EEMGS BRATISLAVA 2-5 JUNE 2025

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BOOK OF ABSTRACTS

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KEYNOTE LECTURES

Building Trust in Advanced Models: A Path to Standardization and Validation for Future Risk Assessment

Maria Dusinska[1], Naouale El Yamani[1], Eleonora Marta Longhin[1], Sivakumar Murugadoss[1], Tanima SenGupta[1], Ann-Karin Hardie Olsen[1], Tatiana Honza[1], Sergey Shaposhnikov[2], Andrew Collins[2,3], Elise Rundén-Pran[1]

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New Approach Methods (NAMs) offer a promising alternative to traditional animal-based hazard and risk assessment. Their ability more accurately to simulate human biology using 3D cultures, co-cultures, air-liquid interface systems, and organ-on-chip techniques, allows for more realistic assessment of cellular responses and specific toxicity such as DNA damage, gene expression, mutagenicity, clastogenicity and aneugenicity. These innovations are especially valuable in evaluating chemicals, nanomaterials, and advanced materials, aligning with the 3Rs principle and supporting EU Directive 2010/63/EU.

For regulatory acceptance, NAMs require formal validation, standardization, and international harmonization. Regulatory validation—such as through OECD Test Guidelines (TGs)—is critical to ensure reproducibility, transferability, and global data acceptance under the Mutual Acceptance of Data (MAD) framework. Without such validation, NAMs remain limited to research use, slowing their integration into regulatory frameworks.

Methods such as the comet and micronucleus assays have been adapted for 3D models, enabling their incorporation into Integrated Approaches to Testing and Assessment (IATA) and Next-Generation Risk Assessment (NGRA). These models also capture secondary genotoxicity mediated by inflammation or oxidative stress, emphasizing the need for validation of co-culture models.

To advance these models for regulatory use, key steps include developing human-relevant exposure scenarios, refining test systems, validating *in silico* tools, and targeting endpoints such as genotoxicity in key organs (e.g., lung, liver, gut, skin). Micronucleus and comet assays applied to the reconstructed human skin model are progressing within the OECD TG program. The skin model provides a robust platform for assessing dermal exposures, supporting its validation as an alternative method.

Overall, NAMs can revolutionize risk assessment by enhancing mechanistic insight, improving human relevance, and reducing reliance on animal testing. Building trust through standardization and validation is essential to fully harness their benefits in future safety assessments.

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From Validation to Innovation: The Evolving Landscape of New Approach Methodologies in Regulatory Toxicology and Beyond

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The landscape of regulatory toxicology is undergoing a profound transformation, driven by the development and adoption of New Approach Methodologies (NAMs). These methodologies—including advanced *in vitro* models, computational tools, and integrated assessment strategies—are reshaping how we evaluate chemical and product safety. While innovation is often viewed as a precursor to validation, this lecture will explore the equally powerful inverse: how the validation process itself can generate innovation.

Historically, validation has served as a gatekeeper for regulatory acceptance, requiring robust evidence of scientific reliability, reproducibility, and relevance. These processes have traditionally followed established frameworks, such as those developed under OECD and ISO guidance. However, validation today is not merely a final hurdle; it is an evolving, iterative process that actively drives methodological refinement, fosters interdisciplinary collaboration, and encourages the development of more predictive and human-relevant tools.

This presentation will examine how validation and innovation are increasingly intertwined. The challenges encountered during method validation often illuminate knowledge gaps, spark novel solutions, and promote the integration of emerging technologies. Moreover, the shift toward performance-based validation criteria and flexible regulatory frameworks is accelerating the transition from concept to application.

By looking at the broader trends shaping this evolution—from the growing role of mechanistic understanding to the emergence of systems thinking in risk assessment—we will consider how NAMs are not only replacing traditional models, but also transforming the very foundations of toxicology. The lecture will also reflect on what is needed to sustain this momentum: enhanced scientific and regulatory cooperation, robust education and training initiatives, and a shared commitment to innovation grounded in rigorous science.

Horizontal Mitochondrial Transfer: A New Hallmark of Cancer with Clinical Relevance

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Horizontal mitochondrial transfer (HMT) has emerged recently as a new phenomenon of cell biology. After documenting acquisition of mitochondrial DNA (mtDNA) from the stroma using a mouse model of cancer, we showed that it moves between cells contained in whole mitochondria. Our further research revealed that cancer cells lacking mtDNA import mitochondria from the stroma in order to restore the respiratory process. While ATP can be formed by cancer cells efficiently by glycolysis, respiration is critical for de novo pyrimidine synthesis, a process mandatory for efficient cell cycle transition and tumour progression. Recent research has revealed that HMT is more common than initially thought and that it involves tumour cells and a variety of cell of the stroma, including immune cells. The process has been shown for more than 20 types of tumours, and the means of mitochondrial transfer present various contact-dependent and contact-indepedent means such as tunnelling nanotubes or extracellular vehicles. Due to the importance of HMT in neoplastic diseases, we propose that this process presents a new hallmark of cancer (1).

Berridge MV et al (2025) Horizontal mitochondrial transfer in cancer biology: potential clinical relevance. Cancer Cell, doi: 10.1016/j.ccell.2025.03.002.

Molecular mechanisms of cancer cells' adaptation to hypoxia and acidosis in tumour microenvironment

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During solid tumour growth, subpopulations of cancer cells are exposed to stresses caused by the irregular local and temporal supply of oxygen, nutrients, and signalling molecules by aberrant vasculature. These physiological stresses support the development of highly heterogeneous tumour tissue with a microenvironment characterised, among other features, by regions of hypoxia and/or acidosis. Hypoxia is a biologically and clinically important phenomenon associated with aggressive tumour phenotype, cancer progression, and therapy resistance. Reduced oxygen availability stimulates cellular adaptive processes that include a shift to oncogenic metabolism, which generates an excess of lactic acid, protons, and carbon dioxide. Intracellular accumulation of these acidic metabolites is incompatible with survival, and thus cells activate constituents of pH regulating machinery, including ion exchangers and transporters that mediate acid extrusion resulting in extracellular acidosis. Among these molecules, a key role is played by the carbonic anhydrase IX (CA IX), which is expressed in tumours in response to hypoxia. CA IX is functionally involved in the protection of tumour cells from hypoxia and acidosis (through its ability to regulate pH and support survival) as well as in the promotion of metastatic phenotype (through its ability to regulate adhesion-migration-invasion and support immune suppression in tumour tissue). For these reasons, CA IX is used as an intrinsic biomarker of tumour hypoxia and an indicator of poor response to treatment and is evaluated as a target for therapy aimed against hypoxia- and acidosis-driven cancer progression.

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ORAL PRESENTATIONS

Development of a Detailed Review Paper and a Retrospective Performance Analysis for the *in vitro* γH2AX/pH3 method

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The development of efficient, high-throughput *in vitro* assays for genotoxicity assessment is a major priority. In addition to hazard identification**,** quantification of the phosphorylated histones H2AX and H3 biomarkers (gH2AX and pH3) provides insights into the genotoxic mode of action (MoA). In 2023, the Organisation for Economic Co-operation and Development (OECD) approved the development of a Detailed Review Paper and Retrospective Performance Analysis for the *in vitro* gH2AX/pH3 method, i.e., as a new project in the Test Guidelines Programme workplan. For this purpose, we reviewed 300 studies performed in the last 15 years. In total, 815 chemicals were included in a dataset comprising 41 aneugens (5%), 416 clastogens (51%), 17 aneugen/clastogen (2%) and 341 non-genotoxic chemicals (42%). The dataset effectively covered different MoAs for aneugenic (kinases inhibitors, tubulin stabilizers and destablizers) and clastogenic substances (oxidative stress, bulky DNA adducts, topoisomerase inhibitor, inter-strand crosslink, etc.). Moreover, “irrelevant” positive genotoxins (apoptosis or p53 inducers) were also included. Results from commonly used *in vitro* genotoxicity tests for each chemical have also been collected. Chemical space analysis of the dataset using multiple descriptors was conducted; it demonstrated similar coverage to the REACH and Drugbank datasets. The performance of the *in vitro* gH2AX/pH3 method (sensitivity, specificity and accuracy) to detect genotoxicity was calculated and compared to the bacterial Ames (TG471) test, the mammalian cell *in vitro* mutation tests (TG476 and TG490), and the *in vitro* micronucleus (TG487) and chromosome aberration (TG473) tests. The analyses demonstrated high predictivity of the γH2AX/pH3 *in vitro* genotoxicity method for *in vitro* genotoxicity determination (91%), and a high concordance of the method with the MLA/HPRT and the MN/CA assays (85%). Moreover, the method was able to precisely define the genotoxic MoA.

Periostin – another piece of puzzle in renal fibrogenesis

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Chronic kidney disease (CKD) and its progressive nature leading to end-stage renal significantly impacts quality of life and high mortality rates of patients. This high mortality is related to tight connections of renal and cardiovascular systems. Renal impairment is one of the major factors for cardiovascular disease. Despite intensive research and known renal structural and cellular changes, the mechanisms responsible for the initiation and progression of renal fibrosis are not completely understood. Also, there is no cure to renal fibrosis, yet. Considering that the development of efficient therapeutics aimed at preventing the progressive loss of renal function would require in-depth understanding of the complex phenomena detailed pathophysiology of fibrotic human diseases is necessary. From a clinical point of view, CKD is defined by the reduced glomerular filtration rate (GFR) and increased albuminuria, however, CKD is silent and usually undetected until advanced stages. Advances in functional genomics, proteomics and biofluid profiling have uncovered several new candidates not only for prediction of CKD progression but also as potential targets for CKD treatment. The utilization of these candidates for clinical practice, however, remains yet unknown.

Periostin has recently been identified as a key player in CKD development. It is one of the four candidates that has been identified thank to their potential to constitute future therapeutic targets against CKD. Periostin expression is increased during tissue development and is then reduced in differentiated adult tissues. In case of injury, or a tissue remodeling, expression of periostin is *de novo* induced. The active participation of periostin in fibrogenesis has already been described and support the presumption that periostin could be a suitable candidate for selective inhibition aimed at the prevention of renal fibrosis and progression of CKD.

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From Uptake to DNA Damage: How Nanoparticles Interact with Human Peripheral Blood Mononuclear Cells?

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Nanotechnology is rapidly becoming one of the fastest-growing markets globally, significantly revolutionizing various industries. Due to their small size, composition, shape, and surface, nanoparticles (NPs) possess unique physicochemical properties. As a result, the number of anthropogenic NPs being synthesized and applied in various fields, such as medicine, food science, cosmetics, pharmaceuticals, and electronics, continues to rise. In 2022, the global market for nanomaterials was valued at USD 10.88 billion and is expected to grow at a compound annual growth rate of 14.8 % from 2023 to 2030. This exponential growth raises concerns about the potential negative effects of NPs on living organisms.

This study investigated the genotoxicity of 9 types of nanoparticles, including 10-30 nm and 50 nm $Co3O_4$-, 35 nm Ag-, 5 nm and 40 nm Au-, 80 nm polystyrene (PS)-, 13 nm and 50 nm Al_2O_3-, and 10-20 nm SiO_2-NPs. Using human peripheral blood mononuclear cells (PBMCs) and established cytotoxicity and genotoxicity assays, such as alkaline comet assay and cytokinesis-block micronucleus (CBMN) assay, the study contributes to a better understanding of the DNA damage that NPs induce.

Based on the results, we identified three distinct mechanisms of nanoparticle-induced genotoxicity. Nanoparticles that were efficiently internalized and induced ROS formation (e.g., Co_3O_4-, Al_2O_3-, and PS-NPs) caused primary DNA damage and significant chromosomal damage. In contrast, nanoparticles that did not induce ROS (e.g., Ag-, SiO_2-NPs) resulted solely in primary DNA strand breaks. Interestingly, Au-NPs, despite limited uptake and ROS induction, still caused a statistically significant increase in primary DNA damage, suggesting alternative genotoxic mechanisms.

The results of this study provide theoretical insights into nanoparticle-induced genotoxic mechanisms and underscore the importance of comprehensive testing in the field of nanogenotoxicology. They may also contribute to developing new recommendations for nanoparticle research and safety assessment

Investigating limit dose and identifying genotoxic hazard of common pharmaceutical solvents with ToxTracker

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When testing substances in early phases of development, it is not always possible to avoid residual solvent in API. To determine tolerability, we assessed 19 commonly used solvents in ToxTracker and then compared observations to published toxicological findings. ToxTracker is a new approach method that provides insight into MOA using GFP-gene expression and can discriminate direct-acting genotoxicants from indirect genotoxicants. The selected solvents were tested as high as possible (up to 5% v/v) using a 24h exposure in the absence or presence of S9. GFP reporter activation and cell survival were assessed using flow cytometry and ~half (10/19) of the solvents induced genotoxic effects (Rtkn-GFP) with concomitant increases in oxidative stress (Srxn1-GFP) in the absence of S9. To investigate causality of oxidative stress, solvents were retested in the absence and presence of ROS scavengers (NAC and GSH) and 3/10 became non-genotoxic. For the remaining solvents, we back calculated molarity and evaluated dose-response using benchmark dose (BMD) analysis in PROAST. A benchmark response (BMR) of 100 was used and BMD limits (95% CI) showed that most (9/10) of the solvents had been tested far above 10 mM, indicating they would be deemed negative if applying this OECD recommended limit dose, and aligning with reported *in vivo* genotoxicity and carcinogenicity findings. Finally, using BMRs up to -20%, cell count data were used to quantitively assess reasonable limits of solvent in ToxTracker.

Can we conclude on mutagenicity classification based on *in vitro* data only?

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The increasing number of new chemical substances covering various industry sectors has raised concerns about their potential to harm human health and the environment. Criteria for hazard classification of chemicals are implemented by the UN Globally Harmonised System for Classification and Labelling (GHS) to facilitate chemical risk management and transport. Currently, the GHS criteria for germ cell mutagenicity are being revised to clarify issues that have brought to difficulties in their interpretation, consider current state of science and facilitate hazard classification using non-animal methods. To support these discussions we explored whether there are situations where the standard *in vitro* tests alone can be sufficient to classify substances as mutagenic. Currently, the genotoxicity testing strategy involves a step-wise approach, starting with *in vitro* tests that cover gene mutations, clastogenicity and aneugenicity. If *in vitro* tests yield positive results, follow-up confirmatory *in vivo* tests are required. In our study, we analysed the EURL-ECVAM genotoxicity database which includes over 700 chemicals, exploring six combinations of *in vitro* tests that cover all the three key endpoints. We then compared the *in vitro* results with overall *in vivo* assay outcome. We observed that most combinations of *in vitro* results that were positive for all endpoints (gene mutation, clastogenicity and aneugenicity) were confirmed *in vivo*, with at least one positive study. Additionally, the number of compounds that were negative for genotoxicity *in vivo* was very low (>3%). Interestingly, for the most part of these outliers there is a clear insight of why they are misleading positives. These findings indicate that it may, in some cases, be possible to classify a chemical as a somatic cell mutagen using *in vitro* assays alone. Novel *in vitro* assays that address specific biological mechanisms providing supporting information in a weight of evidence may help increasing confidence in the results.

New technologies for sound *in vitro* toxicology

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The *in vitro* micronucleus test is a key tool for the detection of aneugenic, i.e. spindle-apparatus mediated genotoxins which cannot be detected by bacterial systems such as Ames or umu chromotest. We have developed a new testing approach based on brain derived, strictly epithelial growing fish stem cells. These cells exhibit embryonic features as arylhydrocarbon receptor expression, the related inducibility of metabolic enzymes and the expression of the epithelial mesenchymal transition signalling pathway. Our H2B-GFP fusion protein based variants of these fish cell lines enable the detection of DNA damage using high throughput automated kinetic live imaging applied to 96 well-plate format. In addition to micro- and small nuclei, the technology can detect lost chromosomes, nucleoplasmic bridges, nuclear buds and karyorrhexis features *in situ*. GFP labelling of nuclear structures also allows allocation of the cell cycle status of any individual cell. The causes of reduced cell growth in terms of cytotoxicity, slowed proliferation and inhibition of cell division are visible. Impacts on monolayer morphology during a 72-h cultivation period can be recorded. The technology enables a highly efficient performance of valid micronucleus tests according to OECD 487. Known aneugens as well as clastogens are reliably recognised. Procarcinogens (cyclophosphamide, aflatoxin, 3,4 dichloroaniline), that act in standardised procedures exclusively after the introduction of (mostly animal derived) metabolic enzymes (S 9 mix technology), proved to be genotoxic in the test variant without S9 mix. Our new technology therefore has great potential to increase the efficiency of the micronucleus test at significantly lower cost, while avoiding the need for animal treatments to produce enzymes for the metabolic activation of the test compounds.

Altered Tryptophan Metabolism and AhR activation in IBD: Association with DNA Damage and Gut Health

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Inflammatory Bowel Disease (IBD), including Crohn's disease and ulcerative colitis, comprises a group of chronic inflammatory disorders affecting the gastrointestinal tract characterized by chronic inflammation. Inflammation may lead to DNA damage and a subsequent increased risk of colorectal cancer. The aryl hydrocarbon receptor (AhR) plays a pivotal role in regulating the intestinal immune response, and its activation by tryptophan metabolites is increasingly recognized as a key factor in the pathogenesis of IBD. In this study, we therefore analyzed fecal water (FW) from 80 IBD patients and 20 healthy controls for their potential to induce DNA damage in relation to their ability to activate the AhR signaling pathway. DNA damage in Caco-2 cells exposed to FW for 30 minutes was assessed using the comet assay, while AhR activity was quantified by the Ethoxyresorufin-O-deethylase (EROD) assay. Targeted metabolomic profiling was employed to quantify tryptophan- metabolites in fecal water. Using fecal calprotectin (FCP > 100 µg/g) as a marker of active intestinal inflammation, we observed that DNA damage was significantly higher in Caco-2 cells that were exposed to FW of active IBD patients, compared to FW of subjects with FCP <100 µg/g. AhR signaling was significantly lower in Caco-2 cells that were exposed to FW from active IBD patients. However, the indole/kynurenine ratio negatively correlated with DNA damage and positively correlated with AhR activity. These findings suggest that under inflammatory conditions, indoles reflect protective effects on the intestinal epithelium through the reduction of DNA damage and activation of AhR. Since indoles are predominantly formed by the gut microbiome, further investigations are needed to identify the specific microbial taxa responsible for modulating DNA damage, AhR signaling and influencing IBD pathogenesis.

In vitro Comet Assay Test Guideline – Getting There!

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While the *in vivo* comet assay has been supported since 2016 by an OECD Test Guideline, TG489, the *in vitro* version of the assay, which is also widely used in genotoxicity testing, has not had this official backing. However, after a failed attempt to rectify this in 2018, we are trying again, and have passed the first hurdle, approval of our submission by the OECD Working Party for the Test Guideline Programme. Our proposal includes both the standard alkaline comet assay for DNA strand breaks (and alkali-labile sites), and the enzyme-linked comet assay. Including an incubation with a lesion specific endonuclease (most often formamidopyrimidine DNA glycosylase, or Fpg) extends the scope of the assay to cover damage to DNA bases; Fpg converts oxidised purines to breaks, but also acts on alkylated bases and bulky adduct lesions. Enzymes with other specificities can be employed.

Working towards our TG, phase 1 is the preparation of a validation report, based on a review of the literature. Relevant studies include concentration response experiments with agents of known genotoxicity, tests with cytotoxic but non-genotoxic chemicals, tests with non-cytotoxic and non-genotoxic chemicals, and inter-laboratory studies (ring trials). Phase 2 will be to prepare the TG, based on standard protocols developed in the course of the COST Action *hCOMET*.

Impact of exposure time and cell line on the outcomes of transcriptomic-based biomarkers for genotoxicity

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In vitro transcriptomics has gained increasing interest as a tool in genotoxicity assessment due to its high-throughput potential and the mechanistic information provided. Application of transcriptomics data in chemical risk assessment is however hampered by the lack of harmonization in data analysis, reporting and interpretation. Transcriptomic biomarkers for genotoxicity (e.g. GENOMARK and TGx-DDI) aim to address these issues and previous studies have shown their potential for classification, potency ranking and derivation of transcriptomic points of departure (tPODs) for genotoxic compounds. However, especially for the latter application, several challenges remain as most quantitative analyses of transcriptomic datasets rely on data collected at a single exposure time, overlooking the dynamic nature of cellular responses to genotoxic substances. Moreover, the selected exposure time and cell type can vary significantly between experimental designs. For example, GENOMARK has been developed in HepaRG cells after 72 hours of exposure whereas the initial development of TGx-DDI was based on data collected in TK6 cells after three hours of exposure. Our study therefore investigates the impact of both exposure duration and cell line selection on biomarkers' predictions and tPODs derivation. Gene expression data generated in HepaRG and RPTECT-TERT1 cells exposed to a panel of genotoxicants across multiple concentrations and time points were analyzed. Biomarker tPODs for GENOMARK and TGx-DDI were derived using benchmark dose (BMD) modeling and compared over time and between cell lines. Our results confirm our previous findings that GENOMARK and TGxDDI yield similar trends in classification and tPoD values for the same experimental conditions, despite differences in biomarker development and sharing only two genes. However, our results also highlight that classification and tPOD outcomes are affected by exposure time and cell type. Both factors thus need to be taken into account when designing *in vitro* toxicogenomics experiments in support of chemical risk 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 chemical risk assessment.

