotoxic effects were observed at non-cytotoxic concentrations. Overall, 2-chloroethanol should not be classified as genotoxic based on the currently available data.

## O-10
# Threshold concentrations for BPDE-induced cell death are characterised by altered DNA damage signalling and associated with unrepaired double-strand breaks

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The environmental carcinogen benzo(a)pyrene (B[a]P) is a product of incomplete combustion and therefore can be found in tobacco smoke, exhaust fumes and at barbecues. B[a]P is metabolised to its activated form benzo(a)pyrene-9,10-diol-7,8-epoxide (BPDE) in the liver. So far, we have shown that exposure of human VH10tert fibroblasts to BPDE causes dose-dependent p53 responses. Non-toxic concentrations induce p53-dependent transcriptional activation of nucleotide excision repair as well as p53/p21-dependent induction of senescence, leading to cellular survival. In contrast, at high BPDE concentrations, p53-mediated cell death *via* apoptosis is activated, suggesting the existence of specific thresholds at which the p53-dependent pro-survival signalling turns into p53-dependent pro-death signalling. The initial activation of the DNA damage response does not differ between toxic and non-toxic BPDE concentrations. At later time points, however, toxic concentrations cause complex changes in the DNA damage response, leading to cell death. In summary, protective ATR-CHK1-p53$^{Ser15}$-p21-dependent signalling changes upon toxic concentrations into ATM-CHK2-p53$^{Ser46}$-NOXA-dependent signalling, mediating the induction of apoptosis. Preliminary data further suggest that this threshold is caused by unrepaired DNA double-strand breaks. Nevertheless, contrary to cell death induction, no threshold was detected with respect to DSB induction and γH2AX formation.

*Invited lecture*

# IL-7
# Gastric cancer: potential carcinogens, biomarkers, chemoprevention and drug repurposing

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Gastric cancer is the 5th most common malignant disease and the 4th leading cause of cancer-related mortality worldwide. Potential carcinogens include bacteria (particularly *Helicobacter pylori* and *Streptococcus anginosus*), tobacco, red meat, and smoked food. Gastric intestinal metaplasia (GIM) is a precancerous lesion for gastric adenocarcinoma (GA) which comprises 95% of the total number of malignancies in the stomach. Biomarkers associated with the progression from GIM to GA could provide valuable insights into identifying a subset of patients with a much higher risk of GA. By multi-bioinformatics in patients, we have identified potential biomarkers for GIM including 110 canonical pathways and constructed a biomarker network consisting of 865 proteins with 23 hub proteins. Accordingly, more than 15 potential chemopreventive compounds (including repurposed drugs) have been identified. The cardiopreventive effect of dietary phenethyl isothiocyanate was approved *in vitro* with human GA cell lines and *in vivo* with mouse models. Drug repurposing was focused on the inhibition of GA tumorigenesis by targeting acetylcholine (ACh) muscarinic receptor-3 receptor (M3R)-mediated WNT/β-catenin signalling in the stem cells with vagotomy, botulinum toxin A (SNAP25 inhibitor) injection, or M3R blockade. The *in vivo* results showed that the denervation and/or blockade of nerve growth factor (NGF) signalling inhibited GA tumorigenesis in M3R-dependent manner through suppression of WNT/$\beta$-catenin-YAP function, suggesting that the feedforward ACh-NGF axis stimulates GA tumorigenesis and might offer a compelling target for GA prevention and treatment. Inhibition of nerve-cancer metabolism by injection of botulinum toxin-A with systemic administration of RAD001 (mTOR pathway inhibitor) and CPI-613 (PDP1/*a*-KGDH inhibitor) reversed the metabolic reprogramming and increased overall survival *in vivo*, pointing to the importance of neural signalling in modulating GA tumour metabolism and providing a proof-of-concept for clinical validation in the future. Ivermectin inhibited GA tumorigenesis through WNT/$\beta$-catenin signalling pathway, cell proliferation pathway and cell death signalling pathway *in vitro* and *in vivo*, suggesting a potential repurposing drug for GA prevention and treatment. Taken together, these studies have contributed to the development of proof-of-concept of GA prevention, early diagnosis, and treatments.

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# O-11
# Proteomics-based system modelling for studying pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer with the lowest 5-year survival rate of all cancers due to late diagnosis and lack of effective treatment. One of the challenges of developing molecular diagnosis and molecular targeted therapy for PDAC is the "mismatch" between research models and patients. This study aimed to identify common proteins and signalling pathways among different research models and patients. Mouse PDAC cells (derived from a genetically engineered mouse model with KrasG12D/Pdx1-Cre), PDAC orthotopically tumours in mice, mouse PDAC spheroids, mouse pancreatic organoids, mouse PDAC organoids, normal mouse pancreatic tissues, human PDAC organoids, and human PDAC tumour tissues after chemotherapy were included. Mass spectrometry-based proteomics, RStudio, Cytoscape and ingenuity pathways analysis, which also incorporated human PDAC transcriptomic data, were used. In the comparison of PDAC models and patients, shared proteins were identified as follows: 60 % between the mouse PDAC cells and the mouse PDAC spheroids, 78% between the mouse PDAC cells and the mouse PDAC tissue, 56% between the mouse PDAC spheroids and the mouse PDAC tissue, 66% between the mouse PDAC cells and the mouse PDAC organoids, 54% between the mouse PDAC spheroids and the mouse PDAC organoids, 38% between the mouse PDAC and the human PDAC tissues, 31% between the human PDAC tumour and the human PDAC organoids, and 6 % between the mouse and the human PDAC organoids. A total of 1,367 common proteins were identified within all the PDAC models. There was a correlation in terms of z-score regarding signalling pathways between the mouse PDAC and human PDAC. In patients, central hub proteins (14-3-3 protein zeta/delta, $\beta$-catenin and SRC) were dysregulated after chemotherapy. The system modelling identified the common proteins among the research models and patients, suggesting the potential translational targets of PDAC.

# O-12
# Mutational signature of dietary acrylamide/glycidamide in renal cancer genomes

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Acrylamide (ACR) is a probable human and established rodent carcinogen (IARC Group-2A) that forms in heated starchy foods and tobacco smoke. Epidemiological studies have suggested an elevated non-significant risk of clear-cell renal cell carcinoma (ccRCC) associated with dietary ACR intake. Next, our previous findings showed that 70% of ccRCC from the ICGC PCAWG collection carried the mutational signature SBS_GA linked to ACR's reactive metabolite glycidamide (GA)[1]. Here we aimed to establish a molecular link between ACR intake and ccRCC by analysing mutational signatures in a unique tumour set with well-documented dietary ACR exposure history. Within the prospective Netherlands Cohort Study on Diet and Cancer involving 120,852 subjects, 654 cases were diagnosed with RCC. For 487 cases, FFPE tumour tissue was available after 22.3 years of follow-up. From this population, we selected a set of never-smokers with ccRCC and a history of high versus low dietary ACR intake (10 cases/group), as assessed by a food frequency questionnaire reflecting chemical analysis of relevant Dutch foods. Genomic DNAs from tumour and non-tumour FFPE tissue pairs were whole-genome sequenced and somatic mutations were analysed by the SigProfilerExtractor tool to identify mutational signatures. The Mutational Signature Analysis (MSA) tool was used for optimised per-sample signature assignment. The NLCS ccRCC genomes harboured COSMIC signatures SBS1, SBS5, and SBS40 in all samples, in proportions irrespective of the ACR exposure groups. In contrast, the MSA-based per-sample signature assignment showed relative enrichment of the SBS_GA mutational signature in the high-exposure (6 of 10 [60%] cases) compared to the low-exposure group (3 of 10 [30%] cases). Our results indicate for the first time ACR-related genome-scale mutagenesis represented by the SBS_GA signature in ccRCC with documented dietary ACR exposure history. This molecular evidence may have important implications for the reduction of human exposure to ACR and related cancer prevention measures.

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[1] Zhivagui et al. 2019. https://doi.org/10.1101/gr.242453.118

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# O-13
# Mutational signatures of tobacco-specific nitrosamines NNN and NNK in cells, animals and humans

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Tobacco use is linked to about 20 cancer types and 25% of cancer deaths, with tobacco smoke containing numerous mutagenic chemicals such as polycyclic aromatic hydrocarbons (PAH). However, the mutagenicity of many chemicals in tobacco products remains underexplored. The tobacco-specific nitrosamines NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNN (*N'*-nitrosonornicotine), both IARC Group 1 carcinogens, form pre-mutagenic pyridyloxobutyl (POB) DNA adducts, yet little is known about the associated genomic mutation landscape(s). We report POB-induced mutational signatures in mammalian cell lines and rat tumours, based on standard and error-corrected next-generation sequencing approaches, and examine their role in human cancer. POB signatures generated in experimental exposure models share profiles dominated by T>N and C>T alterations, with transcription strand asymmetry indicative of damage on thymidines and cytidines. The T>N-dominated mutation profiles can be explained by $O^2$-POB-dT adducts, detected in the exposed cells, while the C>T mutations may correspond to the known adducts 3-POB-dC or $N^4$-POB-dC. Stringent screening of 2,780 ICGC PCAWG cancer genomes revealed the presence of a genome-scale POB signature in ~180 tumours from sites predominantly linked to tobacco smoking, that were distinct from cancer sites typically linked to known smoking-related signatures SBS4 and SBS92. These included haematological malignancies and cancers of the kidney, breast, prostate and pancreas, among other sites. In contrast and as expected, tobacco-smoking signatures SBS4 and SBS92 were jointly or individually predominant in cancers of the lung, liver, head-and-neck or bladder. The study is the first report showing the presence of a tobacco-specific nitrosamine mutational signature in human cancers. It provides relevant information for the prevention of tobacco-associated cancers, by adding valuable insight into the contribution of individual chemicals to the mutation landscapes associated with tobacco consumption, and by raising important questions regarding the potential role of certain tobacco smoke chemicals in tobacco-linked cancer types lacking SBS4 and SBS92.

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# O-14
# Oral tongue cancer infectome in patients with no identified risk factors

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The incidence of oral tongue squamous cell carcinoma (OTSCC) is rising, including in individuals with no identified risk factors (NIRF) who are nonsmokers, nondrinkers and human papillomavirus (HPV) negative. To address possible mechanisms of NIRF OTSCC formation, we combined *in silico* analysis of large multi-omics data, microbial profiling in dedicated OTSCC collections and functional validation assays. We comprehensively analysed public oral cancer multi-omics data for relevant mutational signatures, gene expression and methylome profiles, identifying pathways associated with antiviral and antibacterial responses operative in the NIRF OTSCC subjects (PMID:38168303). This provided a basis for viral and bacterial infectome profiling of ~100 NIRF and nonNIRF OTSCC samples using LUMINEX-based high throughput virus detection and bacterial 16S rRNA sequencing. To assess the host cell-microbial interactions, spatial histopathology analysis was used to map gram-negative bacteria, markers of immune (CD8, CD66b), structural (Pan-cytokeratin) and antibacterial responses (S100A7 antimicrobial peptide). NIRF OTSCCs exhibited keratinization and antimicrobial responses, alongside the presence of several bacteria and viruses (*Porphyromonas gingivalis* in 95% of the screened OTSCC samples, *Fusobacterium nucleatum* in 27%, Epstein-Barr virus in 38%, HPV-16 in 21%, and human herpesvirus-6B (HHV-6B) in 12% of samples). We observed mutual exclusivity of HPV-16 and *F. nucleatum* in the OTSCC samples, and the presence of oral gram-negative bacteria within tumour mass cells, on the membranes of nested tumour cells, in lamina propria's lymphocytes and in striated muscle cells. The analysis of the immune microenvironment is underway. Next, a 3D oral epithelium, with and without ethanol exposures, was employed to test the invasion potential of selected bacterial candidate strains (*P. gingivalis, F. nucleatum, Neisseria mucosa,* and *Rothia mucilaginosa).* Our integrated analysis delineates the viral and bacterial infectome of NIRF OTSCC, providing novel insights into OTSCC formation and host immune responses, and suggesting targetable pathogens and biomarkers for oncological treatment.

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# O-15
# Changes in mitochondrial DNA in colorectal cancer patients

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Mitochondrial DNA (mtDNA) changes have been observed in multiple cancers, including colorectal cancer (CRC). They occur more frequently than alterations in nuclear DNA due to factors such as proximity to reactive oxygen species or fewer DNA repair pathways. Our study's primary hypothesis was that mitochondrial dysfunctions resulting from mtDNA changes play a role in CRC carcinogenesis and could serve as potential CRC biomarkers. To test this hypothesis, we measured mtDNA damage, mtDNA content, and the expression of selected DNA repair genes in both tumour and adjacent non-malignant mucosa. Initially, we conducted the study on 7 patients by RNA sequencing and qPCR (a Pilot part), with subsequent validation on 50 patients by qPCR (a Validation part). Our findings revealed that adjacent mucosa had more mtDNA damage compared to tumour tissue in both the Pilot set (10.267±0.40 and 7.435±0.43, respectively, $p=0.047$) and Validation set (10.549±0.97 and 7.975±0.79, respectively, $p\leq0.0001$). Moreover, most of the DNA repair genes that correlated with mtDNA damage were more expressed in tumour tissue ($p\leq0.0001$). We identified a significant association between low expression of the *BRCA1* gene ($p=0.029$) in tumours and improved patient survival. MtDNA content showed no significant difference between tumour and adjacent mucosa in both the Pilot set (6.858±1.00 and 6.235±0.61, respectively, $p=0.071$) and Validation set (7.125±1.02 and 7.072±0.62, respectively, $p=0.728$). Overall, our results suggest that tumours display less mtDNA damage than adjacent mucosa, with the upregulation of DNA repair genes in tumours potentially contributing to efficient mtDNA damage repair. In conclusion, we demonstrated that mtDNA changes, therefore, play a role in CRC carcinogenesis, so our hypothesis was confirmed. Further research is warranted to assess whether these changes are a cause or a consequence of CRC.

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# New Approach Methodologies

## O-16
## Impact of the ICH S2(R1) guideline on the frequency of irrelevant positive *in vitro* mammalian cell assays

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The present work investigates the impact of the ICH S2 (R1) guideline revision on genotoxicity on the frequency of irrelevant positive *in vitro* mammalian cell assays and consequently the number of follow-up tests needed. Data on genotoxicity tests submitted between 1998 and 2020 in centralised procedures available at the Federal Institute for Drugs and Medical Devices (BfArM) were analysed regarding their frequency and outcome of the *in vitro* and *in vivo* assays. A comparison between the guideline versions was made in regard to the frequency of positive *in vitro* mammalian cell tests, the overall number of *in vivo* tests and the number of additional *in vivo* tests to follow up positive *in vitro* results in the mammalian cell assays. The data analysed included 986 genotoxicity tests comprising of 578 *in vitro* mammalian cell tests and 408 *in vivo* tests conducted for 275 drug products with 413 different compounds tested. Tests for fifty-six compounds (13.56%) of the data set were conducted under the revised guideline version ICH S2(R1), whereas tests for 357 compounds (86.44%) were tested according to the old guidelines ICH S2A and S2B. ICH S2(R1) option 1 of the standard battery was used for all compounds. Within the data set analysed, no statistically significant decrease in positive *in vitro* mammalian cell assays under the revised guideline was detectable. Additionally, no significant differences in the number of follow-up tests or changes in *in vivo* testing were detected between the two guideline versions. Due to the small data set for tests according to the revised guideline ICH S2(R1), in this analysis, only preliminary assertions can be made. Nevertheless, no decrease in the number of irrelevant positive *in vitro* mammalian cell tests was detectable thus no significant effects resulting from the guideline revision could be identified yet.

## O-17
## Application of modifying agents to a multiplexed DNA damage assay provides mechanistic information on genotoxicity and molecular targets

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The base MultiFlow system enables the categorisation of compounds with regard to activity profiles for clastogenicity, aneugenicity or non-genotoxicity. In an effort to go beyond this genotoxicity mode of action (MoA) assessment, we incorporated several DNA damage and repair modifying conditions to selectively alter biomarker response patterns and thereby reveal additional information on clastogenic mechanisms and molecular targets. For this proof-of-concept study, we exposed TK6 cells to 30 reference clastogens for 24 continuous hours. Other cells were pre-treated with the modifying agents: olaparib (PARP inhibitor), MK-8776 (CHK1 inhibitor), NU7441 (DNA-PK inhibitor), or a cocktail of reactive oxygen species (ROS) scavengers and then exposed to clastogens for 24 continuous hours. Finally, other cells were exposed to the clastogens for 4 hours, washed and then allowed to recover for an additional 20 hours. Flow cytometric analyses were performed to measure γH2AX as a marker of DNA double-strand breaks and p53 as a marker of genotoxic stress. All clastogens induced elevations in γH2AX and p53 and showed varied patterns of response following washout or in the presence of the modifying agents. Whereas alkylating agents showed persistence of γH2AX at 24 hrs after washout, non-alkylators exhibited near-baseline values. ROS generators showed greatly diminished responses in the presence of the scavenger cocktail. Treatment in the presence of DNA repair pathway inhibitors provided additional mechanistic insights. The resulting biomarker response data were processed with Benchmark Dose software, and these potency metrics were used in a decision-tree framework for categorising compounds into like-performing classes. Based on this approach, we were able to correctly categorise the vast majority of clastogens into a priori-established classifications.

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## O-18
## Dynamic alterations of H3K27me3 and its role in sensitivity to physiological adaptation at elevated temperature: lesson from *Caenorhabditis elegans*

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This study aimed to assess the impact of elevated temperatures on the physiological adaptation of animals and the role of epigenetics in this process. *Caenorhabditis elegans* (*C. elegans*) was employed as the model organism under two temperature conditions: 20 °C (optimal for *C. elegans*) and 23 °C (higher temperature). The reproductive capacity (brood size) exhibited adaptation from the F5-F6 generation onwards, following significant changes in earlier generations exposed to 23 °C. Concurrently, histone modification emerged as a key mechanism of temperature sensitivity, demonstrated by evaluating germline GFP fluorescence in the let-858:gfp strain. Subsequently, up-regulation of reproduction was observed in the mes-2 (H3K27 methyltransferase) mutant, persisting in the following generation (F0-F1), while a significant decrease in reproduction occurred in the utx-1 (H3K27 demethylase) mutant exposed to higher temperatures (23 °C). Conversely, reproduction in mutants related to other histone methylation processes (met-1, H3K36 trimethylase; met-2, H3K9 trimethylase; set-2, H3K4 methyltransferase; spr-5, H3K4me2 demethylase) did not exhibit significant alterations due to exposure to elevated temperatures (23 °C). Among the mono/di/tri-methylation of the H3K27 mark, only H3K27me3 protein demonstrated differential expression (demethylation) due to exposure to elevated temperatures. Additionally, histone demethylase (utx-1) enzyme activity decreased significantly. The results highlight that exposure to higher temperatures induces H3K27me3 demethylation, playing a crucial role in the elevated temperature sensitivity and physiological adaptation of *C. elegans*.

## O-19
## Detecting a new class of carcinogens by testing their ability to activate endogenous L1 elements

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Cancer is the second leading cause of death in the European Union after cardiovascular diseases, currently taking 1.2 million lives a year. The incidence of cancer has increased in recent years in the EU among other factors due to the increased risk from chemicals in the environment. At the same time, the importance of cancer prevention is also growing, which includes reducing human exposure to carcinogenic chemicals. However, environmental carcinogens are difficult to identify, and exposure to already known carcinogens is often not confirmed for individual cancer patients, suggesting major gaps in our knowledge on the initiation of cancer. Genotoxic chemicals induce DNA damage resulting in mutations in somatic cells, the accumulation of which in turn can lead to malignant transformation. In recent years it has been evidenced that genotoxic effects can even be mediated through the activation of endogenous L1 (LINE1) retrotransposons. Instead of point mutations, genotoxic lesions induced by L1 elements activity are thousands of base-pair-length insertions and genome rearrangements, and L1 activity is present in more than 50% of cancers while it is mostly undetectable in normal somatic tissues. In line with this, several cancer driver mutations have by now been identified in different tumour types that were caused by novel somatic L1 integration events. This also implies that chemicals that act on retrotransposon activity may be potentially carcinogenic and should therefore be screened in the standard toxicological screening palette. However, current standard screening methods cannot detect such types of carcinogens. Our aim is to provide a tool to detect genotoxins acting by this unconventional non-DNA-reactive mechanism, by inducing somatic L1 retrotransposition. Any chemical coming into contact with humans in the environment may be targeted for testing. Therefore, the social benefits of the implementation of such a toxicological assay in terms of cancer prevention would be immense.

# O-20
# Reference chemicals mode-of-action assessed *in vitro* by HCS micronucleus assay after acute and subacute exposures

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Genotoxicity of chemical compounds is of high concern for human, animal and environmental health. The micronucleus (MN) assay is well recognised to assess genotoxicity and classify clastogens or aneugens compounds. MN also has great potential for automation and allows measurement of both *in vitro* and *in vivo* samples. While *in vivo* assays are useful to characterise the effects of metabolites, new hepatic cell models may help and reduce the number of animals in toxicity tests. Here we bring innovation in the field of MN assay by developing an *in vitro* MN assay, associating HepaRP (metabolically competent HepaRG-derived hepatic cell line) and high-content screening after acute or sub-acute exposures to reference genotoxic compounds. We revisited ten ECVAM-listed genotoxic compounds, among them clastogens (aflatoxin B1 and benzo(a)pyrene known to be pro-genotoxic, *i.e.* giving rise to genotoxic metabolites) and aneugens (Taxol and Nocodazole). After acute exposure (24 h of exposure), we analysed different endpoints (MN formation, apoptotic cells, mitotic index, colocalization of γH2AX and 53BP1 to characterise the persistence or repair of DNA damage) at different timepoints (until 3 days of release). We found significant MN increase for clastogens at low and subtoxic doses (0.1 µM) on differentiated HepaRP cells, whereas no significant or low significant results were found using differentiated HepaRG cells, certainly due to the high hepatocyte proportion in differentiated HepaRP cells. The kinetics of MN formation were different for aneugens. Sub-acute analysis (five repeated 24 h of exposures to 0.02 µM followed by 3 days of release) also showed the importance of HepaRP metabolim. To conclude, this approach shows that HepaRP cells are useful for genotoxic studies and assess subacute/subchronic exposures at low subtoxic doses, which are challenging issues to evaluate *in vitro*. It offers an alternative to *in vivo* experiments, achieving a higher throughput and combining different endpoints (cell viability, persistence or repair of DNA damage, MN).

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# O-21
# Assessment of biomarkers in exhaled breath condensate of workers with occupational lung disease

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Inhalation is an important route wherethrough occupational exposure can reach and potentially affect pulmonary function. In this context, exhaled breath condensate (EBC) is a promising non-invasive matrix to assess lung-related biomarkers mainly coming from the lower respiratory tract. To assess the feasibility of EBC, in twelve healthy participants, IFNγ, IL-1β, 2, 4, 6, 8, 10, 12p70, and 13, TNF-α, MMP-1, 2, 7, and 9, VEGFR-1/Flt-1, E- and P-selectin, α-amylase activity (not in plasma) in addition to untargeted proteomics (only EBC) were measured twice six months apart in EBC and plasma samples. A-amylase activity in EBC was more than 10-fold lower than in saliva. Immune marker measurements in EBC were performed and concentrations were noted. EBC markers in general showed more values below detection than plasma or saliva. 29,606 peptides were found in EBC samples of which 179 could be linked to 28 proteins. Nineteen of them were keratins. Thereby, we showed that EBC could be collected with only limited, if any, saliva contamination and we showed the feasibility of measuring immune markers and the proteome in EBC. As part of the EU EPHOR project, 147 workers with lung disease were followed up over one week, questionnaire data, EBC, plasma, urine and spirometry measurements were collected on Monday and Friday. Inflammatory markers and untargeted proteomics are being assessed in addition to cotinine as a measure for smoking exposure and urinary PAHs. This will result in a better insight into how the lung function is linked to the internal exposome and how they can be followed up. This research will contribute to better follow-up of occupational exposures and thereby improve the prevention of occupational lung disease by giving means to assess biological exposure and effects in minimally invasive matrices such as EBC.

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## O-22

# High-content *in vitro* micronucleus assay highlights novel links between epigenetic changes and genotoxic outcomes

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Many diseases are influenced by epigenetic mechanisms. Modulating these complex structural modifications within DNA and the nucleosome is therefore of great interest in the pharmaceutical sector. However, such modifications may induce genotoxic damage. We investigated the relationship between epigenetic modulations and genotoxicity using our high-throughput image-based in vitro micronucleus assay (MEGA Screen). Over 200 epigenetic targeting compounds were dosed onto A549 cells, and effects were observed after a 24-hour exposure and a 24-hour recovery period. Through confocal imaging and computational analysis, we quantified multiple endpoints including micronuclei and DNA double-strand breaks. Results showed that inhibiting certain types of epigenetic modifications led to increases in genotoxic endpoints. Notably, bromodomain and HDAC inhibitors increased micronucleus frequency whereas KDM and HAT inhibitors had little to no effect on micronucleus frequency. The data demonstrated a correlation between inhibition of specific classes of bromodomain-containing proteins and genotoxic response. Inhibition of the bromodomain and extra-terminal-domain (BET) protein family led to a significant increase in micronuclei (range: 2.07–7.60-fold increase) and showed a higher cytotoxicity at lower concentrations compared to other classes of bromodomain inhibitors. Interestingly, inhibitors that target multiple members of the BET protein family led to a higher micronucleus induction. Multiple BRD4 selective inhibitors induced a significant increase in micronuclei (range: 3.38-to-5.83-fold increase), interestingly (+)-JQ1, a BRD4 selective inhibitor, resulted in a 5.83-fold increase in micronucleus frequency. Whereas its enantiomer (*R*)-(-)JQ1), which has no inhibitory effect on BRD4, did not increase micronucleus frequency. The capabilities of the MEGA screen allow us to accumulate and interrogate data on multiple types of epigenetic modulators on a large scale, furthering our understanding of their contribution to genotoxicity. This will provide a wealth of data and through its interpretation will support the development of genetic toxicology safety strategies specifically for epigenetic modulators, ensuring the development of safer drug candidate.

## O-23
## The *in vitro* micronucleus multi-biomarker image stream (ISMN-MB) assay

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The development of new approach methodologies (NAMs) and the ability to quantitatively link key events to adverse outcome pathways (AOPs) have the potential to aid risk assessment and support read-across decisions for similar chemical classes. Understanding mode-of-action (MoA) is vital to identifying the basis for genotoxic potential and the risk of translation into the clinic. Incorporation of DNA-damage biomarkers with the micronucleus (MN) assay is well established. Here we describe the development of a NAM, the *in vitro* micronucleus multi-biomarker Image Stream (ISMN-MB) assay, with a simple, robust protocol for staining p53-proficient TK6 cells with antibodies against *γ*H2AX, p53, and pH3S28 along with DRAQ5™ DNA staining that enables analysis of un-lysed cells. The Cytek® Amnis® ImageStream®X Mk II platform was used for analysis of TK6 cells treated with Ara-c, vinblastine, methyl menthane sulphonate, carbendazim, or crizotinib (i.e., chemicals with varying MoA). The ISMN-MB assay leverages the advantages of high-throughput imaging flow cytometry and retains the spatial context of fluorescence microscopy whilst allowing for high-content data capture (7+ channels) and simultaneous analysis of multiple parameters using gating strategies for extraction of *γ*H2AX, pH3 and p53 biomarker metrics. To quantify MN, a nuclear image masking strategy was developed using IDEAS software to develop templated gating, automated MN extraction, and an easy user interface for batch processing data files with limited bias. The prototype ISMN-MB assay was used to quantitatively assess dose-dependent increases in MN and concordant biomarker responses to provide definitive fingerprints for aneugenic and clastogenic profiles associated with the test compound MoA. The results of these studies will be presented. Future work will focus on the application of AI-enabled tools to further improve data acquisition and assessment.

# New Investigators Session

*Invited lecture*

# IL-8
# Reviewing comet assay as a tool in marine ecotoxicology

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Every year several chemicals that may cause biological effects are released into the environment, especially in the marine environment which is considered the final destination of the contaminants. Direct and indirect toxic effects of these chemicals have potential risk factors, which can be evaluated using some of the marine ecotoxicology tools that provide information about the adverse effects of stressors. For a marine ecotoxicology assessment, different tests are required and several techniques, including the comet assay, can be used. A comet assay was developed for mammals and has been successfully transferred to aquatic organisms to evaluate the genotoxic potential of environmental contaminants in the laboratory and in the field. The most commonly used organisms are fish and mollusks, but the protocol was also applied to crustaceans, worms, cnidarians, echinoderms, marine mammals, and seabirds. Most studies were performed using circulating blood cells, probably due to the practical advantage of processing tissues containing already separated nucleated cells. Solid tissues, such as gill, hepatocytes or digestive gland, can also be used and studies using sperm and embryo cells are rarer. When comparing cell types, it is usually reported that circulating cells are usually less sensitive than hepatocytes or gill cells. Comet assay has faced some criticism due to its lack of ecotoxicological relevance, but it can detect damages at lower organisational levels (molecular) that can impair the whole-organism level. Thus, in ecotoxicological studies, it is important to link comet assay response with other biological responses to discriminate how the observed effect can impair normal physiology. Therefore, it is important that the comet assay shows correlation or links with other well-established biological responses. Another consideration is that the comet assay in the aquatic field has generally lacked coordination, with low standardised procedures, which can be improved to generate more comparable results.

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# O-24
# Mutagenic effects of ethanol and acetaldehyde in oral cancer: an experimental modelling approach

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Alcohol is associated with oral cavity cancer and various other anatomical sites. It is responsible for ~4.1% of all cancers worldwide, 10% of which are lip and oral cavity cancers. The exact mechanisms of ethanol carcinogenicity in the oral cavity remain unclear, even though ethanol's main reactive metabolite acetaldehyde (AcA) may play a crucial role. The COSMIC mutational signature SBS16 has been tentatively linked to alcohol drinking, yet it is also found in alcohol-unrelated pathologies. The direct DNA-damaging effects of ethanol/AcA in well-controlled experimental settings have not been clearly established. We hypothesised that AcA resulting from ethanol metabolism contributes to oral carcinogenesis through the formation of exposure-specific DNA adducts and subsequent mutagenesis. Using a unique collection of 23 alcohol-fixed-paraffin-embedded (AFPE) tumour samples from carcinogenicity bioassays on Sprague Dawley rats exposed to ethanol and AcA, we characterised their mutational signature landscapes by whole-genome sequencing (WGS). We observed an interesting enrichment of SBS17 in one cheek tumour, one larynx tumour, and in 30 % (5 of 16) tumours of the Zymbal gland, an auditory gland considered a direct exposure site. The SBS17 presence suggests roles of inflammation and oxidative stress in the ethanol/AcA-driven cancers. Our WGS results appear to exclude exposure-specific formation of signature SBS16 or of other direct single-base mutagenesis, while the analysis of higher-level mutation types (indels, copy number and structural variants) is ongoing. Next, we used a novel, powerful error-corrected/duplex WGS approach to analyse mutation spectra in AcA-treated immortalised non-cancer oral cell lines. Analysis of DNA adduct formation following AcA treatment is conducted in parallel to explain any mutagenic effects of AcA in the oral cells. Our study generates new mechanistic evidence and innovative molecular markers of mutagenicity and will provide a relevant basis for new strategies for prevention and detection of alcohol-associated oral cancers.

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# O-25
# Quantitative genotoxicity assessment of mycotoxin mixtures

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Global warming and the globalisation of food markets are anticipated to increase mycotoxin contamination across European regions in the near future. As multiple mycotoxigenic fungi can colonise the same food or feed material and produce different mycotoxins simultaneously, the co-occurrence of mycotoxins and the related mixture toxicity, even at low concentrations, has raised concerns about the impact on human and animal health. Moreover, several of these mycotoxins are (suspected) genotoxic compounds and little is known about their possible interactions in cases of coexposure. For non-genotoxic endpoints, the principle of additivity is assumed to generally apply, although other types of combined effects have been described as well. To investigate whether the principle of additivity remains justified for genotoxic mycotoxins, an appropriate strategy to assess the combined effects of genotoxicants using the *in vitro* micronucleus (MNvit) assay was therefore first developed. To this extent, two types of mixtures were assessed for their combined effects. The first consisted of two genotoxicants with a similar mode of action (MoA), *i.e.* ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS), two DNA-alkylating agents. The second mixture contained two genotoxicants with a different MoA, *i.e.* MMS and etoposide (ETP, a topoisomerase II inhibitor). The results of these tests demonstrated that the principle of additivity holds true for both types of mixtures. In the next step, the strategy was applied to assess more complex mixtures consisting of mycotoxins for which the genotoxicity profile is less characterized. The results obtained with a mixture of the mycotoxins aflatoxin B1 (AFB1) and alternariol (AOH) revealed additive effects. Further tests are currently performed to evaluate if the genotoxic effects of other mycotoxins also follow the principle of additivity.