Frozen whole blood can be used to detect DNA repair activity

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Introduction The comet assay is a widely employed method for assessing DNA damage and DNA repair activity by using tissue or blood. While the use of frozen whole blood (WB) samples without cryopreservation for analyzing DNA repair remains largely unreported. This study aims to optimize the application of frozen WB in evaluating DNA repair, improving the assay's feasibility for biomonitoring studies.

Method and Materials Samples (n=82) were utilized to investigate two main objectives: i) To refine the DNA repair assay to examine differences in base excision repair (BER), with a specific focus on utilizing white blood cells (WBC) isolated from frozen WB. WBC were isolated by washing the frozen WB with cold phosphate buffered saline. For comparative analysis, further separation into mononuclear cells (MNC) and granulocytes (GR) was achieved using density gradient separation. Protein extracts from WBC, MNC, and GR were utilized for refining the DNA repair assay.

ii) Validation of experimental feasibility by assessing the impact of genome instability on cancer using samples from individuals with (n=10) or without a family history of cancer (n=19).

Results: Regarding DNA repair, we show for the first time that BER activity can be detected in WBC isolated from frozen WB using a modified comet assay. Interestingly, higher BER activity was detected in GR compared to MNC. Moreover, subjects with a family history of cancer showed significantly lower levels of BER activity (P=0.00235) compared to those with no family history of cancer. Moreover, this decrease was associated with an increase in DNA damage

Conclusion: Frozen whole blood can be used to assess BER activity using the comet assay-based DNA repair assay.

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Validation of MutaTracker, a novel approach method for the detection of gene mutations using error-corrected NGS

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Mutagenicity is an apical endpoint in hazard identification. Traditionally, mutational events are measured by counting surviving clones phenotypically, after environmental selection of a mutation at a known genetic locus. A limitation of this approach is that mutants outside of the target locus are not enumerated, which underestimates true induced mutation frequencies (MF). With the introduction or error-corrected next generation sequencing (ecNGS), it is now possible to resolve precise MFs, without selection, across the genome. MutaTracker is a combination of the ToxTracker assay with single-molecule mutation sequencing (SMM-seq) to couple genotoxicity prediction with actual mutation detection in a rapid, compound sparing, all-in-one mammalian cell-based assay.

ToxTracker is a GFP reporter-based assay that provides insight into chemical mode-of-action (MoA), thereby discriminating direct-acting genotoxicants from indirect genotoxicants. SMM-seq is a highly sensitive, ecNGS technique that detects single nucleotide variants utilizing rolling circle amplification and consensus strand calling. Here, we validated MutaTracker by testing a combination of genotoxic, non-genotoxic, and cytotoxic substances based on the revised ECVAM list. The ToxTracker assay was initially used to classify substances as genotoxic or cytotoxic and determine their mode of action (MoA). Subsequently, genomic DNA from exposed cultures was subjected to SMM-seq to determine mutation frequency and concentration-induced mutation spectra.

ToxTracker correctly classified all genotoxic and non-genotoxic compounds while SMM-seq identified mutation signatures that supported the known MoA for most substances. For example, alkylating agent ENU induced a dose-dependent increase in the mutation frequency, generating a broad mutation spectrum with T>A, T>C and T>G mutations.Potassium bromate induced oxidative DNA damage represented by an increase in C>A mutations that are mainly caused by 8-oxo-guanine lesions. Together, this demonstrated that MutaTracker can be a valuable tool for determining MoA and the combination compliments each NAM, with ToxTracker being able to identify genotoxicity, and SMM-seq identifying mutational fingerprints.

A comparison of oesophageal epithelial cell micronucleus (MN) formation and presence of MN in lymphocytes: Exploring the mechanistic role of reactive oxygen species (ROS)

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Oesophageal adenocarcinoma (OAC) development is linked to chronic inflammatory conditions such as gastro-oesophageal reflux disease (GORD) and Barrett's oesophagus (BO). Inflammation triggers the production of ROS which can lead to oxidative stress, and contribute to DNA damage, MN formation and the subsequent development of oesophageal diseases. Lymphocyte MN levels increase throughout the GORD/BO/OAC histological progression. Previous research on GORD, BO and OAC patients revealed that those with elevated baseline lymphocyte MN levels exhibited a lower sensitivity to treatment with ROS inducers, indicating an adaptive response to oxidative stress, potentially involving DNA repair. Additionally, a positive correlation was found between plasma concentration of oxidative DNA damage biomarker 8-OHdG and lymphocyte MN frequency (MN%).

We aim to further explore the mechanisms by which oxidative stress influences MN formation in the lymphocytes of patients with oesophageal disease and healthy volunteers and investigate the relationship between lymphocyte MN levels and DNA damage in actual oesophageal tissue.

Oesophageal epithelial cells (OECs) and lymphocytes were collected from GORD and BO patients undergoing routine endoscopies, to compare blood and tissue levels of DNA damage. To obtain a clear monolayer of cells suitable for MN scoring, the processing of cytology brush samples was optimized. Results show a positive correlation between lymphocyte MN% and OEC MN% (R value=0.792, p=0.033). Further optimization of OEC staining is required to achieve a more accurate analysis of MN levels.

Peripheral blood lymphocytes from healthy volunteers, GORD and BO patients underwent 24-hour treatment with TH5487, an inhibitor of the oxidative DNA damage repair enzyme OGG-1. Results showed a significant decrease in CBPI ($p \leq 0.001$) and a slight but not significant increase in MN% for all patient groups. Whilst these results suggest that inhibition of OGG-1 is having some effect on MN induction, testing of more patient samples is required to clarify this.

Performance Validation of a Compact High-throughput Vertical Electrophoresis Tank for the Comet Assay

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The Comet assay is a widely used method for evaluating both DNA damage and repair at the single-cell level. To enhance its efficiency for high-throughput screening, a vertical electrophoresis tank was developed which can run 50 microscopic slides at the same time. In this study, we compared the performance of the vertical electrophoresis system with the widely used horizontal electrophoresis system for detecting DNA damage using the Comet assay.

Experiments were conducted using the HepG2 cell line exposed to five increasing concentrations of methyl methanesulfonate (MMS), which is an established positive control for the comet-assay. The comet-assay was performed according to Collins, *et al. Nat Protoc*, 2023, with both the vertical and the horizontal electrophoresis tanks in parallel. Comparative analyses included both the 2-gel and the 12-gel format. Following a minor adjustment to the slide holders of the vertical electrophoresis system to enhance electrical flow, we observed a strong correlation between the vertical and the horizontal system (R^2=0.99, P<0.001), with a slope of 1.00 and 0.92 for the 2-gel and the 12-gel format, respectively. These findings were regardless of placement of samples within the tank. Additionally, the throughput of samples in the vertical system is further supported by the user-friendly set-up of 4℃ cold buffer and ice packs that eliminate the need for external cooling equipment or a cold room.

In conclusion, the vertical electrophoresis performs equally well when compared to the horizontal comet assay. Its compact tabletop design makes it highly user-friendly and suitable for high throughput analyses in most laboratory settings.

Genotoxicity and cytotoxicity assessment in Pathology professionals through the buccal micronuclei assay

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In Histopathology laboratories, Volatile Organic Compounds (VOC) are used, such as formaldehyde and xylene, classified as carcinogenic, mutagenic or reprotoxic, which is why their handling poses risks to human health. The oral micronucleus (MN) assay is a non-invasive, useful and simple method to detect genotoxic and cytotoxic effects in exposed individuals. The main objective was to evaluate the risk of genotoxicity and cytotoxicity of VOC in Pathology professionals on S. Miguel Island, Azores, Portugal. The study involved two groups: one exposed group (n=21) from three laboratories on the island, and a reference group (n=50) randomly chosen from other hospital services that were not exposed to VOC. All procedures were performed, using ThinPrep® monolayer cytology, stained with modified Feulgen technique, and visualized 2000 cels per case under an optical microscope and digitalization.

It was evaluated genotoxicity and cytotoxicity based on damage caused to DNA, accounted for through MN, and other nuclear anomalies (ONA), such as karyorrhexis, pyknotic and karyolytic cells. As a result, VOC proved to have a predictive significance for the frequency of MN cells, leading to the conclusion that it is an increased risk factor for the health of these professionals, approximately four times greater than that of the unexposed group. Despite existing legislation, combined protection measures, individual and collective biomonitoring, as well as investment in facilities are necessary to control this hazardous environment.

Ingested titanium dioxide nanomaterials: new approach to investigate intestinal genotoxicity and key cellular/molecular effects

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Oral exposure to titanium dioxide nanomaterials (TiO_2NMs) is due to their presence in food, food contact materials, medicines and cosmetics. The gastrointestinal tract (GIT) represents primary site of contact, that may result in systemic exposure, if biological barriers are surpassed. The INGESTnano project aimed to investigate nano-bio interactions at the cellular/molecular levels within the context of the intestinal tract and digestion processes, for understanding potential effects on human health.

A group of three TiO_2NMs (NM-102, NM-103, NM-105) was selected as case study using a new approach methodology (NAM), incorporating the *in vitro* human digestion simulation prior to biological assays in Caco-2 and HT29-MTX-E12 intestinal cells. The endpoints included cyto- and genotoxicity, cell uptake, intestinal permeability, GIT transport and epigenomic modifications.

The results showed a more pronounced cytotoxicity in HT29-MTX-E12 cells for digested NM-105, as compared to undigested, concomitantly with subtle changes in hydrodynamic-size. DNA-damage induction was more relevant for NM-105, and the micronucleus assay showed chromosomal damage in HT29-MTX-E12 cells for all TiO_2NMs, especially after *in vitro* digestion. All NMs, digested or not, were internalized by intestinal cells, but did not affect transepithelial resistance, nor the epithelial markers in polarized enterocytes. NM-102 was retained in lysosomes, while NM-103 and NM-105 showed transcytosis, a potential gateway for systemic distribution. Using Reduced Representation Bisulfite Sequencing, several differentially methylated genes were identified for the TiO_2NMs, either digested or not. Pathway and Gene Ontology analyses showed that each TiO_2NMs has a different functional impact on intestinal cells, probably linked to specific physicochemical properties, and digestion seems to reduce this impact. A trend towards CpG hypermethylation was observed upon NM-105 exposure, unlike for the other TiO_2NMs.

This integrated approach enabled the identification of key events and molecular pathways elicited by TiO_2NMs, highlighting the importance of considering the digestion on the induction of adverse outcomes.

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Interlaboratory Validation of the Cell Transformation Assay (CTA) for Carcinogenic Assessment of BPA Alternatives

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Bisphenol A (BPA) has long been used in various plastic products, resins and coatings, making human exposure to this chemical inevitable. Due to its harmful health effects, including endocrine disruption, and immunotoxicity, BPA has been increasingly replaced by several alternative compounds. However, there are still significant gaps in research regarding the safety of these BPA alternatives, particularly concerning their potential carcinogenicity. One of the *in vitro* assays to assess carcinogenic potential of chemicals is the Bhas-42 cell transformation assay (CTA). The assay can detect both genotoxic and non-genotoxic carcinogens It is valuable in identifying potential cancer risks before widespread exposure occurs, contributing to the development of safer chemicals and products, as well as better regulatory standards while adhering to the 3R concept. The EU-Partnership for the Assessment of Risks from Chemicals (PARC) project is addressing these research gaps to enhance the risk assessment of BPA alternatives.

BPA and some alternatives, including BPZ, BPE, BPAP, BPA-MAE, BPP, and TCBPA, were selected for evaluation of their carcinogenic potential using the *in vitro* 2-stage Bhas-42 CTA. A key objective of the project is to validate the CTA as a reliable *in vitro* method for assessing carcinogenicity. To ensure consistency and accuracy across participating labs, an interlaboratory comparison was initiated and a standardized SOP was developed, including concentration ranges for controls and BPA analogues, in alignment with OECD guidance document. The first results from the protocol harmonization, using the selected controls, were consistent across all participating labs. BPA and its analogues are being tested, and the results are under evaluation. The data generated will contribute to the overall weight of evidence on the hazards posed by these chemicals and, when combined with findings from other endpoints, will provide a solid basis for refining their regulation.

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Advancing Mutagenicity Assessment with Error Corrected Sequencing

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Error-corrected sequencing (ECS) is revolutionizing genetic toxicology by enabling highly accurate detection of low-frequency, chemically induced mutations. This transformative technology leverages consensus sequencing and advanced bioinformatics to achieve error rates comparable to baseline somatic mutation frequencies, offering significant advantages over traditional methods. The ability of ECS to define detailed mutational spectra opens new avenues for mechanistic understanding of mutagenesis. As highlighted by Marchetti et al. (2023)[1,2] and the International Workshop on Genotoxicity Testing (IWGT) position paper (2025, in press), substantial progress has been made in validating ECS for regulatory use. Interlaboratory studies, including those using Duplex Sequencing (DS), have demonstrated robust concordance between ECS and established transgenic rodent assays (e.g., OECD TG 488). This concordance, coupled with the enhanced sensitivity and 3R's benefits of ECS, supports the ongoing efforts to amend existing OECD Test Guidelines (TG 488, TG 490, and TG 471) and incorporate ECS methodologies. A Detailed Review Paper (DRP), under development by international experts including US and UK OECD national coordinators, will consolidate these findings and help guide the amendment process. This initiative is driven by a Standard Project Submission Form (SPSF) accepted by the OECD in 2024 and informed by significant contributions from the genetic toxicology community, including extensive research documented in published papers. Strategies and key considerations for the integration of ECS into regulatory mutagenicity testing paradigms will be described. Together, these efforts will provide a firm basis for integration of ECS technologies into regulatory genetic toxicology testing.

References: [1]PMID: 36646809; [2]PMID: 37643677

The impact of high-frequency electromagnetic fields (1950 MHz) on DNA damage in buccal and peripheral blood mononuclear cells

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High-frequency electromagnetic fields (HF-EMF) have been classified by IARC as possibly "carcinogenic to humans". Numerous genotoxicity studies have been performed to clarify if the radiation causes damage to the genetic material, which plays a key role in neoplastic transformation. Many human studies on HF-EMF fields' effects focused on buccal cells due to their direct exposure during phone use. Other cell types (e.g., lymphocytes) have also been investigated. The results of micronucleus (MN) studies, which reflect numerical and structural chromosomal aberrations, are highly controversial, probably due to shortcomings caused by inadequate use of stains and invalid exposure assessment. We realized the first human intervention trial with headsets. 41 participants were exposed to HF-EMF under controlled conditions in Austria. No induction of MN was observed. However, we found evidence for cytotoxic effects, which were increased in the cells of cheeks that were directly exposed to the radiation. Furthermore, we conducted an ex vivo study in which we investigated the impact of demographic factors on DNA damage and co-exposures to occupationally relevant genotoxic factors in peripheral lymphocytes. We found no differences in basal damage of the three groups, (young obese, young normal and old normal). In combined experiments with the 4NQO, $NiCl_2$, CrO_3 and BPDE, we found no evidence for synergistic effects in the lymphocytes from the three demographic groups. In conclusion, the results of the bio-monitoring studies indicate that HF-EMF does not cause chromosomal damage in the mucosa of mobile phone users. However, we found evidence for cytotoxicity and cell cycle dysregulation. The ex vivo experiments showed that older individuals may be more sensitive to high HF-EMF, but did not provide evidence for an increase of chemically induced DNA damage in humans as a consequence of the radiation.

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The *in vivo* Comet Assay: Uncovering DNA Damage in Testicular Germ Cells

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Considering the increasing global production of and exposure to potentially hazardous chemicals, new and easily implementable methods are urgently needed. The *in vivo* comet assay is widely and increasingly used in regulatory toxicology. However, the 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, the demonstration of germ cell genotoxicity and interaction with the germ cell genome can serve as supportive evidence to distinguish between classification categories Muta1B and Muta2 in cases with positive *in vivo* somatic mutagenicity findings. An approach to specifically assess testicular germ cells within TG489 is thus highly demanded. We developed a method (proof-of-concept [1]) that selectively addresses haploid spermatids and primary spermatocytes. Recordings of DNA damage (% tail intensity) and 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 and physical appearance. The haploid spermatid comet populations are identified by setting a DNA content threshold (manually or by modelling) of DNA content distribution plots as described (protocol [2]). Data from two experimental phases in rats testing genotoxic and non-genotoxic agents will be presented. Biomaterials (testis, liver) were collected to facilitate demonstration of inter-laboratory proficiency and transferability in a ring-study. Valuable information regarding effect levels in germ cells *per se*, compared with levels in somatic cells, and biodistribution of substances to the male gonad will be presented. The method adds a versatile, sensitive, rapid and resource- and animal-efficient assay to the currently limited toolbox for regulatory germ cell mutagenicity assessment. The framework proposed herein may contribute to the assessment of male germ cell mutagenicity.

[1] Dirven et al., 2023. DOI: 10.1002/em.22527

[2] Olsen et al., 2025. DOI: https://doi.org/10.1101/2024.12.22.624648

Superior sensitivity of error-corrected sequencing for ultra-rare mutation detection in T-cell lymphoma case after CAR-T treatment

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Chimeric antigen receptor (CAR) T-cells are produced by collecting the patient's PBMCs during the apheresis process and transducing the autologous T-cells with a lentiviral vector harboring the CAR transgene. A fatal, clonal, CD4−CD8− (CAR)+ peripheral T-cell lymphoma (PTCL) occurred one month after a patient received treatment with tisagenlecleucel (Kymriah)[1]. The PTCL presented pathogenic mutations in DNMT3A (p.C652Y), TET2 (p.R1167T) and TET2 (p.Q644*). While the first two were somatic mutations already present in the apheresis material, the third mutation was – according to whole genome sequencing – exclusive to the tumor. TET2 (p.Q644*) introduces an early stop codon and could convey a functional TET2 knock-out in combination with p.R1167T on the second allele. TET2 loss would provide a growth advantage to T-cells and contribute to lymphomagenesis. This sparked an investigation to determine whether the mutation TET2 p.Q644* and, in consequence, a clone carrying this pathogenic mutation was already present at ultra-low frequency in the apheresis specimen or could have appeared during CAR-T cells manufacturing. In a parallel approach, we applied three methods of increasing sensitivity: ddPCR, PacBio long-read sequencing and the error-corrected sequencing method Duplex-seq. Among these, Duplex-seq proved to be the only method with sufficiently high sensitivity and low background to confidently detect the mutation in the apheresis at a frequency of 0.0012%. Our data therefore provided evidence for early presence of a clonal population carrying both mutations in TET2 and DNMT3A that later evolved to a PTCL.

[1] Kobbe et al., Aggressive Lymphoma after CD19 CAR T-Cell Therapy. N Engl J Med. 2024 Oct 3;391(13):1217-1226. doi:10.1056/NEJMoa2402730.

Applying the modified comet assay with DNA repair inhibitors in the detection bulky adducts. What will its specificity be?

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The standard alkaline comet assay is a straightforward and cost-effective genotoxicity test commonly used in genetic toxicology to detect strand breaks and alkali-labile sites in DNA. Furthermore, other altered bases such as oxidized and alkylated bases and cross-links can also be detected by adding several modifications to the assay. However, the detection of bulky adducts, a significant DNA lesion formed by the attachment of a chemical to DNA, is not possible. Given that he main cellular mechanism responsible for the repair of bulky adducts is nucleotide excision repair (NER), the modification of the comet assay by adding blockers of the reparation process, lead to the accumulation of incision break intermediates. This modification has been applied for the detection of bulky adducts but it is not a validated technique.

In order to internally validate this technique, the combination of hydroxyurea (HU) and cytosine arabinoside (Ara-C) or aphidicolin have been added to the comet assay. Both MTS assay and comet assay were performed to determine cytotoxicity and DNA damage, respectively. TK6 were exposed for 3h to 1) BPDE, as an inducer of bulky adducts, to 2) MMS, EMS, KBrO3, H_2O_2, CisPt or Mit-C, as genotoxic agents with different mechanisms, or to 3) DMSO or triton X-100, as cytotoxic agents. These exposures were always accompanied with the DNA repair inhibitors (HU/Ara-C or APC).

Results showed that the use of HU/Ara-C or APC in combination with the comet assay can increase the sensitivity of the comet assay for the detection of DNA damage. Nevertheless, it was also concluded that the modification of the comet assay by adding blockers of the reparation process is not specific for the detection of DNA bulky adducts. Furthermore, these findings question the idea that distinct DNA lesions are repaired by separate mechanisms or the specificity of NER inhibitors.

Skin wound healing: the impact of treatment with antimicrobial nanoparticles and mesenchymal stem cells

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An investigation of the biological mechanisms initiated in wounded skin following application of mesenchymal stem cells (MSCs) and nanoparticles (NPs) (Ag, ZnO), alone or combined, was performed in mice, with the aim to determine the most optimal approach to accelerate the healing processes. The samples were collected seven days after injury. When compared with the wounded untreated animals (controls), combined (MSCs+NPs) treatment induced the expression of *Sprr2b*, encoding Small proline-rich protein 2B, which is involved in keratinocyte differentiation, response to tissue injury and inflammation. The pathways associated with keratinocyte differentiation were also affected. Ag NP treatment (alone or combined) modulated the DNA methylation changes in the genes involved in desmosome organization. The percentage of activated regulatory macrophages at the wound site was increased by the MSC and Ag alone treatment, while the production of nitric oxide, an inflammatory marker, by stimulated macrophages was decreased by MSCs/Ag alone and MSCs+Ag treatment. Ag induced the expression of *Col1*, encoding collagen-1, at the injury site. In summary, MSC and NP treatment of skin wounds (alone or combined) suggests induction of the processes accelerating the proliferative phase of healing; MSCs-NP interactions are a key factor affecting global mRNA expression changes in the wound.

Toward the epigenetic memory: a story of long-term human biomonitoring research

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Effects of air pollution, nanoparticles, or radiation exposure on human health, particularly on genetic material, are frequently studied topics in genetic toxicology. The dynamicity of knowledge in this field is also given by the rapid methodological development allowing transition from traditional to high capacity -omics biomarkers.

Presented data will summarize the results from almost twenty years of the Czech biomonitoring research and bring a comprehensive view on the mechanisms of the effects of the chronic/acute exposure on the structural and functional DNA changes.

A story of this research will show: (i) our paradoxical unexpected results obtained in early phase of the research (cytogenetic results in agreement with/opposite to exposure levels); (ii) finding the explanation by investigation of transcriptome and epigenome; (iii) suggesting the versatile mechanisms of epigenetic adaptation; and (iv) current investigation of the maintenance of epigenetic settings by epigenetic memory.

This knowledge will be documented by individual results from the above-mentioned topics, with focus on the cytogenetic and epigenetic data. Results of micronuclei and/or stable chromosomal aberrations analyses as well as whole human genome DNA methylation data, obtained by comparing different population groups with various exposure profiles during the life, will be reported.

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In vitro high-throughput transcriptomic strategy accompanied by GENOMARK & TGx-DDi as powerful tools for genotoxicity evaluation in 3D HepaRG Spheroids

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Evaluating genotoxicity represents an essential practice in chemical safety assessments, as the induction of DNA damage is associated with, amongst others, malignant transformation and heritable mutations. Although different *in vitro* and *in vivo* methodologies are available to study chemical genotoxicity, they lack specificity, and the extrapolation to humans is complicated by the species-specific responses. Therefore, high-throughput *in vitro* New Approach Methodologies (NAMs) for genotoxicity are being developed to support human-relevant chemical safety assessment. For example, two transcriptomic biomarkers GENOMARK & TGx-DDi have demonstrated high accuracy in classifying (non-)genotoxicants based on gene expression data collected in 2D HepaRG cell lines, and thus represent a promising approach for genotoxicity assessment.

In this context, we aim to investigate the predictivity of the two transcriptomic biomarkers on high-throughput transcriptomic data collected in 3D models of the metabolically active HepaRG cell line to classify Genotoxic (GTx) and Non-Genotoxic (NGTx) Chemicals.

Human hepatic HepaRG 3D spheroids were treated for 72h with three different groups of chemicals at concentrations corresponding to the IC10 & IC10/2 including A) five genotoxic chemicals: Lasiocarpine (PA), Cyclophosphamide (CPA), Ethyl methanesulfonate (EMS), Aflatoxin B1 (AFB1), Benzo[a]pyrene) (BaP); B) Five non-genotoxic chemicals: Aflatoxin B2 (AFB2) 2-Deoxy-D-Glucose (2DDG), D-Mannitol, Caffeine, Ampicillin trihydrate; and C) five non-genotoxic carcinogenic chemicals: Perfluorooctanoic acid (PFOA), Diethanolamine (DEA), Thioacetamide (TAA), Phorbol-12-myristate-13-acetate (TPA), di(2-ethylhexyl) phthalate (DEHP)). The gene expression profiles of the treated spheroids were determined using the high-throughput TempO-seq technology and analyzed with GENOMARK and TGx-DDI. Overall, the classifications obtained with both biomarkers based on the HepaRG 3D spheroid data were in line with the existing knowledge for all tested groups.

Overall, the strategy applied in this project along with the HepaRG 3D spheroid model could be promising in genotoxicity characterisation of chemicals, and thus better protecting human health and the environment.

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Potency ranking and mode of action assessment of nine (suspected) genotoxic mycotoxins using the *in vitro* micronucleus and ToxTracker assay

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Mycotoxins are fungal secondary metabolites that can pose significant risks to human and animal health. However, despite extensive research, the genotoxic potential and/or the underlying modes of action (MoA) of several mycotoxins remain insufficiently understood. To address this, the potential genotoxicity of nine mycotoxins—aflatoxin B1 (AFB1), alternariol monomethyl ether (AME), alternariol (AOH), altertoxin I (ATX-I), beauvericin (BEA), citrinin (CIT), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEN)—was evaluated using two complementary *in vitro* genotoxicity assays. The *in vitro* micronucleus (MNvit) assay was performed in TK6 cells to assess chromosomal damage, both with and without metabolic activation (S9). Additionally, the ToxTracker assay was used to gain insights into their MoAs. Benchmark dose (BMD) analysis was applied to rank the genotoxic compounds based on their potency, and a cross-system correlation analysis was performed to compare the MNvit and ToxTracker data. The overall genotoxicity assessments were largely consistent between both assays. Without metabolic activation, AFB1, AME, AOH, and ATX-I tested positive in both assays, whereas with S9 activation, AFB1, AME, and AOH remained positive, but ATX-I did not for both assays. For DON, OTA, and ZEN (without S9), the two assays yielded conflicting results, as these compounds were positive for genotoxicity in the MNvit assay but negative in ToxTracker. Application of the BMD covariate method showed that potency rankings were generally consistent across assays, except for ATX-I, which was more potent in the MNvit. Notably, all positive results in ToxTracker were primarily associated with Rtkn reporter activation, indicating formation of DNA double-strand breaks. While some mycotoxins induced oxidative stress, this was not the primary cause of the observed DNA damage. These findings provide valuable insights into the genotoxic potential and MoA of mycotoxins and serve as a basis for further genotoxicity studies on mixtures of co-occurring mycotoxins.

Transcriptomic profiling of mouse mesenchymal stem cells exposed to nanoparticles with antimicrobial properties

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Mesenchymal stem cells (MSCs), i.e., adult stem cells with immunomodulatory and secretory properties, contribute to tissue growth and regeneration, including healing processes. Nanoparticles (NPs), i.e. particles with at least one dimension less than 100 nm, are due to their unique properties applied in many areas of human life. Antimicrobial properties of some metal nanoparticles (NPs) may further potentiate the tissue healing caused by MSCs. To investigated the potential of combined MSCs+NPs treatment of tissue injuries, we studied molecular mechanisms underlying NPs effects on MSCs: mRNA and miRNA expression changes, deregulated processes and mRNA-miRNA interactions after *in vitro* exposure of mice MSCs to Ag, CuO and ZnO NPs. While all the tested NPs mediated immunomodulatory effects on MSCs, including processes involved in generation of extracellular vesicles, the most pronounced response was detected for Ag NPs. These NPs impacted the expression of the highest number of mRNAs, including those encoding interferon-ϒ-stimulated genes, as well as genes involved in drug metabolism/cytochrome P450 activity. This suggests immunosuppressive effects of Ag NPs, supporting therapeutic efficiency of MSCs, and response of the cells to potential toxicity of Ag NPs (oxidative stress). Importantly, inhibited osteogenesis and enhanced adipogenesis was evident after the treatment to all NPs. The expression profiling of miRNAs revealed highly interacting MiR-126 and MiR-92a. MiR-126 was downregulated by all NPs suggesting the link to cancer development in which MSCs might be involved, while upregulation of MiR-92a was observed after the ZnO NP treatment only and might be associated with lowering of MSCs healing potency. Overall, our results demonstrate positive effects of NPs on MSCs, although NP impacts on miRNA expression may the limit the therapeutical potential of the combined MSCs+NPs treatment.

Case Study: Nitroso-bisoprolol – How to use ecNGS *in vivo* mutagenicity data and quantitative BMD analysis to derive safe limits for human risk assessment

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N-Nitroso-bisoprolol (NBP) is a nitrosamine drug substance related impurity (NDSRI) of bisoprolol, a beta-adrenergic receptor blocker that has been used to treat cardiac diseases for decades. The mutagenic potential of NBP was investigated using an integrated approach by combining *in silico*, *in vitro* and *in vivo* methods. NBP showed mutagenic effects in an Enhanced Ames Test (EAT) in the presence of 30 % induced hamster S9 only, confirming that the most stringent conditions of the EAT are appropriate to detect the mutagenic activity of weak mutagens, such as NBP. In a mammalian cell gene mutation (HPRT) assay, nitroso-diethylamine (NDEA) relevantly induced the mutation frequency, but not NBP. In a 28-day repeat-dose study in wild-type NMRI mice, a weak induction of mutation frequencies was detected by error-corrected next generation sequencing (i.e., duplex sequencing) in the liver and the bone marrow. Mutation frequency dose-responses were subjected to benchmark dose analysis (BMD). The *in vivo* modeling was further supported by *in-silico* calculations using the validated Computer-Aided Discovery and RE-design (CADRE) tool to predict the potency of NBP and further differentiate its metabolic activity from the anchor nitrosamine NDEA via quantum mechanics (QM) calculations and CYP-binding predictions. Outcomes of this analysis were consistent with *in vivo* studies. By utilizing the lower limits of the BMD confidence interval and applying the methodology outlined in ICH Q3C to calculate Permissible Daily Exposure (PDE) limit, a lifetime PDE of 400 µg/person/day was derived. Additionally, following the ICH M7 framework for deriving Acceptable Intake (AI) limits, an AI of 64 µg/person/day was determined. The integrated *in vivo-in silico* investigation provides a data-based determination of safe limits, supporting NBP is a low-potency mutagen outside the cohort-of-concern and suggesting that the AI based on structural considerations might be over-conservative and should not be capped at the TTC.

Occupational exposure to engineered nanoparticles produced by machining and welding may affect immune response and induce cancerogenesis

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Aim: To investigate the effect of acute inhalation exposure to nanoparticles (NPs) on the transcriptomic profile of chronically exposed male nanocomposite research workers.

Materials & methods: Whole genome mRNA and miRNA expression changes were analyzed from blood samples collected before and after machining or welding. Exposure from the working environment was assessed using stationary and personnel monitoring. Post- and preexposure samples were compared in the exposed group with/without consideration of the type of work (welding/machining of the epoxide resins enriched by SiO2).

Results: In the Welding group, a higher number of mRNA deregulations than in the Machining group were observed, which correlates with a higher individual PM0.1 exposure dose. A significant decrease in the expression of DDIT4 and FKBP5, genes involved in the stress response, in exposed workers was detected after exposure. In the group Machining, the expression of DDIT4 correlated with the exposure dose. The increased levels of miR30-d-5p and miR-3613- 5p (both involved in carcinogenesis) in Welders were associated with NPs exposure dose, suggesting their suitability as an inhalation exposure marker. The results indicate that inhaled NPs may present an occupational hazard to human health. However, further investigation is needed to confirm these suggestions.

Conclusion: The results from transcriptomic analysis (mRNA and miRNA) show that exposure to welding fumes and nanocomposite dust from machining affects the immune system and alters cancer-related pathways. Thus, the use of protective equipment is highly recommended. Personal protection equipment, measures reducing exposure, and sufficient ventilation systems should be used to avoid nanocomposite dust and welding fumes based on safety principles.

Keywords: occupational exposure; nanoparticles; transcriptome changes; welding; machining

Revision of the OECD 487 MN test for a more reliable prediction of genotoxicity/ carcinogenicity

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The *in vitro* micronucleus (MN) test with mammalian cells is a key tool for predicting genotoxicity and the associated carcinogenicity. The MN-test recognises not only DNA strand breaking compounds (clastogens) but also aneugenic toxins affecting the spindle apparatus of eukaryotic cells. We present the use of non-immortalised, strictly epithelial brain derived fish stem cell lines to improve the predictive power of the *in vitro* micronucleus test OECD 487. Confirmed by DAkkS evaluation, H2B-GFP transgenic variants of these cells enable the detection of micro- and small nuclei, lost chromosomes, nucleoplasmic bridges, nuclear buds and karyorrhexis features *in situ* on a 96 well plate format using high throughput kinetic live imaging technologies. Due to the intrinsic metabolic capacities of the cells, there is no need to add animal derived enzymes to the test systems to mimic metabolism. Thus, aneugenic procarcinogens can be recognized, as cell cycle exceeding exposure times are feasible and thereby enabling the frequent display of the spindle apparatus as toxicological target. We recommend to include test compounds with different mode of actions as positive controls in the test guideline OECD 487, since the H2B transgene allows conclusions on the mode of micronuclei formation. In view of the poor separation between cytotoxicity and genotoxicity of the gold standard 4-nitroquinoline, we suggest aneugenic vanadium salts with low cytotoxicity and the procarcinogens cyclophosphamide (clastogenic) and 3,4 dichloroaniline (aneugenic) as positive controls. Robust non-mammalian stem cells with high sensitivity to a variety of embryonic signalling transduction pathways and the resulting metabolic competence have a high potential to reduce false negative genotoxicity outcomes and thereby to avoid subsequent animal testing of apparently non-genotoxic carcinogens. Therefore, the technology presented here should be included in OECD Guideline 487 as a valid variant of the test procedure.

Expanding Assay Controls: Using Multi-Species PBMC and Whole Blood in the Enzyme-Modified Comet Assay Following Potassium Bromate Exposure

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Background: The enzyme-modified comet assay is a widely used tool to assess oxidative DNA damage. Potassium bromate ($KBrO_3$) has been identified as a suitable agent for generating assay controls using monocytic THP-1 cells. However, the high cost of cell cultures and the need for specialized expertise and facilities may hinder its widespread adoption, especially in hospital-based laboratories. Peripheral blood mononuclear cells (PBMC) and whole blood (WB), both commonly used in comet assay, could offer practical alternatives.

Objective: This study evaluates whether cryopreserved PBMC and WB from different species are suitable substrates for generating $KBrO_3$-induced oxidative DNA damage, enabling their use as standardized assay controls in the Fpg-modified comet assay.

Methods: WB was collected from humans, pigs, rabbits, and mice using K_2EDTA tubes. PBMC were isolated via density gradient centrifugation and divided into three experimental sets. Cells were incubated with increasing $KBrO_3$ concentrations (0mM, 3mM, 6mM, 9mM, 12mM) and then cryopreserved. The Fpg-modified comet assay was performed using Ro19-8022-treated HCT116 cells and untreated cells as assay controls. Tail intensity was measured via semi-automated scoring.

Results: Linear regression analysis of porcine and rat PBMC showed slopes ranging from 7.9 (±1.0) to 8.5 (±0.4) with $p < 0.004$ and R^2 values between 0.95 to 0.99. Porcine control PBMC (RPMI-1640 only) exhibited mean Fpg-sensitive sites of 0.20 (±0.7) and 63.67 (±0.76) after 12 mM $KBrO_3$ exposure. Rat PBMC from one experiment showed Fpg-sensitive sites of 3.59 in RPMI-1640 and 67.78 after 12mM $KBrO_3$ exposure.

Conclusion: These preliminary findings suggest porcine and rat PBMC are suitable for $KBrO_3$-based assay controls. Further validation and testing of human and rabbit PBMC, is ongoing to confirm their broader applicability.

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Exploring the Telomere-Mitochondrial Axis in Colorectal Cancer Patients

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Telomere shortening and mitochondrial dysfunction are key players in many diseases, yet emerging research suggests they are not only independent contributors but also interconnected processes. This so-called telomere-mitochondrial axis has been linked to aging, but its role in. colorectal cancer (CRC) remains largely unexplored. This study investigates this axis in CRC by assessing the association between relative mitochondrial DNA copy number (mtDNA-CN) and relative telomere length (RTL) and their relationship with clinicopathological data, recurrence risk, and mortality.

This retrospective study included 166 CRC patients who underwent surgery at Thomayer University Hospital (Prague, Czech Republic) between 2010 and 2020, alongside 10 healthy controls. mtDNA-CN and RTL were measured in peripheral blood collected at diagnosis, as well as in intestinal mucosa and tumor tissues obtained during surgery, using qPCR. Peripheral blood and intestinal mucosa collected from healthy individuals during routine colonoscopies were also analyzed.

In TNM stage I patients, mtDNA-CN and RTL strongly correlated in intestinal mucosa (ρ=-0.77, $p<0.0001$), tumor tissue (ρ=-0.41, $p=0.0316$), and tumor-to-intestinal mucosa ratio (ρ=-0.39, $p=0.0461$), but these correlations weakened and disappeared in advanced stages. Higher blood mtDNA-CN was linked to a lower risk of disease recurrence (HR=0.47; 95% CI 0.22-0.97), even after covariate adjustment. In CRC patients, mtDNA-CN correlated between intestinal mucosa and tumor tissue (ρ=0.38, $p<0.0001$), while RTL correlated between blood and intestinal mucosa (ρ=0.37, $p<0.0001$), blood and tumor tissue (ρ=0.29, $p=0.0002$), and intestinal mucosa and tumor tissue (ρ=0.45, $p<0.0001$). RTL was tissue-dependent in both CRC patients and controls.

This study provides novel insights into the telomere-mitochondrial axis in CRC, highlighting its potential role in disease progression and prognosis.

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Non-invasive detection of circulating mRNA biomarkers in colorectal cancer patients

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Colorectal cancer (CRC) remains the second leading cause of cancer-related deaths worldwide, with over 1.9 million new cases diagnosed annually. Early detection of CRC and pre-cancerous lesions such as colorectal adenomas (CRA) significantly reduce mortality, improves prognosis and patient outcomes. There is an urgent need for identification of non-invasive clinically relevant biomarkers to enable early CRC detection.

This study is structured into three phases: Discovery Phase, Validation Phase1, and Validation Phase2. In Discovery Phase, whole-transcriptome profiling was performed on fresh-frozen tissue samples (CRA, CRC and paired adjacent mucosa) from three groups: CRA patients (n=16), CRC patients (n=8), and healthy controls (n=10) to identify key mRNA markers that distinguish between these groups.

In Validation Phase1, candidate genes identified from sequencing data were validated by RT-qPCR in larger cohorts of paired tissue samples. A total of 10 deregulated genes were selected for CRA patients (n=48) and 8 genes for CRC patients, including 48 samples each from TNM1, TNM2, TNM3, and TNM4 stages. All candidate genes were also evaluated in normal mucosa from healthy controls (n=51).

In Validation Phase2, the top 4 significantly upregulated genes in CRA patients (n=30), and the top 3 genes in CRC patients (n=37) were assessed using RT-qPCR in longitudinal plasma samples (at the time of diagnosis and after surgery). Additionally, we plan to evaluate the expression of these candidate genes in stool samples.

Notably, *CEMIP1* is upregulated in the tissue and plasma samples of both CRC and CRA, suggesting its potential to accurately distinguish patients from healthy controls. We anticipate that these identified mRNA candidates may serve as robust, non-invasive diagnostic markers for early CRC detection.

Keywords: Non-invasive biomarkers, mRNA profiling, colorectal adenoma, colorectal carcinoma

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Biological Dosimetry of Chronic Radiation Exposure in Medical Radiologists using Dicentric Assay and Bayesian Statistical Framework

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Various occupational human cohorts are chronically exposed to radiation and thus require cytogenetic monitoring to assess radiation-related health risks. However, the correct interpretation of such cytogenetic data is challenging and demands the use of advanced biodosimetric methodology.