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# O-26
# Effect of a personalized intensive dietary intervention on DNA damage and repair in colorectal cancer patients

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Accumulation of DNA damage resulting from oxidative stress may influence the risk of cancer recurrence and susceptibility to various comorbidities in colorectal cancer (CRC) patients. Bioactive compounds found in plant foods have the potential to protect against DNA damage; however, there is limited evidence of how dietary factors may affect DNA stability in CRC patients in remission after surgery. In this study, we aimed to investigate the effect of a one-year personalised intensive dietary intervention on DNA damage in post-surgery CRC patients diagnosed with stage I-III. The present study included patients from the ongoing randomised controlled trial, the Norwegian Dietary Guidelines and Colorectal Survival (CRC-NORDIET), enrolled 2–9 months after surgery. The intervention group received individualised nutrition counselling focusing on foods and drinks suggested to dampen oxidative stress and inflammation, aligning closely with the prudent diet recommended by the Norwegian food-based dietary guidelines (NFBDGs). The control group received only standard oncology care advice. The standard comet assay, modified with the lesion-specific enzyme formamidopyrimidine DNA glycosylase (Fpg), was applied to measure DNA damage in peripheral blood mononuclear cells (PBMCs) at 6 and 12 months. Statistical analyses were conducted using a gamma generalised linear mixed model (GLMM) for repeated measurements, with patient IDs defining random effects. A total of 156 CRC patients were included, 78 in the intervention group and 78 in the control group. After 12 months, a significant intervention effect in DNA base oxidation was observed. No intervention effect was observed after 6 months, nor for DNA strand breaks at any of the time points. In conclusion, our results suggest that adherence to a prudent diet, emphasising antioxidant-rich and anti-inflammatory foods and drinks, may serve as a potential moderator for DNA protection against oxidation damage.

## O-27
# DNA adduct formation associated with specific environmental, dietary, and lifestyle habits among kidney transplant patients

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Chronic exposure to certain dietary and environmental chemicals may result in the formation of covalent DNA modifications referred to as DNA adducts, which may contribute to a plethora of secondary medical comorbidities, including cancer. This study aimed to profile DNA adducts among kidney allograft transplant recipients (KTRs) with strict medication routines, additionally monitored dietary habits, lifestyle behaviours, and other external exposures. DNA adduct profiles from 33 kidney transplant recipients were analysed using ultrahigh-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). Targeted DNA adduct levels across categorical external exposures were compared and unpaired group comparisons using Student's t-test for parametric data or Mann-Whitney U-test for non-parametric data were conducted. Specific external factors, such as ageing and smoking, were associated with elevated levels of oxidative stress-related DNA adducts (*i.e.*, 8-Oxo-guanine). Additionally, we noted that specific lifestyle habits (*i.e.*, alcohol consumption) and predisposition to certain secondary medical comorbidities (*i.e.*, (pre-)diabetes) were closely linked to oxidative DNA damage, as marked by increased levels of lipid-peroxidation adducts (1, $N^2$-propano-guanine and *γ*-HOP-guanine). Moreover, immunosuppressive medications such as cyclosporine and mycophenolic acid were linked to higher levels of DNA alkylation (*i.e.*, $N^3$-methyl-adenine and $N^2$-ethyl-guanine). Our study helps further unravel how the external exposome influences the internal exposome (*i.e.* DNA adducts) among KTRs. The elevated levels of 1, $N^2$-propano-guanine, *γ*-HOP-guanine, and 8-oxo-guanine adducts among KTRs in relation to specific exposures is of specific interest as increased levels of these particular adducts have been implicated in different types of mammalian cancers and neurodegenerative disorders. Their presence in KTRs presents an opportunity for monitoring external health hazards among high-health risk individuals. Nonetheless, validation of these findings and their implications towards KTR management (and improving quality of life) requires further research and confirmation in larger cohort designs.

European Centre for Ecotoxicology and Toxicology of Chemicals Session

# O-28
# ECETOC's transformational program
# A framework to incorporate NAMs in regulatory toxicology

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ECETOC has developed a framework [1], incorporating new approach methods (NAMs), designed to meet regulatory requirements, in which both hazard and exposure can be assessed in a tiered approach. The framework allows a transparent and phased introduction of NAMs in chemical safety assessment and enables science-based safety decisions and was successfully tested using a few case studies, which will be presented by the 2nd speaker. In the 1st tier *in silico* data are used for an initial scan of potential hazards. In the 2nd tier, *in vitro* methods are used to obtain a broader spectrum of hazard information data, considering output from tier 1. Exposure is likewise assessed in a tiered approach allowing for a stepwise refinement of the assessment. Should, at the end of tier 2, risk or required information be considered unacceptable, then targeted *in vivo* studies may be performed in tier 3. It is envisioned that these *in vivo* studies will be of short duration (*e.g.* 28 days). Our investigations comparing 28- and 90-day studies will be presented by the 3rd speaker. Smart 28-day studies will consider current OECD guidelines and be enhanced with modern technologies such as 'omics and parameters to obtain information on the kinetics of the chemical studied. In addition, the information from *in silico* and in vitro studies will be used to include specific parameters to better assess the hazard potential. The inclusion of new technologies will be presented by the 4th speaker. This framework should use fewer animals, take less time, less financial and expert resources, while allowing NAMs to be incorporated as they develop.

[1] Ball et al. 2022. https://doi.org/10.1007/s00204-021-03215-9

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## O-29
## Toxicological effects in 28-day studies compared to 90-day studies – what do we miss after short term exposure?

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One aspect of the tiered testing strategy proposed by ECETOC is to conduct an extended or smart 28-day *in vivo* study to close relevant data gaps with regard to STOT-RE classification if the 90-day study is omitted. The aim of this study was therefore to identify potential information gaps by comparing the outcomes of 28-day studies to those of 90-day studies. In order to achieve this goal, we evaluated the extent to which short-term studies with 28-day exposure can or cannot forecast the target organs and effects relevant to the classification of substances according to STOT-RE. High quality rodent studies with either 28- or 90-day exposure were extracted from the two databases RepDose and ToxRef. Studies testing the same chemical, same species, strain and route were paired resulting in 86 comparisons for 78 compounds. Organ weights as well as pathological effects were analysed per study, a LOAEL was derived to assess sensitivity differences. Initial analyses show, that similar target organs are observed in both study types, however, at generally higher dosing in the shorter-term study. Overall, frequently observed target organs like the kidney and liver are usually observed in both study types, whereas haematological findings and effects in the adrenal gland are more frequently missed. When limiting the analysis for missed target organs to those observed at effect levels relevant for STOT-RE classification (below 100 mg/kg bw/d or 10 mg/kg bw/d) the predictivity of 28d studies increases. The analysis is being expanded and will be refined by taking into account differences in dose selection and dose spacing as well as the occurrence of biomarkers like *e.g.* creatinine in clinical chemistry. This work will provide a rational basis for which additional parameters to be included in the smart 28-day study are most useful to bridge the gaps in the 90-day study.

# O-30
# Examples of the ECETOC framework incorporating NAMs in risk assessment

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The ECETOC framework [1] incorporates a tiered approach for both exposure and hazard assessment. The first step is to estimate exposure based on the use of the chemical and its physicochemical properties using the Targeted Risk Assessment Tool. In two examples exposure was predicted to be above the TTC limits. Tier 1 (*in silico*) indicated that both chemicals would be non-genotoxic. Tier 2 consists of *in vitro* assays for biological activity, genotoxicity, absorption and metabolism. *In vitro* assessment is a three-part process. Part 1 is determining what biological activity the chemical may have, using a range of *in vitro* alerting assays. Part 2 is to use more specific assays to follow up on the activity indicated by part 1. Part 3 is to bring in assays and models based on kinetics and metabolism to provide an estimate of *in vivo* effect and no effect levels (IVIVE). The no effect levels are compared with the exposure estimates in a first risk assessment. The assessment can stop at this stage or continue with the use of higher tier exposure models or the use of targeted *in vivo* studies (Tier 3). The examples to be presented will show the outcome of the Tier 2 studies and in one case Tier 3 studies and compare the results with conventional studies. For both chemicals the toxicological modes of action were correctly identified, providing a sound bases for hazard assessment as well as classification and labelling. IVIVE provided the necessary quantitative information for risk assessment. Thus, in both cases, the framework provided equivalent outcomes to a conventional assessment but in one case used no animals and in the other used a tenth of the number of animals.

[1] Ball et al. 2022 https://doi.org/10.1007/s00204-021-03215-9

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# O-31
# Smart *in vivo* studies: using new technologies in the 28-day rat study

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In the ECETOC framework for the incorporation of NAMs in a tiered approach, targeted and 'omics-enhanced *in vivo* studies named Smart studies is the final tier 3 step. These short-term *in vivo* studies will take into account knowledge from tier 1 and 2, will consider current OECD guidelines and be enhanced with modern technologies. Organ specific transcriptomics and plasma-based metabolomics are considered as sufficiently developed technologies to be introduced in such Smart studies. For both technologies, OECD working groups have started to draft guidance for matrix sampling. The comparison of 28- and 90-day studies indicated that enhanced blood analysis for hormones and haematology is helpful in improving the prediction of organ toxicity for 90-day studies. Blood can also be used for genotoxicity assessment. The Smart study should also provide data for the transcriptional changes in some specific tissues as well as toxicokinetic assessment, to account for potential accumulation of parent compounds or metabolites. To achieve the latter, blood micro-sampling technologies will have to be introduced. In addition to the general improvements of the study design, it is also envisioned that compound specific parameters and/or intermediate time points could be included, based on the results of tier-1 and -2 information.

# Next-Generation Sequencing Session

*Invited lecture*

# IL-9
# Advancing quantitative genetic toxicology and genomic technologies to reduce and replace conventional rodent mutagenicity tests

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Regulatory genetic toxicology testing is essential for identifying mutagenic hazards. Existing methods to detect mutations are limited to: (i) specific, genetically modified organisms; (ii) single target genes that may not be representative of a health outcome; and (iii) hazard calling without complementary information on mechanism or potency. There is growing interest in developing and integrating refined approaches to measure multiple types of DNA damage predictive of adverse health outcomes and quantify how these effects change with dose and exposure duration. We conducted a time-series and dose-response analysis of MutaMouse males exposed to benzo(b)fluoranthene, a polycyclic aromatic hydrocarbon, to: 1) demonstrate that Duplex Sequencing (DS), an error-corrected sequencing technology, is an effective *in vivo* mutagenesis test; 2) compare DS performance to conventional mutagenicity tests (*e.g.*, *lacZ* assay; Micronuclei, MN) using benchmark dose-response (BMD) modelling for potency estimation; and 3) evaluate the impact of exposure duration on potency estimates. Our analyses focused on 28-, 90- and 180-day exposures because they represent designs commonly used in OECD test guidelines to assess toxicological effects. We observed strong dose-responses in the liver and bone marrow (BM) using both the *lacZ* assay and DS. Mutation frequency was higher in the liver than BM and increased with exposure duration. A strong dose-response was also observed with the MN assay, although the response was similar across time. Using BMD modelling, we observed a larger increase in potency (*i.e.*, lower BMDs) over time in the liver than in BM. BMDs for MN induction in blood were lower than those for mutation induction in the liver. These studies demonstrate how *in vivo* mutation analysis can be improved to transform regulatory genetic toxicology and replace existing tests that require more time, resources, and animals.

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# O-32
# Extended analysis of NDMA mutagenicity using Duplex Sequencing on an *in vivo* Muta™Mouse mutation assay

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*N*-nitrosamines are a key issue facing the pharmaceutical industry and there is a need to have a better understanding of the *in vivo* carcinogenic risk posed by *N*-nitrosamines. An OECD TG-488 compliant Muta™Mouse transgenic rodent mutation assay was conducted with 7 doses of *N*-nitrosodimethylamine (NDMA), a small potent *N*-nitrosamine, over a 28-day oral dosing regimen, including both acute and repeat-dose groups. The mutation burden at the transgenic lacZ locus and the endogenous Pig-a gene-locus was assessed in a range of tissues, along with micronucleus frequencies in peripheral blood. Results indicated that NDMA did not induce either Pig-a gene mutations or micronucleus frequency when tested up to 4 mg/kg/day but, in the liver, induced a non-linear dose-dependent increase in mean LacZ mutant frequency following 28-day repeat dosing (0.02–4 mg/kg/day). Error-corrected sequencing methods, such as TwinStrand Duplex Sequencing, are highly sensitive and can detect rare mutations in genomic DNA. To enhance our understanding of the mutations induced by NDMA *in vivo*, duplex sequencing was performed on genomic DNA extracted from liver tissue from the Muta™Mouse study to generate mutation frequency and mutation type data along with trinucleotide spectra. A dose-dependent increase in mutation frequency was detected by duplex sequencing in the liver, with no observed genotoxic effect level of 0.07 mg/kg/day, a result supported by analysis of trinucleotide mutation spectra. Duplex sequencing mutation frequency data from both the LacZ gene and the wider mouse genome generally aligned with the LacZ mutant frequency data from the MutaMouse study and benchmark dose analysis revealed concordance between duplex sequencing and the MutaMouse study. The results of this study provide greater clarity on the *in vivo* mutagenic potential of NDMA and highlight the utility of duplex sequencing for *in vivo* mutagenesis assessment.

# O-33
# Exogenous and endogenous carcinogens driving different mutational signatures in uveal and skin melanoma

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Melanocytes, responsible for producing the UV-absorbing pigment melanin, are a common cell-of-origin for both uveal and skin melanoma. Despite this shared origin, the two cancers exhibit distinct mutational landscapes, resulting from various mutagenic processes [1]. These processes imprint specific combinations of somatic mutations onto the genome that are called mutational signatures. In recent years, mutational signatures have proven to be valuable clinical biomarkers. Understanding the relationship between mutagens and the mutations driving carcinogenesis in each melanoma type is essential yet remains incomplete. To better understand underlying mutagenic processes in different melanoma types, we conducted a comprehensive analysis of a large cohort of whole-exome sequencing (WXS) and whole-genome sequencing (WGS) data of uveal and skin melanoma samples from The Cancer Genome Atlas (TCGA). We identified driver genes and mutations using the oncodriveCLUST algorithm and insights from previous studies. We used Palimpsest tools to assign probabilities of etiologically annotated COSMIC mutational signatures to driver mutations. Our findings revealed distinct mutational landscapes driving carcinogenesis in uveal and skin melanoma. Notably, UV mutational signatures dominated in skin melanoma, whereas they were largely absent in uveal melanoma cases of choroid or mixed choroid tissue sites. Similarly, examination of driver genes implicated UV radiation as the primary driver mutation especially in *BRAF* and *NRAS* in skin melanoma, while endogenous processes such as the clock-wise signature SBS1 in *SF3B1* in uveal melanoma. In conclusion, our research provides valuable insights into the mutagenic processes underlying the development of different melanoma cancer types, enabling us to identify exogenous carcinogens that impact specific driver mutations and genes.

[1] Johansson et al. 2020. https://doi.org/10.1038/s41467-020-16276-8

*Invited lecture*

# IL-10
# Genomics in coastal oceanography

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DNA-based methods and approaches are today's cornerstones of marine coastal environmental research. Marine coastal ecosystems are characterized by increasing complexity in the vicinity of the coastline. Modern environmental research approaches try to match this complexity with the increased resolution of the applied methodologies. DNA and RNA-based methods promise to deliver this increased resolution. With the rise of respective high throughput methodologies concerning sampling, sample analysis (e.g. sequencing) and downstream bioinformatic pipelines, we are several steps closer to source the information density of genomics in environmental research and match the required spatio-temporal resolution. We present strategies technological advancements and approaches to deliver environmentally relevant information from genetics towards genomics data and discuss how the respective results inform research design across disciplines.

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# O-34
# MutaTracker, a novel approach method to measure gene mutations using error-corrected NGS to gain understanding of the genotoxic mode of action

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Traditionally, mutagenicity is measured by counting surviving clones phenotypically, after determining resistance (or survival) to a mutation at a known genetic locus. The limitation of these approaches is that only mutants that survive selection are enumerated, which underestimates true induced mutation frequencies (MF). With the introduction of error-corrected sequencing, it is now possible to resolve precise MFs without selection. Herein, a case study on MutaTracker, where single-molecule mutation sequencing (SMM-seq) is multiplexed within the ToxTracker Assay. SMM-seq a highly sensitive technique for detecting single nucleotide variants utilising rolling circle amplification. ToxTracker is a reporter assay that can discriminate between primary and secondary (i.e., indirect) genotoxicity. As a pilot, we evaluated six genotoxic substances with different MoA in MutaTracker: *N*-ethyl-*N*-nitrosourea, potassium bromate ($KBrO_3$), cisplatin, aristolochic acid, benzo[a]pyrene and *N*-nitrosodimethylamine. All six compounds were classified as genotoxic: five with direct DNA damaging signatures and $KBrO_3$, which demonstrably induced secondary genotoxicity via oxidative stress. ENU induced a dose-dependent 14-fold increase in MF at the maximum tested concentration, generating mostly T>A, T>C and T>G mutations. AA induced a 5-fold increase in MF with primarily A>T mutations and B[a]P a 3-fold MF increase with C>A mutations. $KBrO_3$ was classified as an indirect genotoxicant due to oxidative stress, which resulted in a selective 8-fold increase in C>A mutations that often result from oxidative DNA damage, mainly 8-oxoG lesions. Sequencing of DNA from NDMA-treated cultures is ongoing. Taken together, MutaTracker provides evidence that multiplexing ToxTracker MOA information with mutation spectra from SSM-seq can accurately predict primary genotoxic potential and provide deep insight into genotoxic MOA.

## O-35
## Embarking on decoding stem cells: ecNGS of hIPSCs exposed to environmental mutagens during trilineage differentiation using the ReproTracker assay

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The objectives of this experiment were to 1) understand the teratogenic potential of exposure to environmental agents, 2) investigate mutagen sensitivity of human-induced pluripotent stem cells (hIPSC) as they differentiate through the three lineage-specific cell types into advanced cell types while being exposed to environmental mutagens. hIPSC were continuously exposed to potassium bromate ($KBrO_3$) or benzo(a)pyrenediolepoxide (BPDE), or intermittently exposed to X-rays during a differentiation period of 13–21 days. Throughout the exposures, differentiation into three different cell types (mesodermal cardiomyocytes, endodermal hepatocytes and ectodermal neural rosettes) was characterised using the ReproTracker assay. Thalidomide/retinoic acid (human teratogen) and saccharin (non-teratogen) were included as controls. First, cytotoxicity testing was performed to identify appropriate exposure levels for each test condition. Differentiation into the three lineage-specific cell types was assessed by morphological/functional assessment combined with expression patterns of the selected biomarker genes. DNA from each differentiated cell type following exposure to $KBrO_3$ or BPDE were subjected to error-corrected NGS. $KBrO_3$ exposure disrupted the development into neural rosettes and decreased expression of the neural rosette-specific biomarkers (*PAX6* and *NESTIN)*, indicating developmental toxicity. Moreover, $KBrO_3$ induced increased mutation frequencies in all cell types, although statistically significant only in hepatocytes. However, the sample size is small for Mf-analyses analyses limits the power to detect significant changes. Our results suggest that exposure of hIPSC differentiating towards specialised cell types, mimicking early embryonic development, to oxidising agents such as $KBrO_3$, may suggest the induction of both teratogenic and mutagenic effects. This approach holds promise as a "new approach method = NAM" both regarding teratogenicity and mutagenicity.

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# 2nd Partnership for the Assessment of Risk from Chemicals SYNnet Forum

PARC Partnership FOR THE Assessment OF Risks FROM Chemicals

SYNnet

*Invited lecture*

# IL-11
# Hazard assessment in PARC – first outcomes and synergies

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Current approaches for the assessment of environmental and human health hazards due to exposure to chemical substances have served their purpose reasonably well. However, the assessment schemes in place for the various chemical regulations are faced with numerous challenges, ranging from a large number of chemicals and mixture aspects to changes in the types of chemicals and materials produced. This has triggered awareness of the need for a paradigm shift, notably appearing in the EU Chemical Strategy for Sustainability (CSS), requiring new concepts for chemical risk assessment complementary to existing schemes and (partially) replacing them. As a result, new approach methods (NAM) and next-generation risk assessment (NGRA) are commonly regarded as the way forward. However, incorporating new scientific insights and innovative approaches into hazard assessment in such a way that regulatory needs are adequately met has appeared to be challenging. The European Partnership for the Assessment of Risks from Chemicals (PARC) has been designed to address various challenges associated with innovating chemical risk assessment [1]. Its overall goal is to strengthen the European research and innovation capacity for chemical risk assessment to protect human health and the environment. With around 100 participating organisations from all over Europe, including three European agencies, and a total budget of approximately 100 million euros, the PARC work package (WP) on hazard assessment is one of the largest projects of its kind. It has a duration of seven years and is coordinated by ANSES, the French Agency for Food, Environmental and Occupational Health & Safety and BfR, the German Federal Institute for Risk Assessment. The WP hazard assessment focuses on closing data gaps for substances where data are lacking, developing new approach methods as well as increasing their regulatory readiness.

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[1] Marx-Stoelting et al. 2023. https://doi.org/10.1007/s00204-022-03435-7

# O-36
# Closing regulatory data gaps on the genotoxicity and non-genotoxic carcinogenesis of natural toxins and bisphenols

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The global cancer burden is significant and will continue to rise due to several factors, among which increased exposure to harmful chemicals in the environment, workplace, consumer and food products plays a major role. Not only industrial synthetic chemicals, *e.g.* bisphenols and phthalates, but also natural compounds, *e.g.* mycotoxins produced by fungi contaminating food and feed, may be carcinogenic. To prevent or reduce exposure to those substances, it is crucial to investigate and characterise their hazard, in order to support their risk assessment and regulation. The Partnership for the Assessment of Risks from Chemicals (PARC) is joining efforts from around 200 European partners and 3 European Agencies with the major goal of investigating and innovating in chemicals risk assessment and translating the knowledge generated into policies, to protect human and environmental health. Two projects are currently underway in PARC intending to close data gaps on the hazard of mycotoxins, including *Alternaria* toxins and enniatins, and several bisphenol A alternatives. Genotoxicity and non-genotoxic carcinogenesis are among the endpoints selected to assess their toxicological profile, being immunotoxicity, developmental neurotoxicity, and endocrine disruption the other major endpoints addressed. The genotoxicity and carcinogenicity of BPA alternatives and mycotoxins will be primarily assessed through a battery of assays performed according to OECD guidelines, which will facilitate data acceptance by regulatory bodies. The battery consists of the Ames test complemented, for mycotoxins, with a mammalian gene mutation assay, the in vitro micronucleus assay and, for BPA alternatives, a cell transformation assay. In the second tier, more innovative approaches, e.g. omics-based methodologies or 3D cell models will be used to produce confirmatory data, contributing also to the development of new approach methodologies. The results generated are expected to provide a knowledge basis for the implementation/ refinement of the tested substances' regulation and contribute with mechanistic evidence to Adverse Outcome Pathways development, which will further support decision-making.

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# O-37
# New approach methodologies to facilitate and improve the hazard assessment of non-genotoxic carcinogens

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Carcinogenic chemicals, or their metabolites, can be classified as genotoxic or non-genotoxic carcinogens (NGTxCs). Genotoxic compounds induce DNA damage, which can be detected by an established *in vitro* and *in vivo* battery of genotoxicity assays. For NGTxCs, DNA is not the primary target, and the possible modes of action (MoA) of NGTxCs are much more diverse than those of genotoxic compounds, and there is no specific *in vitro* assay for detecting NGTxCs. Therefore, the evaluation of the carcinogenic potential is still dependent on long-term studies in rodents. This 2-year bioassay, mainly applied for testing agrochemicals and pharmaceuticals, is time-consuming, costly and requires very high numbers of animals. More importantly, its relevance for human risk assessment is questionable due to the limited predictivity for human cancer risk, especially with regard to NGTxCs. Thus, there is an urgent need for a transition to new approach methodologies (NAMs), integrating human-relevant *in vitro* assays and *in silico* tools that better exploit the current knowledge of the multiple processes involved in carcinogenesis into a modern safety assessment toolbox. Here, we will describe an integrative project that aims to use a variety of novel approaches and cell models to detect the carcinogenic potential of NGTxCs based on different mechanisms and pathways involved in carcinogenesis. The aim of this project is to contribute suitable assays for the safety assessment toolbox for an efficient and improved, internationally recognised hazard assessment of NGTxCs, and ultimately to contribute to reliable mechanism-based next-generation risk assessment for chemical carcinogens.

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# O-38
# Development of an AOP-based IATA for genotoxicity: Building an AOP network for genotoxic events leading to permanent DNA damage

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The available genotoxicity assessment approaches and tools do not adequately address current regulatory challenges, including the need for quantitative analysis of genotoxicity data and the shift toward animal-free toxicity testing. The integration of detailed genotoxic mode of action (MoA) information has been proposed as a way forward to advance genotoxicity assessment. Over recent years, several new approach methodologies (NAMs) have been developed to provide insights into the MoA of genotoxic agents and/or increased throughput to generate dose-response information. Together with traditional tests, these assays could serve as the building blocks for designing Integrated Approaches for Testing and Assessment (IATAs) for genotoxicity assessment. To enhance regulatory uptake, selecting NAMs to be included in the IATA should be structured and science-driven, a process in which adverse outcome pathways (AOPs) play an important role. Within this context, we have drafted an AOP network based on all the AOPs with genotoxic key events (KEs) available in the AOP-Wiki and scientific literature. To simplify this complex network, duplicated KEs were removed. Moreover, permanent DNA damage, *i.e.* gene. mutations and structural and numerical chromosome aberrations was considered as the 'adverse outcome', and more downstream KEs were excluded from the network. Following these refinements, a simplified AOP network was obtained, consisting of eight AOPs, each triggered by a different Molecular Initiating Event (MIE). Although strategies for AOP development have been described in the literature, there is no strict guidance on collecting the information to characterise KEs and KERs in the AOP, especially in data-rich areas. Consequently, not all of the KEs and Key Event Relationships (KERs) in the AOP network have been thoroughly documented. In the first step, the focus is on the characterisation of the KEs and KERs in the AOP linking "Formation, bulky DNA adducts" to "Increase, Mutations" and a systematic search strategy to document KE(R)s is presented. The following step will apply the lessons learned from characterising the AOP on bulky DNA adduct formation to other KEs and KERs in the AOP network. In parallel, an inventory of the available NAMs in the field of genotoxicity has been compiled. By mapping the NAMs against the MIEs/KEs/AOs in the AOP network, IATAs for genotoxicity will be designed and evaluated through case studies to ultimately assist decision-making.

## O-39
# Meta-analysis for quantifying a genotoxicity AOP linking DNA alkylation to increase in mutations and chromosomal aberrations

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Chemical genotoxicity assessment currently relies largely on qualitative approaches in which the chemicals of interest are classified as either genotoxic or non-genotoxic. Such approaches may not properly capture the nuances of the underlying biology, including potency, mode of action and dose-response relationships. Furthermore, the few quantitative approaches for genotoxicity testing that are currently in use, may be hampered by limited understanding of the relevant temporal dynamics. Indeed, genotoxic adversities are typically the result of a cascade of key events (KEs) triggered by a molecular initiating event (MIE) and occur at various time scales. Integrating the time-dependent activation of genotoxic events as well as response-response relationships between the subsequent events can improve our mechanistic understanding of the genotoxic mechanism of action and in turn, improve regulatory decision-making. In order to achieve this goal, we are developing response-response relationships in order to quantitatively link KEs in an Adverse Outcome Pathway (AOP) modelling the progression towards permanent DNA damage. Within the Partnership for the Assessment of Risk from Chemicals (PARC), a draft AOP network has been constructed, summarising the core concepts of eight AOPs with genotoxic KEs that are described in the literature. For the quantification of this AOP network, we are focusing on a data-rich subsection, linking 'DNA alkylation' to 'increase in mutations' and 'increase in structural chromosome aberrations' through several KEs. Each KE within this AOP has been linked to an *in vitro* assay that can quantitatively assess the endpoint of interest: the DNA adduct assay for measuring byproducts of DNA alkylations, the comet assay and γH2AX assay for measuring DNA strand breaks, the micronucleus assay for measuring structural chromosome aberrations and several mutation assays to measure mutation frequency. Next, existing information related to these assays was collected, including both in-house raw data amongst different partners and publicly available data identified through a large-scale systematic literature search. In order to reduce potential variability across cell systems and types of alkylation, we have restricted our search to studies performed on the frequently used TK6 cell line and a selection of alkylating agents, with our current focus being on Ethyl Methane Sulphonate (EMS). The extracted data will be used to quantify response-response relationships between KEs and assess their variability across compounds. This proof of concept study marks the first endeavour in the field of genotoxicity assessment to link together all previously mentioned genotoxic endpoints.

# O-40
# Genotoxicity biomarkers in occupational biomonitoring studies: linking exposure to preclinical effects

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Biomarkers of effect, also called biomarkers of biological response, are observable and quantifiable biological changes in an organism that result from exposure to chemicals. These early biological changes can occur at biochemical, molecular, or cellular levels, on processes, structures, or functions and may be associated with the later development of disease. In human biomonitoring studies, particularly in occupational settings, the advantage of using exposure and effect biomarkers is assessing exposure from all routes, while simultaneously addressing the concerns about its potential health effects, such as carcinogenesis. The identification of biological effects, in an early stage, allows actions to be taken for the prevention of later occupational disease. Amongst the most commonly characterised effect biomarkers, those targeting genotoxic effects, oxidative stress or inflammation are of utmost importance because they are closely related to carcinogenesis. The micronucleus analysis in human lymphocytes has been widely used as a biomarker of genotoxicity that associates exposure and increased risk of cancer. In addition, the micronucleus assay can be used on buccal cells for addressing site-of-contact effects associated with, e.g. exposure to volatile compounds or to hand-to-mouth exposure. Conversely, the comet assay in blood white cells has been used as a versatile biomarker of DNA damage. In our previous work under the HBM4EU project, the application of the micronucleus assay in human biomonitoring occupational studies demonstrated that genotoxicity biomarkers are relevant for detecting effects from exposure to very low doses of chemicals and mixtures and identifying specific groups of workers potentially at an increased risk of adverse health outcomes. In the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC; https://www.eu-parc.eu/), two multi-centric human biomonitoring studies on occupational exposure to hazardous chemicals are underway: a study on exposure to electronic waste (E-waste) and plastics comprising several waste management industries and another study in health care workers. Groups of non-exposed individuals are included as controls. In both studies, biomarkers of genotoxic effects are assessed, including micronucleus in blood and buccal cells and the Comet assay in blood cells. The findings are expected to provide valuable information on biological effects and putative future health outcomes related to occupational exposure, promoting the implementation of more efficient risk management strategies.

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# O-41
# Set up of a human 3D multi-cellular liver model for non-genotoxic chemical carcinogen detection

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Carcinogenic compounds can be classified as genotoxic or non-genotoxic carcinogens (NGTXCs). For these particular chemicals, DNA is not the primary target, and the possible mechanisms of action (MoA) of NGTXCs are much more diverse than those of genotoxic compounds. Up to now, no specific *in vitro* assay has been permitted to detect NGTXCs efficiently. The classical 2D cell model with one acute 24-hour treatment protocol currently used, may not be the most adapted to identify these specific NGTXCs MoA. An option may be to use three-dimensional (3D) cell models since multiple studies have shown that they performed better than traditional 2D cell models in toxicological studies through a more integrated microenvironment and advanced functions. In this study, we construct a human multicellular liver spheroid including immune, liver, and Kupffer cells. For this purpose, we used magnetic beads and low attachment plates and included in the spheroids metabolically competent human HepaRG cells (hepatocyte cells) with hTERT-HSC (stellate cells) and THP-1 cell line (immune cells). We were able to apply repeated chemical treatments, perform indirect antibodies immunofluorescence staining, and capture confocal images on the same plate with four spheroids per well. Then an image analysis workflow on 3D reconstructed images was developed. Results for different biomarkers related to specific NGTXCs MoA will be presented and differences with the 2D cell model will be highlighted. This new 3D model may permit in the future to identify efficient chemicals with NGTXCs potential.