Twelve interventional radiologists with occupational exposure durations ranging from 5 to 42 years and 14 unexposed controls were examined by classical Dicentric (Dic) assay. Mean Dic yield in the radiologists group exceeded the control level 2,7 times, but individual Dic frequencies didn't show a statistical correlation with the duration of service (r = 0,268; p = 0,1998). The over-spontaneous Dic yields were corrected ('unfolded') by working time to account for lymphocyte elimination; the resulting values showed a strong positive dependence on the exposure duration (r = 0,843; p = 0,0003). Corrected Dic yields were transformed into the posterior probability density of 'possible true yield' within the Bayesian statistical framework. Aberration data were converted into mean or most probable radiation dose estimates and their confidence limits using a linear coefficient of a dose-response calibration curve, constructed *in vitro* specifically in the low radiation dose range.

The estimates of total accumulated dose in studied radiologists ranged from 49 to 593 mGy. Confidence limits for annual doses with a probability of >50% (i.e., having a greater chance of being "true" than "not true") ranged from 0 to 39 mGy, exceeding 20 mGy per year in 10 of 12 cases. Thus, the 'unfolding' of Dic yield revealed a dose-response relationship masked by the partial elimination of aberrant lymphocytes, whereas Bayesian analysis brought additional flexibility and plausibility to cytogenetic biodosimetry of chronic exposure.

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Notes to the microbiome in tumor tissue and adjacent mucosa from CRC patients

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Dysbiosis of bacterial and fungal communities in the bowel has been associated with inflammatory diseases and cancer. Since the most studies deal with the luminal samples of the gut content, we focused on the microbes closely associated with the mucosa. We collected samples of colorectal cancer tissue and adjacent non-affected mucosa from 125 patients and processed them for sequencing of 16S rRNA and ITS1 genes for bacterial and fungal microbiota profiling, respectively. Tumor-associated microbiota was enriched with potential pathogens, such as genera *Fusobacterium*, *Treponema*, *Campylobacter* and *Selenomonas,* whereas adjacent tissue exhibited increased relative abundance of order Bacteroidales and genera *Blautia*, *Faecalibacterium*, *Odoribacter* and *Dorea*. Tumor tissue was markedly resided by fungal genera *Pseudopithomyces* and *Peniophora,* suggesting environmental origin. Stratification to gastrointestinal tract compartments showed that tumor tissues from the left side of the colon and rectosigmoideum had the highest relative abundance of genus *Fusobacterium* and *Streptococcus*, respectively. Genus *Selenomonas* was significantly and specifically enriched in the tumor tissue from the right side of colon. We found marked positive correlation of genus *Parvimonas* with *Peptostreptococcus* ($r=0.85$, $p=7.6\times10^{-15}$), *Campylobacter* ($r=0.82$, $p=4.6\times10^{-13}$), *Dialister* ($r=0.55$, $p=4\times10^{-5}$) and *Fusobacterium* ($r=0.54$, $p=5.4\times10^{-5}$) in adjacent tissue. In the biopsies from CRC patients, we have investigated relative telomere length (RTL), general and oxidative DNA damage. Whereas we found the only significant difference in RTL between tumors (shorter RTL) and adjacent mucosa, there was no association with either localization or microbial settlement. Interestingly, the levels of both general and oxidative DNA damage were significantly higher in tumor tissues than in adjacent mucosa. In our study we evaluated the relationships among DNA damage, RTL and microbiota/fungi, as analysed in intestinal mucosa and CRC tumors.

Colorectal cancer-associated dysbiotic microbiome differs between colon compartments, and certain genera, such as *Fusobacterium*, *Campylobacter*, *Parvimonas* and *Selenomonas* have potential to improve colorectal cancer detection.

In Vivo Mutagenicity Assessment of Benzo[b]fluoranthene Using Error-corrected Next-Generation Sequencing (ecNGS)

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In vivo mutagenicity assessments typically employ assays conducted using transgenic rodents (TGRs). However, TGR-based assays are gradually being supplanted by error-corrected next-generation sequencing (ecNGS) technologies, which can determine induced mutation frequency at any locus in any animal. We conducted a large study of the polycyclic aromatic hydrocarbon (PAH) benzo[*b*]fluoranthene (BbF), examining tissue-, dose- and time-dependent effects using the Benchmark Dose (BMD) covariate approach. MutaMouse males were exposed to 5 doses of BbF for 28, 90, and 180 days; mutagenicity was assessed using both Duplex Sequencing (DS) and *lacZ* transgene scoring. The results revealed strong dose-related responses in liver, lung, and bone marrow; the induced mutation frequency was generally higher in liver. There was an excellent correspondence between TGR results and DS results (i.e., r>0.73), and BMD analyses did not reveal any significant differences between the TGR and DS responses. Interestingly, the DS BMD values were generally more precise. The DS results showed that intergenic loci were generally more susceptible to BbF-induced effects, and that the primary mutation types were C>A transversions and C>T transitions. The former is consistent with the known effects of mutagenic PAHs. The results also showed an increase in potency (i.e., BMD decrease) with increasing treatment duration; the magnitude of the BMD decline from 28 to 180 days permitting a quantitative evaluation of the duration effect. All BMD analyses employed a Benchmark Response (BMR) of 50%; however, the suitability of that fractional increase above background is not known. To address this issue, we collected and analysed dose-response data from 35 DS studies, and, using the Slob (2017) effect size theory, determined a DS-specific BMR of 36%. The results demonstrate the utility of the ecNGS approach, the influence of treatment duration on chemical potency, and the appropriate BMR for analysis and interpretation of DS dose-response data.

In vitro (geno)toxicity evaluation of MFe2O4 (M=Fe, Zn, Mn) magnetic nanoparticles in a 3D HepG2 model

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Nanosized spinel-type ferrites have gained recognition as a unique class of engineered nanomaterials with diverse applications across various disciplines. While they hold great promises, their safety considerations remain largely overlooked. Iron (Fe), zinc (Zn), and manganese (Mn) are biologically essential and can integrate safely into labile pools. However, the biomedical application of Zn and/or Mn ferrite oxide nanoparticles (NPs) requires a thorough toxicity evaluation. In this study, we aimed to assess the potential (geno)toxic activity of three types of ferrite oxide nanoparticles – Fe_3O_4 (N4), $Zn_xFe_{3-x}O_4$ (N8), and $Mn_xFe_{3-x}O_4$ (N9). *In vitro* cyto- and genotoxicity were assessed in a 3D cell model (spheroids) formed from the human hepatocellular carcinoma cell line (HepG2) using the CellTiter-Glo® and the Comet Assays, respectively. In addition, the induction of oxidative stress was examined by measuring reactive oxygen species (ROS) generation using the DCFH-DA fluorescence probe, while malondialdehyde (MDA) was used as a marker of lipid peroxidation. Three-day-old spheroids were exposed to graded concentrations of the tested NPs (up to 250 µg/mL) for 2, 4, 24 and/or 96 hours, depending on the assay performed. The results showed that samples N8 and N9 exhibited greater cytotoxicity in HepG2 spheroids compared to N4 at 24- and 96-hour exposure time points. Comet assay revealed minimal or no significant DNA damage after 24 hours of exposure (5-50 µg/mL). On the contrary, a significant dose-dependent increase in DNA damage was measured after 96 hours of exposure (5-50 µg/mL). A significant dose-dependent increase in ROS generation (DCFH-DA Assay) was observed only for sample N4 after 4-hour exposure, while no significant MDA production was detected after 24 and 96 hours of exposure. Our study highlights health risks, guiding the safe use of these nanomaterials in biomedical applications.

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POSTER PRESENTATIONS

Oxidative Potential as a cell-free assay for predicting of toxicity of fine particles from environmental dust and possible predictions of human health effects

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Several studies suggest that surface area and the potential to form reactive oxidants are promising metrics for predicting the toxic potency of fine and ultrafine dusts. Therefore, two lignite dusts with different fractions (PM2.5 and PM10) originating from a coal mine were investigated for the observed effects on the hydroxyl radical (OH-)-forming activities of these samples. The approach is based on aligned electron paramagnetic resonance (EPR) spectroscopy with 5.5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trap and hydrogen peroxide as substrate and is particularly sensitive to the Fenton reaction-mediated formation of hydroxyl radicals. The results showed that the two lignite dust samples investigated generate ROS in a concentration-dependent manner, with a similar potency as the two reference substances quartz and fly ash (CFA). The investigated coal mine samples and two reference substances with known ingredients show that the method of intrinsic hydroxyl radical generation is a sensitive tool for predicting adverse health effects.

Mechanistic Profiling of Genotoxicants Using Biological Effect Modifiers in a Multiplexed Flow Cytometry Assay

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Mechanistic interpretation of genotoxicity data is increasingly necessary for regulatory and chemical safety decision-making. To enhance mechanistic resolution beyond standard genotoxicity assays, we integrated the In Vitro MicroFlow® micronucleus assay with the multiplexed MultiFlow® biomarker panel, incorporating biological effect modifiers that target DNA damage response pathways. TK6 cells were pretreated with talazoparib (PARP inhibitor), MK-8776 (CHK1 inhibitor), AZD-7648 (DNA-PK inhibitor), or a reactive oxygen species (ROS) scavenger cocktail, followed by treatment with 26 chemicals, including aneugens, nongenotoxic cytotoxicants, and clastogens. Biomarkers measured included micronucleus (MN) frequency, γH2AX, phospho-Histone H3 (p-H3), p53, polyploidy, membrane integrity, and cytotoxicity at 4 and 24 hours. Data were analyzed using benchmark dose modeling and area-under-the-curve calculations, followed by hierarchical clustering to categorize response profiles. Aneugens clustered together based on increased MN, p-H3, and polyploidy. Clastogens formed distinct mechanistic subclasses; alkylating agents exhibited persistent γH2AX, and ROS-inducing agents showed attenuated responses in the presence of scavengers. Topoisomerase I and II inhibitors were clearly distinguished, as were DNA synthesis inhibitors. Nongenotoxic cytotoxicants induced MN but did not activate other biomarkers, allowing for clear separation from genotoxic compounds. Replicate experiments confirmed clustering reliability. This work demonstrates that multiplexed biomarker panels combined with targeted pathway modifiers can accurately classify genotoxicants and disentangle mechanism-specific responses. Importantly, known false-positive compounds in MN assays were clearly distinguished from true genotoxicants. These findings support the development of fit-for-purpose workflows to inform Integrated Approaches to Testing and Assessment (IATA) and quantitative Adverse Outcome Pathways (qAOPs). Ongoing efforts include integrating additional endpoints and exploring visualization methods such as ToxPi to further streamline data interpretation.

The *in-vivo* Comet Assay: Considerations for validation of optimal study design in a regulatory setting

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The *in vivo* alkaline comet assay is a rapid and sensitive technique for detection of single and double strand DNA breaks and is regularly used in the regulatory setting to assess genotoxicity in tissues exposed to a variety of substances. At Gentronix, we are validating the comet assay in Han Wistar rat.

For the *in-vivo* comet assay optimal tissue preparation is pivotal to obtaining robust reliable data for regulatory submission. Liver as the primary metabolic organ, stomach and duodenum as initial contact sites for orally administered compounds were validated to capture both systemic and local DNA damage and techniques for obtaining single cell suspensions were assessed.

Focus was also given to reducing any external factors that may increase cytotoxicity in terms of mechanical dissociation methods employed for tissue preparation. Comet slides were analysed for 'hedgehogs' and neutral diffusion slides were prepared and analysed for % diffused nuclei.

Variability in the comet assay has been widely reported and to this end, study design and validation was focussed on reducing variability within the assay. Specifically, the number of gels on slides, the layout of slides in the electrophoresis tank and buffer volume in the tank. In a regulatory setting scaling laboratory work is also a critical aspect during validation of a new assay, and therefore the time taken for tissue processing as a contributor to variability in the assay and potential DNA repair are important factors that were considered. Optimisation of methodology was initially performed in L5178Y cells.

All considerations were taken into account for the first stage of validation and standard procedures were implemented. Further work will be performed in order to combine the Comet and *in-vivo* micronucleus assays, thereby fulfilling the principle of the 3R's. A laboratory historical control database will also be generated which is essential for regulatory studies.

Unraveling the Role of Mitochondrial DNA in Colorectal Carcinogenesis: From Healthy Mucosa to Tumor Progression

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Mitochondrial DNA (mtDNA) plays a crucial role in cellular metabolism, and its stability is essential for maintaining homeostasis. During colorectal carcinogenesis, progressive changes occur in mtDNA copy number (mtDNA-CN), integrity, and repair mechanisms, potentially influencing energy metabolism and stress responses.

This study aimed to investigate alterations in mtDNA-CN, mtDNA damage, and DNA repair genes expression along the healthy-adenoma-carcinoma sequence to determine their role in tumor development and progression.

We measured mtDNA-CN and mtDNA damage using qPCR and assessed DNA repair gene expression through RNA sequencing and qPCR in colon tissues from healthy individuals, patients with colon adenomas, and colorectal cancer (CRC) patients.

Our findings revealed that mtDNA-CN was significantly elevated in colon adenomas compared to colon mucosa from healthy individuals ($P = 0.002$), adenoma-adjacent mucosa ($P = 0.009$), tumor tissue ($P < 0.001$), and tumor-adjacent mucosa ($P < 0.001$). Moreover, mtDNA-CN was higher in adenoma-adjacent mucosa compared to colon mucosa from healthy individuals ($P = 0.002$), tumor tissue ($P = 0.002$), and tumor-adjacent mucosa ($P < 0.001$). MtDNA damage was significantly higher in tumor-adjacent mucosa than in tumor tissue ($P < 0.0001$), while no significant differences were observed among other tissue types. Additionally, most DNA repair genes were upregulated in both adenomas and tumors compared to tissues from healthy individuals.

These findings suggest that mtDNA-CN and damage levels change during the progression from healthy mucosa to adenomas and carcinomas, underscoring their role in colorectal carcinogenesis. The observed overexpression of DNA repair genes may represent a compensatory response to mtDNA damage.

In conclusion, our results highlight the importance of mtDNA alterations in CRC development and suggest their potential as biomarkers for CRC.

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Does 5G millimeter wave affect skin health? An *in vitro* study on primary juvenile human keratinocytes

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A more stable, faster mobile communications is nowadays increasingly important. The latest innovation is the fifth generation of mobile telephony (5G New Radio). A major advancement in 5G technology is the introduction of a second frequency range (FR2) between 24.52 GHz and 52.6 GHz in the millimeter wave (mmWave) spectrum, which exhibits low-penetration depth. Thus, skin represents the main target organ for potential adverse FR2 effects. Therefore, the EU-funded SEAWave project aims, among others, to investigate biological effects of FR2 on human and rodent skin.

Here, we investigated potential mmWave-mediated induction of DNA-strand breaks and oxidative DNA-lesions as well as transcriptomic changes in low-pigmented primary human juvenile epidermal keratinocytes (NHEK) using the hOOG1-modified alkaline comet assay and microarray technology, respectively. After characterization of cells (growth behavior, chromosome number), cells were exposed blinded to mmWave at three power densities, i.e., sham, 3.33, or 10 W/m^2 for 24 h, with subsequent analysis of DNA integrity and gene expression. Cytotoxicity was determined in parallel by automatic cell counting.

After 24 h of exposure to mmWave, no cytotoxicity and direct DNA damage, i.e., increase in median-based mean tail intensity (maximum fold change of 1.09), was noted. When comparing results ± hOOG1 treatment, no oxidative DNA damage was present in NHEK cells. Transcriptome analysis showed that after 24 h of exposure at 3.33 W/m^2, some pathways were shown activated, including mitochondrial dysfunction and sonic hedgehog signaling pathway, and some pathways were inhibited, such as triacylglycerol biosynthesis at 3.33 W/m^2 and renin-angiotensin signaling at 10 W/m^2.

In conclusion, after 24 h of exposure, mmWave did not induce DNA damage or oxidative DNA lesions in NHEK cells, but nevertheless activated and inhibited certain pathways, some of which are important for cell growth. Further analyses will investigate DNA damages, gene expression, epigenetic landscape and telomere length in additional skin cell models.

Machine Learning Approach for MultiFlow DNA Damage Assay Data to Support the Evaluation of Compounds' Genotoxicity Potential and Mode of Action

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Genotoxic substances damage genetic material and are categorized as mutagens, aneugens, and clastogens. The early identification of genotoxicants and the characterization of their Mode of Action (MoA) is therefore key to ensure drug safety. The genotoxic MoA is an important criterion to progress a drug candidate since it informs about potential risk and drives regulatory risk-benefit assessments and drug labeling.

Due to human health implications of understanding the genotoxic MoA, regulatory safety assessment programs routinely employ mammalian cell and whole-animal tests to investigate a chemical's potential to cause DNA damage (ICH, 2011).

The *in vitro* MultiFlow DNA Damage Assay (MFA) multiplexes different biomarkers into a single flow cytometric analysis, allowing for the distinction between clastogens, aneugens, and non-genotoxicants. While threshold-based approaches assess individual biomarker changes, machine learning (ML) approaches seem to allow for a more granular and more precise evaluation by identifying complex biomarker patterns associated with genotoxic effects.

In this study, we applied ML techniques to classify genotoxic MoA based on an existing MFA dataset. Various ML algorithms were trained and optimized using curated biomarker data, with additional bio- and physicochemical descriptors incorporated to refine predictive performance. The best ML model demonstrated high accuracy in both training and independent validation datasets. A model that integrated MFA biomarker with physicochemical parameters correctly classified all 17 test compounds, including Nutlin-3, a compound with a non-genotoxic p53 pathway activation mechanism. External validation using 49 non-overlapping compounds from a recent dataset demonstrated high model accuracy at 92%. Furthermore, we developed an interactive R Shiny tool to support data visualization and facilitate broader application in genotoxicity assessment workflows.

Our results highlight the potential of ML-driven approaches to support the interpretation of MFA data, help substantiate genotoxicity predictions and streamline risk assessment in pharmaceutical development (Trairatphisan, Dorsheimer et al., Environmental and Molecular Mutagenesis, 2024).

Multifunctional Filters with High Filtration Efficiency and Biocompatibility

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The increasing demand for air quality, health protection, and environmentally responsible materials is accelerating the development of advanced filtration systems that combine high efficiency, biocompatibility, and sustainability. This study presents the design and characterization of fibrous membranes composed of recycled poly(ethylene terephthalate) (r-PET) and silk fibroin (SF), fabricated via electrospinning for use in air filtration applications. The integration of r-PET, a widely available and eco-friendly polymer, with SF, a natural biopolymer known for its biocompatibility, biodegradability, and modifiability, enables the development of multifunctional filtration materials. The influence of silk fibroin content on membrane morphology, fiber diameter, pore size, surface wettability, and thermo-mechanical properties was systematically investigated. These structural features were further correlated with key functional parameters, including air and water vapor permeability, filtration efficiency (FE), and quality factor (Qf), evaluated using aerosol particles ranging from 120 nm to 2.46 µm. While fiber diameter affected both FE and Qf, basis weight was identified as a critical parameter for enhancing FE. Selected membranes fulfilled the FFP1 classification under EN149 and achieved class H13 performance according to EN1822 standards.

In addition to their filtration performance, the membranes exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, which was found to be dependent on both material composition and surface wettability. Cytocompatibility assessments using HaCaT keratinocytes confirmed the membranes' non-cytotoxic nature after 48 hours of incubation.

Overall, the results demonstrate the potential of r-PET/SF electrospun membranes as sustainable, biocompatible, and efficient filtration materials suitable for personal protective and healthcare applications.

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Validation of 3D mammoaggregates as alternative model to study Non-Genotoxic Carcinogens

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The move toward animal-free testing has led to the development of New Approach Methodologies (NAMs), designed to provide more human-relevant data for chemical safety. At the heart of NAMs is the use of advanced *in vitro* models that better reflect human biological responses. Traditional two-dimensional (2D) models often fail to replicate the complexity of *in vivo* tissue. In contrast, three-dimensional (3D) models have emerged as a promising alternative, offering a more physiologically meaningful platform for studying drug response, toxicity, and disease mechanisms, particularly in cancer research. These models mimic tumor multicellularity providing a more accurate representation of *in vivo* tissue behavior. In addition to their value in evaluating genotoxic agents, 3D models are increasingly used to assess non-genotoxic carcinogens (NGTxC) that promote cancer through mechanisms other than direct DNA damage, such as by inducing cell proliferation, oxidative stress, or modulating cellular signaling pathways.

As part of the European PARC project, this study compares 3D mammoaggregate of two distinct breast cancer cell lines—JIMT-1 and T-47D—representing different molecular subtypes. The cell lines were cultured in both 2D and 3D conditions to assess eight reference substances (including both positive and negative controls). Cell viability (alamarBlue assay) was measured in all models, while oxidative stress (DCFDH) and immunotoxicity (ELISA) were measured only in JIMT-1 cells.

Preliminary results showed that JIMT-1 3D mammoaggregate were more sensitive to positive controls, exhibiting a greater decrease in cell viability compared to T-47D (3D) suggesting that the JIMT-1 (3D) model is more sensitive to certain stimuli and is a promising model for investigating further the non-genotoxic mechanisms of chemicals.

Integrating 3D models aligns with NAMs' goals, providing a more human-relevant and ethically responsible platform for toxicological studies and improving the predictive power of *in vitro* hazard assessments and our understanding of the non-genotoxic pathways contributing to cancer development.

Establishing Ex Vivo Toxicity Testing in PDAC Using KPC-Derived OTSCs

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Although many anticancer therapies exert cytotoxic effects by design, their clinical success hinges on achieving a balance between efficacy and tolerability. Toxicity remains a key determinant in the development and clinical implementation of oncologic therapies, particularly with the emergence of immunotherapies and combination regimens that can elicit severe, systemic, or immune-mediated adverse effects. Traditional preclinical models often lack the complexity needed to predict these toxicities accurately.

Organotypic slice cultures (OTSCs) were established from the KPC cell line (Kras^G12D/+; Trp53^R172H/+; Pdx1-Cre)-derived mouse model of pancreatic ductal adenocarcinoma (PDAC). Xenograft tissue offers a physiologically relevant *ex vivo* platform for the implementation of the OTSC method. The tumour slices preserve the native tumour microenvironment (TME), including stromal components and immune cell populations, allowing for functional assessment of therapy-induced toxicity in a spatially and immunologically intact system.

Using KPC-derived OTSCs, we were able to model tissue-specific and immune-related toxicities in response to experimental therapies. Importantly, this system enabled us to distinguish between tumour-targeted effects and collateral damage to non-malignant components of the TME. Moreover, by controlling exposure duration and drug combinations, we can simulate therapeutic schedules and investigate the dose–response relationship in a highly reproducible manner. Upon full implementation of the method in our laboratory, we will evaluate a comprehensive range of therapeutic strategies, with a particular focus on combining epigenetic agents with immunotherapy.

This model supports both early-phase toxicology screening and mechanistic studies, providing a crucial link between efficacy and safety in preclinical oncology research. In future studies, integration of OTSCs with patient-derived immune cells and cytokine profiling may further enhance their utility for personalized immunotoxicity assessment and biomarker discovery.

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Comparative analysis of glyphosate and glyphosate-based herbicide effects in human bronchial epithelial cells NL20

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Glyphosate-based herbicides (GBH) are among the most widely used pesticides worldwide, typically composed of the active ingredient glyphosate (GLY) and various co-formulants such as adjuvants and solvents. Increasing concern over the indiscriminate use of GBHs has led to numerous studies regarding their potential cellular and genotoxic effects, which are diverse and sometimes contradictory, making their regulation difficult. In this study, we evaluated and compared the impact of highly pure GLY (>98%) and GBH "*Faena Fuerte*®" (equivalent to Roundup full ®) on the human bronchial epithelial cell line NL20 exposed to 8, 16, and 32 µg/mL at 24 h. Our findings, which align with previous studies, suggest that neither GLY nor GBH significantly increased the frequency of micronuclei or γH2AX signal. However, both compounds increased chromatin condensation due to enhanced DNA methylation as evidenced by elevated MBD2 signal ($p<0.01$) and *DNMT3A* gene expression. Notably, treatment with 5'-azacytidine, a DNA methylation inhibitor, significantly increased γH2AX-positive cells ($p<0.05$). The GBH formulation produced a more pronounced chromatin condensation. Our findings suggest that glyphosate induces subtle genetic damage due to DNA methylation in NL20 cells. This effect may be enhanced by GBHs, probably due to co-formulants' presence. These results contribute to the ongoing discussion about the safety of glyphosate and GBHs and may inform future regulatory decisions.

Biopriming Micro-Tom Tomatoes with *Paraburkholderia phytofirmans* PsJN: A Strategy for Reducing Nickel Uptake in Ripped Fruits

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Tomato (*Solanum lycopersicum* L.) is a widely cultivated crop of global importance. However, its ability to accumulate nickel (Ni) in fruits poses a health risk for individuals with Ni sensitivity and allergies. Biopriming may have the potential to limit Ni translocation to fruit while also enhancing overall plant health. Benefits of plant-microbe interactions depend on the optimal combination of the bacterial strain, plant species and heavy metal. The aim of our research was to investigate compatibility between Micro-Tom tomato and *Paraburkholderia phytofirmans* PsJN under Ni stress with primary focus on Ni accumulation in different plant tissues. The seeds were transferred into priming medium with P. phytofirmans cells resuspended in sterile H2O, and incubated for 24h. After incubation, bioprimed seeds were dried, surface-sterilized and sown in soil. Non-primed, imbibed seeds were used for the control. The seedlings were maintained in Hoagland solution until the end of experiment. For acute stress, nickel was applied in the form of NiSO4 at a concentration of 50 µM for three weeks, whereas for chronic exposure, 10 µM Ni was supplied in Hoagland solution throughout the experiment. Ni content in roots, leaves, green, and red fruits was analyzed using flame atomic absorption spectrometry (FAAS) at the end of the tomato's phenological cycle. One-way ANOVA was applied to analyze variance, followed by the Tukey (HSD) test for post hoc analysis. Ni was not detected in the tissues of bioprimed control plants. However, a significantly higher ($p<0.05$) Ni concentration was recorded in roots of bioprimed plants regardless of the type of exposure. Conversely, significant decrease ($p<0.05$) in Ni concentration was recorded in bioprimed plants in leaves, green and red fruits compared to non-primed plants. These findings indicate that biopriming promotes Ni sequestration in roots while minimizing its presence in edible parts, highlighting its potential for sustainable agricultural applications.

Impact of Real Driving Emissions from Light-Duty and Heavy-Duty Vehicles on Gene Expression in Human Cell Models

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Mobility connects people and resources worldwide but contributes significantly to greenhouse gas emissions and air pollution, particularly via PM2.5, which harms human health and ecosystems. This study investigates the toxicity of real-world emissions from light-duty (LD) and heavy-duty (HD) vehicles using advanced air-liquid interface (ALI) systems and human respiratory cell models (MucilAir™).

LD vehicle emissions were tested under Real Driving Emissions (RDE) protocols and laboratory conditions in Poland, while HD vehicles were tested in Finland under winter conditions using EN590 and HVO fuels. Emissions were directly applied to cell cultures via a patented ALI exposure system. Transcriptomic responses were evaluated using mRNA sequencing and differential gene expression analysis.

In the LD campaign, emissions from five vehicles under different temperature conditions (+23°C and -9°C) were assessed. While overall gene expression changes were minimal, cold-start conditions (-9°C) triggered a notable immune response. Specifically, 1563 genes were upregulated and 916 downregulated, with 72 genes commonly affected across vehicle types. Key upregulated genes (e.g., IFI44L, IFIT2, CXCL10) were linked to antiviral defense pathways, particularly under cold conditions.

The HD campaign showed even lower transcriptomic responses. Only 50 genes were significantly affected by EN590 fuel emissions and seven by HVO fuel. The most notable biological process affected was the negative regulation of viral transcription (GO:0032897) for EN590 fuel.

Cold-start emissions from LD vehicles had the strongest impact on gene expression, particularly involving antiviral defense mechanisms. In contrast, HD vehicles, especially using HVO fuel, showed limited cellular response. These findings highlight the importance of environmental conditions and fuel type on emission toxicity and support the development of more targeted emission regulations for public health.

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Mutagenicity Assessment of Nitrosamines Using The hprt Assay and Automated Scoring using Image Analysis with Machine Learning

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Nitrosamines, a chemical class with notable mutagenic and carcinogenic potential through DNA alkylation, are now required to be tested in an *in vitro* mammalian cell mutation assay, as well as an enhanced Ames test (EAT) to support an acceptable intake of 1500 ng/day, under recently updated FDA guidance. This study details the setup and validation of the hypoxanthine-guanine phosphoribosyl transferase (hprt) mutagenicity assay using L5178Y cells, while also utilising automated plate image-capture with machine learning (ML) to establish its utility within the pharmaceutical industry for comprehensive nitrosamine mutagenicity assessments. Four Ames-positive nitrosamines were analyzed: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), and 1-cyclopentyl-4-nitrosopiperazine (CPNP). Concordance was found with the mouse lymphoma assay (MLA) for three compounds; NDMA and NDEA showed positive results, while NDIPA was negative. Due to significant inter-replicate variability a statistically significant increase in mutation frequency for CPNP was not detected using the hprt assay whereas CPNP was positive by MLA. Additionally, LOGELs for positive nitrosamines were higher than those in the MLA, indicating sensitivity variances between assays. The automated scoring of mutation plates from vehicle and positive control plates (576 well-images), showed promising accuracy of ~96% when compared to manual methods and potentially higher throughput, thus enhancing assay reliability and efficiency in drug development.

Identification of periostin in kidney tissue

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The kidneys represent one of the most important pathways for the excretion of xenobiotics, thus damage to kidney tissue leads to the accumulation of dangerous substances in the body and further damage to other organs and systems. Chronic kidney disease (CKD) is multi symptomatic condition characterized by progressive damage and loss of kidney function resulting from fibrosis of renal tissue. The process of renal fibrosis is associated with persistent accumulation of extracellular matrix (ECM) proteins and their excessive deposition in the tissue. Scaffold of ECM is made up of proteins such as fibronectin, collagens, elastin, and many others. Periostin belongs to matricellular proteins which serve as regulators of ECM modulation. Its expression in tissues is highest during the body development, then it is reduced to minimum. In specific instances, especially pathological ones, such as tissue injury or metastasis, the expression of periostin is induced again. It has been observed that the level of periostin in kidneys increases with the degree of damage, therefore it has been identified as a perspective marker for identifying kidney damage.

In presented study, we used the unilateral ureteral obstruction (UUO) murine model to study the presence of periostin in kidney tissue during the process of tissue fibrosis formation. We utilized C57BL/6 mice and UUO was performed at three time intervals – 3,7, and 10 days. Using immunohistological and immunofluorescence analyses, we identified areas of periostin presence in damaged kidney tissue.

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The impact of night shifts on oxidative stress parameters and inflammatory cytokines in nurses

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Night shifts are an integral part of healthcare professions, including nursing. However, long-term exposure to night shifts can have adverse effects on the health of these workers. This complex impact includes disruption of the circadian rhythm, increased oxidative stress, and inflammatory responses, which may contribute to chronic health issues.

The study focuses on analyzing the impact of night shifts on the levels of oxidative stress parameters and inflammatory cytokines in nurses working night shifts (n = 140) compared to nurses not working night shifts (n = 100). Specifically, the activities of antioxidant enzymes (CAT, SOD, Gpx, MDA, TAC) and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) were evaluated. Nurses working night shifts showed reduced activity of the antioxidant enzymes CAT, SOD, and GPx ($p < 0.001$), suggesting that their bodies had a lower ability to neutralize reactive oxygen species (ROS) generated during oxidative stress.

Nurses working night shifts also had higher levels of TNF-α and IL-6 ($p < 0.001$), and slightly elevated levels of IL-1β ($p < 0.05$). These pro-inflammatory cytokines are commonly associated with inflammatory responses and may be responsible for weakened immune function due to oxidative stress and disrupted circadian rhythms. Elevated MDA levels ($p < 0.01$) in nurses working night shifts indicated a higher level of lipid peroxidation and cell membrane damage caused by oxidative stress. This increased MDA served as a marker of cellular damage due to excessive exposure to free radicals.

Our study highlights the importance of a balanced work schedule and the need to monitor the health status of healthcare workers performing night shifts to minimize negative health consequences.

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Antibacterial Properties and Biocompatibility of Electrospun Silk Fibroin Mats with Incorporated Seed´s Oils

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The raspberry (*Rubus ideaus*) and blackcurrant (*Ribes nigrum*) cold pressed oils – loaded electrospun nano/microfiber mats based on silk fibroin were investigated as biocompa-tible and antibacterial mats with the potential in medical applications to inhibit bacterial infections. The study presents the production and characteristics of the fibrous mats made by electrospinning. Oils-loaded silk fibroin nanofibrous mats were characterized using scanning electron microscopy, attenuated total reflectance-Fourier transform infrared spectrometry, and thermogravimetric analysis. *In vitro* biocompatibility of the selected samples was studied, and it was proven to have the non-toxic effect on the human keratinocyte cell line (HaCaT). The antioxidant activity of the studied samples was also determined by the DPPH assay. The results show that the selected samples affected the DPPH radical scavenging activity in a dose-dependent manner. Moreover, the significant activity of oil-loaded nanofibers against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) have been exterminated.

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Bilateral renal carcinoma in a foundry worker – a precedent occupational disease in the Czech Republic

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Objectification of exposure in work-related diseases is a very complex matter. Not only must there be appropriate and valid methods for monitoring the working environment, but also methods for assessing the risk of health damage. In oncological diseases, it is known that a number of factors contribute to their etiology. These include genetic predisposition, type of factor, portal of entry, dose, metabolism, method of detoxification, excretion, etc. And all this in the mutual interactions of individual factors with genotoxic potential and with the original "setting" of the exposed person. The determination of individual permissible exposure values thus loses its justification in the case of multifactorial etiology and stochastic dose-effect dependence. In this context, biomarkers of exposure and effect are becoming increasingly important, which assess the complex effect including for the possibility of assessing long-term effects. The importance of this monitoring has been repeatedly confirmed in the Czech Republic. These studies confirmed an increased genotoxic risk at all monitored iron and steel metallurgical workplaces using a range of genotoxicological methods. Within the framework of the IARC assessment, iron and steel industry is associated with an increase in the relative risk of lung cancer of 1.5-2.5 times compared to the normal population. A relationship has also been demonstrated between occupational exposure in these operations and an increased incidence of cancers of the stomach, urogenital tract (except for the kidneys) and some forms of leukemia (IARC, 1987). This information is provided by the organ map of the incidence of cancer in relation to exposure to a specific pollutant or environment. In their work, the authors present information about the first report of professional bilateral oncological kidney disease in a long-time foundry worker. This is a precedent case not only in the Czech Republic, but also an incentive to supplement the IARC organ map.

Immunotoxicological Assessment of Novel 4-Thiazolidinone-Based Anticancer Agents and Their PEGylated Complexes Using Functional Immune Assays in Human Leukocytes

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The immunotoxic potential of drugs is a critical parameter in their preclinical evaluation. This study assessed the immunotoxicity of two 4-thiazolidinone-based water-insoluble anticancer agents, Les-3288 and Les-3833, and their water-soluble complexes with PEGylated polymeric carriers (A24-PEG550 and A24-PEG750), using established immunological methods. Human peripheral blood was used as a source of immune cells to evaluate cellular and humoral components of the immune response.

A panel of *in vitro* functional immune assays, including lymphocyte proliferation, phagocytic activity, respiratory burst, and cytokine production (IFN-γ, TNF-α, IL-6, IL-4), was employed to assess potential immunotoxic effects. Free Les-3288 suppressed lymphocyte proliferation and reduced IFN-γ and TNF-α levels. PEGylation with both A24-PEG550 and A24-PEG750 reversed these effects, indicating reduced immunotoxicity. Neither the free drug nor its complexes affected the phagocytic function or oxidative burst of monocytes and granulocytes.

Due to high cytotoxicity, Les-3833 was tested at reduced concentrations. Interestingly, free Les-3833 enhanced T-dependent B-cell proliferation, and this effect was maintained upon PEGylation with A24-PEG550. However, its complexes with both PEG carriers decreased granulocyte phagocytic activity and respiratory burst, while monocyte function remained unaffected. Cytokine analysis revealed no impact of Les-3833 on IFN-γ or IL-6, while PEGylation slightly improved TNF-α and IL-4 responses compared to the free drug.

These findings highlight the value of immune assays in detecting subtle immunotoxic effects of anticancer candidates. PEGylation of 4-thiazolidinone-based drugs reduced their adverse effects on immune response, supporting its potential as a strategy to improve safety profiles of cytotoxic agents.

Enhancing Efficacy of Chronic Kidney Disease Treatment via Gold Nanoparticles

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The rising prevalence of chronic kidney disease (CKD) and its associated mortality rates represent a growing global health burden. Despite advances in understanding CKD pathophysiology, no specific therapies exist to effectively reverse its progression. Several independent studies suggest that periostin, extracellular matrix protein, could be a promising target for CKD-specific therapy, as its inhibition leads to improved health characteristics in *in vivo* models. The efficacy of the used periostin-inhibiting approaches could be enhanced by employing gold nanoparticles as carriers for targeted drug delivery, facilitating the delivery of therapeutic agents to the most affected kidney regions. However, this hypothesis requires thorough testing, because although numerous studies have shown that nanoparticles can deliver therapeutic agents to disease site, the long-term fate of these carriers, particularly their persistence and effects over time remains elusive.

Jakič (2023) reported that 10 nm polyethylene glycol-coated gold nanoparticles (10 nm PEG@AuNPs) were detected in mice even 120 days post-administration, with about 20% of the applied dose accumulating primarily in the liver, and also in the spleen and kidneys. This raises concerns about biosafety as exogenous material persists in vital organs long after treatment. To address these concerns, the expression of genes related to inflammation and fibrogenesis (Col1a1, Col3a1, Cxcl2, Fn1, Postn, Tnfa) was assessed at the mRNA and protein levels in the liver, spleen and kidneys of male C57BL/6 mice at 60 and 120 days after a single intravenous dose of 10 nm PEG@AuNPs (1 mg/kg b.w.). The findings indicate that nanoparticles affect tissues at the molecular level even months post-injection, highlighting the need to further investigate their mechanism of action and potential long-term toxicity.

References:

Jakič, K. (2023). Distribution, accumulation and biological effects of gold nanoparticles *in vivo* (Dissertation Thesis).

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Evaluating chemical genotoxicity using multiple cell culture models to advance quantitative Adverse Outcome Pathway (qAOP) development

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The investigation of adverse outcomes caused by chemical toxicity supports human and environmental health as genotoxic events can initiate carcinogenicity. Genotoxicity is measured using hazard-based assessments, whereby *in vitro* approaches inform *in vivo* testing, but are unable to predict carcinogenicity. Adverse Outcome Pathways (AOPs) link molecular initiating events to key events that ultimately lead to an adverse outcome linked to cancer. AOPs span multiple levels of biological organisation, although are currently qualitative and the progression to quantitative (qAOPs) will increase understanding on how different modes of action (MoAs) impact the adverse outcome. This study aimed to generate genotoxicity data using the cytokinesis-blocked micronucleus assay (CBMN) for chemicals with varying modes of action: electrophiles (MMS & (B[α]P)), radical species generators (hydroquinone, sodium arsenite) & topoisomerase II inhibitors (dexrazoxane). TK6 cells, 2D HepG2 cells and 3D HepG2 spheroid models were dosed with the compounds for 1.5 cell cycles and analysed for DNA damage. In TK6 and HepG2 spheroid models, the lowest observed adverse effect levels (LOAEL) for MMS were 9.08μM and 45.4μM, whereas B[α]P showed no genotoxicity in TK6 and 2D HepG2 models, but a LOAEL of 15μM in HepG2 spheroids. Hydroquinone gave LOAELs of 50μM in both TK6 and 2D HepG2 cells but no genotoxicity in HepG2 spheroids, and sodium arsenite gave a LOAEL of 3.75μM in TK6 cells but no genotoxicity in HepG2 spheroids. Additionally, dexrazoxane exhibited a LOAEL of 10μM in TK6 cells. This data indicates the variation in sensitivity of different cell culture models to chemical genotoxicity depending metabolic capabilities and the chemical MoAs. It will also inform the development of qAOPs for more accurate risk assessments and guide ongoing mechanistic investigations.