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15th International Comet Assay Workshop

*Invited lecture*

# IL-12
# The comet assay in middle age

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The comet assay is 40 years old. It has proved its worth as a method for measuring DNA lesions, in genotoxicity testing, human and environmental biomonitoring, and basic research into mechanisms of action of DNA damage and repair. It is grown-up enough to have an OECD guideline, though so far only for the standard *in vivo* assay for strand breaks in animal testing. It is versatile; it has been adapted to allow the detection of different DNA modifications, including oxidised and alkylated bases and cross-links, as well as to monitor DNA repair. Long-standing issues with the assay were addressed in the COST Action hCOMET, resulting in the publication of standard methods in Nature Protocols. If widely adopted, these should reduce the problem of inter-laboratory variability. The assay is sensitive, and can reveal small differences between treatments or between people that are statistically significant – but there can be a question mark over the biological significance of low levels of DNA damage. I will discuss recent work on DNA repair, and briefly refer to my hopes for the future of the assay.

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*Invited lecture*

# IL-13
# Biomarkers of effect: A journey from exposure monitoring to predictors of adverse health outcome

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A biomarker of effect is *any measurable biochemical, physiologic, or other alteration within an organism that, depending on the magnitude, can be recognised as an established or potential health impairment or disease.* The presence of a continuum from exposure, early biological events and disease is the unifying mechanism that justifies this conclusion. In recent years, a progressive increase of interest in assessing the link between early damage and the risk of disease has been observed, with the main aim of making more efficient the prevention of cancer and other chronic diseases, especially in subjects exposed to harmful agents in the environment or at work. An extensive effort to standardise the validation process of effect biomarkers will be discussed, and a suitable multi-level road-map driving biomarkers of effect towards clinical practice is presented. Most examples will focus on the micronuclei assay since literature exists demonstrating the role of micronucleation in the pathogenesis of cancer and other non-communicable diseases, and the link with clinical outcomes is substantiated by epidemiological evidence and a strong mechanistic basis. The validation of micronuclei is also the main topic of an OECD project, aimed at developing specific mixture threshold levels for regulatory use, through an integrative effect-biomarker AOP project which started in October 2022. The project starts from the assessment that effect-biomarkers are the only option to assess known and unknown exposures and mixtures in an integrative way, and that validated effect biomarkers can be used to address relevant health effect endpoints and modes of actions in humans. A systematic understanding of both the relevance and interpretation of effect-biomarker data will lead to increased protection for workers and populations exposed to genotoxic agents. Preliminary documents from the project will be discussed.

*Invited lecture*

# IL-14
# Search for the most reliable genotoxicity test

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Chemicals are rutinously tested with various validated/standardised genotoxicity tests. Most currently used assays are 30–50 years old and not reliable. The majority of in vitro tests have only a specificity and sensitivity between 60 and 70%. The most widely used in vivo procedures (CA and MN tests with bone marrow cells) have a very low sensitivity (50–60%). Therefore, there is a clear need for the development of improved tests. In the past years, new procedures have been developed (the PigA gene mutation assay, *γ*H2AX-based experiments, reporter tests that are based on the measurement of activation of p53 and the Tox Tracker Assay). Furthermore, attempts have been made to develop cell lines that reflect the metabolism of xenobiotics in humans. Several human liver-derived cell lines (HepG2, HepaRG) were found that can activate promutagens. It was also claimed that the metabolism of mutagens is reflected in MN experiments with hen eggs which may be an alternative to experiments with rodents. We attempted recently to find a new human-derived cell line that possesses active phase I and phase II enzymes that activate/detoxify mutagens. Therefore, we tested 12 human-derived liver lines in comet experiments with representatives of different groups of mutagens. The most sensitive line was Huh6, which had never been used in genotoxicity studies before. We studied in subsequent experiments the metabolic capacity of the cells (measurement of drug-metabolising enzymes) and developed a protocol for MN experiments. This procedure was used in validation experiments with a panel of different chemicals. The selection of chemicals was based on the ECVAM table. The results show that the sensitivity/specificity of MN assays with this cell line is substantially higher than tests with conventional cell lines that are currently used and also higher than most *in vivo* procedures.

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*Invited lecture*

# IL-15
# Revolutionising DNA repair analyses: Latest enhancements to the comet-based *in vitro* DNA repair assay

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The comet assay is widely used as a biomarker assay in human population studies - primarily to measure DNA damage, but increasingly also to assess the capacity of cells for DNA repair. Originally, DNA repair measurement involved tracking the removal of DNA damage over time post-exposure, a method that proved laborious and impractical for high-throughput biomonitoring. The comet-based *in vitro* DNA repair assay emerged as an alternative approach [1], employing protein extracts incubated with agarose-embedded DNA substrates containing specifically induced DNA lesions. DNA incisions produced during the incubation reaction are quantified as strand breaks after electrophoresis, reflecting the extract's incision activity. Recent advancements have optimised this assay for more precise applications. A modified protocol now allows the assessment of base excision repair (BER) activity in mitochondrial extracts, distinguishing it from nuclear DNA repair. This refinement was demonstrated using liver tissue from Zucker fatty and spontaneously hypertensive rats, revealing reduced mitochondrial BER activity in obese rats compared to lean counterparts. This modification promises insights into mitochondrial DNA repair mechanisms and their metabolic implications. Further enhancements have addressed the assay's logistical feasibility. Studies have shown that frozen whole blood samples can be used to assess DNA damage and oxidation, simplifying sample handling for extensive biomonitoring. Preliminary data suggest that BER activity can be effectively detected in white blood cells isolated from frozen blood. These findings support the applicability of the DNA repair assay in large-scale biomonitoring and clinical studies. Moreover, the assay has been adapted for non-invasive biomonitoring using human placental tissue. An optimised protocol for placental protein extracts, validated in pre-eclamptic samples, showed significantly lower BER activity compared to controls, highlighting the assay's potential in clinical investigations of prenatal health. Collectively, these advancements revolutionise the comet-based *in vitro* DNA repair assay, enhancing its applicability, and practicality for diverse research and clinical contexts.

[1] Vodenkova et al. 2020. https://doi.org/10.1038/s41596-020-0401-x

*Invited lecture*

# IL-16
# Is automation the next innovation for the comet assay?

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The comet assay is a trusted and widely used method for assessing DNA damage in individual eukaryotic cells. Various innovations, such as the whole blood comet, the HTP comet assay and the CometChip, have only increased the assay's popularity. However, the method remains time-consuming and requires regular monitoring and sample manipulation by the user. This limits the throughput of the assay, increases the risk of errors, and contributes to intra- and inter-laboratory variability. We have developed a device which automates high-throughput sample processing for the comet assay. This device is based upon our patented, high-throughput, vertical comet assay electrophoresis tank, and incorporates our novel, patented combination of assay fluidics, temperature control, and a sliding electrophoresis tank to facilitate sample loading and removal. This automated device performs at least as well as our "manual" high throughput system but with all the advantages of a fully "walkaway" device, such as a decreased need for human involvement and a decreased assay run time [1]. Our automated device represents a valuable, high-throughput approach for reliably assessing DNA damage with minimal operator involvement, particularly if combined with the automated analysis of comets.

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[1] Karbaschi et al. 2023. https://doi.org/10.3390/ijms24087187

## O-42
## The *in vivo* comet assay: uncovering DNA damage in testicular germ cells

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The *in vivo* comet assay is widely and increasingly used in regulatory toxicology. However, OECD test guideline 489 (TG489) does not recommend obtaining testicular germ cell data, as testicular cell suspensions contain a mix of germ cells and somatic cells. According to CLP demonstration of germ cell genotoxicity and interaction with the germ cell genome can act as supportive evidence to discern between classification categories Muta1B or Muta2 in the presence of positive somatic mutagenicity *in vivo* findings. An approach to specifically assess genotoxicity of testicular germ cells within TG489 is thus highly demanded. We developed a method (proof-of-concept [1]) to selectively analyse haploid spermatids and primary spermatocytes. Recordings of DNA damage (% tail intensity), as well as DNA content (total fluorescence intensity) of individual comets, are combined with visual comet identification to distinguish testicular comet populations based on their different DNA content/ploidy and physical appearance. The haploid spermatid comet populations are identified by setting a DNA content threshold (manually or by fitting a normal three-mixture distribution function) in DNA content distribution plots. Data from two experimental phases in rats testing genotoxic and non-genotoxic agents will be presented. Biomaterials (testis, liver) were harvested to facilitate the demonstration of inter-laboratory transferability. Valuable information regarding effect levels in germ cells as such, compared with levels in somatic cells, and distribution of substances to the male gonad have been obtained. In accordance with the 3R-principle both somatic and germ cell data are obtained from the same animals. Our adaptation adds a versatile, sensitive, rapid and resource- and animal-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 contribute to improving the assessment of male germ cell mutagenicity.

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[1] Dirven et al. 2023. https://doi.org/10.1002/em.22527

# O-43
# DNA Damage in Patients with End-Stage Renal Disease

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End-stage renal disease (ESRD) is an irreversible renal failure in which patients need dialysis due to the severity of nephron injury and functional loss. Changes in prooxidant and antioxidant capacity begin in the early stages of renal damage and very evident in dialysis patients. DNA damage secondary to inflammation and chronic course is also expected to develop in ESRD. In this study the changes in genotoxic damage in ESRD patients both undergoing or not undergoing hemodialysis compared to healthy controls were evaluated in detail. DNA damage in the peripheral lymphocytes of the subjects were determined by the alkaline Comet assay. 8-OHdG levels indicating the oxidative stress related DNA damage were measured in the plasma samples using ELISA kits. The patient group with ESRD consisted of 44 patients undergoing hemodialysis and 31 patients not undergoing hemodialysis. The control group consisted of 40 healthy volunteers similar to the patient group in terms of age, gender, lifestyle, and smoking habits. DNA damage expressed as %DNA tail moment increased significantly in ESRD patients with or without dialysis moreover DNA damage in ESRD patients on dialysis increased more dramatically compared to the control group and the levels of 8-OHdG levels of ESRD patients on dialysis (4.1 fold) and ESRD patients without dialysis (2.1 fold) were significantly higher than the healthy controls. The duration and the severity of disease was moderate positively correlated with increased DNA damage. Approaches to reduce oxidative-stress related DNA damage in ESRD patients are highly recommended.

# O-44
# Impact of different electromagnetic fields on DNA stability *in vitro*

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Numerous studies suggest links between electromagnetic fields (EMFs) and cancer risks in humans. It is known that long-term exposure to high-frequency electromagnetic fields (HF-EMF) from mobile phones may increase glioma risks, while exposure to low-frequency electromagnetic fields (LF-EMF) from power lines has been associated with childhood leukaemia. In the present study, we investigated how HF-EMF (1950 MHz, UMTS signal) and LF-EMF (50 Hz, from power lines) might affect DNA stability in human-derived astrocytoma (1321N1) and lymphoma (Jurkat) cell lines, respectively. Additionally, we assess whether these EMFs influence DNA damage when cells are co-exposed to chemicals that can mimic occupational hazards. These four substances (4-nitroquinoline 1-oxide (4NQO), benzo[a]pyrene diolepoxide, $NiCl_2$, $CrO_3$) cause various types of DNA damage, such as pyrimidine dimers, bulky base adducts, and both DNA-DNA and DNA-protein crosslinks or oxidative damage. Our assessment, conducted through a single-cell gel electrophoresis assay, revealed that while exposure to HF-EMF reduced both basal and 4NQO-induced DNA damage in astrocytoma cells, it did not significantly alter chemically induced DNA migration in other test conditions. These findings could potentially indicate adaptive cellular responses. Our results suggest that acute exposure to mobile phone and power line-specific EMFs does not exacerbate genotoxic effects from chemical exposures that are relevant in occupational settings.

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## O-45
## Enzyme-modified CometChip: Detection of enzyme-specific DNA damage with a high-throughput comet assay

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The Comet assay is a widely utilised technique for assessing DNA damage at the single-cell level. To enhance its application for high-throughput screening, the Comet assay was adapted into the CometChip, which has seen significant advancements. This study focused on validating an enzyme-modified version of the CometChip, tailored to detect enzyme-specific DNA damage, particularly DNA oxidation damage. We conducted experiments using the formamidopyrimidine DNA glycosylase (Fpg) enzyme to quantify DNA oxidation lesions. This was performed on two cell lines, HepG2 and THP1, exposed to potassium bromate (KBrO3), which is an established positive control for the FPG-comet assay. Various parameters of the assay were tested, including cell loading temperature and density, buffer washes, and the optimisation of incubation times for alkaline unwinding and neutralization. Additionally, three concentrations of Fpg were evaluated. The results showed good comparability with other formats of the comet assay, such as the commonly used 2-gels or 12-gels formats. Moreover, the enzyme-modified CometChip demonstrated reproducible detection of DNA oxidation in a 96-well microarray format. The enzyme-modified CometChip facilitates efficient screening of large sample volumes, making it suitable for basic research and applied studies, including biomonitoring and drug development. However, the large sample size that can be processed by the CometChip would benefit from an efficient high-throughput image analysis system. Currently, there is a shortage of reliable AI or software for automated image analysis of the comet assay. Overall, our research highlights the potential of the enzyme-modified Comet Chip, with numerous applications in genotoxicity assessment.

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# O-46
# Antigenotoxic effects of melatonin in obese mice

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Overweight and obesity are increasing dramatically in recent years, due to the increase in the consumption of fast foods. Obese individuals have a decrease in antioxidant defences, which can lead to DNA damage. Thus, studies are being conducted in order to improve complications caused by obesity. Therefore, animal research is using the cafeteria diet (CAF) as a model for inducing obesity, since it is based on the consumption of ultra-processed foods. Complementary dietary strategies are currently being studied to prevent and/or improve complications caused by obesity, such as natural compounds with anti-obesity effects. Among these compounds, melatonin (MEL) has been standing out due to its antioxidant and anti-inflammatory properties. Thus, the aim of the present study was to evaluate the effect of melatonin supplementation on biochemical and genotoxic parameters in CAF-fed mice. For that, 60 male Swiss mice were used, divided into six different experimental groups. Blood samples were collected from the animals at 17 and 21 weeks in order to evaluate damage to DNA, lipid (triglycerides, total cholesterol and HDL), and glycemic profile. At the end of the experiment, the animals were euthanised, and the liver, kidney, and bone marrow were removed to carry out the other tests. Thus, it can be observed that CAF-induced changes in the lipid and glycemic profile, in addition to leading to DNA damage in the tissues evaluated. When the animals were supplemented with MEL it was possible to observe that there was a reversal of these changes. CAF diet led to oxidative stress and damage to the liver, kidney and bone marrow but MEL was effective in attenuating these changes present in obese mice. In conclusion, the results demonstrate that MEL is an efficient antioxidant and may be a candidate for reducing the biochemical and genetic changes present in obesity.

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# O-47
# An important limitation of the comet assay

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Is it possible to measure initial DNA damage levels in plants (*Arabidopsis*) with the comet assay? One of the limitations of the comet assay is the fact that there is always a delay between the time of treatment and the fixation of nuclei in agarose. This means that very rapid events after treatments might be missed because they are completed before the nuclei are fixed. For example, if *Arabidopsis* plants are irradiated with X-rays, it reasonably takes at least 6 minutes to slice leaf tissue and embed nuclei in agarose on a slide. During this short time, single-strand (ssb) breaks in DNA are completely repaired. Then, the DNA damage that remains consists of ATM- and BRAC1-dependent repair of double-strand breaks (dsb). So, DNA damage after X-irradiation consists of ssb and dsb. Ssb are very rapidly repaired. Dsb repair is delayed in plants because expression of at least BRAC1 and Rad51 has to be induced and complexes for dsb repair have to be formed [1]. Given any data set of comet results, one should ask: What does the DNA repair curve look like? At what point during the repair curve were measurements made?

[1] Arnould et al. 2023. https://doi.org/10.1038/s41586-023-06635-y

# O-48
# Age-related DNA damage in middle-aged hospitalised COVID-19 patients

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Unfavourable changes in DNA damage, oxidative stress, and inflammation have been linked to viral infections, but there is limited data on COVID-19 and DNA damage. This case-control study aimed to investigate whether hospitalised COVID-19 patients (n=48) differ from healthy age- and sex-matched controls with respect to DNA damage, oxidative stress markers, unconjugated bilirubin (UCB) and 92 different inflammatory markers. We analysed oxidative DNA damage by using formamidopyrimidine DNA glycosylase (FPG) and by challenging whole blood samples with $H_2O_2$ using the Comet assay. UCB was analysed with HPLC. An olink proteomics panel was used for inflammation assessment. The results regarding DNA damage revealed that middle-aged COVID-19 patients (n=24, mean age 55.7 years) but not older COVID-19 patients (n=24, mean age 83.5 years) showed a significant increase in DNA damage (%DNA in tail, $p<0.05$) after formamidopyrimidine DNA glycosylase (FPG) treatment. Unexpectedly, markers of oxidative stress remained unchanged (FRAPmalondialdehyde) compared to healthy controls. COVID-19 patients had significantly higher levels of C-reactive protein and 55 different inflammatory proteins ($p<0.001$) in the serum. Interestingly, UCB levels were significantly reduced in COVID-19 patients, especially in middle-aged COVID-19 patients ($p<0.05$), compared to healthy controls. These results indicate that middle-aged hospitalised COVID-19 patients exhibit higher levels of DNA damage, which might be caused by changes in inflammatory pathways rather than oxidative stress.

[1] Draxler et al. 2023. https://doi.org/10.1016/j.redox.2023.102914

## O-49

# Evaluation of nanoparticle-induced genotoxicity in human peripheral blood lymphocytes using CBMN and comet assays: An *in vitro* study

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The growing use of nanotechnology has escalated the exposure of organisms, including humans, to nanoparticles, triggering concerns regarding their genotoxic potential. Genotoxicity encompasses various forms of genetic damage, including cytotoxicity, genetic mutations, and chromosomal abnormalities. While substantial data on nanotoxicology exists, a comprehensive understanding of the risks, mechanisms of action, and interactions of diverse nanoparticles with biomolecules and tissues across different organisms remains elusive. To address this knowledge gap, our study investigates the genotoxicity of four different nanoparticles: aluminium oxide ($Al_2O_3$ NPs), iron oxide ($Fe_3O_4$ NPs), silicon dioxide ($SiO_2$ NPs) and titanium dioxide ($TiO_2$ NPs). The genotoxic potential of these nanoparticles was evaluated on human peripheral blood lymphocyte culture using Cytokinesis Block Micronucleus (CBMN) assay and the comet assay. In the CBMN assay, binucleated cells (BN) with micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD) were scored along with mono and multinucleated cells. The results showed a moderate-to-highly significant frequency of genotoxic events when compared with the untreated negative control. The comet assay also resulted in positive genotoxicity in nanoparticle-treated samples, with a significant increase in comet tail length and percentage of tail DNA compared to the untreated control groups. The study observes that nanoparticles have a dose-dependent adverse effect on human peripheral blood lymphocytes. The study will be useful to elucidate the mechanism of action by closely analysing the endpoints.

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# O-50
# Evaluation of potassium bromate as a positive control in the *in vivo* Fpg-modified comet assay for the detection of oxidised bases

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The *in vivo* comet assay is included in the ICH and EFSA strategies for genotoxicity testing. Its correspondent OECD guideline (OECD TG 489) only contemplates the standard version of the assay which detects DNA strand breaks and alkali labile sites (ALS). However, the mechanistic information is limited as strand breaks are quite unspecific lesions caused by different agents and occur as intermediates in the repair of most premutagenic lesions. In this regard, the assay has been modified to detect other DNA lesions using different strategies, the most common one being the use of DNA repair enzymes, such as formamidopyrimidine DNA glycosylase (Fpg), to detect oxidised lesions. This modification in the OECD guideline will allow to increase in the information extracted from an animal with just a small change in the comet assay protocol. The aim of this work is to test potassium bromate ($KBrO_3$) as a possible positive control for the *in vivo* Fpg-modified comet assay. To do so, Wistar rats were orally administered twice with different doses of $KBrO_3$ (0, 100, 200 and 300 mg/kg bw) or once with 300 mg/kg bw of EMS as a standard positive control. Liver, duodenum, kidney, brain and whole blood were obtained 3 hours after the last administration and analysed. Samples for histopathology analyses were also collected. Significantly increased levels of net Fpg-sensitive sites were found in the liver, duodenum, kidney and whole blood of all the animals. Moreover, Fpg-sensitive sites were also detected in the brain of the animals administered with EMS. DNA strand breaks and ALS were only detected in the animals administered with EMS, with the exclusion of whole blood. These results suggest that $KBrO_3$ may be a good positive control for the detection of oxidised bases in the Fpg-modified comet assay in several tissues.

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## O-51
## Study of genotoxic effects on exocrine pancreas after chronic dietary exposure to cocktail of pesticides

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Pancreatic ductal adenocarcinoma (PDAC) is projected to become the third leading cause of cancer-related death by 2030. One reason could be the environmental exposures, including pesticides, which could induce or alter cellular mechanisms such as genotoxicity, microenvironment remodelling and autophagy. The aim of our work was first to study the genotoxic effects of pesticide exposure on the exocrine pancreas by comet assay. To avoid the DNA damage caused by the tissue autodigestion and/or isolation method, we optimised the protocol of acinar cell isolation to reach very low basal DNA damage (<5 % of tail DNA). Pancreatic tissue was grinded mechanically using a dounce in HBSS-EDTA 0.02M buffer supplemented with STI (Soybean Trypsin Inhibitor). Acinar cell isolation was confirmed by flow cytometry before performing an alkaline comet assay, analysed with Instem Comet IV software. We studied thereafter the effects of five-month exposure to a cocktail of pesticides (Imazalil, Thiabendazole, Boscalid, Lambda-Cyhalothrine) called ITBC on the exocrine pancreas of mice fed with those components at low doses (acceptable daily intake (ADI) or 10x ADI of the pesticide cocktail). The levels of DNA damage were assessed in both the control chow diet (CD) and the obesogenic western diet (WD). These effects will be illustrated by statistical graphs, some based on a logit transformation of the tail intensity, and evaluated through statistical tests. To complete this study, we performed histological analysis on the pancreas with hematoxylin/eosin colouration. Autophagy markers were also studied such as the autophagy inducer Vps34, expressed mostly in pancreatic acinar granules. Regeneration and remodelling of the tissue were studied by analysis of proliferation markers, fibroblast activation and fibrillar collagen composition. In conclusion, we developed a suitable method for isolating fresh acinar pancreatic cells without damaging their DNA for comet assay analysis. We applied this novel methodology to analyse the genotoxic effects of chronic dietary exposure to pesticides.

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# Posters

# P-1
# Urinary benzene metabolites and DNA damage in children living in Riyadh, Saudi Arabia

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Benzene is a widespread environmental contaminant and a well-known human carcinogen. While its adverse health effects are significant across the general population, children are considered a high-risk group due to their greater vulnerability compared to adults. Aim: to assess environmental exposure to benzene by measuring two of its urinary metabolites and to evaluate the potential link between benzene exposure and DNA damage in peripheral blood. A cross-sectional study was conducted on 226 healthy children attending Pediatric Clinics at King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia. The levels of two benzene metabolites, trans, trans-muconic acid (t,t-MA) and *S*-phenylmercapturic acid (S-PMA), were quantified in spot urine samples using UPLC. DNA damage in peripheral blood was assessed using the alkaline version of the comet assay. S-PMA was detected in 80.5% of urine samples, while t,t-MA was detected in 44.7%. S-PMA had a mean concentration of 31.02 μg/g creatinine and a median concentration of 2.5 μg/g creatinine, with a range from 0.42–681.96 μg/g creatinine. For t,t-MA, the mean concentration was 8.78 μg/g creatinine, median 0.9 μg/g creatinine and the range was 0.047 to 136.28 μg/g creatinine. Spearman's rho correlation analysis showed a significant positive correlation between S-PMA and t,t-MA excretion (Rs=0.602, $p<0.001$). However, no significant associations were observed between the levels of urinary benzene metabolites and any of the measured comet parameters. This study demonstrates prevalent benzene exposure among children but finds no significant correlation between benzene metabolite levels and DNA damage. The absence of a significant association may be due to DNA repair processes that mitigate strand breaks, highlighting the need for applying alternative biomarkers of DNA damage to better understand the health impacts of benzene in children.

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# P-2
# The impact of environmental pollution on oxidative stress in mothers/newborns (the update results, HAIE)

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The aim of the study was to analyse contaminants that affect oxidative DNA damage and lipid peroxidation in non-smoking mothers and newborns. 147 pairs of mothers/newborns originated from Ceske Budejovice (CB; an agricultural region) and 201 pairs were recruited from Karvina (an industrial region). Personal and medical data, external (PM2.5, B[a]P) and internal (PCBs, OCPs, BFRs, PFASs and OH-PAHs) exposures, antioxidant mechanisms (SOD, CAT, GPx and AOX), and cytokines concentrations (IL-1β, IL-6) were investigated as parameters potentially affecting the markers of DNA oxidation (8-oxo-7,8-dihydro-2'-deoxyguanosine) and lipid peroxidation (15-F2t-isoprostane). The localities differed in the type of ecological burden. Significant levels of PCBs, OCPs, and PFASs were detected in probands from CB ($p<0.001$). In contrast, significantly increased ambient air levels of B[a]P and PM2.5 were confirmed by OH-PAHs detected in probands from Karvina ($p<0.001$). In mothers, multivariate analysis suggested that the extent of oxidative stress before and after delivery was relatively the same. In Karvina, 15-F2t-isoprostane was positively associated with IL1β, contaminants (PCB28, BDE153 and PFTrDA) and cotinine. Unexpectedly, no association between 15-F2t-isoprostane and OH-PAHs were seen in mothers. This link has only been observed in newborns. The comparison between the maternal and neonatal IsoP model for Karvina showed agreement in some predictors (IL1β, cotinine, sampling period, PCB28). Additionally, neonatal 15-F2t-isoprostane was linked to vacuum extraction, allergy and inflammation. Multivariate analysis didn´t show neonatal IsoP difference between localities but confirmed significantly elevated 8-oxodG levels in Karvina ($p=0.05$). 8-oxodG was also positively linked with naphthalene exposure, gestational diabetes and AOX. Results of AOX indicated that the antioxidant mechanisms were induced but they failed to eliminate ROS efficiently. In newborns from CB, 8-oxodG levels were more affected by cotinine, sampling period and PFOA. In conclusion, the transplacental transfer of PAHs, PFAS and some POPs in low levels may affect the neonatal oxidative state in early life.

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## P-3
## Genoprotective effect of *Scutellaria altissima* L. extracts against $H_2O_2$-induced oxidative damage

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Oxidative injury of biomolecules arising from the presence of reactive oxygen species is a major factor in the development of different human disorders. One of the approaches to protect human health is to use natural compounds with antioxidant potential that can be applied in the diet. Therefore, the increasing interest in compounds originating from plants has been noted. Taking into account all the above, the aim of this study was to examine the antioxidant and antigenotoxic effects of *Scutellaria altissima* 70% aqueous-ethanolic extracts (labelled as SaB, SaP and SaS) originating from plants collected at different localities. By applying colorimetric assays total phenolic, flavonoid and phenolic acids were determined. SaS exhibited the highest phenolic and flavonoid content (79.71 mg GAE/g DE; 39.74 mg QH/g DE), while SaP mostly contained phenolic acids (57.84 mg CAE/g DE). Antioxidant potential determined in DPPH, ABTS and FRAP assays revealed the highest activity for SaP ($IC_{50}$ values were 0.89 mg/mL and 1.14 mg/mL for DPPH and ABTS; reduction potential detected at 124.38 μmol $Fe^{2+}$/g DE in FRAP). In order to test the protective effect of extracts, it was necessary to determine the non-cytotoxic concentrations of extracts by MTT assay, which revealed cell survival reduction in the range of 27–82%. Further on, non-genotoxic concentrations of extracts were determined in an alkaline comet assay. By applying the same assay, the antigenotoxic potential of extracts against oxidative damage induced by hydrogen peroxide was recorded. A dose-dependent genoprotective effect was observed in the cases of SaB and SaP (inhibition of genotoxicity up to 60% and 53%). Interestingly, for SaS a hormesis effect was observed since the lowest non-genotoxic concentration exhibited the most potent protective effect (inhibition up to 44%). These findings underscore the potential health benefits of *Scutellaria altissima* extracts highlighting their potential as therapeutic agents in combating oxidative stress-related diseases.

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## P-4
## Genotoxic effect of a pesticide mixture by the comet assay

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Assessing the risk of exposure to pesticide mixture remains challenging, potentially leading to overestimation or underestimation of toxicity. This study aims to establish a possible link between exposure to a pesticide mixture of glyphosate, nicosulfuron, *S*-metolachlor, terbuthylazine, captan, spinosad, deltamethrin, acetamiprid and its genotoxic effect. Human cell lines AGS and Hep G2 were exposed to the pesticide mixture for 24 hours by the three concentrations based on the acceptable daily intakes (ADIs) of the selected pesticides: ADIs/5, ADIs, 5ADIs. Hydrogen peroxide was employed as a positive control. The length of the comet's tail is shortest at the highest concentration, indicating that this concentration has resulted in the breaking of larger DNA pieces than any other used concentration. Concerning the AGS cell line, no statistically significant variations were across concentrations, indicating that the pesticide mixture did not produce DNA breaks in these cells. The medium concentration resulted in the greatest intensity of the comet tail and a statistically significant increase in the tail moment compared to the negative control. This suggests that the mixture of the pesticide concentrations equal to the ADIs led to the creation of a greater number of smaller fragments, *i.e.* DNA breakage of the HepG2 cell line compared to the control, implying that this pesticide mixture concentration causes a genotoxic effect. Integrated toxicological studies are necessary to understand the specific mechanisms involved in the genotoxicity of the studied mixture and the consequent carcinogenic effects.

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## P-5
## Machine learning for automatic analysis of comet assay outcomes

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The comet assay, a widely used technique for detecting DNA damage at the cellular level, has become a fundamental tool in toxicology. However, the evaluation of assay results can be subjective and labour-intensive, relying heavily on the analyst's expertise. In this study, we faced the application of machine learning techniques as an efficient tool for the automation and optimisation of comet assay analysis, providing a more accurate, consistent, and reliable methodology. Using a convolutional neural network trained with an extensive set of comet assay images, we developed a model capable of accurately segmenting the comets, being able to complete massive analysis of the assay results in just a fraction of the time required with conventional methods. The model will be validated by comparing its results with manual segmentations performed by toxicology experts. Preliminary results show correlation and demonstrate its potential to reduce inter- and intra-observer variability. Furthermore, segmented comets obtained with the trained model are morphologically analysed using traditional algorithms of image processing in order to find metrics that could act as new damage indicators, apart from the tail moment and tail intensity, that correlate well with the DNA damage. The integration of machine learning, specifically convolutional neural networks, into comet assay analysis not only enhances the accuracy and consistency of DNA damage detection but also significantly reduces the time and variability associated with traditional methods, paving the way for more efficient and reliable toxicological assessments.

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## P-6
## A genetic screen for *blm* suppressors in *Ustilago maydis* identifies novel proteins affecting DNA repair and recombination

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The maintenance of genome integrity is a fundamental cellular process, and it is highly conserved among all domains of life. Since the DNA molecule is under constant threat from endogenous and exogenous factors that cause its damage, organisms have evolved several DNA repair mechanisms. Homologous recombination (HR) is essential for the error-free repair of DNA double-strand breaks, which are the most deleterious lesions. Despite extensive research on HR in different organisms, not only all functions of known HR factors and interactions among them are still unknown, but the question also arises as to whether there is a possibility of the existence of unknown factors. The focus of our research is to uncover novel cellular factors that regulate HR by isolating suppressors of *blm* in *U. maydis,* a unicellular phytopathogen that is extremely resistant to radiation and has a DNA repair system similar to that in humans with highly conserved BRCA2 (named Brh2). We have identified 4 novel factors of unknown functions (named Rec3, Zdr1, Bls9 and Bls2), and 3 known factors: Rad51, Dna2 and Mph. Mutations in each of these genes suppress the hydroxyurea sensitivity of *blm*. Rec3 is a member of the family of Rad51 ATPases. It plays a critical role in induced allelic recombination and is crucial for the completion of meiosis. We have shown that there is a close functional connection between Brh2 and Rec3. Zdr1 is Cys2-His2 zinc finger (C2H2-ZF) protein whose loss doesn't cause a detectable change in HR, but it is involved in DNA repair. Bls9 doesn't have any characterised domains. It is involved in DNA repair and HR between chromosome homologs. Bls2 is an uncharacterized protein that displays a slow-growth phenotype that can be suppressed by truncated Bls9. These novel factors can provide insights into HR regulation, interactions among HR participants, and relations to other cellular processes.