Preclinical evaluation of the therapeutic potential of CXCR4 Antagonist WZ811 in multiple myeloma

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Multiple myeloma (MM) is a complex and heterogeneous hematologic malignancy characterized by the clonal expansion and uncontrolled proliferation of neoplastic plasma cells within a dynamic bone marrow microenvironment that supports tumorigenesis through multifaceted cellular and molecular interactions. The CXCR4-CXCL12 axis mediates the dynamic interplay between malignant plasma cells and the bone marrow microenvironment, thereby driving MM progression through enhanced cellular adhesion, survival signaling, and tumor-supportive interactions. In our study, we show that CXCR4 protein expression is markedly elevated in MM cell lines, as determined by flow cytometry and western immunoblotting. Concurrently, quantitative real-time RT-PCR revealed increased CXCR4 mRNA levels, indicating transcriptional upregulation in MM cells. A selective small-molecule antagonist of CXCR4, WZ811 reduced the viability of MM cell lines, with EC50 values ranging from 17 to 84 μM at 72 hours. *In vivo*, administration of WZ811 significantly diminished tumor burden while enhancing survival outcomes. Mechanistic investigations revealed that WZ811 induces MM cell death through disruption of mitochondrial transmembrane potential, externalization of transmembrane phosphatidylserine, and activation of key caspases, including caspase-3 and caspase-8. Moreover, WZ811 triggered a significant increase in the G0/G1 phase of the cell cycle in MM cells, accompanied by modulation of critical regulators such as ATM, Chk2, H2AX, and phosphorylated H2AX. Notably, WZ811 effectively eliminated the myeloma stem cell-like side population, despite encountering slight resistance in the presence of supportive stromal cells. These findings collectively underscore the therapeutic potential of WZ811 in modulating critical oncogenic pathways in MM, providing a compelling basis for further clinical investigation.

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Evaluation of bioactivity of essential oils: cytotoxic and genotoxic effects on colorectal cancer cell lines

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Colorectal cancer (CRC) ranks among the most frequently diagnosed malignancies. Cancer prevention using phytochemicals obtained from biologically active plants is a new attractive and reasonable strategy.

In our study, we evaluated the effect of six essential oils (EOs) – peppermint (P), oregano (OR), tea tree (TT), lemon (LE), lavender (L), frankincense (F), and two EO blends – Zengest (ZG) and OnGuard (OG) on human CRC cell lines HT-29 and HCT-116. The cytotoxic (MTT assay), genotoxic effects (comet assay), and reactive oxygen species (ROS) generation (ROS-Glo™ H_2O_2 Assay) in CRC cells exposed to EOs and EO blends for 24h were determined.

Our results showed that the 24h-treatment with EOs and EO blends affected cell viability in a dose-dependent manner. The viability (IC_{50}) is growing according to the following sequence: ZG < OR < F < OG < L < TT < LE < P for the HT-29 cell line, and ZG = F < OR < OG < LE < L < TT < P for the HCT-116 cell line.

For all selected oils, we observed a significant genotoxic effect in both cell lines. The most prominent were OG, P, and LE, which demonstrated genotoxicity at concentrations causing ~80% viability. OR proved to have the highest genotoxicity at a concentration of 0.033 µg/mL in the HT-29 cell line. Selected oils, at concentrations that increased DNA damage, significantly increased ROS production in both studied cell lines. The significantly highest ROS production was shown by OG at a concentration of 0.26 µg/mL for HCT-116 cells.

EOs compounds have been found to exert anticancer activity against numerous human neoplastic cell lines and our results confirm the significance of EOs using in the treatment of neoplasia.

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Plasma-derived Biomarkers and Their Cellular Impact in Oesophageal Disease Progression

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Human plasma contains a complex array of bioactive molecules that can influence cellular responses, including genotoxicity. In this study, we investigated the effects of plasma from healthy individuals and patients with gastro-oesophageal reflux disease (GORD), Barrett's oesophagus (BO), and oesophageal adenocarcinoma (OAC) on two cell lines: the oesophageal adenocarcinoma line OE33 and the human lymphoblastoid line TK6. Cells were exposed to 10% human plasma (replacing foetal bovine or human serum) for 4 and 24 hours. We assessed DNA damage using the micronucleus assay, cell viability and proliferation via cytokinesis-block proliferation index (CBPI), cell cycle distribution by flow cytometry, and cytokine secretion (IL-8 in TK6 and IFN-β in OE33) using ELISA. Invasion assays were also performed on OE33 cells post-treatment.

Our results revealed inter-individual variability in the genotoxic potential of plasma. Notably, plasma from some individuals increased micronucleus frequency in TK6 cells, while others exhibited anti-genotoxic effects. On average, plasma from BO patients significantly elevated micronucleus formation in TK6 cells compared to healthy controls ($p = 0.0019$). In OE33 cells, plasma from BO and OAC patients induced cell cycle arrest at the 24-hour time point, significantly reducing S-phase populations ($p = 0.0182$ for BO; $p = 0.0320$ for OAC). Plasma exposure had a comparatively modest effect on TK6 cell cycling. Treatment with the antioxidant N-acetyl cysteine (NAC) did not alter plasma-induced micronucleus formation, suggesting oxidative stress-independent mechanisms. Additionally, OAC-derived plasma elevated IL-8 and IFN-β secretion in TK6 and OE33 cells, respectively, compared to untreated controls.

These findings demonstrate that human plasma exerts both genotoxic and immunomodulatory effects *in vitro*, which vary depending on disease status. This work supports the potential utility of plasma-based biomarkers in characterising disease states and highlights the importance of understanding individual plasma bioactivity in translational and toxicological research.

Advancing PFAS-Free Coatings Through a Safe and Sustainable-by-Design (SSbD) Approach – Insights from the PROPLANET Project

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Poly- and perfluoroalkyl substances (PFAS) have long been utilized due to their outstanding water- and oil-repellent properties, as well as their high thermal stability and durability. However, their persistence in the environment and potential risks to human health have prompted strict regulatory measures and efforts to phase out PFAS compounds. The Horizon Europe project PROPLANET aims to develop innovative PFAS-free coating materials that tackle environmental and health concerns while fostering chemical advancements and circular value chains. The project focuses on developing sustainable PFAS-free coatings for the textile, glass, and food-packaging machinery industries by implementing the principles of Safe and Sustainable by Design (SSbD).

SSbD integrates safety throughout the development and life cycle of PFAS-free coatings, supporting the shift to sustainable chemicals in line with the EU's Chemicals Strategy for Sustainability. Therefore, in the first step, we reviewed existing toxicological data on coating components and identified potential data gaps. These gaps have been assessed, and necessary gap-filling approaches have been applied in a tiered approach tailored to the material's development and life cycle stages.

Our gap-filling strategy relies on new approach methodologies (NAMs), including *in vitro* and *in silico* methods such as QSAR models, with a particular focus on Carcinogenicity, Mutagenicity and Reproductive toxicity (CMR). In cases of high uncertainty in predictions originating from *in silico* methods, early-stage formulations will undergo *in vitro* screening for cytotoxicity (Alamar Blue), genotoxicity (comet), inflammation (pro-inflammatory markers by ELISA), mutagenicity, carcinogenicity (cell transformation assay) and reproductive toxicity (using *C. elegans*). Traditional 2D models are used in initial assessments, while final products are evaluated using advanced 3D models.

In conclusion, coatings are assessed for technical performance while ensuring safety and sustainability. This work contributes to global efforts in advancing NAMs for next-generation risk assessment (NGRA) and strengthening the SSbD framework.

Antibacterial properties of silk fibroin-based membranes as potential protection against respiratory diseases

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Interest in fully biodegradable “green” materials worldwide is rapidly increasing, mainly because of the catastrophic situation with plastic waste contamination due to the accumulation and contamination of microplastics in the whole ecosystem. This is especially true after the pandemic, when the use of disposable packaging was preferred to prevent the spread of the virus in any form, and the use of disposable protective equipment, such as masks and respirators, also resulted in a surge in waste. A possible solution is the choice of biodegradable/compostable materials as an environmentally acceptable alternative to plastics produced from fossil sources. Great attention has been paid to polylactic acid (PLA) due to its thermoplastic behaviour, which combines biodegradability, biocompatibility, and the combination with poly(3-hydroxybutyrate) (PHB) at a certain weight composition can lead to a synergistic effect, resulting in significantly improved mechanical properties. In this study, the electrospun nanofibrous mats with an enhanced filtration efficiency were prepared using a PLA/PHB blend containing silk fibroin (SF), which acts as a biocompatible agent. The effect of SF on fiber diameter, wettability, filtration performance, and comfort properties, such as air/water permeability, was investigated. SEM showed a smooth, continuous bead-free nanofiber structure with good compatibility between all components. The filtration performance was calculated from measurements of penetration through the membranes using DEHS aerosol particles ranging from 120 nm to 2.46 µm. The filtration characteristic of PLA/PHB/SF membranes, with a base weight of approximately 2 g/m^2, showed excellent filtration efficiency, reaching approximately 99%, comparable with commercial filters classified as P3. The PLA/PHB/SF composites significantly enhanced antibacterial efficiency, even though neat SF and PLA/PHB show negligible antibacterial activity against *S. aureus* and *E. coli* bacteria. Biocompatibility increases with the increasing content of silk fibroin.

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Genotoxicity testing using the micronucleus and comet assays in normal human cell based 3D epithelial models

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Safety assessment of new products for human use requires genotoxicity testing to ensure non-carcinogenicity. Current *in vitro* assays have low specificity resulting in a high rate of false positives. To determine the biological relevance of positive *in vitro* genotoxicity results, *in vivo* assays are conducted. However, *in vivo* genotoxicity testing of cosmetic ingredients was banned by 7th Amendment to the Cosmetics Directive. Three dimensional reconstructed human tissue models, which have *in vivo*-like barrier function and metabolism, are considered as models with improved biological relevance compared to 2D cultures. Toward this end, the reconstructed skin micronucleus (RSMN) and comet assays (CA) that utilize MatTek's highly differentiated EpiDerm™ tissue model have been adapted for use with tracheal, vaginal, oral, and corneal tissues. EpiDerm is a 3D normal human cell-based epidermal model that is highly reproducible, contains an *in vivo*-like barrier, and possesses *in vivo*-like biotransformation capabilities. RSMN assay results show statistically significant dose-dependent increases in cells containing micronuclei (MNC) for 9 direct genotoxins and 6 genotoxins that require metabolic activation, and no increases for 4 non-genotoxins. In addition, CA results show statistically significant increases in % tail DNA after treatment with a model genotoxin. Utilizing the RSMN protocol with tracheal, vaginal, oral, and corneal tissue models, statistically significant increases in MNC (0.3 to 1.2%) were observed after treatment with genotoxins. Similarly, CA results with tracheal, vaginal, oral, and corneal tissue models showed statistically significant increases in % tail DNA. Hence, the EpiDerm RSMN and CA assays can be applied to other *in vitro* human epithelial tissue models to predict genotoxic effects following real life exposure conditions. Together, RSMN and Comet assays for skin, tracheal, vaginal, oral, and corneal tissue models will identify a wide spectrum of genotoxic hazards and will increase confidence in the veracity of *in vitro* test results.

Screening of the Genotoxicity of Samples Collected from Rivers and Reservoirs in Northern Spain During Cyanotoxin Blooms

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Cyanobacterial harmful algal blooms (cyanoHABs) pose significant environmental and public health risks due to their potential to produce toxins. Numerous cases of animal toxicity and mortality, including aquatic birds and cattle, have been documented. In humans, exposure to cyanotoxins has led to severe health incidents, with the most notable being the Caruaru dialysis center tragedy in Brazil, which resulted in over seventy deaths.

This study aims to evaluate the cytotoxicity and genotoxicity of a series of water samples collected from two rivers and six reservoirs in Spain, with an average sampling frequency of every six months. Composite samples were obtained from the photic zone at each monitoring site, stored in cold conditions, and transported to the laboratory for freezing until analysis.

To assess genotoxicity, the UMU-test was applied using Salmonella typhimurium TA 1535. Cytotoxicity was evaluated in HepG2 cells after three hours of exposure using three complementary assays: MTT assay, LDH determination, and ATP production. Additionally, ELISA tests were performed to quantify a selection of cyanotoxins, aiding in the interpretation of the genotoxicity results.

Data analysis is currently underway. Based on the findings, further assessments of selected samples and isolated cyanotoxins will be conducted to deepen our understanding of their toxicological impacts.

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The influence of genetic and epigenetic changes of the AH receptor on cytogenetic damage and DNA adducts

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Approximately 4–10% of oncological cases are attributed to occupational exposure. Therefore, biomonitoring the delayed effects of clastogenic agents, such as polycyclic aromatic hydrocarbons (PAHs), is essential for assessing the risk of developing cancer. Chromosomal aberrations (CAs) have long been recognized as reliable biomarkers for detecting the genotoxic effects of clastogenic substances, with increased CA frequency being closely associated with elevated cancer risk.PAHs activate the aryl hydrocarbon receptor (AhR), a transcription factor that regulates the expression of genes involved in xenobiotic metabolism and detoxification. Genetic polymorphisms and epigenetic modifications in the *AhR* gene can influence metabolic processes and modulate individual sensitivity to PAH exposure. These variations may contribute to differences in the formation of PAH-DNA adducts and the induction of chromosomal aberrations. The objective of this study is to investigate the influence of genetic and epigenetic alterations in the *AhR* gene on the formation of PAH-DNA adducts and the frequency of chromosomal aberrations. The study population will consist of individuals occupationally exposed to PAHs, alongside a control group composed of individuals working in environments with no documented increased risk of mutagenic or carcinogenic exposure. Cytogenetic analysis of peripheral blood lymphocytes will be conducted to evaluate the cytogenetic impact of PAH exposure. Single nucleotide polymorphisms (SNPs) and DNA methylation of the *AhR* gene will be assessed using real-time polymerase chain reaction (PCR). Quantification of PAH-DNA adducts in leukocytes will be performed using enzyme-linked immunosorbent assay.

The Quail Chorioallantoic Membrane Assay as an *In Vivo* Model for Thymol-Based Treatment of Colon Tumor

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Cancer diseases remains one of the leading causes of death worldwide and represents a major public health problem. One of the most frequently diagnosed malignancies is colorectal carcer, and its treatment requires the development of novel, more effective, and less toxic therapeutic strategies. In this context, there is increasing interest in exploring natural compounds with potential anticancer properties. In the present study, we utilized the chorioallantoic membrane (CAM) model of the Japanese quail embryo (*Coturnix japonica*) as a rapid, cost-effective, and ethically acceptable *in vivo* platform for testing the antitumor effects of potential therapeutic agents. Our focus was on thymol – a natural monoterpenoid phenolic compound that is one of the main bioactive components of the essential oil derived from *Thymus vulgaris*. Due to its pharmacological properties – including radioprotective, antimicrobial, and antibacterial effects, and protective action against chromosomal aberrations – thymol is considered a promising candidate for further investigation of its anticancer potential and possible application in oncology. We applied thymol in various concentrations to tumor xenografts developed by implanting human colorectal carcinoma cells onto the CAM. Several parameters were evaluated, including tumor presence and post-treatment changes, tumor cell infiltration through the CAM, necrosis of both, tumor and infiltrating cells, stromal edema, and the inflammation the CAM stroma. Another objective of this study was to develop a standardized system for the histopathological changes in the CAM model, since strandardized classification criteria currently do not exist. Our results support the use of the CAM model, combined with the proposed histological scoring system, as an effective platform for assessing the anticancer activity of compounds. Implementing a standardized evaluation method enhances the diagnostic and therapeutic potential of this model in preclinical cancer research.

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The therapeutic potential of isosilybin B for treating liver diseases

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Liver diseases affect millions of people worldwide, accounting for 4% of global mortality. In Slovakia, liver cirrhosis is the leading cause of death among individuals aged 35 to 44. With increasing therapeutic needs, alternative treatments derived from traditional medicine are investigated. One of the candidates is silymarin, extracted from Milk Thistle (*Silybum marianum*), containing multiple flavonolignans. Silibinin, the main constituent, is already known for hepatoprotective effects, however, other silymarin components may have superior therapeutic potential.

This study compared hepatoprotective, anticancer, and antifibrotic properties of isosilybin B, silibinin, and silymarin *in vitro* using hepatic cancer cells (Hepa1-6, HepG2) and non-cancerous hepatocytes (AML12). Isosilybin B demonstrated higher cytotoxicity to cancer cells and significantly lower toxicity to non-cancer cells compared to silibinin. At non-toxic concentrations, isosilybin B selectively induced G1 cell-cycle arrest in cancer cells without affecting healthy hepatocytes. Further, isosilybin B displayed comparable or superior antifibrotic and hepatoprotective properties compared to silibinin, effectively reducing pro-fibrotic gene expression and alanine aminotransferase (ALT) levels in AML12 cells stimulated with TGF-β1.

Overall, isosilybin B demonstrated superior anticancer effects, selective toxicity towards liver carcinoma cells, and antifibrotic and hepatoprotective activities. These findings underscore the therapeutic potential of IB, positioning it as a promising candidate for further research and development in liver disease treatments, potentially surpassing silibinin in therapeutic efficacy.

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Assessing the Genotoxicity of Botanicals Using Standard Assays – an Update from the Botanical Safety Consortium

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Thirteen botanicals selected by the Botanical Safety Consortium as data-rich case studies were tested in four standard genotoxicity assays covering mutation and chromosome damage endpoints. The goal of this exercise was to determine whether the results from the four selected assays provided an assessment of genotoxicity consistent with the existing data for each case study, thereby supporting their use in generating genotoxicity profiles of botanicals. The four assays included the bacterial reverse mutation assay (Ames test), the *in vitro* micronucleus (MN) assay in TK6 cells and in HepaRG cells, and the ToxTrackerÒ assay. The Ames and MN tests were conducted in accord with their respective OECD Test Guidelines.

Based on existing information for the 13 botanicals tested, aristolochia, comfrey, green tea, and milk thistle were expected to be positive in the Ames test and/or were expected to induce MN. Our results were consistent with the existing literature. Four Ames-positive botanicals were identified. The MN test in TK6 cells, conducted +/- S9, identified one botanical as positive while the MN test conducted using metabolically competent HepaRG cells identified four botanicals as positive or equivocal. ToxTrackerÒ assays identified four positives, consistent with previously published data.

Our results suggest that currently available *in vitro* genotoxicity assays are suitable for testing botanicals. Currently, we are evaluating all the data to determine a recommended testing scheme. In addition, our test data will be compared with in silico predictions of genotoxicity that were made for each botanical based on their identified constituents to determine how to combine in silico data, *in vitro* test data, and human exposure data to produce a comprehensive assessment of genotoxicity.

Selective inhibition of NF-κB signaling by QNZ in multiple myeloma

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Multiple myeloma (MM) is a monoclonal B-cell neoplasia characterized by the clonal expansion of plasma cells within the bone marrow (BM). In the disease pathogenesis, both canonical and non-canonical nuclear factor kappa B (NF-κB) pathways are frequently dysregulated, thereby fostering tumor cell survival, proliferation, and chemoresistance. To investigate the role of NF-κB signaling in MM, we demonstrated elevated activity of NF-κB pathways in MM cell lines. Consequently, we evaluated the anti-myeloma effects of the selective NF-κB inhibitor QNZ (EVP4593) in MM models. QNZ significantly reduced the viability of MM cell lines *in vitro*, as well as ex vivo-isolated malignant plasma cells, compared to healthy cells. To assess the protective influence of the stromal microenvironment in MM, QNZ was shown to inhibit MM cell proliferation both in monoculture and in co-culture with BM stromal cells, although a diminished cytotoxic effect was observed in the co-culture system. In addition, QNZ exhibited potent anti-MM activity *in vivo* in a xenograft mouse model. Delineation of the molecular and cellular anti-MM mechanisms of QNZ revealed mitochondrial- and caspase-dependent induction of apoptosis, involving activation of caspase-8 and caspase-3, accompanied by a reduction in anti-apoptotic proteins Apaf-1 and Mcl-1, and an increase in pro-apoptotic protein Bax. Moreover, QNZ induced cell cycle modulation, characterized by downregulation of Chk2, its phosphorylated form (p-Chk2), and cyclin B1. Importantly, evaluation of QNZ effects on NF-κB-mediated signaling pathways demonstrated decreased expression of NF-κBp105, NF-κBp65, and both isoforms of I-κBα and I-κBβ. These preclinical findings provide a framework for the further clinical evaluation of the selective NF-κB inhibitor QNZ in MM.

Composition and genotoxic activity of methanolic extracts of *Teucrium montanum* L.

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Teucrium montanum L. (Lamiaceae) is a perennial plant rich in biologically active components such as polyphenolic compounds and terpenoids. Previous studies have confirmed the beneficial effects of phenolic acids and flavonoids, which are known for their strong antioxidant activity, as well as the antimicrobial, antifungal, antiproliferative and anti-inflammatory effects of *T. montanum*. In this study, methanolic extracts of *T. montanum* from the flowering aerial parts collected from two ecologically different localities were used. The composition was determined by high performance liquid chromatography (HPLC). The potential genotoxic activity of different concentrations (12.5, 25, 50, 100 and 200 μg/mL) of both extracts on human peripheral blood mononuclear cells (PBMCs) was tested *in vitro* using the comet assay.

The HPLC results showed the presence of various phenolic acids, chlorogenic and caffeic acid, the flavonoids luteolin and luteolin glucoside, quercetin glucoside and quercetin heteroside, and apigenin. The dominant component in sample 1 was phenolic acid, which was not identified in the second sample. In sample 2, the flavonoid luteolin glucoside was the most abundant component, which could not be detected in sample 1. Quercetin heteroside was found in sample 1, while it was only present in trace amounts in sample 2. The results of our study show that there was no increase in DNA damage in the samples of PBMCs treated with both extracts of *T. montanum* at any of the concentrations used. The results obtained suggest further investigation of the composition of *T. montanum* extracts and the quantification of their components, as well as an investigation of their antigenotoxic potential.

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia through the Grant Agreement with the University of Belgrade – Faculty of Pharmacy [No 451-03-136/2025-03/ 200161].

Clinical and Technical Aspects of Circulating Tumor DNA as a Predictive Marker for New Therapeutic Approaches in Locally Advanced Rectal Cancer

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Total neoadjuvant therapy (TNT), which combines radiotherapy and chemotherapy followed by surgical resection, is a novel approach to treating locally advanced rectal cancer (LARC). TNT offers several benefits, including improved local disease control and a higher rate of sphincter preservation. However, not all patients respond equally to TNT – some may experience limited or no benefit, potentially leading to delays in surgery or the need for alternative treatments. Predicting which patients will benefit most from TNT remains a challenge but identifying new predictive biomarkers – particularly in the field of liquidy biopsy, such as circulating tumor DNA (ctDNA) – could improve this process.

Our study aims to determine whether ctDNA levels change during the course of TNT in LARC patients and whether ctDNA can serve as a predictive marker for treatment outcomes. Plasma ctDNA was measured at four time points in patients undergoing TNT: T1= before TNT, T2 = the day after TNT, T3= before surgery (6 weeks after TNT) and T4= 6 weeks after surgery). CtDNA will be quantified using several methods including spectrophotometry, fluorometry, capillary electrophoresis and qPCR). Another objective of the study was to compare these methods, evaluate correlations among them, and recommend the most suitable one. Detailed results will be presented at the meeting.

Predictive biomarkers are essential for optimizing LARC and TNT management by enabling accurate patient selection and minimizing unnecessary toxicity. We believe our findings will help clarify whether plasma ctDNA can serve as a predictive biomarker for TNT responses in LARC and should be further validated in large, independent studies.

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Cellular responses to oxidative stress-inducing chemicals in HepG2 spheroids: Effects on viability, proliferation, and genotoxicity

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Potassium bromate (KBrO3) and Diethyl maleate (DEM), though chemically distinct, both induce oxidative stress and share common mechanisms of action. KBrO3, applied in the food industry, and DEM used in pesticide production are increasingly restricted following KBrO3's classification as possibly carcinogenic to human (IARC 2B) and EU restrictions on DEM. Both chemicals deplete glutathione, leading to disturbed redox balance and uncontrolled reactive oxygen species (ROS) generation. KBrO3 acts as an oxidizing agent indirectly depleting glutathione via ROS increase, while DEM forms glutathione conjugates, inhibiting its function. In the present study, we investigated the cellular responses to KBrO3 and DEM using an *in vitro* HepG2 3D cell model (spheroid). The effects of DEM (400 – 800 µM for 24h, and 100 – 400 µM for 96h) and KBrO3 (625 – 3500 µM for 24h and 25 – 200 µM for 96h) on cell viability (MTS assay), cell cycle distribution (Hoechst 33258), cell proliferation (Ki67), DNA double-strand break (gH2AX) and mitotic cell (histone H3 positive events) formation were investigated upon exposure to 3-day old spheroids. The results showed that both chemicals decreased cell viability and altered cell cycle distribution at both time points. Cell proliferation was slightly decreased by KBrO3 after 24h, but not by DEM. DEM reduced proliferation after 96h, while KBrO3 did not impact it. Both chemicals induced DNA double strand breaks after both time points. After 24h, both compounds increased pH3-positive cell counts at lower concentrations but induced a significant decline at higher concentrations. After 96h, only DEM significantly influenced mitotic activity by dose-dependently suppressing pH3-positive events with higher concentrations. These findings indicate that both chemicals disrupt cellular homeostasis through multiple adverse effects, including oxidative stress related genotoxicity, emphasizing the need for further research and regulatory measures.

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Evaluation of the genotoxic and inflammatory effects of benzo[b]fluoranthene and benzo[g,h,i]perylene in a co-culture lung model at pseudo-air-liquid Interface

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Indoor air pollution (IAP) is an emerging public health concern linked to respiratory infections, chronic diseases, and cancer, contributing to 3.2 million premature deaths annually. Among the most harmful airborne pollutants are polycyclic aromatic hydrocarbons (PAHs), such as benzo[g,h,i]perylene (BGP) and benzo[b]fluoranthene (BBF) classified as priority pollutants due to their widespread presence and high toxicity. Since inhalation is the primary route of exposure to airborne pollutants, assessing their effects on the respiratory system is crucial. We investigated the genotoxic and inflammatory effects of BGP and BBF using an *in vitro* co-culture of human alveolar epithelial (A549) and differentiated (d.THP-1) macrophage-like cells at the pseudo-air-liquid interface. Following a 24-hour exposure at non-cytotoxic concentrations, BGP (≤18.1 µM) and BBF (≤39.6 µM) did not induce DNA double-strand breaks (gH2AX) and mitotic cells (pH3), nor did they increase micronuclei frequency in the cytokinesis-blocked micronucleus (CBMN) assay, affect cell proliferation (Ki67), or cell cycle distribution (flow cytometry). BGP increased IL-6 secretion and the percentage of cells expressing TNF-α, IL-6, and IL-1β (ELISA, flow cytometry), while BBF increased the percentage of cells expressing TNF-α, IL-6, and IFN-γ. Transcriptomic analysis revealed that neither BGP nor BBF upregulated DNA damage-responsive genes, except for *CDKN1A*. However, both PAHs upregulated inflammation-related genes, including *TNF-α*, *IL-10*, *IL-17d*, *IL-8*, *IL-6*, and *IL-1β*. Under the study's conditions the respiratory inflammatory potential of BGP and BBF was demonstrated. The lack of genotoxicity may be attributed to the low metabolic activity of A549 cells, which limits their ability to metabolize BGP and BBF into their genotoxic metabolites. The upregulation of pro-inflammatory cytokines and related genes suggests that these PAHs may contribute to chronic airway inflammation and associated respiratory diseases. Further research is needed to assess their long-term health effects and their relevance to IAP.

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Genotoxic and antigenotoxic assessment of functionalized TiO2 powder with Dihydroquercetin

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Titanium dioxide (TiO_2), a widely studied wide-bandgap photocatalyst, has broad applications in the biomedical field. Dihydroquercetin (DHQ), a naturally occurring flavonoid, is an efficient scavenger of reactive oxygen species, with a wide range of therapeutic effects. This study's objective was to screen potential adverse/beneficial impacts on the genome sensitivity of TiO_2-based ICT complex with DHQ. Genotoxicity assessment, by using the comet assay, demonstrated that pristine TiO_2 at various concentrations (1, 2, 5, 10, and 20 mg/mL) induced statistically significant DNA damage in MRC-5 cells, while TiO_2/DHQ did not, indicating that DHQ mitigates the genotoxic potential of TiO_2. Furthermore, 1, 2, 5, 10, and 20 mg/mL TiO_2/DHQ showed antigenotoxic effects by reducing H_2O_2-induced DNA damage in MRC-5 cells and by supporting its protective role against oxidative stress. These findings suggest that TiO_2-based ICT complex with DHQ can potentially serve as a safe, non-toxic agent. Further assessment of its bioactivity is required.

SPECIAL SESSIONS

Organized by Special Interest Groups and not through the standard abstracts submission process and the submitted abstract selection.

EceToc (The European Centre for Ecotoxicology and Toxicology of Chemicals) on Dose Selection

The Role of Toxicokinetics and Metabolism for *in vivo* Dose Selection

Bennard van Ravenzwaay[1], Phil A. Bothham[2], Richard Curry[2]

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Toxicity is the result of the combination of toxico-kinetic (TK) and -dynamic (TD) properties of a compound. Here, we will focus on TK.

TK factors are particularly critical if the rate for excretion is slower than the rate of absorption. Continuous administration under these circumstances will result in an accumulation over time of the compound in the blood, resulting in an increase of the maximum concentration in the blood (Cmax) and the area under the plasma concentration curve (AUC). In TK studies this can be observed as a break in the linearity of the dose – Cmax/AUC curve. This may result in a severe increase of toxicity if the duration of administration is extended. Examples for this type of toxicokinetic mediated toxicity are reported for the phenoxy herbicides. Species specificity may play an important role (for phenoxy herbicides dogs are particularly sensitive, diclofenac being responsible for high mortality of vultures in India). Prolonged, high AUC values may also lead to the formation of new metabolites. This happens when high affinity metabolic pathways become saturated, resulting in the use of lower affinity metabolic pathways. For risk assessment it is important to realize that metabolites formed under such conditions may not be encountered under relevant human exposure conditions.

Another challenge is saturation of absorption when increasing dose levels will not result in an increase of Cmax or AUC. Although this would normally not be associated with a negative outcome on animal welfare (except for compounds causing local irritation), data obtained using doses higher than the limit of absorption would not be useful for relevant risk assessment and would result in unnecessary high dose animal testing. Therefore, selection of dose levels above the limit of absorption should, in our opinion, be avoided.

Considerations for dose level selection for developmental and reproductive toxicity studies: 3Rs and scientific perspectives

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Following advice on dose selection issued by the European Chemicals Agency (ECHA) in 2022 for reproductive toxicity studies conducted under REACH, this presentation will cover the implications for, and impact on, study outcomes and interpretation for different chemicals and sectors. The ECHA advice followed an evaluation of existing extended one generation reproductive toxicity (EOGRT) studies with respect to design, conduct and toxicological findings, and concerns that potential hazardous effects could be missed due to inadequate dosing. The resulting advice specified that the highest dose tested should *"demonstrate an aim to induce clear evidence of reproductive toxicity without excessive other toxicity and severe suffering in parental animals (e.g. prostration, severe inappetence (lack of appetite), excessive mortality as signs of severe suffering) that would compromise the interpretation of co-occurring reproductive effects."* The recommendations have been evaluated by experts from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), in relation to existing Organisation for Economic Cooperation and Development (OECD) test guidelines and guidance documents, including on humane endpoints and the maximum tolerated dose, and consideration of other factors such as maternal clinical signs of toxicity, food consumption/nutritional intake, clinical chemistry parameters, circulatory/cardiovascular changes, target organ toxicity, maternal stress and toxicokinetics. The group recommend that dose selection should be based on a biological approach that considers all these factors to ensure a high level of human health protection is achieved whilst balancing the scientific aims and impact on animal welfare. Excessive dose levels should be avoided as they can give rise to effects that are not relevant to human health assessments. The results of recent discussions with ECHA and the wider scientific community on this topic will also be presented, highlighting the challenges in balancing animal use and welfare with regulatory requirements and the need for continued dialogue on this topic.

Setting Concentrations of *In Vitro* Toxicity Tests using toxicokinetic information

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In vitro assays are increasingly utilized in the toxicological evaluation of chemicals, employing high concentrations where the criteria for setting an upper concentration are primarily based on solubility and cytotoxicity, or a predefined default concentration. However, it is imperative that the upper concentrations *in vitro* also account for the equivalent dose *in vivo*. A concentration that induces an effect *in vitro*, which surpasses the internal concentration at the maximum external dose *in vivo*, may not be pertinent for toxicological assessment, as the cellular effect observed *in vitro* cannot manifest *in vivo*. This discrepancy highlights the need for a more integrated approach to toxicological testing that bridges the gap between *in vitro* and *in vivo* data.

To address this issue, we propose the utilization of measured or modeled toxicokinetic data to extrapolate the top dose *in vivo* to an upper concentration in vitro. This *in vivo* to *in vitro* dose extrapolation (IVIVE) employs pharmacokinetic/toxicokinetic modeling to derive an *in vitro* concentration (cmaxIVIVE) that mirrors the internal dose at the maximum external dose *in vivo*.

The cmaxIVIVE can be employed to (1) establish a relevant upper concentration for *in vitro* testing (integrated approach) and (2) interpret the results of an *in vitro* test, even if the top concentration exceeds the cmaxIVIVE (post hoc approach). We delineate a workflow that integrates solubility and cytotoxicity data, along with *in vivo* and *in vitro* toxicokinetic data, to derive cmaxvivo or cmaxIVIVE. This workflow not only ensures the relevance of *in vitro* testing but also provides a robust framework for interpreting the results in the context of *in vivo* exposure.

By incorporating IVIVE, researchers can enhance the predictive power of *in vitro* assays and ensure that the concentrations used are biologically relevant, thereby improving the overall quality and reliability of toxicological assessments.

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Dose Selection for 'Omics Studies

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'Omics technologies provide a wealth of information, important in understanding modes of action (MOA) and hazard identification. For regulatory *in vivo* studies all aspects presented by the first and second speakers will apply. For investigative studies doses selected usually cover a broader range and do not need to include a dose without effect. Multiple blood sampling may aggravate toxicity and should be selected with caution. Terminal study sampling of organs and fluids should be done in a consistent way, guidance is currently developed for submission to OECD.

In vitro 'omics studies provide early hazard information and can used in next generation risk assessment. Concentration selection for such *in vitro* studies should establish dose-response relationships while avoiding artefacts due to the physical-chemical properties and overt cytotoxicity, confounding results. Therefore, concentrations should be selected to a range that spans from No Observed Effect Concentrations (NOEC) to moderate cytotoxicity, whilst avoiding precipitation. This ensures reliable dose response modelling for quantification of the point of departure (PoD), the concentration at which adverse effects begin. Benchmark Concentration (BMC) methods are preferred over the NOEC because it accounts for variability in dose-response data and is not solely dependent on preselected concentrations. BMC values are derived from modeling dose-response curves, providing a more accurate estimate of the concentration at which a specific response occurs. This is important for continuous data, such as transcriptomic and metabolomic changes, where defining a PoD is challenging. To increase relevance of findings *in vitro*, BMC values should be compared with expected/calculated human internal concentrations (e.g. plasma Cmax). *In vitro* studies have the advantage that the number of concentrations and times selected are not limited by animal welfare considerations. Concentration range finding studies should be performed using multiple cytotoxicity assays to consider various cytotoxic MOAs and also be used in the main study.

Mutamind: Evaluation and Assessment of Mutagenicity of N-Nitrosamines using *In Vitro* Tests

Chairs: *Anke Londenberg (ITEM, Hannover, Germany) & Roland Frötschl (BfArM, Bonn, Germany)*

Session description:

In risk assessment the class of N-nitrosamines (NAs) belong to the "Cohort of concern" as some of them are highly potent mutagenic carcinogens. If toxicity data are unavailable, the newly released carcinogenic potency categorisation approach (CPCA) developed by an international workgroup of regulatory experts is used. This concept categorises NAs according to their structural features in 5 groups and derives daily intake thresholds.

NA impurities can originate from manufacturing or storage processes of active pharmaceutical ingredients (APIs) or be endogenously formed. Recently, these N-nitrosamine drug substance-related impurities (NSDRIs) lead to recalls of some pharmaceuticals. Individual NA toxicity categorisation and refinement of *in-vitro* testing strategies leading to NA specific thresholds is of high interest.

The Mutamind project encompasses several aspects of N-nitrosamine mutagenicity, their formation and analysis with suitable and reliable assays. These include endogenous formation, toxicokinetics, metabolic activation of NDSRIs and identification of involved enzymes, DNA adduct formation, kinetics and repair, optimisation of *in vitro* mutagenicity tests (AMES test, alkaline comet assay), and the use of benchmark dose (BMD) modelling. The results were compared to CPCA categories and are discussed with regards to their regulatory assessment.

Estimation of bioavailable concentration of endogenously formed NAs by PBK modelling

Max Spänig

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

Endogenous formation of N-nitrosamines under gastric conditions contributes next to direct ingestion to the overall exposure to NAs. Formation kinetics measured under gastric conditions and *in-vitro* derived ADME properties parametrized N-nitrosamine PBK models. Exposure to CPCA daily thresholds and endogenously formed N-nitrosamines was modelled and both estimates are compared.

Finding an optimized AMES test design for N-nitrosamines

Steffi Chang

ICCR-Roßdorf GmbH, Germany

The sensitivity of the Ames test, as tested under OECD TG471 conditions, for N-nitrosamines has been questioned, due to conflicting results published for the same NA species and the dependency of the test on S9-mix. Therefore, a panel of N-nitrosamines was tested using different types of S9 mix, focusing on the use of 30% non-induced hamster S9 mix, to find an improved test design. The panel of N-nitrosamines was compiled displaying the wide spectrum of the chemical variability, including NDSRIs.

The alkaline *in vitro* comet assay with liver cell models as a tool for genotoxicity screening of N-nitrosamines

Christina Ziemann

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Currently, mutagenicity testing of N-nitrosamines is mainly based on N-nitrosamine-adapted Ames testing in the presence of S9-mix. However, bacteria and mammalian cells exhibit marked differences in cell characteristics and genome. Therefore, it seems desirable to also introduce mammalian cell-based tests in genotoxicity screening of potentially mutagenic N-nitrosamines. Predictivity of the short-term alkaline comet assay with liver-based model systems (HepG2 cells with S9-mix, primary rat liver cells, primary human liver cells, and cultured human liver slices) was thus evaluated using a panel of known, mutagenic N-nitrosamines and NDSRIs with the different model systems found to exhibit more or less potential for detection of mutagenic N-nitrosamines.

DNA alkylation damage by nitrosamines and DNA repair in liver cell models

Jörg Fahrer

Division of Food Chemistry and Toxicology, RPTU Kaiserslautern-Landau, Germany

This talk deals with the genotoxicity of N-nitrosamines including NDSRIs in human and rodent liver cells. Furthermore, the relevance of different DNA repair pathways in the protection against N-nitrosamine -induced DNA adducts will be illustrated in genetically engineered DNA repair-proficient and –deficient liver cell models.

Use of Benchmark modelling for NA ranking of *in vitro* mutagenicity and DNA repair data

Anke Londenberg

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

BMD modelling was used to support the analysis of Ames and comet data regarding the sensitivity of the respective assays and model systems. Using BMD confidence intervals (BMD CIs), various N-nitrosamines including NDSRIS tested in the AMES test were ranked and compared to CPCA categories.

Potential *In Vitro* Approach for Assessment of Mutagenic Risk of NDSRIs

Roland Frötschl

BfArM, Bonn, Germany

A high number of new NDSRIs has already been discovered and more will potentially be discovered in the future. Testing all these NDSRIs in *in vivo* mutation assays is not considered feasible and also not desirable. The EMA CPCA approach currently used for setting NDSRI limits only allows maximum AIs of 18 ng/d, 100 ng/d, 400 ng/d and 1500 ng/d depending on the class assignment and justification of higher limits usually requires costly *in vivo* testing usually by a mutation assay intransgenic rodents GR. The results of the different Mutamind sub-projects have delivered valuable insights into mechanistic aspects of N-nitrosamines mutagenic activity and mutagenic potency prediction using *in vitro* testing. These data can be used to develop potential strategies for mutagenic potency prediction using *in vitro* testing only.

WORKSHOPS

HESI GTTC Workshop:
Developing Consensus on the Use of Quantitative Dose-response Analysis for Risk Assessment of Mutagenic Substances

Co-chairs: *George Johnson (Swansea University, Swansea, UK) and Paul White (Health Canada, Ottawa, Canada)*

Although interpretation of genetic toxicity data has traditionally been restricted to qualitative hazard identification, the last decade has seen a concerted movement toward quantitative interpretation of genotoxicity dose-response data for risk assessment and regulatory decision-making. The most robust method for quantitative analysis of mutagenicity dose-response data is the Benchmark Dose (BMD) approach, which determines the dose that elicits a set fractional increase in response over background. Standardised methods and software have been developed for effective BMD analysis; the current approach advocates model averaging and, where necessary, covariate analyses to investigate effects related to compound potency, treatment duration, cell type, repair capacity, etc. For human health risk assessment, quantitative methods have been developed to extrapolate below *in vivo* BMD values to determine exposure limits below which the likelihood of effect can be deemed negligible. Nevertheless, there is a pressing need to quantitatively define relationships between mutation and disease (e.g., cancer); moreover, determine how mutagenicity risk assessment should account for inter- and intra-species variability, exposure duration, and effect severity. Importantly, and despite significant advancements, quantitative methods for human health risk assessment of environmental mutagens have hitherto not been accepted by regulatory authorities. This workshop will provide an overview of current approaches for quantitative interpretation of mutagenicity dose-response data, an overview of applications for evaluations of drug impurities and polycyclic aromatic hydrocarbons (PAHs), an overview of novel *in vitro* approaches for quantitative assessment of mutagenic hazard, and lastly, an overview of issues and concerns regarding risk assessment and regulatory decision-making. The workshop will include five 35-minute presentations, followed by 40 minutes of open discussion.