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## P-7
## Evaluation of *in vitro* genotoxicity of differently sized cobalt oxide nanoparticles

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Cobalt (II, III) oxide nanoparticles (Co3O4-NPs) have shown promise for various technological and medical applications, such as drug delivery and potential anticancer therapy tools [1]. However, widespread acceptance of these applications could lead to increased direct human exposure to $Co_3O_4$-NPs. Therefore, this study aimed to assess the cytotoxic and genotoxic potential of differently sized cobalt oxide NPs (10–30 and <50 nm) to human peripheral blood mononuclear cells (PBMC) *in vitro*. Nanoparticle uptake analysis by PBMCs was assessed using flow cytometry light scatter analysis. While induction of reactive oxygen species (ROS) generated by Co3O4-NPs was evaluated using the cell-permeable oxidant-sensitive fluorescent dye 2,7-dichlorodihydrofluorescein diacetate ($H_2$DCFDA). Three standard genotoxicity tests were applied: alkaline comet assay and sister-chromatid exchange (SCE) assay for primary DNA damage evaluation and cytokinesis-block micronucleus (CBMN) assay for chromosomal damage analysis. Results showed that larger nanoparticles, with a primary size of <50 nm, were more efficiently uptaken by human PBMCs and induced higher levels of ROS compared to smaller nanoparticles (1030 nm). Furthermore, both sizes of $Co_3O_4$-NPs caused significant DNA and chromosomal damage, often in a dose-dependent manner. These findings suggest that Co3O4-NPs possess genotoxic properties and can induce significant amounts of DNA damage in human PBMCs *in vitro*. This raises concern about the safety of Co3O4-NPs and underscores the importance of conducting thorough genotoxicity analyses of Co3O4-NPs in various cell cultures to ensure their safe use in technological and medical applications.

[1] Huang et. al. 2021. https://doi.org/10.3390/pharmaceutics13101599

## P-8
## Investigation of nitrosamines using miniaturised Ames tests

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The presence of genotoxic impurities in pharmaceuticals and packaging materials poses a significant health risk, necessitating the development of reliable test methods to ensure product safety. The emerging concern about nitrosamines propelled an international joint action for developing and refining methods to achieve an appropriate sensitivity towards this class of chemicals. Regulatory authorities, including the EMA and the FDA, recommend the implementation of Enhanced Ames Test conditions to elucidate the genotoxic potential of nitrosamines, which involves the application of a high percentage (30%) of liver microsomal fractions to bioactivate the nitrosamines. Studies to date apply the Agar Plate test for the assessment of nitrosamine-related genotoxic potential, however, miniaturised versions of the traditional Ames test could be applied to achieve a robust sensitivity while using significantly fewer test samples, S9 microsomal fraction and plasticware. Our approach involves two miniaturised versions of the Ames assay: the agar-based MicroAmes6 in a 6-well plate format and the liquid microplate fluctuation format assay known as the Ames MPF with the 3 strains TA100, TA1535 and *E.coli* uvrA[pKM101]. Short alkyl-chain nitrosamines are tested with a modification of the original protocols. We conclude that miniaturised Ames tests offer a resource-efficient approach for assessing the mutagenicity of nitrosamines, therefore large-scale adoption of these miniaturised Ames assay formats is strongly encouraged across industries.

## P-9
# Analysis and evaluation of genotoxicity and carcinogenicity assessment across legislation towards the regulatory implementation of NAMs

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Genotoxicity and carcinogenicity are key endpoints for the risk assessment of all types of substances. Research on alternatives to animal testing for these endpoints has been active for decades and has led over time to the development of many short-term *in vitro* tests for the identification of mutagens and carcinogens. While these tests are integrated into current testing strategies, resulting in a reduction of animal testing, high relevance is still devoted to data from *in vivo* animal studies. The case study presented here is part of the collective effort carried out within the European Partnership for the Assessment of Risks from Chemicals (PARC). It addresses the challenges associated with innovation in chemical risk assessment, including the phasing out of animal testing through the introduction of New Approach Methodologies (NAMs) [1]. To fully understand the feasibility of introducing NAMs in regulatory practices, the present PARC case study analyses and compares how genotoxicity and carcinogenicity are evaluated in the different EU legislative domains and how these interact. Gaps and inconsistencies in risk assessment procedures are highlighted, as well as needs and opportunities for NAM development and implementation. Furthermore, available NAMs and emerging models for genotoxicity and carcinogenicity evaluation are identified. The results of this work will help in clarifying the scope for NAM implementation within current regulatory frameworks and will support the identification of short- and long-term goals toward phasing out animal testing for genotoxicity and carcinogenicity.

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[1] Marx-Stoelting et al. 2023. https://doi.org/10.1007/s00204-022-03435-7

## P-10
## DNA damage in sperm cells of a marine crustacean assessed by the comet assay

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Few studies explore DNA damage in sperm cells using marine invertebrates. Comet assay has already been used in mollusc spermatozoa for monitoring aquatic environments and in prawns to evaluate different toxicants. However, no information was found about marine amphipods. *Parhyale hawaiensis* is a marine amphipod with a circumtropical distribution and is considered a model for evolution, development, and ecotoxicological studies. We aimed to develop a methodology to collect sperm cells of *P. hawaiensis* and evaluate their DNA damage using the comet assay. To test the protocol, we directly exposed the sperm cells to different mutagenic agents (ethyl methanesulfonate – EMS, $H_2O_2$, and UV radiation) and compared their response to hemocytes (somatic cells from the hemolymph that have an already validated protocol) response. Sperm cells showed good results and higher DNA damage than hemocytes under exposed conditions. Then, as a proof of concept, we exposed adult organisms to mutagenic compounds (EMS, benzo[a]pyrene (BaP), azo and anthraquinone dyes) at non-lethal concentrations to analyse sperm cells' DNA damage. Organisms exposed to EMS presented a clear concentration response in DNA damage after 24 h of exposure. We also observed that BaP was able to induce a statistically significant increase in DNA damage of sperm cells after exposing the organisms to 96 h. For the two dyes, although DNA damage increased, statistical differences were not observed after 96 h of exposure. We believe we successfully optimised a protocol to detect the genotoxicity of chemicals in sperm cells using *P. hawaiensis*. The test can be performed in the laboratory and has the advantages of employing organisms that are easily cultivated in reduced space, using simple laboratory resources, and using a reduced amount of material and reagents. The protocol could also be used in field studies to monitor the presence of germ cell mutagens in the marine aquatic environment.

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## P-11
## DNA damage and oxidative stress biomarkers in healthy male volunteers after a repeated bolus and continuous glucose infusion

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Glucose variability (GV) is a phenomenon that describes fluctuations in blood glucose levels over the course of a day. As part of the investigation of risk factors for coronary heart disease, GV is increasingly becoming the focus of scientific attention. Scattering GV can contribute to the development of metabolic syndrome and type 2 diabetes. Hyperglycemia can lead to oxidative stress, which results in molecular damage due to an accumulation of reactive oxygen species (ROS). To obtain more information on the immediate effects of GV, 10 healthy men aged 21–30 years were administered intravenous glucose continuously or as a repeated bolus over a 48-hour period in a crossover study design. Whole blood was analysed for DNA damage using the Comet assay using three different incubation solutions (lysis buffer, $H_2O_2$ and the lesion-specific enzyme formamidopyrimidine DNA glycosylase (FPG)) and plasma for various markers of oxidative stress (protein carbonyls (PC), unconjugated bilirubin (UCB) and total antioxidant potential (FRAP). A significant time effect was found for the three incubation solutions for DNA damage, PC and UCB. This can potentially be attributed to circadian changes. However, no differences were observed for any of the markers between the two intervention groups. In conclusion, it was shown that bolus glucose administration had no significant acute effect on DNA damage and oxidative stress markers in healthy men compared to continuous administration.

[1] Bragagna et al. 2023. https://doi.org/10.3390/ijms241713608
[2] Feldbauer et al. 2022. https://doi.org/10.1371/journal.pone.0279308

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## P-12
## Using ToxTracker and DNA repair-deficient cell lines to determine the genotoxic mode of action of *N*-nitrosamines

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N-Nitrosamines (NAs) are considered probable human carcinogens and were recently detected as impurities in pharmaceuticals, leading to a concern for human health. NAs require metabolic activation before they become mutagenic. There are many differently sized NAs and not all NAs are mutagenic. Understanding which NAs are genotoxic and their mode of action (MoA) will improve our understanding of the mutagenic potential of these drug impurities as it relates to their structure. While NAs are potent mutagens in vivo, in vitro metabolization is generally less efficient. We first optimised a hamster S9-based protocol for in vitro NA metabolization. Next, we assessed the genotoxic potential of 8 different NAs to which humans are commonly exposed using the ToxTracker assay. ToxTracker is a reporter assay that can provide insight into the MoA of genotoxicity. All tested NAs were classified as genotoxic in ToxTracker in the presence of hamster S9 with a clastogenic MoA. The smaller NAs did induce DNA strand breaks but no detectable inhibition of DNA replication, likely related to the size of the induced DNA lesions. To further investigate the MoA of these NAs, we applied the DNA Repair Profiler, a collection of mammalian cell lines with deficiencies in the major DNA repair pathways. For the smaller NAs, there was no increased cytotoxicity in base excision (BER) and nucleotide excision repair (NER)-deficient cells compared to wild-type cells. Currently, we are exploring the repair of DNA damage from the larger NAs as well as the relevance of DNA mismatch repair to reduce NA-induced gene mutations.

## P-13
## Genotoxicity assessment of a promising contrast agent candidate: dependence on concentration and exposure time

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Hafnium(IV)-containing Wells-Dawson polyoxotungstate (Hf-WD) is a polyanion exhibiting significant capabilities to attenuate X-rays. Thus, this tungsten-based nanocluster is considered a promising contrast agent candidate for computed tomography. Since this nanocluster has the potential to be applied as a medicinal agent, the evaluation of its potential toxicity is of key importance. Accordingly, the effect of exposure time and Hf-WD concentration on the values of tail DNA as a genotoxicity marker was studied. Tail DNA was determined using the standard procedure for alkaline comet assay using an epifluorescence microscope (Zeiss, Göttingen, Germany) connected through a camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd., UK). Human blood samples were taken from young, healthy male and female individuals and treated *in vitro* with Hf-WD at concentrations of 1, 10, and 100 μM for 4 and 24 hours. The obtained tail DNA values were expressed as the mean value±standard deviation. The concentration of 1 μM resulted in a tail DNA value of 1.30±0.19 after 4 hours, whereas 24-hour exposure induced a significantly lower value of 0.96±0.16. Similarly, significantly different values were also observed for 4 and 24 hours of treatment at Hf-WD concentrations of 100 μM (1.05±0.12 and 2.53±0.35, respectively). On the other hand, the results of tail DNA for 10 μM Hf-WD were not a function of the exposure time. In accordance, it can be concluded that the monitored genotoxicity evaluation was dependent on the concentration of the studied compound and the exposure time of blood samples.

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## P-14
## Bioactivity assessment of graphene quantum dots on HEK293T cells

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Graphene quantum dots (GQDs) are emerging as a promising carbon-based material with remarkable properties, including strong quantum confinement and edge effects. This zero-dimensional material, composed of graphene sheets smaller than 100 nm, exhibits stable fluorescence, low toxicity, and good water solubility. These characteristics enable GQDs to be utilized in diverse fields such as optoelectronics, sensors, bioimaging, drug delivery, energy, and environmental detection. Furthermore, the versatility of GQDs is increased by their amenability to modification, facilitating the tailoring of their properties to suit specific application requirements. In our study, we analysed the effects of commercially available blue (BGQDs) and green (GGQDs) graphene quantum dots on cell viability and cell death mechanisms in the HEK293T cell line (ATCC® CRL-3216™). BGQDs and GGQDs were applied 24 hours after cell sub-cultivation at the final concentrations of 2.5, 5 and 10 μg/mL Detection of apoptosis, necrosis and autophagy was assessed by fluorescent microscopy, while MTT assay was used for cell viability assessment. Our results revealed a significant concentration-dependant reduction ($p<0.001$) of cell viability following exposure to both BGQDs and GGQDs compared to negative control. Differential ratios of early and late apoptotic cells were observed between BGQDs and GGQDs treatments, with statistical significance ($p<0.0001$) noted. The difference in detected autophagosome signals between treatments and controls was not found. These results underscore the cytotoxicity of commercial BGQDs and GGQDs in applied concentrations predominantly favouring apoptosis over autophagic cell death as a potential underlying mechanism for their effects on cell viability.

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## P-15
# Assessing the potential of transcriptomics-based biomarkers to predict genotoxicity in different cell lines

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Transcriptomics-based biomarkers such as GENOMARK and TGx-DDI have been shown to be powerful tools for predicting the genotoxicity of chemicals [1]. Respectively developed in HepaRG cells after 72 hours of exposure and in TK6 cells with a 4-hour exposure period, these biomarker sets consist of 84 and 64 genes each. Although they differ in the compounds used for their development and only have 2 genes in common, both biomarkers sets result in similar classifications and potency rankings of genotoxic compounds in HepaRG cells [1]. To further investigate their prediction performance in different cell systems and at multiple exposure times, both biomarker sets were applied to quantitative and time-scaled datasets for cisplatin generated in RPTEC-TERT1 and HPPTEC cells. Cisplatin, a known genotoxic drug, was correctly classified by the two biomarkers in the two cell lines, with TGx-DDI predicting DNA damage induction at earlier time points than GENOMARK, aligning with their respective development exposure times. In addition, benchmark concentrations were modelled with BMDExpress2 for both biomarkers. Interestingly, benchmark concentrations demonstrated highly comparable consistent trends over time for both GENOMARK and TGx-DDI in the two cell lines, highlighting the robustness of the biomarkers across different cell systems. These results will be further compared to those obtained with HepaRG-Cisplatin data in order to assess the robustness of the BMC values in cell systems from different origins. Overall, the results obtained so far highlight the power of transcriptomics-based biomarkers and their usefulness in chemical genotoxicity assessment. This project is performed under the funding of the Partnership for Assessing Risks from Chemicals (PARC) and aims to support the integration of transcriptomics into Integrated Approaches to Testing and Assessment (IATA) for genotoxicity.

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[1] Thienpont et al. 2024. https://doi.org/10.1021/acs.chemrestox.3c00318

## P-16
## Early warning signals of genotoxic compounds in native and invasive fish: a case study from Sava Lake

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The Sava Lake was formed in the late 1960s, when the Sava River island was turned (artificially) into the peninsula in Belgrade, Serbia. It represents the most organized sports and tourist complex in Belgrade and an active water recreational area for citizens. Throughout the year, a large number of tourists visit the lake, and this increases pollution pressure. Additionally, the presence of the invasive fish black bullhead (*Ameiurus melas*) may disrupt water quality and lead to habitat degradation, potentially facilitating the successful establishment of viable populations of allochthonous, invasive species. However, ecogenotoxicological studies on this lake have never been conducted. Therefore, this research aims to detect "early warning signals" of organisms' exposure to genotoxic compounds, focusing on the native European perch (*Perca fluviatilis*) and the invasive species – black bullhead, to determine which species is under greater pollution/contamination/anthropogenic pressure. The comet and micronucleus tests were applied, as DNA damage could be a useful biomarker for detecting "early warning signals" of exposure to genotoxic compounds. Individuals were examined during the summer and winter seasons. The comet assay revealed statistically significant differences in DNA damage between native and invasive individuals, with invasive individuals exhibiting significantly less DNA damage (*P. fluviatilis* with average TI%: 18.06; 23.23 and *A. melas* with TI% 5.47; 7.51 for July and December, respectively). Lower levels of DNA damage were detected during the summer season for both species. The number of nuclear abnormalities and micronuclei also differed between species, with a significantly higher number detected in the native species and for both species in the summer month. Based on the aforementioned findings, the selected tests proved to be effective for early detection of DNA damage and showed good discriminatory potential for assessing the resistance of different species to genotoxic compounds, highlighting the resistance of the invasive species.

## P-17
## Methylation status of *RB1* gene and parameters of oxidative stress and inflammation in sarcoma patients – a preliminary study

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The aim of this study was to investigate the methylation status of the *RB1* gene and the level of oxidative stress and inflammation in sarcoma patients. Blood samples (N=51) were collected from newly diagnosed sarcoma patients admitted to University Hospital Centre Zagreb (Zagreb, Croatia). Based on tumour burden the patients were stratified into (1) undetectable (no signs of tumour mass); (2) medium (primary tumour <7 cm); and (3) heavy (primary tumour >7 cm and/or metastases). Copy number variations (CNVs) and methylation of the *RB1* gene were assessed in DNA isolated from peripheral blood leukocytes using MS-MLPA. In blood samples, oxidative stress parameters (reactive oxygen species, malondialdehyde, glutathione and activity of superoxide dismutase) and inflammation parameters (C-reactive protein, leukocyte count and neutrophil count) were followed. CNVs and aberrant methylation of the CpG106 promoter region of the *RB1* gene were not detected. However, in one sample hypermethylation (by approximately 10%) of imprinted locus CpG85 in intron 2 of the *RB1* gene was found. In addition, a very good correlation between the tumour burden and level of C-reactive protein and tumour burden and level of glutathione was found. This single-centred study on a cohort of consecutive sarcoma patients indicates that sarcoma patients can have aberrant germline DNA methylation and confirms the relationship of tumour burden to inflammation and oxidative stress.

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## P-18
## Effects of graphene quantum dots on HepG2 spheroids: A study on biocompatibility and potential biomedical applications

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Graphene quantum dots (GQDs) are a type of carbon nanomaterial characterised by their diminutive size, which gives them a tunable bandgap and exceptional physical and chemical attributes. Typically comprising multiple atomic layers and measuring less than 10 nm, GQDs exhibit admirable biocompatibility, minimal toxicity, and promising potential for biomedical applications. They have excellent photoluminescence and are utilized in a myriad of applications, including optical and electrical sensors, biological imaging, and tumour therapy. GQDs can also be combined with other materials to create nanocomposites with superior performance. This study aimed to investigate two types of commercially available graphene quantum dots (GQDs). Tested blue and green GQDs, mainly differ in their optical properties, particularly in the colour of the emitted light, determined by their bandgap. The cytotoxic and genotoxic effects of GODs (up to 250 μg/mL corresponding to 100 μg/cm$^2$) were evaluated in an advanced *in vitro* 3D model system, developed from human hepatocellular carcinoma (HepG2) cells, after 24 hours of exposure, using the luminometric CellTitter-Glo assay, the comet assay, and the analyses of *γ*H2AX and p-H3 expressions using flow cytometry. GQD samples induced similar dose-dependent cytotoxic effects in HepG2 spheroids. At non-cytotoxic concentrations, the two GQDs induced a dose-dependent increase in DNA damage, as detected by the comet assay. However, no induction of DNA double-strand breaks (*γ*H2AX) or increased expression of pH3 was observed, indicating no clastogenic or aneugenic activity, respectively. While our results show a certain degree of cyto- and genotoxicity of tested GQDs, there was no evidence of induction of the most harmful types of damage to the genetic material. Nevertheless, the mechanisms underlying the observed effects have to be further elucidated in order to confirm their safety.

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## P-19
# Assessing combined metal exposure: Insights into cellular bioavailability and genotoxicity of Co(II) and Ni(II) in HepG2 cells

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Cobalt (Co) and nickel (Ni) find widespread applications in alloys, pigments, catalysts, and as components in lithium-ion batteries powering electric vehicles (e.g., scooters, bicycles, or cars). This extensive use led to an increased presence of these metals in the environment and consequently in the food chain. Even if both metals are known to cause detrimental health impacts to humans when overexposed, knowledge about the consequences and underlying mechanisms of toxicity in case of a combined exposure is rare. These shortcomings are addressed in the current study exposing liver carcinoma (HepG2) cells with Co or Ni or a combination of both. Quantifying the cellular amount of Co and Ni pointed out that the bioavailability differs exposing the metals alone or in combination. The combination of Co and Ni resulted in higher Co levels with subsequent decreased amounts of Ni compared to the individual treatment. Since we could verify that both metals also transfer in the cell nuclei we were interested if genotoxicity may be one underlying mechanism of toxicity. Neither single exposure with up to 100 μM Co or 500 μM Ni (already toxic doses) nor a combination (25 μM Co and 150 μM Ni) did cause single strand breaks or elevated amounts of γH2AX (a marker of double-strand breaks). Interestingly, DNA damage response (PARylation) is increased in all tested doses starting at 12.5 μM Co and 75 μM Ni alone or in combination up to 100 μM Co and 500 μM Ni. More studies are needed to assess the potential damage sites and underlying mechanism of toxicity, especially in case of combined exposure.

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## P-20
## Establishment of a method of comet assay using interstitial cells of rat testes

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The comet assay is a method to assess DNA damage. Because this assay applies to various organs, it is useful to evaluate organ-specific genotoxicity. Genotoxicity is one of the mechanisms of actions of carcinogenesis and is considered to have high human relevance. In rats, the testis is the second most common organ of carcinogenesis after the liver, and most of these are Leydig cell tumours. The comet assay using whole testis was previously reported, except the testis contains various kinds of cells such as Leydig cells, Sertoli cells and sperm. To accurately evaluate the genotoxic potential of chemicals in Leydig cells, we established a comet assay using isolated rat Leydig cells from the testis.

# P-21
# Genotoxicity of benzo[ghi]perylene in the human bronchial epithelium NL-20 cell line

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Mexico City and its metropolitan area present serious air pollution problems. Due to its geographical location, it is difficult to achieve the complete combustion of gasoline and diesel in more than five million vehicles that circulate there daily, and the mountains that surround it do not allow an easy dispersal of contaminants. Polycyclic aromatic hydrocarbons are among the most important pollutants present in Mexico City's atmosphere, the most abundant and less studied being benzo[ghi]perylene (b[ghi]p); due to lack of information this compound is currently "unclassifiable as to carcinogenicity in humans" by the IARC. For these reasons, the aim of this work was to evaluate its effect on the human bronchial epithelium cell line NL-20 through several markers. The cells were treated for 24 h at 2, 4, and 8 μM of b[ghi]p, and immunohistochemical staining revealed the markers of H2AX*γ*, 2-oxo-guanosine, 53BP1, BRCA-1, H3K9me3, and TRF2. Likewise, genotoxicity was evaluated using the alkaline version comet assay and a variant with the endonuclease formamidopyrimidyl glycosylase (FPG) for adduct formation. The results showed a significant increase in DNA strand breaks and in nuclei positive for H2AX*γ* in every concentration in comparison to the cells grown without b[ghi]p. No increase in 2-oxo-guanosine and strand breaks via comet assay with FPG was observed. An increase in the nuclear expression of 53BP1 suggests the activation of DNA repair by non-homologous recombination, RRNH, which correlates with the decrease in expression of the H3K9me3 marker that is related to nuclear heterochromatin. Finally, an increase in TRF2 expression indicated important telomere protection activity. The results allow us to conclude that b[ghi]p is genotoxic through analysing several markers; it induces DNA strand breaks, reduces heterochromatin formation, and RRNH mechanisms are activated, and finally, through this evaluation, the compound does not induce the formation of adducts.

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## P-22
## Marine environmental DNA metabarcoding for northern Adriatic phytoplankton monitoring

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Phytoplankton, the photosynthetic microorganisms that form the basis of aquatic food webs, are crucial indicators of water quality and ecosystem health. In addition to conventional techniques for monitoring phytoplankton based on their morphology (*e.g.* light microscopy), environmental DNA (eDNA) metabarcoding has recently been proposed as an approach to assess the overall phytoplankton biodiversity in marine environmental studies based on targeted genetic marker sequence variability. High-throughput sequencing methods targeting a variable region of small subunit ribosomal (SSU) RNA gene were used to detect and identify eukaryotic phytoplankton. The biodiversity patterns revealed by metabarcoding were determined from perennial monthly sampling at the long-term monitoring stations in the northern Adriatic. Among the phytoplankton groups that were analysed, dinoflagellates, followed by diatoms were the most diverse groups in terms of genetic diversity. The results revealed numerous phytoplankton genera not reported in previous northern Adriatic phytoplankton studies. These genera are predominantly smaller or cryptic species that are difficult to identify morphologically. In addition, the comprehensive and long-term metabarcoding dataset enabled methodology benchmarking and guidelines development for the northern Adriatic. eDNA metabarcoding proved to be an important molecular tool that complements standardised light microscopy and advances conventional monitoring and research of phytoplankton genetic diversity and community structure to assess biodiversity and the state of a dynamic, coastal marine environment such as the northern Adriatic.

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## P-23
## Safety strategy for the development of small molecule kinase inhibitors

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Kinases are continuing to be targets of high value for the treatment of various diseases. Low off-target mediated toxicity and high selectivity of kinase inhibitors (KI) is a primary focus in early drug development, especially for lifelong treatments. Undesirable adverse effects reported for KI include genotoxicity and organ-related such as cardiovascular-, hepato-, haemato-toxicities. For a pharmaceutical target in development for a non-life-threatening indication we were faced with the challenge of a kinase family consisting of several isoforms, each related to potentially different kinds of toxicological downstream effects, including carcinogenicity. Thus, we were aiming to develop potent candidate molecules being both highly isoform- and kinome-selective, especially having a low degree of off-target inhibition towards those kinases that are considered safety-relevant and those that could worsen the disease itself upon inhibition. Our strategy evolved with the progression of the project and included testing for: (1) Isoform selectivity of the kinase family using biochemical and cell-based assays; (2) Binding affinity determination in a small panel of safety-relevant kinases and kinases which were regularly hit by the compound series; (3) Kinome selectivity using binding assays at a CRO; (4) Functional cellular assays (micronucleus) as well as markers of DNA damage and repair; (5) Activity-Based Protein Profiling (ABPP) in an intact cellular environment. Overall, this comprehensive assessment considerably increased the confidence in the safety of the compounds. We would like to give an overview of how this approach can be used more broadly, on existing data gaps, and which approaches, such as DNA damage repair pathways, may be further used to increase the safety assessment of small molecule KI.

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## P-24
## High Throughput MultiFlow Assay – evaluation of a drug-centric test set

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The MultiFlow® assay is a multiplexed biomarker assay that evaluates compounds for genotoxic activity while also providing insights into mode of action (MoA). Here, we describe modifications to the standard assay that enable much higher throughput—on the order of several hundred compounds per day. This high-throughput screen (HTS) measures γH2AX, phospho-histone H3-positive events, p53 activation, polyploidization, and cytotoxicity at a single concentration and timepoint (24 h), with and without rat liver S9. A random forest model based on about 80 previously evaluated reference compounds tested at 100 μM was used to convert the MultiFlow® biomarker response data into two classes: genotoxic or non-genotoxic. To begin evaluating the performance of the HTS assay, a test set consisting of 77 coded compounds was supplied in DMSO (37 presumed genotoxicants, 40 presumed non-genotoxicants). An unbiased computational method was used to select the most informative test compounds. The overall genotoxicity calls from the new HTS assay were evaluated by a 2x2 contingency table. Performance metrics, based on the random forest classification model, were as follows: accuracy=0.779 (lower and upper 95% CI in parentheses, 0.661-0.868); sensitivity=0.778 (0.651–0.863); specificity=0.78 (0.669–0.863); PPV=0.757 (0.633–0.849); NPV=0.8 (0.686–0.885). While a negative result in this assay is provisional given the top concentration of 100 μM, this result is likely valuable because false negatives would be, by definition, low-potency genotoxicants. Overall, the modified MultiFlow® methodology, optimised for throughput, appears to be applicable to the pharmaceutical chemical space, providing genotoxic activity predictions with unprecedented efficiencies.

*PPV: positive predictive value*
*NPV: negative predictive value*

## P-25
# Agarose seed coating with PGP bacteria alleviates DNA damage in *Pisum sativum* L. grown on serpentine soils

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Serpentine soils are distinctive ecosystems characterised by low macronutrient availability and high concentrations of heavy metals. Plants adapt by developing a variety of protective mechanisms which include interactions with plant growth promoting rhizobacteria (PGPR). PGPR can improve overall plant health and tolerance to biotic and abiotic stressors through various mechanisms. Given the growing area of heavy-metal contaminated soils, the application of PGPR is investigated in phytoremediation and biotechnological applications. We employed the plant comet assay to examine the ability of PGPR seed coating to reduce heavy metals-induced DNA damage in *P. sativum* L. (pea). The soil was collected from two serpentine sites in Central Bosnia and is rich in Ni, Cr and Fe. Serpentine *Pseudomonas* sp. with known PGP properties and tolerance to Ni, Co, Cu, and Cr was grown overnight in a tryptone soy broth. The cells were collected by centrifugation and mixed with freshly prepared 0.5% agarose. The imbibed seeds were surface sterilized. One batch was transferred into a Petri dish with *Pseudomonas*-agarose mixture for 15 min. The second batch was incubated in *Pseudomonas* sp. only in order to evaluate the efficiency of the agarose coating. The untreated batch was used as a control. Plant comet assay was performed on young, fully developed, randomly selected leaves. The analysis of TI showed statistically significant differences in DNA damage among the three experimental groups ($p<0.0005$). Also, the application of 0.5% agarose coating improved the efficiency of the bacterial inoculant. While agarose provides a physical barrier and better bacterial adherence, *Pseudomonas* sp. may have an indirect role in alleviating DNA damage by enhancing nutrient availability. The results of our experiment demonstrate that PGPR has a significant role in stress alleviation in plants and justify further study of the actual molecular mechanisms that protect plant DNA from heavy-metal-induced damage.

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## P-26
## Steel industry and DNA damage in Bosnia and Herzegovina

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In recent decades, there has been a growing interest in exploring the relationship between air pollution and its impact on health. However, such investigations remain scarce in Bosnia and Herzegovina, despite the persistent issue of poor air quality. In the city of Zenica, heavy industry, particularly steel production, is a primary source of sulfur dioxide and $PM_{10}$ in the air, both notorious for contributing to smog and adverse health effects. We hypothesised that levels of DNA damage would be significantly higher among residents of the Zenica region compared to those in Sarajevo or the rural site of Ribnica. To test this hypothesis, biomonitoring was conducted on isolated oral mononuclear cells from 38 residents of Zenica and 40 individuals from Ribnica. The alkaline comet assay for the analysis of DNA damage was used. Tail intensity, tail length and tail moment comet assay descriptors were registered (CometAssayIV, Instem, Belgium), and descriptors of highly damaged nuclei were calculated. Data for Sarajevo residents were obtained from previous studies. The results revealed a significant increase ($p<0.001$) in DNA damage not only in Zenica but also in Ribnica, which served as a control area. Both localities exhibited significantly higher values compared to Sarajevo. These findings underscore the urgent need for continuous air pollution and biological monitoring to drive systemic improvements in air quality and public health.

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## P-27
# The endosymbiotic bacteria *Wolbachia* modifies gene expression and epigenome in *Chorthippus parallelus* (Orthoptera: Acrididae)

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*Wolbachia pipientis*, an obligatory endosymbiotic alphaproteobacteria, demonstrates a pervasive presence across arthropods and nematodes. The persistence of this symbiotic relationship across diverse taxonomic groups hints at the potential physiological benefits *Wolbachia* confers to its hosts, suggesting a pivotal role in the evolutionary dynamics of the affected organisms, including genetic divergence and potentially even speciation. Within the orthopteran species *Chorthippus parallelus* (Orthoptera: Acrididae), both the Iberian endemic *C. p. erythropus* and the continental subspecies *C. p. parallelus* differ by the strain of *Wolbachia* that infects them, as well as by morphological, behavioural, and other genetic traits. Their geographic distributions overlap within the Pyrenees, forming a hybrid zone (HZ) that represents a suitable system for studying the "key genes" that maintain the genetic boundaries between emerging species. In fact, *Wolbachia* plays a substantial role in reinforcing the reproductive barrier between both subspecies by inducing cytoplasmic incompatibility in them. The analysis of gene expression and global genome methylation has recently been incorporated into the study of this model to improve our understanding of *Wolbachia's* influence. Individuals infected by *Wolbachia* from the *C. p. parallelus* subspecies, compared to uninfected ones, show discernible differences in the transcriptional activity of essential genes involved in different physiological pathways related to energy metabolism, development, or the immune system, alongside epigenomic alterations (DNA methylation). Some of these differences are also influenced by sex. The forthcoming investigation endeavours to deepen our comprehension by extending these analyses to cover the remaining subspecies and its hybrids under the premise of an intricate and nuanced interplay among distinct *Wolbachia* strains, individuals of both sexes and the three orthopteran lineages constituting the HZ.

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# P-28
# ToxLearn4EU: Toxicology Innovative Learning for Europe

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The 2021 EU Action Plan 'Towards Zero Pollution for Air, Water and Soil' highlighted that climate change, environmental pollution, biodiversity loss and unsustainable use of natural resources pose multiple risks to human, animal and ecosystem health and are the four main environmental challenges to be addressed. Toxicologists and ecotoxicologists with solid skills are needed to fulfil the ambitious objectives of this Action Plan as it aims, in particular, to improve the assessment of the toxicity of pollutants (especially emerging ones) and complex mixtures found in air, soil and water, developing innovative methods to evaluate their toxicity and biomonitor our environment. The ToxLearn4EU project comprises a consortium of seven Higher Education Institutes (HEIs), three Research Institutes and several laboratories across Europe. It intends to modernize education in Toxicology and other related areas by developing innovative educational content, such as interactive courses and case studies, which will be freely accessible online at https://toxlearn4eu.eu/. The main goal is to produce quality digital education on current topics in this field. The project meets the needs of: Students – by stimulating their interest and limiting school dropout through active pedagogies, also adapted to digital practice; Teachers – by proposing stimulating online course materials that can be integrated into their courses or by motivating them to generate new innovative courses; Partners – by modernising part of their curricula; Stakeholders in toxicology and ecotoxicology – by developing student and lifelong learner competencies adapted to the job market and keeping up-to-date with recent changes at the European Green Deal; European Commission: by modifying curricula and developing courses adapted to the Green Deal. According to the proposed project plan, we have organized two summer schools that provide more than 120 h of lectures and practical work to more than 50 students. We are currently working on finalising 10 case studies that are intended to improve the quality of toxicology teaching and will be freely available. Moreover, online courses covering emerging pollutants, new approaches, and risk assessment will soon be available, providing basic knowledge to toxicology students. This information is presented on behalf of the ToxLearn4EU consortium: Institute for Medical Research and Occupational Health (Croatia), Institute of Public Health of the University of Porto (Portugal), San Raffaele Scientific Hospitalization and Treatment Institute (Italy), Julius Maximilian University of Würzburg (Germany), University of Navarra (Spain), National Distance Education University (Spain), Hasselt University (Belgium), Maastricht University (Netherlands), University of Sarajevo (Bosnia & Herzegovina), National Polytechnic Institute of Toulouse (France).

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## P-29
## Cellular study of the genotoxic interaction between deoxynivalenol and acrylamide

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Food safety represents a major global concern regarding the exposure of humans and animals to a broad spectrum of food contaminants. Deoxynivalenol (DON) is the most widespread mycotoxin in food, mainly found in cereal products. Around 80% of the population is exposed to DON at concentrations close to or exceeding the tolerable daily intake. Given its high prevalence, it is necessary to define DON toxic interactions with other food contaminants. In this context, although DON is not considered genotoxic, previous studies have highlighted its ability to exacerbate DNA damage induced by various compounds, questioning its potential cocktail effects with other genotoxic contaminants. Among them, acrylamide is a compound formed during cooking processes, found in fried foods and cereal products. Classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer, acrylamide exposure is associated with several toxic effects. The aim of our study was therefore to assess the ability of DON to aggravate the genotoxicity of acrylamide in a normal intestinal cellular model. Our data show that DON exacerbates the cytotoxic and genotoxic activity of acrylamide, observed here through the analysis of the DNA damage biomarker γH2AX and confirmed by comet assays. Furthermore, our results indicate that the DON-mediated increase of acrylamide-induced DNA damage is not related to the apoptotic program. As a consequence of this genotoxic interaction, cells co-exposed to DON and acrylamide exhibit more important cell cycle defects and chromosomal instability, as evaluated by the chromosomal aberration assay. In conclusion, our work shows that DON exacerbates the genotoxicity of acrylamide, with repercussions on the maintenance of genetic stability, potentially implying an increased carcinogenic risk. These results therefore provide crucial information regarding food safety, given the high probability of co-contamination by DON and genotoxic agents such as acrylamide.

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# P-30
# Cytotoxic and genotoxic potential of *Achillea millefolium* herb methanol and dichloromethane extracts

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Cancer stands as the leading cause of mortality worldwide, and multidrug resistance represents a significant obstacle in treating the disease, complicating effective treatment strategies. Today, research is shifting towards identifying new natural agents and strategies due to the decreasing effectiveness of available drugs. Plants are promising sources of such agents because of their broad biological activities and minimal side effects. *Achillea millefolium* L. (yarrow) is commonly used in both folk medicine and modern phytotherapy. The aim of this study was to chemically characterise dried methanol and dichloromethane extracts of yarrow flowering tops (herb) and provide information about their cytotoxicity and genotoxicity. Wild-growing yarrow herb was collected in Serbia; the presence of sesquiterpene lactones in prazulenes was proved by an appropriate colour reaction according to European Pharmacopeia. LC-MS analysis of the methanol extract revealed 28 caffeoylquinic acids and flavonoids. GC-FID and GC-MS analysis showed that among phytosterols and triterpenes of dichloromethane extract, *β*-sitosterol and *α*-amyrin were the most abundant. The cytotoxic effect of tested extracts on lung adenocarcinoma (A549), colorectal adenocarcinoma (HCT116), and normal foetal fibroblast (MRC-5) cell lines was evaluated using the MTT test. The results highlight the dichloromethane extract as the most potent against all tested cell lines, with the highest cell viability reduction seen on A549 cells (up to 94%). Both tested extracts of yarrow demonstrated selective toxicity towards cancer cells compared to normal cells. Dichloromethane extract exhibited the highest selectivity index value towards A549 cells (SI>3). Additionally, the genotoxicity was investigated through the alkaline comet assay on all cell lines. These results revealed that both extracts induced damage to DNA at all tested concentrations in a dose-dependent manner, with 23.9% as the highest observed tail intensity. Based on the obtained results, *A. millefolium* extracts surface as potential candidates for novel anticancer therapeutics, although additional research into their underlying mechanisms is required.

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## P-31
## Assessing DNA repair capacity of hydrogen peroxide-induced oxidative damage using *in vitro* comet assay in 3T3 cells

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Unrepaired oxidative DNA damage can lead to significant biological consequences, including carcinogenesis, mutagenesis, and ageing. Hydrogen peroxide ($H_2O_2$) is a major reactive oxygen species (ROS) that causes oxidative DNA damage by forming hydroxyl-free radicals, which can traverse cell membranes via aquaporins. The comet assay is a sensitive, quick, relatively straightforward, and cost-effective method for detecting DNA damage induced by oxidative stress. It can also be used to investigate DNA repair [1]. Our study aimed to examine the DNA repair capacity over specific time intervals in response to $H_2O_2$-induced oxidative DNA damage in the 3T3 cell line. We used the alkaline comet assay to measure DNA repair. In our previous study, we determined that 50 μM $H_2O_2$ for 30 minutes caused maximum DNA damage. After this exposure, the medium containing $H_2O_2$ was replaced with fresh medium. DNA repair was assessed at specific time intervals: 15, 30, 45 minutes, 1, 2, 4, 6, 8, 16, and 24 hours, with each interval studied in duplicate. For each sample, 100 cells were examined under a microscope to assess comet test outcomes, and DNA tail density data were analysed. Statistical analyses were performed using Repeated Measures ANOVA with SPSS (Version 23.0 for Windows), considering $p<0.05$ as statistically significant. The results indicated a statistically significant reduction in DNA damage at each designated time interval compared to the initial damage induced by 50 μM $H_2O_2$ for 30 minutes. Notably, DNA repair processes began at each time interval and statistically approached the control group from the 6th hour onward. These findings offer valuable insights for future research, including human biomonitoring studies aimed at evaluating intra- and inter-individual variations in DNA repair capacity.

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[1] Kuchařová M et al. 2019. https://doi.org/10.33549/physiolres.93390

# P-32
# Carcinogenic activity of PAHs in indoor air in Croatian households

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Polycyclic aromatic hydrocarbons (PAHs) are semi-volatile compounds containing two or more aromatic rings. PAHs are omnipresent both in outdoor and indoor air. Currently, air quality standards only apply to outdoor air, although studies have shown that people spend 80% or more of their time indoors, where inhabitants potentially interact with pollutants produced by building materials, electronics, toys, furniture, carpets, paints, household chemicals and domestic combustion activities (*e.g.* cooking, heating, smoking). The aim of this study was to determine, for the first time, the mass concentrations of eleven PAHs in the $PM_1$ particle fraction (particles with an aerodynamic diameter less than 1 μm) in the indoor air of Croatian households. Samples were collected over seven days at eight households from March to May 2023. PAH concentrations were determined by an Agilent Infinity 1260 high-performance liquid chromatography (HPLC) using a fluorescence detector. The following PAHs were analysed: fluoranthene (Flu), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(j)fluoranthene (BjF), benzo(b)fluoranthene (BbF), benzo(k) fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DahA), benzo(ghi)perylene (BghiP), and indeno(1,2,3-cd) pyrene (IP). Concentrations of $\sum_{11}$PAHs in Croatian households ranged from 0.664 ng/m$^3$ to 11.479 ng/m$^3$ with a median value of 2.704 ng/m$^3$. The median concentration of BaP, the most widely studied PAH which is often used as an indicator of carcinogenic hazards in polluted environments was 0.240 ng/m$^3$. Flu and Pyr, PAHs specific for biomass burning, ranged from 0.118 ng/m$^3$ to 0.325 ng/m$^3$ and from 0.062 ng/m$^3$ to 0.256 ng/m$^3$, respectively. The highest mass contribution in all indoor air samples came from PAHs with five and six rings. The total carcinogenic activity of PAHs in indoor air was 0.574 ng/m$^3$. BaP had the highest average contribution in total carcinogenic activity (60%), while the other five and six-ring PAHs contributed 38%.

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## P-33
## Are the invasive alien species suitable as bioindicators for the assessment of the genotoxic potential in aquatic ecosystems?

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Due to their wide distribution in ecosystems, invasive alien species draw attention as potential sentinel organisms. The Chinese pond mussel *Sinanodonta woodiana* is an invasive alien species distributed throughout the Danube catchment. Our previous passive biomonitoring study has shown that *S. woodiana* is less sensitive in the detection of water genotoxicity using the comet assay compared to the native representatives of *Unio sp.* The sensitivity in response of invasive and native mussels to pollution was further investigated by comet test in active biomonitoring studies at highly contaminated sites in the Belgrade area. First, it was found that exposure of the native species *U. tumidus* and *U. pictorum* to sites contaminated with untreated wastewater increased DNA damage in hemocytes. In the current study, the genotoxic effect of untreated wastewater on the haemocytes of *S. woodiana* was evaluated. Specimens were exposed to one uncontaminated site (Ada) and two sites close to wastewater outlets but with different levels of pollution (Sajam-highly polluted site and Belgrade Waterfront-less polluted site). The basic chemical parameters and *Escherichia coli* in the water were analysed as indicators of pollution. A statistically significant increase in DNA damage was found only between the laboratory group and the Belgrade Waterfront site. An increase in highly damaged nuclei was also observed at this site. The lack of response at the Sajam site indicates that this species may have certain adaptations to overcome environmental stress at overpolluted sites and that they may include valve closure or filtration rate reduction. Therefore, the *E. coli* concentration in the visceral mass and the intervalvular fluid of mussels at these two sites was quantified and compared. The number of *E. coli* was lower in the specimens at the Sajam site, suggesting that valve closure may indeed be a mechanism of protection against environmental stress.

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## P-34
## Assessing the antioxidant activity of strawberry tree honey using DNA plasmid phiX174 RF1: A pilot study

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Strawberry tree honey (*Arbutus unedo* L., STH), a unique type of monofloral honey with a distinct bitter flavour, is renowned for its abundant phenolic content and powerful antioxidant properties, contributing to various beneficial health effects. The primary objective of this study was to evaluate the antioxidant activity of STH, STH phenolic extract (E), and dominant phenolic acid in STH – homogentisic acid (HGA) using DNA plasmid phiX174 RF1. Oxidative damage was induced by UV light radiation and hydrogen peroxide. To examine the impact of sugars, artificial honey (AH) was also included in the study for comparison. STH, E, and HGA were tested in four concentrations (0.5×, 1×, 2.5×, and 5×) selected based on the typical daily intake of STH by an adult person. Supercoiled and relaxed forms of plasmid were separated by electrophoresis for 60 min at 150 mA on a 1.5% agarose gel. The proportion of relaxed coil DNA, determined using Gel Analyzer® software, indicates the free radical-induced damage to the plasmid. By measuring the intensity and ratio of the supercoiled (undamaged) and open circular (damaged) forms of plasmid, ratios were obtained to determine concentrations at which STH, E, and HGA exhibit antioxidant activity. The strongest antioxidant activity in the concentration-response trend was observed for E and HGA, with the highest investigated concentration of HGA showing a decline in antioxidant activity. The results obtained for the E compared to HGA suggest that the phenolic compounds present in the E contribute to its superior antioxidant effect. STH showed slightly weaker antioxidant activity, while AH did not affect the change in oxidative damage induced by UV light radiation and hydrogen peroxide. The results of this study indicate that the overall antioxidant character of the honey extract is strongly dependent on the specific mixture of bioactive compounds present.

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## P-35
# Genotoxicity of polycyclic aromatic hydrocarbons complex mixture in human circulating blood cells: translation of a real-scenario exposure to *in vitro*

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Polycyclic aromatic hydrocarbons (PAHs) include a diverse group of organic compounds that are increasingly becoming prevalent environmental pollutants, primarily originating from human activities like incomplete combustion and natural events such as wildfires and volcanic eruptions. They are also commonly found indoors, where regulation for air pollutants is generally lacking compared to outdoor environments, except for specific occupational exposure limits. In this study, we investigated the cytotoxic and genotoxic effects of a complex mixture of 11 PAH compounds (fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and chrysene) on human whole blood cells from three donors under *in vitro* conditions. Exposure concentrations were based on the measurements in households and were subsequently determined based on adult breathing frequencies during various activities to mimic real-life exposures. Low (1 h) and medium (8 h) exposure concentrations reflected normal activity, while high (16 h) exposure concentrations included periods of rest, accounting for sleep duration. This approach aimed to translate real concentration exposures into *in vitro* conditions accurately. Cytotoxicity was assessed using a viability assay with differential staining, while genotoxic effects were evaluated through comet and micronucleus (CBMN) assays, examining primary DNA damage and genome instability, respectively. Our findings revealed dose-dependent cytotoxicity, with no significant primary DNA strand breaks detected by the comet assay. However, the CBMN assay indicated increased genomic instability. These results underscore the importance of evaluating mixture toxicity, as individual compound exposures may not fully represent the effects of complex mixtures and can at times be misleading. Such assessments are crucial for accurately understanding the potential health risks associated with exposure to PAHs and other environmental contaminants. Future research should continue to explore the interactions and cumulative effects of these compounds to inform regulatory measures and mitigate adverse impacts on human health and the environment.

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## P-36
## Assessment of the impact of polyamide microparticles in adult zebrafish by multibiomarker response

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The accumulation of microplastics in organisms can trigger numerous reactions, such as inflammation, destabilisation of lysosomal membranes, antioxidant defence, genotoxic effects, impaired reproduction, etc. In the current study, we investigated the effects of polyamide microparticles (particle size 0.05-0.16 mm) in 10-month-old adult AB wild-type zebrafish using a multibiomarker approach. For 7 consecutive days, fish were exposed to 1 mg of the particles together with food (*Artemia salina* nauplii). The specimens were euthanised, the condition index was determined, gut samples were collected for microparticle accumulation. Blood was collected for the comet assay while the liver was used for the determination of the following parameters: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST), total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI). In order to better analyse the accumulation of microplastic particles in the gut, the particles were stained with iDye Poly (Jacquard) dye. The particles were visible in the digestive tract of the exposed subjects; however, a quantitative analysis after the digestion of tissue with KOH was not possible. There was no significant difference in the condition index between the control and treated groups. However, there were differences in most of the biomarkers analyzed. In the treated group, there was a significant increase in the level of DNA damage. Oxidative stress biomarkers showed a significant decrease in GSH-Px and GST and an increase in GR and TOS. Enzymes of the first line of defence SOD and CAT were also decreased but not significantly. The inhibition of antioxidant defences reflects the toxic effect of pollutants, indicating cell damage. GSH-Px is efficient in $H_2O_2$ elimination when its concentration is close to physiological levels. It has also shown that after an initial increase in GST activity due to ROS overproduction, its enzymatic activity gradually decreases.

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## P-37
# Genotoxicity and neurotoxicity biomarkers in the Mediterranean mussels from the Montenegrin and Slovenian Adriatic coast

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Recent trends in climate change, acidification and pollution of seawater in the Mediterranean Sea have become serious threats to benthic communities and the aquaculture sector. Scientific evidence pointed out several reasons for decreased yield in mussel culture as lack of spat, heat waves, predation, This study investigates genotoxic and neurotoxic effects in *Mytilus galloprovincialis* at mussel farms in the Boka Kotorska Bay (Montenegro, sites Cogi and IMB) and in Gulf of Trieste (Slovenia, Bay of Piran). Genotoxicity was measured based on DNA damage induction by Comet assay, expressed as a percentage of tail DNA (tail DNA%) in mussel's haemocytes. The neurotoxic effect was evaluated by acetylcholinesterase (AChE) activity in gills. Tail DNA% showed significant differences between all three sites and higher values in mussels from Cogi and IMB in comparison with Bay of Piran. Higher values of tail DNA% indicate the presence of genotoxic agents. AchE activity showed significantly lower values in mussels from Cogi and IMB in comparison with the Bay of Piran. Inhibition of AchE activities was frequently found in mussels at impacted sites under different types of pollution. Considering significantly higher tail DNA% and significantly lower AchE activities in mussels from the Boka Kotorska Bay, our results showed that the Mediterranean mussels from the Montenegrin coast were exposed to higher stress levels than specimens from the Bay of Piran (Slovenia). The ecological relevance of genotoxic and neurotoxic effects are profound and also can be used in the evaluation of mussel, environmental and human health (One Health initiative).

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## P-38
## Genotoxicity assessment of Vanadium-doped iron-oxide nanozymes in HepG2 cells

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Nanozymes, defined as nanomaterials with enzyme-like properties, pose the potential to overcome the intrinsic limitations of natural enzymes. They are characterised by being inexpensive, highly active and stable, with size-composition-dependent activity, and having a large surface area for further modification and bioconjugation. As such, they are promising candidates for use in various industries such as food processing and medicine. Vanadium ferrite ($VFe_2O_4$) has attracted interest due to its catalytic and enzymatic properties. However, there is a lack of data to assess its safety, particularly regarding its potential use in biomedical and food applications. In the present study, we aimed to investigate the potential (geno)toxic activity of vanadium-containing iron oxide nanozymes VxFe3-xO4 ($0.1 \leq x \leq 0.3$) with octahedral, faceted morphology in average sizes D between 38 nm < D < 80 nm. In vitro cyto-/genotoxicity in the HepG2 (human hepatocellular carcinoma) cell line was assessed using the MTT assay, the comet and micronucleus assay, intracellular ROS detection and flow cytometry, respectively. Cells were exposed to graded concentrations (25–100 μg/cm$^2$) of the tested nanozymes for 4 and 24 h. The results showed that the tested nanozymes were not cytotoxic to cells in the concentration range up to 100 μg/cm2, except for $V_{0.3}Fe_{2.7}O_4$, which reduced cell viability by approximately 30% at all concentrations tested. A significant dose-dependent increase in DNA damage was observed with the Comet assay at both exposure times and for all tested nanozymes. In addition, a 4-hour exposure to nanozymes led to an induction of reactive oxygen species (ROS). On the other hand, no increase in micronucleus formation or induction of DNA double-strand breaks was observed. Although further studies are required to fully clarify the safety profile of nanozymes, this study paves the way for the use of the tested nanozymes in various areas of application.

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## P-39
## Aliens among us: invasive alien fish as bioindicator organisms and food source

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Despite all efforts and initiatives, the loss of biodiversity in freshwater ecosystems is progressing dramatically. After habitat destruction, invasive alien fish (IAF) are a second cause of biodiversity loss in freshwaters. The black bullhead *Ameiurus melas* (Rafinesque, 1820) is a fish species native to North America that was first detected in Serbia in 2005 and has become ubiquitous in the environment. Its use as a bioindicator organism as well as its breeding in aquaculture should be considered in the context of IAF management. This study was conducted at Lake Markovačko, which is exposed to agricultural activities, to investigate the use of black bullhead as a bioindicator organism. A set of biomarkers was analysed during the 2021 summer season, while fish from the recirculating aquaculture system (RAS) were used as control. The genotoxic response was assessed using an alkaline comet assay and a micronucleus test in blood cells. In addition, the concentration of metals and metalloids in fish was analysed to reveal their safety for the human diet. Supplementary analyses included basic physicochemical parameters, faecal indicator bacteria and the analysis of pesticides in lake sediment. The integrated biomarker response (IBR) approach was used to link all biomarkers and present the results concisely. In all months except August, the level of DNA damage was significantly higher compared to the control. The highest number of nuclear abnormalities and micronuclei was observed in June. The highest value of the metal pollution index (MPI) was observed in June. No sample exceeded the maximum allowable concentrations (MAC) according to EU and national regulations. The integrated biomarker response showed that all four months had significantly higher values compared to the control, with the highest values in June. This study emphasises the sensitivity of the black bullhead as a bioindicator organism and its safe use in human nutrition.

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## P-40
## Diamond templates for stimulated growth of various human cell lines *in vitro*

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Materials science offers countless possible ways of using diamonds in many medical fields in various forms. Numerous studies point to polycrystalline diamond thin films as a proven non-cytotoxic and biocompatible material with many possibilities for surface modification and functionalization to improve its interaction with living cells and tissues. A new biomimetic system based on three-dimensional scaffolds covered with a functionalized (boron) doped diamond film capable of electrically stimulating various human cell lines was developed. Two sets of diamond-coated Si samples were fabricated on flat and rough initial Si. In this way, the diamond coating mirrored/copied the initial Si morphology. These two sets were further divided into two subgroups depending on the finish. A hydrogen terminus, known as a hydrophobic surface (water contact angle >70°), and an oxygen terminus, known as a hydrophilic surface (contact angle with water <10°) were used. For culture experiments using model cell lines A549 (lung), HepG2 (liver), and TH1 (kidney), 4 types of diamond surfaces (2 different surface roughnesses (R, F) further modulated by two different terminations) were used. For cytotoxicity studies, H-terminated diamond showed cytotoxicity in HepG2 and A549 cells, especially HepG2 were more sensitive, but further studies are needed to confirm this observation. Moreover, the H-terminated diamond showed genotoxicity in the studied cell lines at both surface roughnesses. The highest value was observed for TH1 cells on the rough surface. Based on the results, rough H-terminated diamond surfaces are more toxic than smooth surfaces. The project should contribute to the development of new materials that support cell adhesion, proliferation, and growth, which is key for a new generation of implantable biomedical devices.

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## P-41
## Usage of fish cell lines as an alternative to experimental animals in eco/genotoxicology – a case study of comet assay in different zebrafish-based models

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Fish cell lines have been successfully introduced for the detection of genotoxic effects and can serve as an alternative to animal testing in preliminary eco-/genotoxicological studies. To demonstrate the sensitivity of the model based on the zebrafish liver cell line (ZFL) we have used 8 concentrated extracts of surface waters prepared by Horizon large volume solid-phase extraction (LVSPE). These samples were selected based on their genotoxic potential determined by comet assay in ZFL. The activity of the samples was investigated *in vivo* using AB wild-type zebrafish embryo technique with a maximum enrichment factor of 100 (REF100). There was no embryotoxicity or teratogenicity recorded at the maximum enrichment factor of REF100 for 96 hours. Genotoxicity in embryos was assessed at 48 hpf using 10 embryos per experimental group for slide preparation. The level of DNA damage in the control group of zebrafish embryos was significantly higher in comparison with the level of DNA damage assessed in the control group of ZFL cells. Also, for zebrafish embryos we have observed higher variation in the level of DNA damage in nuclei scored from the same slide which was expected, keeping in mind that in this case, multiple cell types are present in the analysed group. A significant increase of DNA damage in comparison with the control group of embryos was observed only in the group treated with etoposide. The applied concentration of etoposide (10 μg/mL) was 100x higher in comparison with the concentration used for ZFL cells (0.1 μg/mL), suggesting lesser sensitivity of embryos as *in vivo* model in comparison with ZFL cells as *in vitro* model. Moreover, none of the tested LVSPE extracts induced a significant increase in DNA damage at REF100. It should be emphasised that some of the extracts were genotoxic in *in vitro* model even at lower REF.

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# P-42
# The influence of exposure time on genotoxic properties of a tungsten-based polyanion

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The aim of this study was to investigate the effect of exposure time and concentration on the results of tail DNA values obtained by alkaline comet assay. For this purpose, human peripheral blood samples were incubated at 37 °C in the presence of three different concentrations of tugsten-containing polyanion, $\alpha_2$-$K_6P_2W_{18}O_{62}$ for 4 and 24 hours. Blood samples were taken from healthy young donors who gave their consent to participate in the study in accordance with the Institutional Ethics Committee and the ethical principles of the Declaration of Helsinki and were not previously exposed to potential genotoxic agents. The alkaline comet assay was performed for genotoxicity evaluation. After 4-hour treatment with the lowest concentration of 1 μmol/L tail DNA value was obtained as 0.80±0.12, whereas after 24 hours the same concentration resulted in the value of 1.22±0.21. Then, the concentration of 10 μmol/L induced tail DNA values of 1.06±0.11 and 1.15±0.23 for 4 and 24 hours of exposure, respectively. Finally, 0.66±0.11 (4 hours) and 0.83±0.11 (24 hours) were obtained for 100 μmol/L of the studied polyanion. The results demonstrated that the obtained tail DNA values for 4 and 24 hours were significantly different for the concentration of 1 μmol/L. On the contrary, there were no significant differences between the values obtained for 4 and 24 hours of exposure, for both 10 and 100 μmol/L. Accordingly, it seems necessary to carry out an alkaline comet assay for both 4 and 24 hours of exposure of peripheral blood samples to potential genotoxic agents to perform an adequate genotoxicity evaluation.

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## P-43
# Assessment of DNA damage and oxidative stress of green tea epigallocatechin-3-gallate (EGCG) by the comet assay

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Epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, has been shown to inhibit cancer cell growth and tumorigenesis *in vitro* and in animal models, particularly by eliminating cancer cells through induction of apoptosis but also by protecting normal cells against genotoxic hazards. However, increasing evidence indicates that EGCG produces reactive oxygen species (ROS). The aim of this study was to evaluate DNA damage and oxidative damage measured by comet assay, lipid profile, liver function parameters, and vitamins A and E levels during an interventional study with 90 days duration in 30 individuals with an EGCG intake of 225 mg/day. Peripheral blood from 30 healthy individuals (10 males and 20 females; 18–45 years) was collected at time 0 (T0) and time 90 (T90). During 90 days, participants ingested capsules of green tea extract (225 mg EGCG) daily. Haematological cardiovascular risk factors, including lipid profile and liver function parameters, were assessed using colourimetric methods. Vitamins A and E in serum were quantified by HPLC and analysis of DNA damage and oxidative damage was performed by comet assay. Our results showed an increase in DNA damage and DNA oxidative damage after 90 days of EGCG consumption. Lipid profile and liver function parameters were not affected by EGCG and serum levels of vitamin E increased but not vitamin A. The results suggest that EGCG can induce DNA damage, possibly due to ROS induction, with an associated increase of antioxidant vitamin E, however without altering haematological cardiovascular risk factors.

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# P-44
# A mixture design with nanoplastics and bisphenol A: cytotoxicity and genotoxicity assessment

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The widespread circulation of nanoplastics (NPs) and bisphenols (BPs) in the environment, inevitably results in complex interactions and associated detrimental effects, which have the potential to reach the human body by multiple routes. Considering the levels of plastic oral exposure via ingestion and the absorption of these substances in gastric tissues, NPs endorse hazardous potential given the evidence of toxic effects on a cellular level. Furthermore, the ability to act as a vector for other pollutants and its ability to be internalized by cells potentiate long-term effects. Bisphenol A (BPA) is a xenoestrogen, that exhibits hormone-like properties that mimic the effects of estrogen in the body and is widely used in consumer products. In order to efficiently assess the risk to human health, it is crucial to evaluate exposure responses from complex and realistic scenarios. The aim of this study was to investigate the cytotoxicity and genotoxicity of polystyrene (PS)-NPs and BPA *per se* and in combined exposures on the gastric GP202 cell line. Environmental relevant concentrations previously tested in literature were selected, for PS-NPs - 20, 100 and 200 μg/mL and for BPA – 0.1, 1 ng/mL, and 0.04 ng/mL as the new reference value proposed by EFSA. Cytotoxicity was assessed by Cell Titer- Blue® assay and genotoxicity by cytokinesis-block micronucleus assay, following the 487 OECD Guideline. Our results showed no cytotoxic effects associated with either individual or combined exposures. Genotoxicity was tested for the worst-case scenario of combined exposure, being all the endpoints – micronucleus, nucleoplasmic bridges and nuclear buds, significantly increased (200 μg/mL PS NPs + 1 ng/mL BPA) (69±6.15, 26±2.73 and 37±3.15) in comparison with negative control (40±6.56, 11±1.53 and 4.0±0.58), respectively. These results highlight the importance of further studies with combined exposures of NPs with BPA and other chemicals of concern since NPs are known to act as vectors. Further evidence is needed in order to perform an effective safety risk evaluation of these substances.

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# P-45
# Can we identify safe(r) substitutes for PFAS coatings?

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Poly- and perfluoroalkyl substances (PFAS) are widely used in diverse production sectors for their water- and oil-repellence properties, high thermal stability, and durability. Despite these advantageous properties, PFAS has been heavily debated due to their high persistence in the environment and potential adverse health outcomes. Some PFAS species are subject to restrictions, and the European Chemicals Agency (ECHA) issued a full ban proposal in February 2023. In this context, the EC Horizon Europe project PROPLANET aims to develop novel PFAS-free coating materials for the industrial sectors of technical textile, food-packaging machineries, and low-maintenance glass, by applying Safety and Sustainability by design (SSbD) principles. In this perspective, the safety aspects are addressed starting from the early development phase of the materials. Existing information and data on the toxicological properties of the individual chemicals have been collected, and data gaps identified. New approach methodologies (NAMs) are applied using a tiered approach consistent with the development and life stage of the materials. Focusing on the genotoxicity and mutagenicity aspects, results obtained with *in silico* Quantitative Structure-Activity Relationship (QSAR) models, and *in vitro* assays run on human cell models exposed to the developed materials will be presented. To include safety considerations along the development of new coating materials throughout their life cycle aims at promoting the shift towards safer and more sustainable chemicals, while promoting innovation and competitiveness in the chemical industry, according to the EU Chemicals Strategy for Sustainability.

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## P-46
## Expression of DNA repair enzyme OGG1 in non-cultured and organ-cultured corneal and limbal epithelium

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8-oxoguanine DNA glycosylase-1 (OGG1) is a protein involved in oxidative stress. Our aim is to examine the distribution and expression of OGG1 in non-cultured and organ-cultured human limbal epithelium. Samples of non-cultured and Eye-bank organ-cultured corneo-limbal tissue were processed for immunohistochemistry. By semiquantitative evaluation and using Fiji (Image J), the distribution and intensity of reactions were examined. Nuclei in all layers of non-cultured as well as cultured epithelium showed varying degrees of positive staining for OGG1. However, some difference in the distribution of nuclei with high-density reaction was noted between epithelium in non-cultured and cultured samples. The percentage of nuclei with high-density staining in superficial-, intermediate-, and basal layers was 34/30/47,2 in the cornea, 16,7/22,5/35 in the limbus, and 31,6/20,8/20,9 in organ-cultured epithelium. Organ culture is used for storing tissue for transplantation and as a starting point for ex vivo production of epithelium. Our experiments show that epithelial cells retain the ability to express OGG1 under conditions that are commonly used for such tissue storage/cultivation.

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## P-47
## Ames test assessment of 50 *N*-Nitrosamines covering varying structure, molecular weight, and CPCA categories

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The Ames test is used for evaluating the mutagenicity of pharmaceuticals and impurities, and the assay's reliability is essential for decision-making by regulatory authorities. For *N*-Nitrosamines (NA), the enhanced Ames test (EAT) is recommended to ensure assay sensitivity because legacy data suggests the standard format does not accurately predict the carcinogenic potential of many NAs. Thus a negative Ames Test alone is no longer considered sufficient by regulatory authorities to de-risk the genotoxic carcinogenic potential of NA impurities. Therefore, in the absence of relevant rodent bioassay data, or where read-across is not possible, extensive follow-up testing is now required to discharge concerns regarding the potential genotoxic carcinogenicity of NA impurities. We previously demonstrated the mutagenicity of alkyl-nitrosamines can be detected in the Ames test and identified the assay conditions that contributed most to assay sensitivity [1]. As a follow-up, we have investigated 50 NAs of varying structure, molecular weight, and CPCA category. The studies aimed to clarify their mutagenic potential using an OECD compliant Ames test design sensitive to this class of compound. The results showed 84% of the NAs were positive for mutagenicity when tested in an appropriately designed Ames Test. This figure is substantially higher than reported for non-nitrosamine mutagen compound classes. As expected, the percentage of positive NAs increased with the addition of S9, with 75% positive in the presence of the induced 10% Hamster S9-mix, but only 49% with the induced 10% Rat S9-mix. These data align with previous publications that highlight the base-pair substitution strains are the most sensitive (88% positive +Rat S9, 89% positive +Hamster S9). Our prospective study results agree with retrospective reviews of the Ames Test data and confirm the high sensitivity of the OECD-compliant Ames Test to effectively detect the mutagenicity of NAs.

[1] Thomas et al. 2024. https://doi.org/10.1093/mutage/gead033

# P-48
# Identification of biomarker and therapeutic target for chronic kidney disease: *in vivo* model

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The worldwide trend of increasing prevalence of chronic kidney disease (CKD) represents a serious problem, as there is currently no direct treatment for this progressive pathology, and its advanced stages are associated with reduced quality of life and the need for dialysis and/ or organ transplantation. Besides the physical and mental health of the patient being affected, on a global scale consequences of this phenomenon include escalation of the healthcare and economic burden. Improvement of this unfavourable state can be reached by the identification of a biomarker for the detection of early stages of the disease and a specific target for therapy. Based on the literature research both roles can be stood by periostin, whose expression level correlates with the presence and progression of kidney diseases and which experimental inhibition leads to amelioration of health characteristics. However, there are still many questions that need to be answered specifically in terms of CKD. The first step to properly determine the short- and long-term effect and role of periostin in the fibrogenic process characteristic for CKD as well as to identify structural and functional changes in individual disease stages is the selection and verification of a suitable *in vivo* model. According to the literature, the mouse model of unilateral ureteral obstruction (UUO) should be the best-fitting model for our purposes, in addition to offering the advantage of reducing the number of experimental animals since in this model the non-ligated kidney could be used as a control. Based on our preliminary data, including the observation of increased expression of several profibrotic markers at the mRNA and protein levels and matching histological findings just in the ligated kidney but not in the contralateral one, the UUO model can be considered a suitable model for our next experiments.

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## P-49
## Safety assessment of smoke flavouring primary products by the European Food Safety Authority (EFSA)

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Smoke flavourings primary products (SFPP) are complex mixtures of chemicals, derived from the thermal degradation of wood. They are used as an alternative to traditional smoking and may be added to foods to give them a smoked flavour. The European Food Safety Authority (EFSA) recently evaluated the safety of eight SFPP, currently on the market and submitted for the renewal of their authorisation. The evaluation was concerned only with the chemical characterisation, dietary exposure and genotoxicity of SFPP. EFSA considered the data submitted in the technical dossiers of the SFPP and followed the principles of the 'EFSA guidance for the preparation of applications on SFPP'[1]. In this guidance, a combination of a component-based and a whole-mixture approach is recommended to assess chemical mixtures containing unidentified components, such as SFPP. First, the mixture should be chemically characterised as fully as possible. The identified substances should then be assessed for their genotoxic potential using all available data, including structure-activity relationship information. If the mixture contains one or more substances that are genotoxic *in vivo* via a relevant route of administration and their estimated exposure via the consumption of the SFPP is above the Threshold of Toxicological Concern of 0.0025 μg/kg bw/day, the whole mixture raises concern about genotoxicity. If none of the identified components raises concern for genotoxicity, the genotoxic potential of the unidentified fraction should also be evaluated to complete the assessment of the mixture. EFSA could not rule out concerns regarding genotoxicity for any of the eight SFPP: six SFPP raise a safety concern for genotoxicity since they contain furan-2(5H)-one and benzene-1,2-diol, i.e., two *in vivo* genotoxic substances via the oral route, whose exposure estimates are above the above mentioned TTC. For the other two SPFF a concern for genotoxicity could not be ruled out due to lack of data.

[1] EFSA FAF Panel 2021. Scientific Guidance for the preparation of applications on smoke flavouring primary products. https://doi.org/10.2903/j.efsa.2021.6435

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## P-50

# Evaluation of anti-cancer effect of a novel formulation from umbilical cord blood stem cell-derived exosomes and retinoic acid as a potential therapeutic candidate against malignant melanoma

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In the UK, malignant melanoma is the 5th most common cancer. The treatment of malignant melanoma is a challenge for clinicians because of its metastatic status. Several key therapies are currently available as anti-cancer treatments such as chemotherapy, immunotherapy, surgical excision and nanotechnology-based therapies. However, all these treatments have severe complications that restrict their extensive use. Therefore, this study has focused on two potential therapeutic modalities with optimistic safety profiles, i.e., Retinoic acid and Cord blood stem cell derived exosomes as anti-cancer drug. The current study has evaluated the genotoxicity and cytotoxicity of RA and CBSC-derived exosomes by employing Comet assay and CCK8 assay, either alone or combined, on lymphocytes from healthy and melanoma patients, and also on the CHL-1 melanoma cell line. By using RT-qPCR, molecular mechanisms were explored. The optimal doses of 10 μM RA and 120 μl of $10^7$ particles/ml CBSC-derived exosomes significantly exerted cytotoxic and genotoxic effects on CHL-1 melanoma cells as compared to healthy and melanoma lymphocytes, both treatments showed genoprotective effect (***$p<0.001$) against oxidative stress induced by $H_2O_2$ and (UVA+B). RA rescued DNA damage caused by oxidative stress in both healthy and melanoma lymphocytes but in the CHL-1 melanoma cell line, RA aggravated the genotoxic effect (***$p<0.001$). The similar results were observed with the exosome treatment (***$p<0.001$). The combined treatment (10μM RA + 120μl of $10^7$ particles/ml exosomes) synergistically increased the observed DNA damage in the CHL-1 melanoma cell line. RT-qPCR results showed upregulation of Caspase-3, P53 and P21, therefore significant initiation of apoptosis was evident in all treatments, particularly for the combined treatment of RA and exosomes. RT-qPCR results also suggested downregulation of Bcl-2 and S100 in all treatments demonstrating suppression of anti-apoptotic signalling pathway and therefore causing cytotoxicity of these treatments for melanoma cells and is a beneficial characteristic of new potential cancer therapy.

# P-51
# Predictive modelling of genotoxicity biomarkers in response to air pollution exposure across seasons

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Air pollution, a global environmental and health concern, is being studied for its impact on genome integrity and human health due to the complex composition of urban air and its association with adverse health outcomes. Using alkaline comet assay descriptors and micronucleus frequency from peripheral blood cells as indicators of early biological effects, we investigated the effect of air pollutants on DNA damage and genome stability within a cohort of healthy occupationally unexposed individuals. Conducted across consecutive colder and warmer seasons to account for seasonal variations, the study included 60 volunteers from Zagreb, Croatia, aged 18–55, with a BMI<30 kg/m$^2$. With the exception of particulate matter with an aerodynamic diameter <10 μm ($PM_{10}$) and benzo[a]pyrene bound to $PM_{10}$ during the cold season, all other monitored outdoor air pollution parameters remained below regulatory limits. Additionally, we assessed benzene, toluene, ethylbenzene, *o*- and (*m*- + *p*-) xylene (BTEX) concentrations in blood samples obtained from study participants and found a significant positive correlation between benzene and toluene blood levels with ambient BTEX concentrations. Recorded average values for micronucleus frequency and comet assay descriptors were notably higher during the warmer season but this difference was not statistically significant. A statistically significant positive correlation was observed between ambient pyrene and DNA damage evaluated by the comet assay and the formation of nuclear buds. Predictive models were constructed for biomarkers of DNA and chromosomal damage based on more than 30 air quality biomarkers, highlighting several PAHs (fluoranthene, pyrene, benzo[a]pyrene, benzo[b]fluoranthene, etc.) as the most promising predictors of % DNA tail. As for the micronucleus assay, the benzene level in blood was one of the most significant predictors. Our findings highlight the necessity for further investigation and classification of PAH compounds, especially those with insufficient experimental evidence regarding their genotoxicity and carcinogenicity.

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## P-52

# Apigenin and homogentisic acid protect against benzo[a]pyrene-B[a]P, acenaphthene-Ace and benzo[b]fluoranthene-B[b]F induced genome damage in human lung cancer (A549) cells

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Although air pollutants' correlation with lung cancer incidence has been established, still their mechanisms of action are not fully understood, nor how to protect the cells from their damaging effects. Among polycyclic aromatic hydrocarbons such as B[a]P, Ace and B[b]F, only B[a]P is classified as a human carcinogen (Group 1). Human lung cancer (A549) cells are a good model for evaluating DNA damaging effects in lung tissue since they retain normal type II alveolar cells' metabolic activities, necessary for damaging/toxic effects of some air pollutants. Here, we used MTT (cytotoxicity, proliferation) and wound-healing assay, alkaline and Fpg comet assays (primary and oxidative DNA damage) and micronucleus *cytome* assay (genomic instability) in A549 cells after 24 and 48 h exposure to the environmentally relevant B[a]P (1, 2, 5, 10, 20, 50 and 60 μM), Ace (0.1, 0.2, 0.4, 0.8, 1 and 2.6 μM) and B[b]F (0.04, 0.06, 0.1, 0.25 and 10 μM) concentrations. The protective role of antioxidant homogentisic acid (HO, *Arbutus unedo*, strawberry-tree honey 1.3, 6.4 and 10 μM)) and apigenin (API, a dietary flavonoid 20, 40 and 60 μM) in daily used doses found in one cup of tea with honey was also investigated. B[a]P-induced primary DNA damage did not significantly differ among treatments, with oxidatively damaged DNA observed after 24 h. Similar was not seen for the other two selected pollutants. Moreover, all tested doses induced apoptosis along with the increased micronuclei and nuclear buds frequency indicating dose-dependent genome instability. HO and API demonstrated a protective effect for Ace and B[b]F as well as lower B[a]P concentrations and also slowed wound repair time for lower B[a]P concentrations. API slowed wound-repair-time for Ace and B[b]F. All selected PAHs alone repaired the wounds faster except for higher B[a]P concentrations. The effect of continuous exposure to lower B[a]P concentrations, as well as Ace and B[b]F, can induce serious damage toward A459 cells. However, HO and API demonstrated various protective mechanisms against PAH-induced toxicity warranting further research in this domain.

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## P-53
## Biomonitoring of genotoxic damage in food production due to workplace exposure to pesticides in Mexico

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Biomonitoring of human populations exposed to environmental agents that can act as mutagens or carcinogens can allow the detection of damage and early disease prevention. In recent years, the comet assay (SCGEA, single cell gel electrophoresis assay) has become an important tool to evaluate DNA damage, both in environmental and occupational exposure contexts. In agricultural activities, pest control is essential, and the most effective method is the use of chemical agents that also represent an important source of exposure to potentially toxic compounds. Pesticides constitute a heterogeneous group of substances designed specifically to control different pests; however, some studies have reported an increase in levels of DNA damage after occupational or environmental exposure to these compounds, evidenced by several cytogenetic and molecular biomarkers. However, even though Mexico is a country with extensive agricultural activity with reports of excessive use of pesticides, genotoxic analyses have been relatively few and, in some cases, contradictory. This project sought to determine DNA damage in agricultural workers from the states of Michoacán (Zamora-Jacona, Los Reyes) and Tlaxcala (Nativitas, Huamantla), occupationally and environmentally exposed to complex mixtures of pesticides, using the comet assay on whole peripheral human blood samples. As in other investigations, the results found were not consistent in all the monitored places, some exposed subjects showed damage (Zamora-Jacona in Michoacán and Nativitas in Tlaxcala), observing statistically significant increases in the tail length, intensity, and moment, while on other places there was no increase in those DNA damage parameters (Los Reyes in Michoacán, Huamantla in Tlaxcala). Confounding factors such as sex, age, BMI, occupational exposure period, level of protection, smoking habit (units of cigarettes per day), alcohol consumption (weekly) and medication were considered in the analyses. The comet assay demonstrated its usefulness in assessing pesticide exposure and health risks and suggests the need for periodic monitoring, education, and training of occupational workers for the safe application of potentially harmful pesticides.

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## P-54
# Assessment of DNA damage induced by green synthesized silver nanoparticles in human lymphocytes using the comet assay

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Due to their antibacterial properties and unique electrical and thermal conductivity, silver nanoparticles (AgNPs) are among the most widely used, both in industry and agricultural fields, as well as in healthcare products. However, their potential to cause adverse biological effects, including genotoxicity, raises significant concerns about health and environmental risks. This study aimed to assess their capacity to cause DNA damage in cultured human lymphocytes, shedding light on the potential risks associated with their use. Blood samples were taken from three healthy donors, who were exposed for 30 minutes to three different concentrations of AgNPs. The AgNPs were synthesized using a green method, through the reduction of $Ag^+$ ions from the precursor $AgNO_3$ (Reasol®, 99.98% purity) by the action of green tea infusions (Lagg's®). The reduction reaction was evaluated by colourimetry. Subsequently, the AgNPs in solution were characterized using UV-Vis spectrophotometry. Genotoxicity was evaluated at three concentrations using the alkaline comet assay to detect DNA breaks and alkaline-labile sites. The results indicate that AgNPs have the potential to induce DNA damage with a clear concentration-response relationship, showing a statistically significant increase. Oxidative stress induced by AgNPs may be an important factor in their genotoxic effects, possibly due to the formation of DNA-protein cross-links, in addition to DNA strand breaks. In conclusion, this study has demonstrated that AgNPs can cause genotoxic damage in cultured human lymphocytes under the study conditions. However, further research is urgently needed to fully understand the extent of their genotoxicity and implications for health. The use of materials containing AgNPs should be closely monitored for potential DNA damage risks, highlighting the importance of ongoing research in this field.

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# P-55
# Biocompatibility of innovative resveratrol micro- and nanoparticles

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Resveratrol (Res) is a polyphenolic compound widely found in fruits and vegetables. Res possesses antioxidative, antimicrobial, anticancer, and other beneficial effects. The challenge in the practical application of Res is its poor bioavailability, caused by low water solubility, rapid metabolism, and its overall instability. Nanotechnology could overcome this issue, significantly improving the inadequate bioavailability and biological activity of Res through nanoparticle synthesis. This study aimed to synthesise and characterise Res particles of defined shape, investigate their cytotoxic and genotoxic potential and ability to induce oxidative stress in cells. Nanoparticles were synthesised by using a physicochemical solvent-nonsolvent method without significantly harmful chemicals or expensive surfactants. The obtained particles were stable and uniform, elongated micro- and nanoparticles (ResNPs), and they were physico-chemically characterized. Biocompatibility of ResNPs was assessed using MTT and comet assay on MRC-5 and A549 human cell lines. MTT assay showed a dose-dependent cytotoxic effect with no statistically significant difference between treated cells, and obtained $IC_{50}$ values were 27.68 and 30.21 μg/mL for MRC-5 and A549, respectively. There was also a visible change in cell density and morphology. In the Comet assay, a low level of genotoxicity was observed in A549 cells, while in MRC-5 cells, DNA damage was below the control level. Further on, testing of superoxide anion levels showed that ResNPs treatment did not induce its increase in the MRC-5, and even lowered the amount of $O^{2-}$ in A549 cells, while catalase levels observed semi-quantitatively, were slightly elevated in MRC-5 whilst in A549, had no effect. ResNPs might achieve beneficial antioxidative effects via several mechanisms, including stimulation of catalase expression, and possibly even genoprotective effects in normal cells. Still, ResNPs possess high bioactivity and a narrow cytotoxic threshold, indicating caution regarding biocompatibility.

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## P-56

# *Frangula alnus* extract and its dominant constituent emodin as potential chemotherapeutics against hepatocarcinoma

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Since plants could be considered an important source of chemotherapeutics, we paid our attention to alder buckthorn (*Frangulla alnus* Mill) anticancer potential and targeted hepatocellular carcinoma (HCC), as the one that is swiftly increasing alongside a high mortality rate. This is of special importance since HCC drug resistance to available chemotherapeutics has become a major obstacle to successful treatments. The aim of this work was to comparatively investigate the anticancer effect of the ethyl-acetate extract of *Frangula alnus* (FA) and its dominant constituent emodin (E) in both hepatocellular (HepG2) and normal (MRC-5) human cells in order to evaluate their potential to be used as an adjuvant in HCC therapy. Searching for the underlying mechanism was provided as well. Cytotoxicity in the MTT assay showed strong selectivity of both agents against HCC cells (inhibition was up to 49% and 53% in treatments with FA and E, respectively, without any effect on MRC-5). However, such selectivity was not observed when further assays were provided. Flow cytometer analysis of cell cycles showed that FA and E induced arrest in the G1 phase and accumulation of cells in the G2/M phase in both cancer and normal cells. AnnexinV-FITC/7AAD dying showed that both substances triggered apoptosis in both cell lines, but the effect was more pronounced in HepG2 than in MRC-5 cells. Percentages of apoptotic cells for the two highest concentrations were 22.92% and 91.27% with respect to 0.94% and 61.53% with FA, and 34.4% and 71.01% with respect to 2.95% and 31.41% with E. Mitochondrial membrane potential was also modulated but no selectivity was achieved. Alkaline comet assay pointed out significant genotoxicity of FA and E in both HepG2 and MRC-5 cells. In conclusion, although FA derivatives induced notable activity against hepatocellular cells, caution is needed due to insufficient selectivity.

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## P-57
## Genotoxic activity of benzo[g,h,i]perylene (B[ghi]P) and benzo[b]fluoranthene (B[b]F) as single compounds and binary mixtures

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Air pollution is one of the most important environmental risk factors for health, associated with 7 million premature deaths annually. Epidemiological studies have found a strong link between air pollution and increased incidence of respiratory infections, respiratory diseases, and even cancer. Among air pollutants, polycyclic aromatic hydrocarbons (PAHs) have a high toxic potential in terms of cytotoxicity, mutagenicity, genotoxicity, and carcinogenicity. These compounds are formed during the incomplete combustion of fossil fuels, wood, coal, tobacco, and even food preparation. Consequently, PAHs can be ingested orally through contaminated food or by inhalation of polluted air. Among PAHs, benzo[g,h,i]perylene (B[ghi]P) and benzo[b]fluoranthene (B[b]F) have been identified as priority air pollutants by various environmental agencies, partly because of their frequent detection and partly because of their high toxicity. Nevertheless, information on their possible genotoxic effects is scarce. Therefore, the present study aimed to evaluate their genotoxic activity in the form of single compounds and binary mixtures (ratio 1:1) in the human hepatocellular carcinoma (HepG2) cell line, which has already been shown to be very sensitive for detecting the (geno)toxic effects of indirectly acting compounds. Cytotoxicity was determined by the MTT assay and genotoxicity by the comet and cytokinesis block micronucleus assays. The study showed that both the PAHs tested and their binary mixture can affect DNA integrity and thus pose a risk to the exposed population. Therefore, it is urgent to further evaluate their risk to a healthy population.

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# P-58
# Harmonization and standardization of data collection methods

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The consistent standardisation of air quality data collection techniques is significant for accurately forecasting, analysing, and assessing air quality and, thus, improving the impact on public health. The data analysis experts of the European IPChem platform [1] consider the following items upon data collection: [i] a clear distinction between different air pollution data, *i.e.* indoor air pollution such as sensor data, health data, biological data, environmental data, human biomonitoring data, survey data, indoor air sampling data, climate data, and toxicological data. [ii] While data collection methods based on surveys are provided by IPChem, we argue that sensorics, sampling, and databases need to be regarded as well. Moreover, [iii] the venues of data collection require a clearer distinction among these three categories: [A] residential/homes/households, [B] public buildings indoor, and [C] outdoor public venues. Most prominently, [iv] the data that are measured need to be categorised within a standardised chart. Therefore, the EDIAQI project has founded a task force to collaborate beyond EDIAQI's four pilots and four campaigns with all seven IDEAL cluster projects to categorise chemical as well as non-chemical data within an air quality data chart comprising air pollution data definitions within highly structured and less granular categories. The first results show that the streamlined definitions make data analysis more concise. Reinforcing the use of this standardised air quality data chart could foster the continuous integration of this scale as a new standard within the Horizon Europe Data Management Plan [2] thus significantly improving data management within health cluster activities and leading to a clearer and more effective collection, categorisation, assessment, and analysis of air quality data.

[1] European Information Platform for Chemical Monitoring: https://ipchem.jrc.ec.europa.eu/
[2] H2020 Template for Data Management Plan:
https://ec.europa.eu/research/participants/data/ref/h2020/gm/reporting/h2020-tpl-oa-data-mgt-plan_en.docx

# P-59
# Croatian vegetarian study: V2.0

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The debate on vegetarianism and non-vegetarianism encompasses various aspects, including health, ethics, environmental impact, and cultural traditions. The health impacts of a vegetarian diet remain argued, as it can increase the intake of certain micronutrients while potentially reducing others, thus influencing various metabolic pathways and health-related biomarkers. Our current study aimed to investigate these differences by comparing apparently healthy individuals (N=162) with different dietary preferences (vegetarians and matched non-vegetarians). We analysed the two groups for inflammatory, oxidative, and DNA damage status as well as their dietary preferences by non-quantitative food frequency questionnaire (FFQ). The results revealed distinct biomarker levels between the two groups. Preliminary results showed that plasma C-reactive protein (CRP) levels were lower in vegetarians compared to non-vegetarians indicating more beneficial inflammatory status in those avoiding meat products. Regarding biomarkers of oxidative stress, the level of antioxidative enzyme superoxide dismutase was comparable between vegetarians and their non-vegetarian counterparts. Additionally, the level of DNA strand breaks was higher in vegetarians compared to non-vegetarians as evaluated by the comet assay indicating a higher level of genomic instability. A vegetarian diet may result in a higher intake of some vitamins and micronutrients, which provide antioxidant defence, but it may also lead to a deficiency in others involved in DNA metabolism and stability. To sum up, our results go in line with our previous study (V1.0) on 80 volunteers, while a growing body of evidence suggests that inflammation, oxidative stress, and DNA damage have a key role in the pathogenesis of various diseases, including diabetes, obesity, cancer, neurodegeneration, metabolic syndrome, cardiovascular disease, liver disease, and other chronic disorders. Therefore, specific dietary patterns could play a role in the modulation of the above-mentioned biomarkers and consequently have either positive or negative health outcomes.

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## P-60
## Application of comet assay to determine the DNA damage in the model organism *Caenorhabditis elegans*

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The comet assay is a widely used method for measuring DNA damage and can be conducted under neutral or alkaline conditions. It is a well-established method in cell culture and in some rodent models. However, the application in the model organism *Caenorhabditis elegan*s (*C. elegans*) is limited. In this study, we aim to use *C. elegans* for genotoxicity testing, bridging the gap between *in vitro* and *in vivo*. First, we started with a well-defined cell culture system to understand crucial steps and their effect on the comet tail. The human lymphoblastoid cell line TK6 was treated with different substances, including alkylating agent methyl methanesulfonate (MMS, 100 μM for 4 h), the oxidative agent tert- butyl hydroperoxide (tBOOH, 25 μM for 1 h) and the cytostatic drug bleomycin (0.5 μg/mL). After treatment, we conducted both neutral and alkaline comet assays under different lysis durations. In the alkaline comet assay, we observed no difference in the comet tail with increasing lysis duration and concluded that lysis is not crucial in the alkaline comet assay. In neutral comet assay, lysis seems to be essential. In another set of experiments, we conducted alkaline and neutral comet assays with and without alkaline unwinding. In the neutral comet assay, there was no difference with and without unwinding, while in alkaline there was a higher percentage of DNA in the tail with all substances. Applying the learned lessons: L4 stage worms were treated with tBOOH (0.75 mM for 1 h) and single cell suspension was prepared for use in both assays. Interestingly, the neutral comet assay did not yield a comet tail and slides could not be scored due to the high background. However, we successfully conducted the alkaline comet assay and currently, we are in the process of preparing a set of comet images and defining visual scoring categories in *C. elegans*.

## P-61
# Assessing the potential synergistic/antagonistic effects of citrinin and cannabidiol on SH-SY5Y, HepG2, HK-2 cell lines, and lymphocytes

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Citrinin (CTN) is a mycotoxin produced by *Aspergillus*, *Penicillium*, and *Monascus* species often a contaminant of food and feed, known for its nephrotoxic properties. Cannabidiol (CBD) is a non-psychoactive cannabinoid found in the cannabis plant which has gained significant attention for its potential therapeutic benefits. If the climate conditions during cannabis plant growth, harvest, storage, and processing are favourable for fungal growth, CTN contamination can occur. Therefore, it is important to study the possible effects of CTN and CBD combination due to their possible co-occurrence in medicinal cannabis. Using human neuroblastoma (SH-SY5Y), hepatocellular carcinoma (HepG2), renal proximal tubular epithelial (HK-2) cell lines, and human peripheral blood lymphocytes we assessed the cytotoxicity after 24 h exposure to CTN when applied alone and in combination with CBD. The rationale was to assess whether CBD at a concentration that corresponds to literature data could counteract the potentially toxic effects of the selected mycotoxin. Viability of SH-SY5Y, HepG2 and HK-2 cell lines was determined by the standard MTS test. The viability of lymphocytes and their genome stability following exposure to the tested compounds was estimated using cytokinesis-blocked micronucleus cytome assay. The exposure to the tested concentration of CTN and CBD, resulted with $IC_{50}$ of 50−100 μM for CTN and 13−40 μM for CBD in tested cell lines, while in lymphocyte cultures we observed slight cytotoxic effects with apoptosis as the dominant type of cell death. Combined exposure to CTN ($IC_{20}$) and CBD ($IC_{20}$) resulted in significantly decreased viability of SH-SY5Y cells, while other cell types were not significantly affected. This indicates a negative synergistic effect on cells. Considering that the extent of cytogenetic changes in lymphocyte cultures treated with CTN and CBD was lower than in those exposed to single CTN, it seems that the tested concentration of CBD was efficient against its cyto-/genotoxicity in lymphocytes.

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## P-62
## Cyclosporin A, a non-genotoxic carcinogen – its possible mechanisms of action

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Non-genotoxic carcinogens are chemicals that can cause cancer without directly targeting DNA and altering DNA sequence. Instead of causing mutations in genetic material, these carcinogens usually interfere with cellular processes or disrupt normal regulatory mechanisms, ultimately leading to uncontrolled cell growth and tumour formation. Unlike genotoxic carcinogens, which directly damage DNA, non-genotoxic carcinogens may act through multiple mechanisms, such as epigenetic alterations, stimulation of inflammation, interfering with hormonal signalling pathways or interfering with cell division processes and migration. Here, we investigated the toxic effects of the non-genotoxic carcinogen cyclosporin A (CYC) and the negative control ampicillin trihydrate (AMP) on an *in vitro* 3D cell model (spheroids) developed from human hepatocellular carcinoma (HepG2) cells. The effects of CYC (0,1, 1, 10 μM) and AMP (10, 100, 1000 μM) on cell viability (MTS assay), cell cycle distribution (Hoechst 33258), cell proliferation (Ki67), DNA double-strand break (*γ*H2AX) and mitotic cell formation (histone H3-positive events) were investigated after exposure of 3-day-old spheroids for 24 h and 96 h. The results showed that neither CYC nor AMP affected cell viability. CYC caused a moderate but insignificant increase in cell number in the G0/G1 phase after 24 h and 96 h, which was due to the accumulation of non-dividing cells (Ki67-) in the G0 phase. Conversely, AMP did not affect the cell cycle distribution. Cell proliferation decreased dose-dependently after 24 and 96 h of exposure to CYC, while AMP only slightly reduced proliferation after 24 h. AMP did not induce DNA double-strand breaks; conversely, a slight, however insignificant, increase in *γ*H2AX-positive cells was observed after 24 h of exposure to CYC. Mitotic cell formation was not impacted, neither by CYC nor by AMP. Non-genotoxic carcinogens are an important research issue due to their complex and not fully understood potential adverse effects, which need to be further investigated.

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## P-63
## Predicting COVID-19 symptom severity based on host genetic predisposition

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In light of the recent COVID-19 pandemic, identifying individuals at risk of developing severe symptoms of this disease remains a challenge. Since its initial occurrence, specific high-risk groups of individuals with an elevated likelihood of developing severe symptoms have been defined. Those conventional risk assessments usually rely on demographic and clinical factors, such as older age, male sex, and previous comorbidities, but the variability in symptom severity in individuals not having these traits underscores the need to also explore underlying genetic predispositions. This study concentrates on elucidating host genetic factors associated with the progression of COVID-19 symptom severity, which may be used in prediction models. Genomic data used in this study was obtained by whole exome sequencing of 191 patients hospitalised with SARS-CoV-2 infection. Based on their health record data, patients were classified into three severity groups moderate, severe, and Critical. For this classification, National Institute of Health classification guidelines were followed. We focused on identifying single nucleotide polymorphisms (SNPs) within genes previously related to the COVID-19 disease pathway and calculating their minor allele frequencies (MAF). SNPs that had statistically significant differences in MAF between severity categories were used as predictors in several machine-learning models, while symptom severity, divided into three categories, was used as the response variable we tried to predict. The model showed high accuracy in predicting symptom severity suggesting that host genetic factors play a role in determining the severity of COVID-19 symptoms and could be used to identify high-risk individuals.

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# P-64
# Mutagenic effect of alizarin, a natural anthraquinone dye, in bacteria and a marine invertebrate

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Alizarin is a natural dye from the anthraquinone class. It has been used as an acid-base indicator and as a dye in several industrial processes including textiles. Therefore, it can end up in the environment and be considered a potential environmental contaminant. Alizarin showed mutagenicity *in vitro* in the *Salmonella*-microsome assay with the addition of S9 metabolic activation. No information was found on the *in vivo* mutagenicity of alizarin in aquatic organisms. The objective of this work was to confirm the *in vitro* mutagenicity of alizarin (97.1% purity) using a miniaturised *Salmonella*-microsome assay (microplate agar, MPA) and to evaluate the mutagenicity *in vivo* in a marine crustacean (*Parhyale hawaiensis*) using the micronucleus test. Alizarin was dissolved at its maximum solubility in dimethylsufoxide (DMSO) (1.93 g/L). MPA was performed with TA1537 (±S9), the most sensitive strain to anthraquinones, in concentration-response experiments (0.15–38.61 ng/µL). Anthraquinones require more than the usual 5% S9; therefore, we tested in two higher concentrations of S9 (10% and 30%) induced by phenolbarbital/5,6-benzoflavone. The negative control was DMSO and the positive controls were 9-aminoacridine (-S9) and 2-aminoanthracene (+S9). Alizarin was mutagenic only with S9 with an increased response using 30% of S9. For the micronucleus test, *P. hawaiensis* (8 months old) was exposed for 96 h at 0.48, 0.97, and 1.93 mg/L of alizarin. Hemolymph was collected and used to prepare the slides for the evaluation of micronuclei frequency. Positive and negative controls were zinc 1.5 mg/L and DMSO, respectively. A total of 500 cells/slide were observed (optic microscope at 1000x) and the number of micronuclei was counted. All alizarin concentrations caused a significant increase in the micronuclei frequency in *P. hawaiensis* hemocytes compared to the control. Alizarin is mutagenic to bacteria and *P. hawaiensis* and even though alizarin is a natural dye, it seems like an unsafe option from a mutagenic perspective.

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## P-65
## Cellular transformation after the exposure to plastic particles derived from 3D-printed objects

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Humans are continuously exposed to micro- and nanoplastics (MNPs) originated from the breakdown of larger plastic waste. MNPs accumulation in tissues and organs raises concerns about their potential impacts on human health, particularly the induction of cancer. Validated carcinogenic studies conducted with rodents present economic and ethical dilemmas. Alternatively, *in vitro* cell transformation assays (CTAs) enable us to simulate the in vivo initiation and promotion stages of carcinogenesis. The objective of this study was to evaluate the cell-transforming potential of MNPs produced during the breakdown and degradation of 3D-printed objects at the end of the lifecycle through the validated Bhas-42 CTA. Polycarbonate particles without and with single-walled carbon nanotubes (PC and PC-CNT, respectively), and polypropylene particles without and with silver nanoparticles (PP and PP-Ag, respectively) were obtained by cryomilling 3D printed objects and sieving below 5 μm. The resulting particles were (0.25–0.30 μm**)** dispersed according to the NanoGenotox protocol and tested following the OECD guidance 231. Bhas-42 cells were exposed to the particles (6.2–100 μg/mL) from day 1 to 4, in the initiation assay, and from day 1 to 14, in the promotion assay. Parallel cultures up to day 7 were conducted to assess cell growth, cellular internalisation and the expression of proliferation and adhesion genes. The initiation assays showed a concentration-dependent cell growth decrease for all the materials, with PP-Ag standing out. By contrast, cell growth decrease was minor in the promotion assays. Despite all materials being internalised by the cells, no increase in the induction of transformed foci in any of the assays was observed for any MNP. Gene expression analyses are still in progress.

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## P-66
## Hepoid 3D cultures allow the study of genotoxic and mutagenic compounds in human liver

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The liver is an essential organ that guarantees many metabolic functions and plays a critical role in xenobiotic detoxification and bioactivation of various pro-carcinogens. Standard *in vitro* genotoxicity assays, particularly those based on mammalian cells, regularly lead to so-called "false positive" results, often necessitating recourse to animal testing. The poor specificity of these tests is therefore a cause for concern in the context of the 3Rs regulation which encourages the development of reliable alternative *in vitro* methods. 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 DMSO which restricts its usefulness for drug-metabolism studies. The DMSO-free advanced 3D model of human hepatocytes Hepoid allows concomitant proliferation and differentiation of human hepatocytes (primary and HepaRG cells). Hepatocytes rapidly organise into characteristic polarised hollow spheroids exhibiting high levels of liver-specific functions and xenobiotic metabolism enzyme expression and activities after a few days and for at least 4 weeks. By various approaches (γH2AX, comet assay, micronucleus test, transcriptomic analysis), we have studied the effects of well-known genotoxic carcinogens (MMS, MMC, colchicine, vinblastine, DMH, $AFB_1$), non-genotoxic carcinogens (DEHP, methylcarbamate) and non-genotoxic non-carcinogen (ethionamide) and demonstrated the relevance of the model to efficiently discriminate the genotoxic effects of various classes of molecules. Once validated, we studied the *in vitro* genotoxic potential of the main heterocyclic aromatic amines (HAAs) AαC, IQ, MeIQx and PhIP, food and environmental contaminants suspected of being involved in the occurrence of hepatocellular carcinoma. Our results showed significant DNA damage after treatment with AαC and IQ. Taken together, our results demonstrate the reliability of the Hepoid model for the *in vitro* assessment of chemical risks linked to genotoxicity and mutagenesis and confirm the capacity of HAAs to cause DNA damage in a highly differentiated human liver model.

## P-67
## An innovative approach to ambient air pollution exposure assessment

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Polluted air consists of a complex mixture of gaseous pollutants, particulate matter (PM) of various sizes and compounds bound to it, and metals. The size of the particles, along with the chemical composition of the complex mixture, determines possible adverse health effects of air pollutants on human health. For comprehensive *in vitro* evaluation of the biological effects of ambient air toxic compounds, treatment of cell models consisting of mixtures of relevant cell types grown at the air-liquid interface (ALI) with a complete mixture of pollutants is crucial. However, a limited number of studies evaluated the impacts of real-world atmospheric pollution using such an approach. We have developed a mobile exposure system suitable for direct exposure to complex mixtures of ambient air pollutants in field conditions. The system consists of an exposure box where the cells are treated at ALI and an incubator that keeps the required temperature, humidity, and $CO_2$ concentration. Additionally, instruments analysing air quality and particle concentrations can be included in the experimental setup. Using the system, we exposed cell models of the lungs and olfactory mucosa (a proxy to brain effects) to the ambient air in four localities of the Czech Republic differing in the quality of air pollution (characterised by concentrations of PM, ozone, nitric oxides, volatile organic compounds, and polycyclic aromatic hydrocarbons). We measured concentrations of these pollutants and correlated them with a panel of relevant biomarkers, including parameters of general toxicity, markers of oxidative stress and inflammatory response, as well as global mRNA and miRNA expression analysis. Our system represents a unique approach to monitoring the direct biological effects of ambient air in field conditions, considering the complex character of environmental air pollution.

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## P-68
## Findings from long-term biomonitoring research in humans with risk of chronic and/or acute inhalation exposure to nanoparticles

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Nanoparticles (NPs), particles of matter of a diameter of 1–100 nm, have been intensively studied in the last two decades due to their many benefits but also possible negative impacts on humans. The effects of their exposure have been studied mostly *in vitro*. Only a limited number of them focused on the real exposure to humans. Among these reports, studies from Czechia published during the years 2015–2024 play a dominant position. The following cohorts were studied with the aim of analysing the effects of chronic and acute exposure to NPs: (i) research workers exposed for a long period of time were followed for six consecutive years and (ii) a group of volunteers with acute exposure to materials used in stomatology. In these complex studies, the following markers were included: (i) markers of oxidative damage; (ii) cytogenetic markers; (iii) DNA damage analysis by comet assay; (iv) telomere length measurements; and (v) -omics markers including whole genome DNA methylation, mRNA, and miRNA analysis. Moreover, aerosol exposure monitoring to assess the differences in particulate matter exposure, including nanosized fraction, was carried out. These general trends were observed: (i) differences between effects of chronic and acute exposure in cytogenetics and epigenetics findings; (ii) different impacts of various exposure profiles for individual working activities on the level of individual biomarkers; (iii) long-term NPs exposure was accompanied by a weaker response or no effects of selected biomarkers; (iv) complex results implicate the possible adaptation to chronic low-dose exposure to NPs; (v) more prominent effects were observed related to acute exposure in selected cytogenetic parameters. In conclusion, the high diversity in the NPs exposure profile and the general lack of this type of human studies, indicate that more research is urgently needed to obtain reliable answers related to NPs exposure risks.

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## P-69
## The influence of development and heat stress on major and minor satellite DNA in the beetle *Tribolium castaneum*

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Satellite DNAs are tandemly repeated sequences preferentially assembled into large arrays within constitutive heterochromatin and their transcription is often activated by stress conditions, particularly by heat stress. In the flour beetle, *Tribolium castaneum* (peri)centromeric heterochromatin is mainly composed of a major satellite DNA TCAST1 interspersed with minor satellites. With the exception of heterochromatin, clustered satellite repeats are found dispersed within euchromatin. In order to uncover a possible satellite DNA function within the beetle genome, we analysed the expression of the major TCAST1 and a minor TCAST2 satellite during the development and upon heat stress. The results reveal that TCAST1 transcription was strongly induced at specific embryonic stages and upon heat stress, while TCAST2 transcription is stable during both processes. TCAST1 transcripts are processed preferentially into piRNAs during embryogenesis and into siRNAs during later development, contrary to TCAST2 transcripts, which are processed exclusively into piRNAs. In addition, increased TCAST1 expression upon heat stress is accompanied by the enrichment of the silent histone mark H3K9me3 on the major satellite, while the H3K9me3 level at TCAST2 remains unchanged. The transcription of the two satellites is proposed to be affected by the chromatin state: heterochromatin and euchromatin, which are assumed to be the prevalent sources of TCAST1 and TCAST2 transcripts, respectively. In addition, distinct regulation of the expression might be related to the diverse roles that major and minor satellite RNAs play during the development and stress response. In other insects, such as the beetle *Tribolium castaneum*, the major (peri)centromeric satellite DNA TCAST1 is expressed into piRNAs in the germline and into small interfering RNAs (siRNAs) in somatic cells. TCAST1 piRNAs and TCAST1 siRNAs are involved in the establishment and maintenance of heterochromatin, respectively, acting in cis at the genomic loci from which they derive.

# P-70
# The impact of real-world ambient air pollution exposure on human airway tissue model

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Ambient air pollution negatively affects human health and can lead to a wide range of respiratory diseases, including chronic obstructive pulmonary disease, various cancers, and aggravated asthma. Air pollution is a complex mixture of particulate matter, volatile organic compounds, sulfur and nitrogen oxides, and many others. Due to this complexity, an investigation of the toxicity of real-world complete ambient air seems to be more relevant than toxicity tests of separated fractions. A 3D model of human upper airway epithelium (MucilAir™) grown at the air-liquid interface was used for direct exposure to complete ambient air under real-world conditions. MucilAir™ tissues were derived from asthmatic and healthy donors and exposed in a specially developed exposure system for 5 consecutive days at four localities of the Czech Republic differing in the concentration of air pollutants: Ostrava (consistently high pollution levels from several sources), Kvasiny (industrial production), Praha (traffic-related air pollution) and Kosetice (background station with low levels of pollutants). We aimed to assess differences in the toxic response among tissues exposed in different localities as well as between healthy and those suffering from asthma individuals. General tests of toxicity included the analysis of transepithelial electrical (TEER) measurement and cytotoxicity tests (activity of adenylate kinase and lactate dehydrogenase). We further analysed markers of oxidative stress (level of 8-isoprostanes) and inflammation (selected cytokines, chemokines and growth factors). Our results indicate a substantial difference in toxic responses among MucilAir™ tissues exposed in different localities. The five-day exposure in Praha resulted in severe cell damage, therefore, no toxicological endpoints were evaluated. We further observed an increased level of several cytokines in tissues exposed in Kvasiny. Overall, the toxicological results correspond with the meteorological situations and the level of air pollution in individual localities.

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# P-71
# Transcriptome changes in humans exposed to nanoparticles

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Epidemiological studies have attracted attention to nanoparticles (NPs) potential negative effects on human health. We investigated the effect of occupational inhalation exposure to NPs in a group of acutely (working with dental nanocomposite material) and chronically (welding on metal surfaces, machining of the nanocomposite) exposed workers on transcriptomic level changes. We analysed whole genome mRNA and miRNA expression profiles by the NGS to identify the most significantly deregulated genes and to compare the profiles between them. Volunteers whole venous blood was sampled for RNA isolation, before and after exposure, followed by RNA libraries preparation. Analyses of the differential mRNA and miRNA expression were performed. In the group of acutely exposed subjects, we detected large interindividual variability in gene expression changes. The significant deregulation of *DDIT4*, *PER1*, and *GPR124* or *hsa-miR-15b-5p, hsa-miR-6861-3p, hsa-miR-548o-3p,* and *hsa-miR-3913-5p* was observed when compared post- and pre-shift sampling. The deregulation of these genes may result in the induction of oxidative stress, synthesis of eicosanoids, or disruption of cell division. The analysis in the group of chronically exposed workers showed mainly downregulation of mRNAs in both exposed subgroups (welding/machining), when after versus before exposure sampling was compared. The opposite situation was observed in the case of miRNA deregulation. The deregulated miRNAs were mainly connected to cancer processes or fatty acid metabolism, the roles of altered mRNAs were related to immune response pathways. The results indicate that the impact of exposure to NPs is dependent on the style of working as well as on the biological variability among study subjects. Acute and chronic occupational exposure to NPs seems to have different effects on changes in gene expression profiles.

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# P-72
# Integrated micronucleus and multi-endpoint screen for identification and classification of *in vitro* genotoxicants

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Screening for *in vitro* genotoxicity can remove potential hazards early in development and identify lead candidates for further testing. The *in vitro* micronucleus assay is routinely used to screen compounds for induction of chromosome damage. However, micronucleus induction can arise via non-DNA reactive modes of action which can be important information when identifying compounds for further development. The *in vitro* MultiFlow® assay is a multiplexed flow cytometric-based assay for the prediction of the genotoxic mode of action (clastogenic, aneugenic or non-genotoxic) based on changes in γH2AX, p53, phospo-histone H3 and polyploidy. Integrating *in vitro* micronucleus and MultiFlow® endpoints can provide genotoxic mode of action information and a better understanding of any apparent genotoxic response. Human TK6 cells were exposed to known clastogens, aneugens and non-genotoxicants for 24 hours in 96-well plates. Cells were sampled at 4 and 24 hours and analysed for compound-induced changes in p53, γH2AX, phospo-histone H3 and polyploidy using the MultiFlow® assay and at 24 hours for induction of micronuclei. The results from the MultiFlow® endpoints correctly predicted the aneugenic and clastogenic mode of action with micronucleus positive results and correctly identified non-genotoxicants. Integrating *in vitro* micronucleus and MultiFlow® endpoints offers a useful tool and provides important mode of action information for early compound screening.

## P-73
## Biosafety of nanocomposite hydrogels developed for skin wound healing

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Any skin impairment leads to a cascade of events, known as wound healing, however, infections or pre-existing conditions, such as obesity or diabetes often delay the healing process, resulting in the development of chronic wounds. An ideal wound dressing material should be able to protect the wound from bacterial infections, prevent excessive fluid loss, maintain a moist healing environment and promote the healing process. Nanoparticles incorporated into scaffolds allow the creation of nanocomposite smart materials with unique physicochemical and biological properties promoting skin regeneration. As the risk assessment of newly prepared nanocomposites is crucial for their potential use in biomedicine, our aim was focused on determining the cytotoxic and genotoxic effect of three hydrogels (Alginate, Pluronic F-127, and Gelatin methacrylate – GelMA) with a different chemical compositions and iron oxide nanoparticles content. A comprehensive evaluation of the biological effects of these nanocomposites has been performed on different types of skin cells (keratinocytes and fibroblasts) as well as a 3D EpiDermFT skin model. A significant increase in both cytotoxicity and genotoxicity after 24 h (nano)hydrogel treatment, measured by LDH assay, micronucleus test, and comet assay, was observed only in higher concentrations of GelMA nanohydrogel. Moreover, we noticed a higher amount of apoptotic and necrotic cells after GelMA exposure, and also the percentage of micronuclei was significantly higher. Using H&E staining, we determined that nanohydrogels exposure did not cause any histopathological changes in skin structure. Moreover, the closure of the wound has been observed upon nanohydrogel application. These results suggest that above mentioned hydrogels loaded with iron oxide nanoparticles could be promising candidates for wound dressings as they do not show any toxic effects, but further investigation is essential for their implementation in practice.

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## P-74

# Novel cellular factors connecting genome stability and population recovery after massive stress in *Ustilago maydis*

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Death is an essential part of the life cycle of all organisms. It is fundamental to biological processes and the evolution of life. In most organisms, the impact of dying individuals on others stops after death. However, it seems that in the microbial community, the way of death can make a significant difference for the surviving population. The unicellular basidiomycete *Ustilago maydis* is a yeast-like organism that can recover population abundance after devastating stress using biomolecules released from dying cells. This phenomenon is called Repopulation under Starvation (RUS) [1]. We have investigated the recovery dynamics of *U. maydis* when using nutrients originating from cells killed with four different treatments (heat, UV radiation, hydrogen-peroxide and hypoxia). Although the cellular ability to recycle biomolecules released from dead cells can be useful in terms of ecological success, the nutrient-rich substrates come with significant risks. We have shown that some of them exhibit a genotoxic nature. To cope with this, *U. maydis* needs to employ complex machinery to protect its genome from DNA damage and mutational changes. By employing two strategies, i.e., transcriptome analysis and mutant hunt, we identified six novel cellular factors important for efficient population recovery after massive stress. Some of these factors are proteins with unknown functions and others are well-known for their role in maintaining genome stability and cytoskeletal organisation.

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[1] Kojic et al. 2020. https://doi.org/10.1111/mec.15680

## P-75
## A strategy for developing a robust framework of genotoxicity assays for the safety assessment of botanicals

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For centuries, botanicals have been used for disease prevention, treatment of various ailments, and to enhance well-being. However, safety evaluations of currently marketed botanicals are often inadequate. Due to widespread use, a pressing need exists for appropriate toxicity evaluations of these complex mixtures, which contain many unknown constituents. The Botanical Safety Consortium, comprising an international group of experts in specialities such as toxicology, chemistry, and pharmacognosy, is developing a toolkit of assays to generate reliable toxicological profiles of botanicals. Importantly, genotoxicity assays are included in this effort, as this endpoint is not readily captured by adverse events and history-of-use reports, and genotoxicity is a critical component of any toxicological profile. The proposed genotoxicity testing strategy for botanicals begins with in silico modelling followed by in vitro guideline studies including the Ames and in vitro micronucleus assays, with options for additional tests to further characterise genotoxicity and mode-of-action when indicated. To evaluate the effectiveness of this approach, 13 botanicals with existing data were chosen as case studies. ToxTracker® assays have been completed; four positives were identified, all of which were expected based on previously published data. Preliminary Ames test results are consistent with the ToxTracker results and the existing literature. In vitro micronucleus assays are underway. These case studies aim to evaluate the suitability of currently available genotoxicity assays for testing botanicals. The final strategy will include published genotoxicity data, in silico and *in vitro* test data, and human exposure data while minimising the need for animal testing.

## P-76
## Genotoxicity assessment of enniatins and *Alternaria* toxins with the *in vitro* micronucleus assay and the SOS/umu test

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Food and feed can be contaminated with a wide variety of mycotoxins produced by fungal strains. No manufacturer is responsible for providing hazard data on these mycotoxins, resulting in many data gaps. This is especially true for emerging mycotoxins such as enniatins and *Alternaria* toxins. Within the work package 'Hazard Assessment' of the European Partnership for the Assessment of Risks from Chemicals (PARC) project (GA No 101057014), toxicity testing is performed to address data gaps of concern for human health for these mycotoxins. Several toxicological endpoints are assessed including genotoxicity. First, a selection out of the large number of enniatins and *Alternaria* toxins was made based on the review of existing data and other criteria such as availability and price. The selected mycotoxins were then tested in the *in vitro* cytokinesis-block micronucleus (MN) assay in mammalian cells (*i.e.* TK6) with and without metabolic activation according to the OECD test guidelines 487. For the enniatins, the SOS/umu test was also used as a screening assay. All enniatins tested so far were negative in the *in vitro* MN test and the SOS/umu, both in the absence and in the presence of metabolic activation. In contrast, tenuazonic acid and alternariol induced a clear positive effect in the MN test after 24 h in the absence of metabolic activation. For compounds showing positive results in one of the *in vitro* assays, additional testing (*e.g.* Fluorescence In Situ Hybridisation (FISH) test) will be performed. When all tests are finalized, the results will be combined with genotoxicity data collected by other PARC partners to assess the genotoxic potential of the enniatins and *Alternaria* toxins in a weight-of-evidence approach. One important strength of this research is that all tests are performed with compounds from the same batch.

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## P-77
# Analysis of *in vitro* cytotoxicity and genotoxicity of polystyrene nanoparticles in human hepatoma cell line (HepG2)

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The extensive use of plastics has led to a growing ecological concern as they can persist in the environment for many centuries. Plastics may be degraded into microparticles, which can be further broken down into even smaller – nanoparticles (NPs). Among the NPs found in the environment, one of the most common is polystyrene nanoparticles (PS-NPs) [1]. Therefore, the potential negative impact of PS-NPs on human health should be explored. This study aimed to assess the cytotoxicity and genotoxicity of polystyrene nanoparticles (70 nm) in a human hepatoma cell line (HepG2) *in vitro*. The cellular uptake and levels of reactive oxygen species generated by PS-NPs were evaluated using 2,7-dichlorodihydrofluorescein diacetate dye ($H_2$DCFDA), which is oxidant-sensitive and fluorescent. The cytotoxicity was assessed using two methods: AlamarBlue assay, based on metabolic activity, and Trypan Blue, based on cell permeability. The potential primary DNA damage under the exposure of PS-NPs was tested by alkaline comet assay and potential induced oxidative DNA damage by enzyme-modified comet assay. The results from flow cytometry analysis of HepG2 cells exposed to PS-NPs indicated an efficient uptake of these nanoparticles into the cells. Additionally, it was observed that an increase in the concentration of PS-NPs resulted in a corresponding rise in the levels of reactive oxygen species. The tested PS-NP concentrations were not cytotoxic to HepG2 cells, but they did induce both primary and oxidative DNA damage. These findings indicate that polystyrene nanoparticles can be absorbed effectively and have genotoxic potential, raising concerns regarding plastic safety.

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[1] Kik et al. 2020. https://doi.org/10.1016/j.envpol.2020.114297

## P-78
## Exploring the link between microplastic and genotoxicity in fish

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Worldwide, reports of MPs in various environmental compartments have been growing. Despite the fact that rivers are thought to be one of the main sources of plastic waste, not much is known about them. Fishes are one the most valuable sources to estimate the level of MP pollution in rivers due to their position in the food chain. Here, we aimed to connect ingested MPs in the intestine to other environmental factors and their effects on genotoxicity in fish blood. A total of 15 specimens of common bream (*Abramis brama*) were collected during 2022 from the Danube River in Belgrade, in the area where the river receives untreated communal and wastewater. Tissue samples were collected: blood for genotoxicity analysis (comet assay) and gastrointestinal tract for MP analysis. After digestion with KOH 10%, the digests were filtered using a vacuum filtration system with a 50 μm stainless steel mesh. The particles were directly visualised under a light stereomicroscope to classify and identify the MPs found according to their shape, colour, and size. A total of 21 MP particles were found in 11 specimens (73%). Regarding the MP shapes, it was identified 20 microfibers (95%) and 1 fragment (5%). Concerning the colour, it seems that blue microfibers were dominant (66.67%), followed by black (9.52%) and purple microfibers (9.52%), followed by red (4.76%) and brown microfibers (4.76%), and white fragment (4.76%). The size of the microfibers ranged between 135.81 μm and 2516.73 μm, with an average of 677.94±337.13 μm. DNA damage in blood ranged between 9.76 and 46.03 TI, with an average of 25.18±13.32 TI. Although there was no direct positive correlation between the number of MPs particles and DNA damage in samples, the high number of MPs could be interpreted as an indirect link to river pollution pressure and strong genotoxic potential.

## P-79
# Adverse (geno)toxic effects of bisphenol A and its analogues BPS, BPAP, BPAF, BPFL, and BPC in a 3D HepG2 cell model

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Bisphenol A (BPA) is a widely used chemical in polymer additives and epoxy resins for manufacturing a range of products for human applications. It is known to be an endocrine disruptor and there is growing evidence that it is genotoxic. The hazardous properties of BPA have raised concern and led the EU to restrict its use to protect human health and the environment. As a result, there has been a gradual shift towards using presumably safer analogues, which consequently led to an increase in their residues in the environment, however, our knowledge of their toxicological profiles is scarce. We investigated the adverse toxic effects of BPA and its analogues (BPS, BPAP, BPAF, BPFL, and BPC) with emphasis on their (cyto) genotoxic activities after short (24 h) and prolonged (96 h) exposure in *in vitro* hepatic three-dimensional cell model developed from human hepatocellular carcinoma (HepG2) cells. The results showed that BPFL and BPC were the most cytotoxic analogues that affected cell viability, spheroid surface area, cell proliferation, and apoptotic cell death. BPA, BPAP, and BPAF induced DNA double-strand break formation (γH2AX assay), whereas BPAF and BPC increased the percentage of p-H3-positive cells, indicating their aneugenic activity. All BPs induced DNA single-strand break formation (comet assay) (BPAP>BPFL>BPAF≈BPS>BPC≈BPA), with BPAP (≥0.1 μM) being the most effective and BPA and BPC the least effective (≥1 μM). The results showed concentration- and time-dependent effects, indicating the problem of long-term and repeated exposure of humans and animals to BPA and its analogues, in which delayed effects may occur. Overall, the present results indicate that BPAP, BPAF, BFL, BPS, and BPC cannot be considered safer alternatives to BPA in terms of their cytotoxic and genotoxic activities, and therefore further studies on their potential adverse effects and mechanisms of action are needed to adequately evaluate the risks of BPA analogues and assess their safety to humans.

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## P-80
## Assessment of the genotoxic and mutagenic potential of a CBD isolate and a *Cannabis sativa* extract *in vitro*

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Cannabidiol (CBD) is a naturally occurring cannabinoid found in hemp, *Cannabis sativa* and *C. indica* plants. Unlike THC, CBD lacks psychotropic properties but exhibits various pharmacological effects. In recent years, the public perception of cannabis and CBD products has shifted, with many countries legalising their medicinal use. This shift has led to a rapid expansion of CBD products marketed as ameliorating many health problems. CBD products are used by patients as well as by healthy people who believe in the claimed benefits. While CBD's therapeutic potential is widely reviewed, its adverse effects are less explored. The present study aimed to explore the potential genotoxic activity of a CBD isolate (CBD CP) and a *Cannabis sativa* extract (CBD EX). The toxic activity of the CBD samples was evaluated using the MTT assay, and the genotoxic and mutagenic activity was evaluated using the comet, micronucleus, γH2AX and p-H3 assays and the AMES test, respectively. We further performed toxicogenomic analyses of selected genes involved in xenobiotic metabolism and DNA damage response. Both CBD samples showed no mutagenic activity in the AMES test (OECD 471). They demonstrated cytotoxicity for HepG2 cells with $IC_{50}$ values of approximately 26 μg/mL (4 hours) and 6–8 μg/mL (24 hours). Noncytotoxic concentrations upregulated genes encoding metabolic enzymes involved in CBD metabolism, indicating HepG2 cells' ability to metabolise CBD. Under the applied conditions, both CBD samples were non-genotoxic, as no DNA damage or influence on genomic stability was observed in any of the applied tests (comet, micronucleus, γH2AX, and p-H3 assays), and no changes were observed in the expression of genes involved in the genotoxic stress response. The obtained results will contribute substantially to the safety evaluation of CBD-containing products and their authorisation, as well as further development in the field of hemp exploitation for medical and nutritional purposes.

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# P-81
# Exploring the SAR of arylboronic acid compounds

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Arylboronic acids (ABAs) are common synthetic reagents used in carbon-carbon bond-forming reactions and are frequently used in the production of active pharmaceutical ingredients (APIs). While ABAs themselves are not currently present in an API, they could be present in the final product at low levels as an impurity which would require an assessment of their genotoxicity, a problem first proposed in 2010 [1]. Consequently, understanding the mutagenicity of ABAs has become increasingly important due to potential human exposure to pharmaceutical products. To investigate the structure-activity relationships, we analysed a dataset of 186 ABAs (including arylboronic esters), sourced from both public and proprietary data, and determined how steric and electronic effects may impact the potential to elicit a mutagenic response in the Ames test. Furthermore, we demonstrated how subclasses of ABAs may be de-risked by the use of Derek Nexus to identify deactivating features that are likely to lead to a negative response in the Ames test, with a predictive performance of 78% balanced accuracy (94% sensitivity, 63% specificity) for a set of 143 boronic acids/benzoxaboroles. For 43 aryl boronate esters, primarily pinacols, while all 17 positive compounds are correctly predicted by Derek Nexus, there is a relatively high number of false positives (18 compounds, 42%). This may be partially due to the negative bias of the relatively small data set. Derek Nexus is therefore highly sensitive for alerting the user to a potentially mutagenic ABA and predictions can be used for regulatory submissions, for example, ICH M7 assessment; however, further *in vitro* testing may help to refine the SAR, particularly in the case of aryl boronate esters, to reduce the instances of false positives.

[1] O'Donovan and Mee. 2010. http://dx.doi.org/10.1093/mutage/geq090

# P-82
# Lung toxicity comparison between actinolite cleavage fragments and asbestos

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Asbestos is hair-like fibres of relatively constant width, whereas non-asbestiform silicate minerals (non-asbestos) are generally shorter and thicker. Asbestos exposure is known to cause health issues like fibrosis and cancer. When mechanical stress is applied to the non-asbestiforms, the fracturing generates more or less elongated particles called "cleavage fragments". Cleavage fragments (CF) of actinolite have been identified in asphalt aggregates. The absence of epidemiological and toxicological data on CF alone led us to design this project, whose objective is to evaluate the pulmonary toxicity of actinolite CF compared to actinolite-asbestiform *in vivo* in rats. A sample of actinolite CF free of asbestos fibres and actinolite-asbestos was administered in the lungs of rats, euthanised 24 hours and 3 months after exposure. In 3 months, with similar particle numbers, cleavage fragments are slightly less efficiently cleared from rats' lungs (62–72% for fragments *vs.* 80% for asbestos). However, with 2 times more fibres (length/diameter >3), asbestos fibres are more efficiently cleared than fragment fibres, leaving about similar fibre numbers after 3 months. Histopathological analysis of lung tissue 3 months after exposure revealed lung damage and fibrosis for asbestos and cleavage fragments. Emphysema was observed 24 hours after exposure for all tested samples, but only for asbestos after 3 months. However, microhemorrhage was present only in cleavage fragments exposed to animals. Dosages in bronchoalveolar lavages (BAL) showed cytotoxicity and inflammation-induced 24 h after exposure to all treatments except controls. Three months after exposure, inflammation was persistent only in asbestos-exposed lungs and cytotoxicity only in cleavage fragments. TGF-®, the most critical pro-fibrotic cytokine, is overexpressed in 3 months after exposure to asbestos-exposed BAL. Finally, apoptosis occurred quickly and persisted for all exposed lungs. Genotoxicity endpoints like micronuclei, γH2AX, and 8-OHdG are being investigated, along with transcriptome and methylome analysis.

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## P-83
## Unravelling the mediating role of DNA repair and mitochondria in the development of obesity

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Animal models indicated an association between base excision repair (BER) deficiency and an increased risk of obesity development. Therefore, we aimed to identify genetic variants in BER genes and subsequently investigate their effect on intracellular lipid accumulation through *in vitro* knockdown models. Additionally, we investigated the role of mitochondria. We hypothesised that BER deficiency could lead to reduced mitochondrial function and enhanced intracellular lipid accumulation. Whole-exome sequencing data from 30 morbidly obese subjects was used to select biologically functional variants in BER-related genes. Transient BER knockdown models were generated through shRNA transfection in HepG2 cells. BER activity was tested via comet assay. Intracellular lipid accumulation was determined by Oil-Red-O staining. Oxidatively induced DNA damage in mitochondrial DNA (mtDNA) was assessed by qRT-PCR, and mitochondrial respiration was studied by Seahorse Mito stress test. Based on the whole-exome sequencing data, functional mutations were detected for *MUTYH*, *NTHL1*, and *OGG1* in obese subjects. The knockdown of these genes in HepG2 cells was confirmed by qRT-PCR and significantly decreased BER activity for *MUTHY*, *NTHL1 and OGG1* compared to *WT* cells. All the knockdown cells were more able to accumulate intracellular lipids than *WT* cells after exposure to oleic acid or a mixture of oleic and palmitic acid. Additionally, significantly increased levels of mtDNA damage were detected in *MUTYH*, *NTHL1* and *OGG1*-deficient cells. Compared to LacZ control cells, the Seahorse assay revealed increased proton leakage in the knockdown cells. Transient knockdown of BER genes impairs BER activity, subsequently increases levels of oxidatively damaged mtDNA, mitochondrial dysfunction, and intracellular lipid accumulation in HepG2 cells. These data suggest that DNA repair deficiency might contribute to obesity development.

## P-84
## Changes in telomere length and mitochondrial DNA copy number in the colorectal adenoma-carcinoma sequence

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Changes in telomere length (TL) and mitochondrial DNA copy number (mtDNA-CN) have separately been proposed as risk factors and prognostic biomarkers for various cancer types. However, results regarding colorectal cancer (CRC) have been conflicting. Emerging evidence also suggests a connection between these two cellular processes. The deregulation of the telomere-mitochondria axis, caused by ageing or other physiological factors, seems to trigger carcinogenesis. We aimed to discern the potential of TL and mtDNA-CN in distinguishing colorectal adenoma (CA) progression to CRC and compare their changes in different biological specimens. The study included 10 control individuals, 145 CA patients, and 256 CRC patients. Peripheral blood and fresh frozen tissues (healthy colorectal mucosa from controls, paired adenoma-adjacent non-affected mucosa from CA patients, and paired tumor-adjacent non-affected mucosa from CRC patients) were used for DNA/RNA isolation, and multiplex RT-qPCR for molecular analyses. CA and CRC tissues exhibited shorter TL than their corresponding adjacent mucosa ($p<0.001$), with healthy colorectal mucosa showing the longest TL among all solid tissues. Elevated mtDNA-CN was detected only in adenomas vs. mucosa ($p<0.0001$), correlating with TL ($p<0.01$). Blood samples showed lower mtDNA-CN than solid tissues irrespective of their pathological condition ($p<0.0001$) and shorter TL than healthy ($p<0.01$) and non-tumor mucosa ($p<0.0001$). There was a moderate positive correlation of mtDNA-CN between the tumor and corresponding mucosa ($p<0.0001$) and of TL between all tissues in CRC patients ($p<0.001$). Our findings hold significant promise in understanding the potential for adenoma formation and progression to CRC, as mtDNA-CN changes and associations with TL were typical of precancerous lesions and were lost with tumour progression. Moreover, blood samples from CRC patients can serve as surrogate tissue for assessing TL changes in solid tissues.

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## P-85
## Evolutionary considerations in *Drosophila* sibling species: geometric morphometric approach

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Evolutionary genetics is interested, *inter alia*, in behavioural changes that may lead to speciation. *Drosophila melanogaster* and *Drosophila simulans* represent a well-known pair of sibling fruit fly species that were subjected to numerous morphological, cladistic and molecular analyses. Although morphologically very similar, those species significantly differ in cuticular chemoprofiles and courtship songs involved in mating. In nature, those species often coexist and share similar habitats, with subtle differences in spatial and temporal distribution. In laboratory conditions, hybridisation was observed in higher frequency when the male was *D. simulans*, while that was not the case in the reverse situation. In this work, we have examined the size and shape of morphological traits involved in mating, using laboratory populations of these two species. Examined traits were both two- and three-dimensional: 1) wings, used as visual stimuli and in courtship song production, marked with 15 landmarks; 2) head, important in orientation courtship phase, marked with 12 landmarks; 3) 1st pair of legs, involved in the male tapping courting phase, marked with 28 landmarks. Our results, reached by the geometric morphometric approach, pointed to significant differences in shape and size of the aforementioned traits between species, emphasising that visual and tactile stimuli, together with olfactory cues and acoustic signals, are involved in behavioural recognition. In that way, visual and tactile stimuli involved in courtship may significantly contribute to conspecific mating and to prezygotic ethological reproductive isolation between *D. melanogaster* and *D. simulans*.

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## P-86
## Interpopulation morphological differences in *Glomeris hexasticha* Brandt, 1833 (Diplopoda: Glomerida: Glomeridae)

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Organisms utilise different strategies (*e.g.* morphological, physiological, behavioural *etc.*) for adapting to diverse environmental conditions. Morphological variation is necessary for evolutionary mechanisms to act. In some millipedes (Myriapoda: Diplopoda), morphological variation has been investigated thoroughly, but not in the order Glomerida. Considering this fact, the aim of our study was to investigate interpopulation differences in some morphological traits in *Glomeris hexasticha* Brandt, 1833, between two populations (Ada Makiš and Hyde Park) from Belgrade, Serbia. Morphological variation in shape and size of the anal shield, the collum, the gnathochilarium, the antennae, the legs, and the telopods was investigated using a geometric morphometric approach. A different number of landmarks and/or semilandmarks has been positioned on analysed structures: the anal shield (two landmarks and 16 semilandmarks), the collum (two landmarks and 12 semilandmarks), the gnathochilarium (30 landmarks), the antennae (28 landmarks), the legs (21 landmarks) and the telopods (50 landmarks). No interpopulation differences were detected in the centroid size of all analysed morphological traits, whereas they were obtained in the shape of the anal shield, gnathochilarium and legs. Shape differences in the aforementioned traits could be explained by their influence on fitness. Namely, the anal shield probably has a role in reproduction, whereas the gnathochilarium processes food, and the legs, used in locomotion, affect the survival of the individuals. Therefore, the differences in the shape of some morphological traits could contribute to the survival and reproduction rates of the studied species.

## P-87
## Sexual shape and size morphological differences in *Glomeris hexasticha* Brandt, 1833 (Diplopoda: Glomerida: Glomeridae)

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Sexual dimorphism (SD), which represents a widespread phenomenon in evolutionary biology, can be divided into two components: shape sexual dimorphism (SShD) and size sexual dimorphism (SSD). These components of SD indicate that both the shape and size of morphological structures may be susceptible to different evolutionary pressures in males and females. Sexual size dimorphism has been well-studied in many animal species, including millipedes (Myriapoda: Diplopoda). However, sexual shape and size dimorphism of certain morphological traits have been investigated using a geometric morphometric approach in some Diplopoda species, but not in representatives of the order Glomerida. The goal of this study was to investigate SShD and SSD in the following structures: the anal shield, the collum, the gnathochilarium, the antennae, the legs and the telopodes. The studied species was *Glomeris hexasticha* Brandt 1833, and the samples were collected from two populations (Ada Makiš and Hyde Park) from Belgrade, Serbia. Obtained results indicated the presence of SShD in the anal shield and the gnathochilarium in both analysed populations, while SShD in the antennae was detected only in the Ada Makiš population. On the other hand, SSD has been observed in all analysed morphological traits in both populations, with female sex possessing higher values. Results of the present study support the presence of the general SSD pattern in millipedes, with females being a larger sex. They also suggest higher intersexual size differences compared to intersexual shape differences. Considering these results, we may assume that evolutionary pressure could affect the shape and size of the morphological structures in different ways in examined glomerid species.

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## P-88
## New evidence for an alternative end-joining mechanism of DNA double-strand break repair in *Deinococcus radiodurans*

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Genome stability in the radioresistant bacterium *Deinococcus radiodurans* depends on RecA, the main bacterial recombinase. Without RecA, gross genome rearrangements (GGRs) occur during repair of DNA double-strand breaks (DSBs). Long repeated (insertion) sequences have been identified as hot spots for this RecA-independent DSB repair, and single-strand annealing (SSA) is postulated to be the most likely mechanism involved in this process. We have recently sequenced five isolates of *D. radiodurans recA* mutant carrying GGRs and detected large deletions in chromosome II in all the sequenced *recA* isolates. The mechanism behind these deletions clearly differs from the classical SSA; it utilised short (4–11 bp) repeats as opposed to insertion sequences or other long repeats, and worked over larger linear DNA distances from those previously tested. Our data are most compatible with alternative end-joining (A-EJ), a recombination mechanism that operates in eukaryotes but is also found in *Escherichia coli*. In wild-type *D. radiodurans*, GGRs were found only at very large amounts of radiation-induced DSBs. However, changes in genome structure may also be possible during propagation and storage of wild-type cultures. We investigate this possibility by listing structural differences between two completely sequenced genomes of *D. radiodurans* strains with a recent common ancestor – the type strain stored and sequenced in our laboratory (of the ATCC 13939 lineage) and the first sequenced strain historically used as reference (ATCC BAA-816). We detected a number of structural differences and found the most likely mechanisms behind them. Most surprising is the finding of deletions between short repeats because it indicates utilisation of a less accurate A-EJ repair mechanism in conditions in which a more accurate one should be both available and preferred. Our results are the first to demonstrate A-EJ as a significant part of the *D. radiodurans* toolbox for genome repair and maintenance.

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## P-89
## Molecular programs directed by arsenic and smokeless tobacco (co)exposure: experimental multi-omics analysis and implications for oral cancer epidemiology

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Chronic exposure to arsenic underlies various health conditions including cancer. Furthermore, co-exposure involving prevalent lifestyle habits may enhance arsenic toxicity. Smokeless tobacco (SLT) products are extensively consumed in South Asian countries, where their use frequently co-occurs with exposure to arsenic from contaminated groundwater. To decipher the oral epithelial cell responses to arsenic (sodium arsenite, SA) and SLT extract, individually or in combination, we performed experimental multi-omics analyses of the DNA methylome and transcriptome reprogramming (RNAseq), and of various biochemical/phenotypic changes induced by SA and/or SLT. Chronic exposures induced pronounced changes in DNA methylation states with the predominant SA and SA+SLT-induced hypomethylation affecting genes involved in DNA damage response, signal transduction, regulation of transcription, cell cycle control, inflammatory and immune responses and apoptosis. The acute-response RNAseq analysis revealed exposure-specific regulation of transcription, signal transduction, protein ubiquitination, apoptotic process, and cell differentiation changes, with the combined SA+SLT treatment inducing processes related to the regulation of transcription, cell proliferation, regulation of IKB/NFKB signalling, and apoptosis. To validate the omics results at the phenotypic level, we observed a dose-dependent decrease in cell viability, induction of DNA damage, cell cycle changes, and an increase in apoptotic cells, with the most pronounced effects observed for SA+SLT co-exposure. The observed DNA damage likely resulted from apoptosis induction, as chronic exposure effects assessed by whole-exome sequencing did not reveal specific mutagenicity due to the SA and/or SLT exposure. Our integrative study provides insights into both chronic and acute responses to SA and/or SLT, revealing convergence on key mechanisms emerging from large-scale epigenomic and transcriptomic reprogramming and manifesting by specific phenotypic outcomes. Future studies are warranted in exposed human subjects, including exposure assessment and oral cancer investigations, to validate the observed mechanisms and to generate *in vivo* evidence for relevant cancer prevention measures.

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## P-90
## CutCancer: A twinning approach to strategically advance research on carcinogenesis and cancer

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The number of cancer cases in Europe is increasing each year, from the current 3.5 million to an estimated 4.3 million in 2035, regardless of age, gender or social status, placing an enormous burden on health systems, patients, families and society at large. The European Commission has prioritised cancer as one of the world's major challenges, by launching Europe's Beating Cancer Plan to improve outcomes by 2030. Central to this plan is the need to tackle the entire cancer disease pathway, emphasising the urgency to develop more efficient therapeutic approaches. Genomic instability and the tumour microenvironment are identified as critical factors in cancer development and progression. However, despite their importance, molecular understanding remains limited. Addressing this gap is crucial for developing effective prevention strategies, treatment approaches and combating treatment resistance in cancer therapy. The CutCancer project aims to accelerate cancer research through interdisciplinary collaboration and innovative technologies, particularly in the fields of preclinical 3D carcinogenesis and cancer research. It focuses on understanding genomic instability and tumour microenvironment to understand cancer initiation, progression, and resistance to treatment. Through advanced *in vitro* 3D model systems and cutting-edge technologies including high-throughput screening approaches for risk assessment of chemicals and nanomaterials and single-cell and spatial biology techniques, the project aims to unravel the complexities of cancer biology. The partnership with the leading research institutions Swansea University Medical School from the UK, Stockholm University from Sweden, and Amsterdam University Medical Center from the Netherlands brings together expertise in genetic toxicology, spatial transcriptomics, and imaging mass cytometry. By integrating data and knowledge generated within CutCancer, we aim to enhance our understanding of cancer complexity. The ultimate goal is to contribute to cancer prevention, accurate prognosis, and overcoming therapy resistance, benefiting both individuals and society as a whole.

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## P-91
## Hepatotoxicity of a mixture of bisphenol A, bisphenol S, and bisphenol F: an analysis of toxicogenomic data

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Bisphenol A (BPA), bisphenol S (BPS), and bisphenol F (BPF) are widely used in the industry for the production of plastics and resins, but their potential hepatotoxicity raises concerns due to possible health risks. The aim of this study was to investigate the hepatotoxicity of a mixture of BPA, BPS, and BPF using an *in silico* analysis of toxicogenomic data. For this purpose, the Comparative Toxicogenomics Database (CTD, https://ctdbase.org/), Cytoscape, GeneMANIA (https://genemania.org/), and the Toppgene tool toppfun function (https://toppgene.cchmc.org/) were used for bioinformatic analysis. In the CTD database, gene sets were identified that could contribute to the development of liver diseases, chemical and drug-induced liver injury due to BPA, BPS, and BPF. A total of 150 common genes for all three bisphenols were identified. Cytoscape analysis highlighted the 5 most significant genes among these 150 common genes, which are *ARG1, KNG1, CP, APOA1,* and *SERPING1*, known to have changes in their expression that can lead to liver function impairment. In the GeneMANIA predictive server, it was found that the dominant type of interaction among the common genes for BPA, BPS, and BPF was co-expression (52.54%). Genes of interest were involved in pathways such as the nuclear receptors pathway, metabolism, and the hydrocarbon receptor pathway, as well as biological processes including the regulation of programmed cell death, regulation of the apoptotic process, and small molecule biosynthetic processes. Additionally, molecular functions such as oxidoreductase activity, signalling receptor binding, and cell adhesion molecule binding were identified, all of which are linked to the development of hepatotoxicity. In conclusion, it was determined that the mixture of BPA, BPS, and BPF can induce hepatotoxicity by modulating key genes (*ARG1, KNG1, CP, APOA1, SERPING1*) and pathways associated with the regulation of cell death processes, oxidoreductase activity, and nuclear and hydrocarbon receptors.

# P-92
# Antioxidant potential of dihidroqercetin alone and in combination with biochaga *in vitro*

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Free radicals, which continuously arise from normal mitochondrial function or exposure to external toxins, play a significant role in the development and progression of various diseases. Antioxidants with low molecular weight, which are non-enzymatic, can be obtained through dietary sources. Dihydroquercetin, also known as taxifolin (3,5,7,3,4-pentahydroxyflavanone), is a flavonoid that exhibits promising properties such as anticancer, anti-inflammatory, and neuroprotective effects. Additionally, it has a strong ability to modulate and prevent oxidative stress, cardiovascular issues, and liver diseases. Another natural remedy is the edible medicinal mushroom Chaga (*Inonotus obliquus*), which has been used for centuries to treat various health conditions. Chaga displays antitumor, anti-inflammatory, hypoglycemic, immunomodulatory, antioxidant, and antigenotoxic effects. To assess their antioxidant capabilities, we measured the scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl), the total antioxidant activity using FRAP (ferric reducing antioxidant power), and the scavenging capacity for hydroxyl radicals. When tested alone, dihydroquercetin (DHQ) demonstrated excellent reducing power ($IC_{50}$=0.3216 mg/mL). When combined with Biochaga (B), the synergistic effect resulted in strong reducing power ($IC_{50}$=0.531 mg/mL). DHQ also exhibited moderate DPPH radical scavenging activity ($IC_{50}$=0.169 mg/mL), while the combination of DHQ and B showed very strong inhibition of DPPH ($IC_{50}$=0.105 mg/mL). Furthermore, DHQ effectively scavenged hydroxyl radicals ($IC_{50}$ =2.501 mg/mL), with the combination of DHQ and B demonstrating moderate activity ($IC_{50}$=8.475 mg/mL). In summary, these investigated substances fall into the category of radical scavengers, efficiently neutralising hydroxyl radicals for which the body's natural antioxidant defences may be insufficient.

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## P-93
## Assessment of air pollution effects on genomic stability: preliminary findings from Zagreb, Croatia

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Air pollution stands as a significant challenge in both environmental and public health realms, acknowledged by global authorities as a risk factor associated with detrimental health consequences. Its impact on human health, whether in ambient environments or workplaces, can manifest through DNA alterations. Exposure to diverse air pollutants has been correlated with the development of cardiovascular and respiratory ailments, premature death, and the onset of cancer, along with its associated economic burden. Mitigating air pollution levels holds the potential to alleviate the disease burden linked to conditions like stroke, heart disease, lung cancer, and respiratory disorders, including asthma. Aligned with these concerns, our objective was to investigate the influence of air pollution on genomic stability and consequent health implications by investigating potential associations between air pollutants and biomarkers of effects. Initially, we conducted a retrospective assessment of genomic instability among the general population (N=130) residing in Zagreb, Croatia. We correlated these instabilities, evaluated by the comet and micronucleus assays in blood samples, with air pollution levels spanning from 2011 to 2015. Notably, apart from benzo(a)pyrene (B[a]P), which exhibited a significant negative correlation, we did not observe any noteworthy positive associations among the assayed parameters. Our findings indicated that the measured air pollution parameters mostly remained below regulatory limits, except for B[a]P. Subsequently, we investigated the potential impacts of air pollution exposure on genomic instability. This investigation encompassed comet and micronucleus assays conducted on blood cells of the general population (N=60) in Zagreb, Croatia, during colder and warmer periods spanning from 2021 to 2022. While all measured outdoor air pollutants generally adhered to previously reported values and remained below regulatory limits, exceptions were found for $PM_{10}$ particles and B[a]P bound to $PM_{10}$, exceeding permissible levels. Nonetheless, we did not observe the major impact of air pollution on cytogenetic biomarkers tested. Given the identified threat of air pollution to public health, further exploration in this domain is imperative. Our future endeavours aim to replicate such studies in other urban areas, where exposure to distinct air pollutants is anticipated.

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# P-94

# The error-corrected next generation sequencing revolution in genetic toxicology has arrived: modernizing OECD test guidelines for mutagenicity testing

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Although several short-term mutation assays are used for regulatory decision-making in genotoxicity testing, they are limited in what they measure. Emerging technologies such as error-corrected Next-Generation Sequencing (ecNGS) use the principle of consensus sequencing of the DNA duplex in conjunction with bioinformatics to remove both polymerase chain reaction and sequencing errors, resulting in error rates of less than one per hundred million bases sequenced, which is similar to the baseline mutation frequency of somatic and germ cells. Thus, ecNGS can directly detect low-frequency mutations induced by chemicals and other substances in DNA from any species and in any tissue. Furthermore, ecNGS provides simple and trinucleotide mutational spectrum data, allowing insight into mutagenic mechanisms of action. To benchmark this technology to support development of a Standard Project Submission Form (SPSF) in support of a proposal to amend Organization for Economic Cooperation and Development (OECD) Test Guidelines for mutagenicity (TG 488, TG 490, TG 471), we reviewed the current ecNGS state-of-the-art in terms of in vivo and in vitro studies to generate foundational data that will build confidence in the use of ecNGS as a valid option for generating mutagenicity data for regulatory submission. OECD National coordinators from the USA and UK, together with a team of subject matter experts, worked to develop the SPSF. The data collated by the team resulted in the approval of the application by the Working Group of National Coordinators (WNT) in April of 2024. A summary of these data will be presented, and the next steps include the establishment of an international Expert Working Group (EWG) to develop a Detailed Review Paper (DRP) to support amending test guidelines designed for the detection of mutagenicity.

*Supported by Marchetti et al. (2023) doi: 10.1016/j.mrrev.2023.108466.*

# Health and Environmental Sciences Institute Workshop

**Where the Rubber Meets the Road:**
Transitioning Academic Research into Regulatory Requirements

Abstracts of the 52nd EEMGS meeting – HESI Workshop
Arh Hig Rada Toksikol 2024;75(Suppl. 1):22-209

# Reflections on the commonalities and differences between academic and regulatory genotoxicity studies

Carol Beevers
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The adoption of an OECD test guideline is the culmination of years of work, starting with the developing laboratory establishing the test system and methodology, through acceptance of the technique in the literature and inter-laboratory validation, and ending with the development of the final guideline recommendations. The purpose of the guideline is to provide clear instruction on critical aspects of study methodology, and studies performed according to the written guideline procedure are accepted as reliable and suitable for regulatory scrutiny. Standardisation of assay procedures can also facilitate comparisons of data generated in different laboratories as the number of methodological variables is greatly reduced. In contrast, deviation from guideline recommendations can lead to questions regarding the reliability of the findings and uncertainty in data interpretation. One consequence of this can be regulatory hesitancy to conclude on the safety of the test material. Recent examples of conflicting study outcomes and their impact on regulatory opinions will be presented, together with recommendations on how to bridge the gaps between academic and regulatory genotoxicity studies.

# Statistics and experimental design: similarities and differences between regulatory and academic studies

David Lovell
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Standard regulatory studies in genotoxicity testing use experimental designs based upon methods developed in the mid-to-late twentieth century. Development of new test methods and mechanistic studies, especially those where multiple variables may be acting, would benefit from the application of Design of Experiment (DOE) approaches as opposed to the 'traditional' one-facto-= at-a-time (OFAT) approach. These methods extract more information from the available biological material. An educational programme will probably be needed to help genetic toxicologists and regulators understand these developments in experimental design and statistical methodologies.

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# What do regulatory agencies look for in studies and how they use academic and regulatory studies

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Preclinical studies are required to evaluate the toxic potential of chemicals and consumer products and the pharmacology and safety of pharmaceuticals. The study data are necessary for defining the conditions for the safe use of these products to ensure consumer and patient protection. Drug regulations usually require study conformity with GLP and OECD TG for acceptance of studies submitted for product approval. For the acceptance of non-GLP/OECD compliant studies (e.g. from publication), regulators are focusing on demonstrated study quality and sufficient description of experimental protocols to assess the reliability of the study results. Data and information demonstrating study quality and validation, performance, robust data detection and evaluation are crucial, such as, but not limited to i) quality of test substance, sources, analytics for identity and purity, dose formulation and preparation, stability; ii) cell culture or animal origin, welfare compliance, dosing regiments and application, treatment duration, sampling and sample preparation and effect detection; iii) data detection, data recording and data evaluation for statistical and biological relevance, data reporting, presentation and storage. It is often problematic to retrieve all necessary information from academic studies only available as published literature. Important data for genetic toxicology studies are e.g. the endpoint, dose range and response, number of dose groups, effect size, controls used, control effects, historical controls, sufficient sample size, exposure data for *in vivo* endpoints as well as all available data on mechanistic background. All data considered as of sufficient quality may be used for a weight of evidence approach to conclude on the (geno)-toxic profile of the substance and the impact on its use.

# Genotoxicity in marine organisms, assessing the potential impacts of offshore oil and gas activities on the marine environment

Steven Brooks
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Genotoxicity assessments in both wild fish and field transplanted mussels that live on and around offshore oil and gas installations in the North Sea have been performed to assess the health status of the populations and to determine the potential impacts of oil and gas activities on the marine environment. The application of three genotoxicity biomarkers (comet, micronuclei and DNA adducts) in marine organisms will be presented and discussed.

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# PigA: lifecycle and lessons learned

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The concept of using the *Pig-a* gene as a reporter gene for somatic cell mutation came from Aratan and co-workers from their findings on paroxysmal nocturnal haemoglobinuria (PNH). The first proof of concept papers was published by the National Center for Toxicological Research (NCTR)/US FDA, and Litron Laboratories using multiple strains of rats. Multiple laboratories were later invited to be part of the validation. The initial assay design used a basic method of acquiring 1 M mutant red blood cells (RBCs) and 0.3 M reticulocytes (RETs); however, this method had limitations for statistical analysis. Eventually, a new method was developed using a magnetic enrichment process to score ~100 M RBCs equivalent and ~3 M RETs equivalent making the assay really high throughput. Genotypic correlation of phenotypic mutants was established using Pig-a mutant hematopoietic stem cells and lymphocytes. Being an indigenous gene, there is a possibility to have a pre-existing mutation in the Pig-a gene, therefore, a predose analysis was added as a regular feature in all studies using the Pig-a assay. An expert workgroup (EWG) was established under HESI-GTTC in 2011. Efforts to develop OECD test guidelines were initiated in 2016 and the guidelines were finally approved in 2022, OECD 470. This assay is now a regulatory assay and is being effectively used to follow up *in vitro* gene mutation (Ames) positive compounds. The life cycle of the assay and the lessons learned through this journey will be presented and discussed during the meeting.

# Duplex Sequencing: the roadmap for error-corrected next-gen sequencing

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Investigating the impact of environmental and occupational exposure on the mutation burden and their health consequences has relied mostly on animal models such as the transgenic rodent (TGR) assay because of the lack of efficient approaches to detect rare mutations directly in the human genome. In early 2012, a ground-breaking study reported the detection of mutations in the mitochondrial genome using Duplex Sequencing (DS), a novel error-corrected next-generation sequencing (ecNGS) technique. DS uses molecular barcodes to tag individual DNA molecules and bioinformatics tools to greatly reduce the error rate from 1/1000, as it happens with standard NGS, to below 1/10,000,000. In 2015, Twinstrand Biosciences was founded to develop DS into a commercial product of interest to a variety of research fields including genetic toxicology. For the latter, commercial kits for humans, mice and rats became soon available and the first demonstration of the power of DS for chemical mutagenesis studies was published in 2020. The gentox community embraced with open arms the technology for its many advantages with respect to the TGR assay, the current gold standard regulatory mutation studies, including the ability to measure mutations in the mammalian genome rather than using bacterial reporter genes; obtaining information about the type of mutations induced concurrently with determining mutation frequencies; and, evaluation the impact of genomic features on mutation susceptibility. Several studies have now been published consistently reporting that DS is well suited for mutagenicity studies and necessitating fewer animals to achieve the same power of the TGR assay. These data formed the support for a standard protocol submission form (SPSF) that was accepted by the Organization for Economic Co-operation and Development (OECD) in April of 2024. The objective of the SPSF is to prepare a detailed review paper to demonstrate the applicability of DS in improving chemical testing with the ultimate goal of including DS into existing OECD test guidelines. In this context, the recent decision of Twinstrand to stop selling the kits came as an unexpected obstacle in an otherwise straightforward road. However, there is optimism that another Company will take over the DS kit business soon, and other ecNGS approaches are now available both commercially (e.g., Single molecule mutation-seq) and from individual research groups (e.g., CODEC). Thus, the roadmap for inclusion of DS and other ecNGS approaches into an OECD test guideline has just taken a little detour but the final destination is still in sight.

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# From bench to OECD validation: the ToxTracker journey

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The first ToxTracker reporters, for the detection of oxidative stress and DNA damage, were developed at the Leiden University Medical Centre (LUMC) and published in 2012. Since then, the company Toxys has been created, and the assay has been expanded with additional reporters to detect non-DNA reactive genotoxic modes of action. ToxTracker has been thoroughly validated across laboratories, most recently in a ring trial that was used as the OECD validation to support test guideline creation. This presentation will describe use cases, where lack of DNA reactivity was pivotal to product stewardship and provide interlaboratory data from the OECD ring-trial.

# Use of quantitative-based genotoxicity risk assessment

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Genotoxicity testing is usually done using batteries of tests, with a selection of test systems and genetic endpoints. Due to the severity of potential consequences and the irreversibility of effects traditionally any effect was considered unacceptable unless counteracted by e.g. the therapeutic benefit of a life-saving medication, so no safe exposure level could be defined. Yet, practical thresholds are applied in situations where e.g. exposure to genotoxic molecules is unavoidable or where sufficient margins can be defined so that the risk, whereas not zero, is considered acceptable. Yet, the situation continues to be unsatisfying from a risk assessment perspective. Approaches have been presented that allow the derivation of health-based guidance values by using quantitative methods combined with uncertainty factors. Yet, the uptake and implementation of such ideas in the different regulations remains challenging. Therefore, a workshop was recently held at BfR in Germany to discuss across different regulatory fields in order to review the status of implementation and identify hurdles, which will be summarized.

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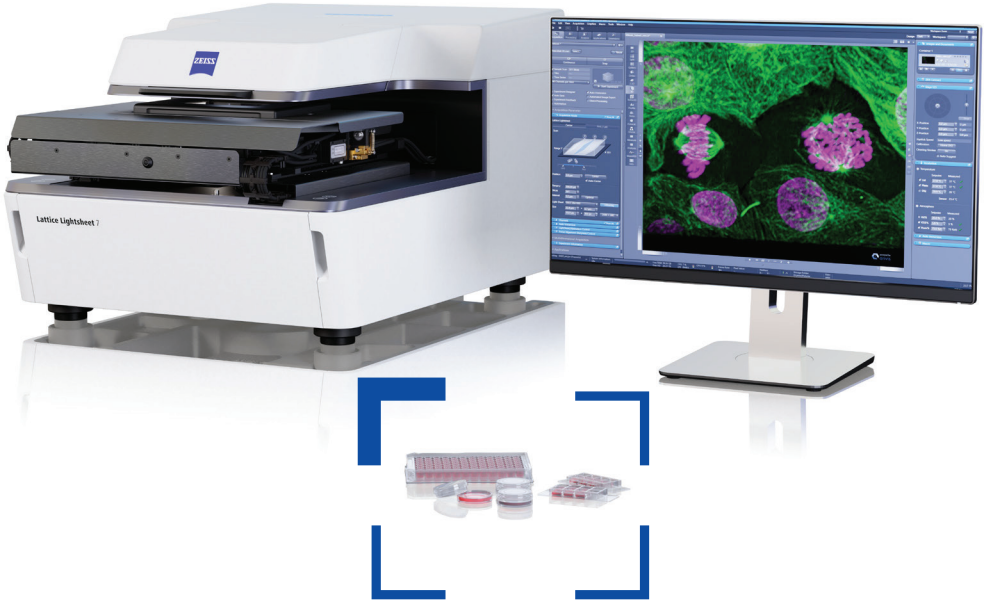


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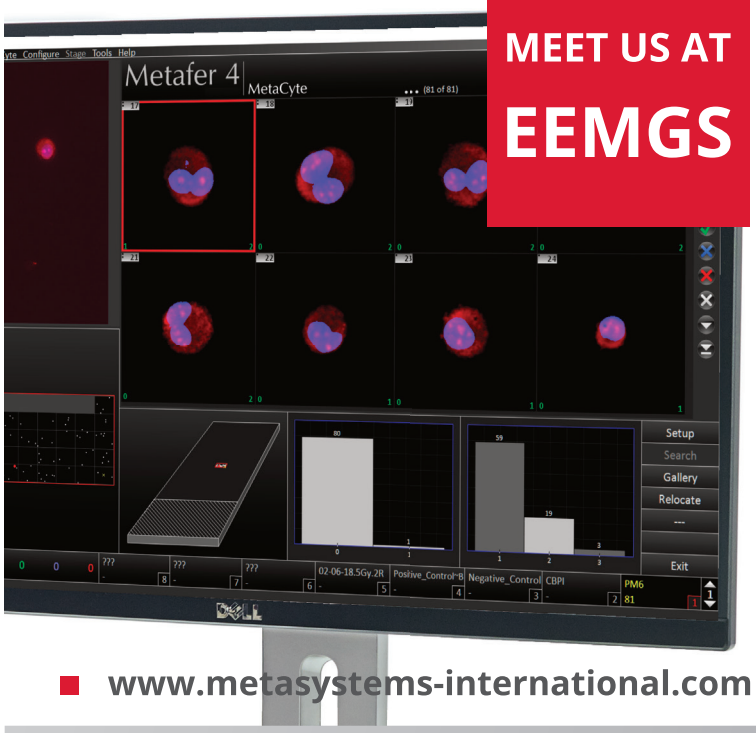


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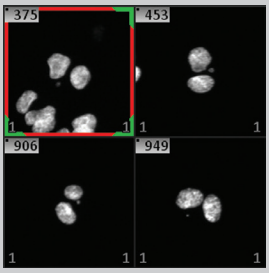


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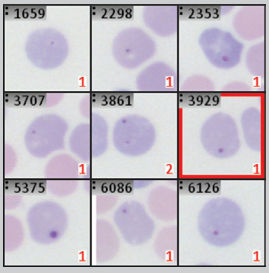


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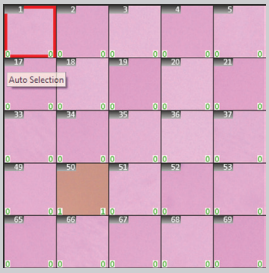


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