Welcome and Introduction to HESI GTTC

Connie L. Chen[1], George Johnson[2] and Paul White[3]

[1]HESI, Washington, DC, USA; [2]Swansea University, Swansea, UK; [3]Environmental Health Science & Research Bureau, Health Canada, Ottawa, Canada

Quantitative Interpretation of Mutagenicity Dose-response Data – Concepts, Considerations, and Concerns

George Johnson

Swansea University, Swansea, UK

Genetic toxicity data has traditionally been used for qualitative hazard identification, but there is increasing utility for these data to be used for risk assessment and regulatory decision-making. Dose-response analysis is required to define a point of departure metric from a relevant *in vivo* endpoint, with preference being to use the Benchmark Dose (BMD) lower confidence with a critical effect size (CES) of 50%. This value is then extrapolated to human risk through conversion to human equivalent dose and division by a series of uncertainty factors for a health based guidance value approach, or by the human exposure level for calculation of a margin of exposure. Additional approaches are being considered to calculate cancer based acceptable intakes by reference to a mutation to cancer potency correlation. The fundamental concept for using mutagenicity dose-response data for human risk assessment includes an understanding that mutation is a key event along multiple adverse outcomes of numerous human diseases, including cancer. There is also a quantitative relationship between mutagenic potency and carcinogenic potency for an expanding number of well characterised substances. Considerations around quantitative assessment of mutagenicity data include standardisation and harmonisation through critical assessment of the approach; progress to date looks very promising. The HESI-GTTC and other expert groups regularly engage with regulatory experts, and their concerns around using genetic toxicity data in this way are addressed and overcome in a data driven and logical manner.

Regulatory Perspective on Quantitative Use of Mutagenicity Dose-response Data for Regulatory Decision-making

Alisa Vespa

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To establish acceptable limits for mutagenic impurities in pharmaceutical drugs, regulatory authorities and the pharmaceutical industry alike take into consideration the recommendations provided in ICH's M7(R2) Guideline, and associated Questions and Answers document, on "*Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*". The guideline provides a framework for establishing acceptable limits for mutagenic impurities, and includes use of rodent carcinogenicity studies to establish compound-specific acceptable limits. While *in vivo* mutagenicity assays can be used to assess the mutagenic hazard of an *in vitro* mutagen, a compound-specific acceptable limit cannot be established from quantitative assessment of the dose-response curve when the outcome of the *in vivo* mutagenicity assay is positive. Since 2018 and continuing today, various *N*-nitrosamine impurities have been identified in medicines, most of which do not have carcinogenicity data to establish an acceptable limit. Consequently, pharmaceutical regulators have had to discuss whether and how other sources of data, including *in vivo* mutagenicity data, can be used to establish compound-specific acceptable limits for regulatory purposes. HESI's Nitrosamines Forum for Advancing Critical Translational Science (NA FACTS) Research Advisory Team has identified research priority areas to facilitate acceptance of quantitative dose-response assessments of *in vivo* mutagenicity data for regulatory applications.

Reference:

ICH M7(R2) Guideline (2023) and Questions and Answers (2022) on Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk.

Mutagenicity Assessment of Nitrosamines and NDSRIs

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The Ames test and Transgenic Rodent (TGR) mutation assay serve as pivotal tools for assessing the mutagenic potential of compounds and contributing to hazard identification and risk assessment. In recent years a multi-sector collaboration under the HESI GTTC Mechanism-based Genotoxicity Risk Assessment Group has sought to harmonize methodologies for evaluating the mutagenic potential of N-Nitrosamines (NAs) and Nitrosamine Drug Substance Related Impurities (NDSRIs) using robust, data-driven science. The *in vitro* Ames test, particularly in its revised form – the Enhanced Ames Test (EAT), indicates hamster induced-liver S9 fractions enhances sensitivity in predicting rodent carcinogenicity, compared with rat S9, and that 30% S9 demonstrates the highest sensitivity rates (90%). Similar conclusions were also reported by FDA and EMA. Moreover, comparative analyses between EAT results and *in vivo* Transgenic Rodent (TGR) mutation assays provide a concordance rate of 79%, affirming the EAT's efficacy in NA and NDSRI hazard identification. Additionally, the strong correlation between TGR mutagenic potency estimates and rodent carcinogenic potency (R^2 = 0.95) offers a pathway for employing TGR data not just in hazard identification, but in establishing Acceptable Intakes (AIs) for NDSRIs. This empirical evidence advocates for implementing biological data into quantitative risk assessments, promising more accurate AI calculations and better regulatory compliance. As these methodologies evolve, refining protocols to better detect and manage risks associated with nitrosamine exposure remains crucial for ensuring pharmaceutical safety. Collaboration between academia, industry, and regulatory bodies continues to play a vital role in advancing mutagenicity assessment practices and public health protection.

Quantitative Interpretation of Polycyclic Aromatic Hydrocarbons (PAH) Mutagenicity Dose-response Data in a Risk Assessment Context

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Quantitative analyses of mutagenicity dose-response data are increasingly being used to support risk assessment and regulatory decision-making. The Benchmark Dose (BMD) approach is a technique for dose-response analysis; the covariate method has been effectively used for comparative potency analysis and chemical potency ranking. Calculation of the carcinogenic risk posed by polycyclic aromatic hydrocarbons (PAHs) employs potency equivalence factors (PEFs), with the values being used to scale the carcinogenic potency of PAHs to that of a reference compound (i.e., benzo[*a*]pyrene). Researchers are endeavouring to refine currently used PAH PEFs; however, lack of carcinogenicity data has restricted progress. Due to cost and animal use concerns, it is unlikely that more carcinogenicity data will ever be generated. We evaluated whether *in vivo* mutagenicity data, coupled with the BMD covariate approach, can be used to determine PAH PEFs for cancer risk assessment. Moreover, whether mutagenicity-derived PEFs (mPEFs) can be used to evaluate the assumption that the mutagenic hazards of PAHs in complex mixtures are additive. We used *in vivo* transgenic rodent (TGR) mutagenicity dose-response data for individual PAHs, and the BMD covariate approach to determine mPEFs. The calculated values were then employed to evaluate the assertion that the mutagenic hazards of PAHs in complex mixtures are additive. Results show that mPEFs are well-aligned with carcinogenicity-based PEFs (cPEFs) recommended by regulatory authorities worldwide. mPEFs for 3 PAHs were lower than corresponding cPEFs; however, values were all within five-fold of those commonly employed for risk assessment. The results further support the assertion that the mutagenic hazards of PAHs in mixtures are additive. Indeed, the mutagenic potency of complex PAH mixtures was found to be well aligned with that calculated using the additivity assumption. The approach constitutes an effective strategy to determine PEFs for any polycyclic aromatic compound (PAC) with existing *in vivo* mutagenicity dose-response data.

Mutagenicity assessment using *in vitro* approaches

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Over recent years, there has been a paradigm shift in genotoxicity assessment—from purely qualitative evaluations (i.e., genotoxic versus non-genotoxic) toward a more quantitative understanding of genotoxicity data. Enhanced insights into the strengths of the benchmark dose (BMD) modeling approach, along with ongoing methodological improvements, have played a key role in this transition. Although most progress to date has been made using *in vivo* genotoxicity data, multiple past and ongoing efforts suggest that quantitative analysis also holds promise for *in vitro* data. In this presentation I will discuss several examples illustrating how quantitative data analysis can advance the field of risk assessment. First, the application of the BMD approach to *in vitro* micronucleus data will be presented, focusing on potency ranking of genotoxicants and the assessment of their combined effects. Next, lessons learned from current efforts to develop a quantitative adverse outcome pathway (qAOP) for alkylating agents—describing the key events and the key event relationships—will be explored. Finally, the added value of integrating high-throughput toxicokinetic modeling with the BMD approach to support extrapolation from *in vitro* (transcriptomics) data to the *in vivo* situation will be demonstrated.

Overall, these examples demonstrate that incorporating quantitative assessment into *in vitro* genotoxicity testing is essential to inform modern risk assessment frameworks, particularly in efforts to reduce animal testing and implement next-generation strategies.

Open discussion

Facilitated by: George Johnson and Paul White

Satellite Workshop in Vienna:
Use of Genotoxicity Methods for Human Biomonitoring: Prevention – Diagnosis and Therapy of Cancer

Importance of DNA damage biomarkers in human biomonitoring

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Damage to DNA is the most fundamental pathology which increases the risk of several human developmental and degenerative diseases. This knowledge has been accumulated over the past few decades using a wide range of DNA damage biomarkers both at the cytogenetic level (e.g. chromosome aberrations, micronuclei) and at the molecular level (e.g. DNA breaks, DNA deletions, point mutations, telomere loss). Many of these biomarkers have been shown to be robust indicators of DNA damage induced by important preventable conditions such as (i) deficiency in micronutrients required as cofactors for synthesis of nucleotides (e.g. folate, vitamin B-12) and for DNA replication and DNA repair (e.g. zinc, magnesium) and (ii) exposure to high levels of endogenous (e.g. methylglyoxal) or environmental genotoxins (e.g. ionising radiation). The importance of DNA damage biomarkers is that their use *in vitro* and *in vivo* makes it possible to determine which dietary and lifestyle factors need to be optimised and which environmental factors should be minimised to increase genome integrity in human populations. Importantly, we also need to use these biomarkers to understand better the interactive effects of chronic sub-optimal nutrition and simultaneous exposure to various genotoxins and build robust *in vitro* models for this purpose. Today's symposium provides an exciting program of presentations that illustrate the great potential of DNA damage biomarkers in identifying and preventing loss of genome integrity.

Biomarkers of early effect in biomonitoring: A long and inspiring journey

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Traditional occupational and environmental health risk assessments often rely on external exposure measurements, such as air monitoring, which may not fully capture the complexity of exposure. Human biomonitoring provides a more comprehensive assessment by measuring internal exposures or biological effects resulting from all potential routes of exposure (i.e., inhalation, oral, and dermal). Effect biomarkers, which quantify stressor-induced biological responses linked to disease mechanisms, offer a valuable tool for evaluating risks associated with chemical mixtures. These biomarkers help bridge the gap between exposure and health outcomes by capturing key events in the pathogenesis of cancer and major non communicable diseases, offering a strategic tool for prevention. Integrating effect biomarkers into biomonitoring programs enables the identification of at-risk subpopulations and supports the prioritization of risk management measures. Statistical analyses incorporating effect and exposure biomarkers, alongside demographic variables, can provide insights into chemical exposure trends, thereby improving risk assessment strategies. Advancing the application of effect biomarkers in regulatory risk assessment will facilitate harmonized approaches to occupational and environmental health protection. The presentation will discuss the development of genotoxic biomarkers, starting with their early utilization in exposure monitoring, exemplified by ionizing radiation, and then examine the growing evidence that identifies DNA damage and genomic instability as central mechanisms in the pathogenesis of many diseases, leading to the definition of biomarkers of effect. The next steps involve the validation of these biomarkers as biomarkers of risk, with possible use in clinical practice or in occupational safety.

Using NanoSeq to examine mutational signatures of chemotherapeutics and carcinogens in human tissue-derived organoids

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Distinct mutational signatures associated with exposure to specific environmental carcinogens (e.g. tobacco smoke) and chemotherapeutics (e.g. temozolomide [TMZ]) can be detected in the DNA of human tumours and normal tissues using next-generation sequencing. In order to better understand the mutations observed in people, we aim to characterise signatures induced by mutagenic exposures experimentally. We are currently using normal human tissue-derived organoids, along with a genome-wide, duplex sequencing technology (NanoSeq), to examine mutations caused by a panel of environmental and chemotherapeutic agents. NanoSeq enables highly sensitive, error-free detection of subclonal mutations, eliminating the laborious and time-consuming step of single cell cloning in experimental mutation assays. Using organoids derived from 4 tissues – stomach, colon, kidney and pancreas – treated with several carcinogens (aristolochic acid I [AAI], benzo(a)pyrene, aflatoxin B1 and 2-amino-1-methyl-6-phenylimidazo[4-5-b]pyridine [PhIP]), we found that all accumulated mutations as detected by NanoSeq. We extracted carcinogen-specific mutational signatures consistent with those previously identified using conventional whole-genome sequencing. Further, using this approach in gastric organoids treated with 30 chemotherapeutic agents, we identified a single base substitution (SBS) signature for TMZ that matches a signature observed in human tumours (COSMIC SBS11), as well as SBS signatures for mitomycin C and nitrogen mustard alkylating agents (e.g. chlorambucil).

Use of Micronucleus Experiments with Buccal Cells in Occupational Biomonitoring

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MN experiments with buccal cells are increasingly used in occupational studies. They have the advantage that the collection of the cells is not invasive and that no cultivation is required. In total, 288 studies have been published with different groups of workers in the last four decades. Most studies were realized with agricultural workers, miners, medical staff, painters, petrol station attendants and metal production workers. The most pronounced effects were observed with metal production workers followed by miners and painters. The analysis of the published articles shows that some of them have methodological shortcomings, in particular lack of the control of confounding factors (nutrition), use of inadequate stains, scoring problems and lack of chemical exposure measurements. The publication of guidelines, picture galleries and inter-laboratory validation experiments has led to a substantial improvement of the quality of the studies in the last years. We realized in Austria a number of human studies with different occupational groups. The findings show that the results depend on the specific occupational settings. No positive results were obtained with electroplaters, workers that are exposed to animal manure and welders, while positive findings were obtained with carpenters. Furthermore, we observed for the first time a pronounced effect in road markers that are inhalatively exposed to silica dust and different reactive chemicals. Micronucleus reflect structural and chromosomal aberrations, which play a key role in the etiology of human cancer. At present the surveillance of workers is based solely on chemical analytical exposure measurements, which do not reflect synergistic effects, which can be detected in experiments.

Use of the Comet Assay in Occupational Studies

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The comet assay is widely used in human biomonitoring as a sensitive method for detecting genetic damage, usually in peripheral blood mononuclear cells, but also in whole blood and buccal epithelial cells. The assay can be applied to frozen cells – meaning that samples do not need to be analysed immediately on collection. Incorporating a digestion with lesion-specific enzymes allows detection of oxidised and alkylated bases as well as DNA breaks. In combination with other biomarkers, comprehensive monitoring of acute and chronic exposure to genotoxins is possible. The capacity of cells to repair DNA damage can also be measured. The assay has been applied across a range of occupational settings, for example in workers exposed to pesticides, lead, formaldehyde, benzene, styrene, microwave radiation, and tobacco-related compounds; a consistent association is seen between specific occupational exposures and increased DNA damage, validating the assay's role in biomonitoring programs.

We assessed DNA damage and repair in 239 workers exposed to asbestos, stone wool, or glass fibres, along with 148 controls, using the comet assay. In 61 asbestos-exposed workers, compared with 21 controls, significantly higher levels of oxidised and alkylated DNA bases were found; DNA damage correlated with years of exposure – indicating persistent genotoxic risk. In 98 stone wool-exposed workers and 43 controls, exposed non-smokers showed more DNA strand breaks, but no specific base damage or change in DNA repair capacity, suggesting limited genotoxic impact. Among 80 glass fibre workers, even low-level exposure was linked to increased strand breaks and oxidation damage compared to 36 unexposed. DNA damage was influenced by antioxidant enzyme activity, while repair capacity showed an inverse relationship with damage. To summarise, mineral fibre exposure, even at low levels, can lead to measurable DNA damage; the comet assay can play an important role in identifying and mitigating genotoxic risks in exposed populations.

Assessment of Occupational Exposures to Combustion-derived Carcinogens by Monitoring Urinary Metabolites of Polycyclic Aromatic Hydrocarbons (PAHs)

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds characterized by two or more fused aromatic rings. They are formed by incomplete combustion of organic matter and ubiquitous environmental contaminants. They are commonly detected in urban air, vehicle exhaust, grilled and roasted foods, tobacco smoke, power plant emissions, forest fire emissions, and a variety of occupational settings. Exposures to PAHs can occur via inhalation, dermal contact, or incidental ingestion, with food being a major source of PAH exposure for the general population. Following exposure and absorption, PAHs are initially metabolised by cytochrome P450 isozymes, with primary oxidation by-products (i.e., epoxides and phenols) being further metabolized to quinones and diols. Secondary metabolites are subsequently conjugated to endogenous substances such as glucuronide and glutathione prior to excretion via the urine or faeces. Importantly, the oxidized metabolites of some PAHs (e.g., benzo[*a*]pyrene) are DNA-reactive, contributing to the formation of bulky adducts and subsequent genotoxic effects (e.g., chromosomal damage and mutations). Additionally, numerous PAHs are known or suspected carcinogens. Biomonitoring to assess PAH exposure and internal dose can be accomplished by monitoring the concentration of secondary metabolites in bile and urine; with the latter being used to assess human exposure. Indeed, monitoring of urinary PAH metabolites is commonly used to assess human exposures to PAHs in occupational settings. For example, monitoring of urinary 1-hydroxypyrene has been used to assess PAH exposures in people engaged in metal refining and founding, roofing and paving, coke production, wood preservation, coal-tar distillation, and firefighting. Exposures during firefighting are of particular interest in light of firefighters' increased cancer risk relative to the general population. Indeed, numerous studies have used urinary hydroxypyrene biomonitoring to assess PAH exposures of municipal and wildland firefighters. Of particular interest is the efficacy of interventions (e.g., dermal decontamination and respiratory protection) to reduce firefighters' PAH exposure levels.

Dietary intervention studies with humans

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DNA damage is a key factor in the development and origin of cancer, neurodegenerative disorders, infertility, and accelerated aging. Optimizing nutrient intake offers a promising strategy to mitigate DNA damage and enhance genome integrity. Over the past 25 years, more than one hundred human intervention studies concerning nutrition and its protective effects against genotoxic damage have been conducted. Most of these studies utilize the single-cell gel electrophoresis (comet assay), which quantifies DNA migration in an electric field. This assay has emerged as a vital tool and has been employed in approximately 90 human intervention trials. Other assays, such as the micronucleus test in lymphocytes or buccal cells, are also used, though less frequently. Overall, the protective effects were observed in half of the studies, with pronounced benefits from plant foods (spinach, kiwi, onions), coffee, green tea, honey, olive oil, and vegetable-rich diets. Small doses of specific phenolics (e.g., gallic acid, xanthohumol) reduced oxidative DNA damage. Randomized controlled trials and longitudinal studies have demonstrated that supplementation with micronutrients such as folic acid, vitamin C, zinc (Zn), and selenium (Se) reduces biomarkers of DNA damage, including chromosomal aberrations, oxidized bases, and telomere attrition. Despite promising results, methodological shortcomings in many studies—such as inadequate controls, uncalibrated repair enzymes, and poor statistical rigor—highlight the need for standardized protocols. Future research should prioritize high-quality human trials to refine dietary recommendations for DNA damage prevention. Collectively, these findings underscore the potential of targeted nutrient intake to improve genome maintenance and reduce disease risk.

Impact of Overweight and Weight Loss on DNA Damage in Humans and Underlying Mechanisms

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Overweight including obesity is a growing global health concern and a well-established risk factor for various cancers and other diseases. While cancer development is a long-term process, taking years to decades to manifest after the beginning of exposures or circumstances with increased cancer risk, DNA damage alterations can be detected without delay, providing a valuable biomarker for assessing cancer risk.

Several established methods in human biomonitoring, including micronucleus formation, comet assay, and γH2AX detection, allow for the assessment of DNA damage at the cellular level in human samples. The most often used cells are human peripheral blood lymphocytes.

Research, including our own findings, consistently shows increased DNA damage in individuals with overweight or obesity compared to those with a healthy weight. This DNA damage is likely driven by oxidative stress, chronic inflammation, hormonal imbalances, the dysregulation of pro-inflammatory cytokines and compromised DNA repair mechanisms. Importantly, for obesity, weight loss has been found to be associated with a significant reduction in DNA damage, which may contribute to a lower cancer risk. Understanding the molecular mechanisms involved could enhance obesity-related cancer risk characterization and prevention strategies.

Genotoxic and cytotoxic effects of electromagnetic fields

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Power frequency magnetic fields (PF-MF) resulting from generation, distribution and utilization of electrical current as well as radiofrequency electromagnetic fields (RF-EMF) used for telecommunication, broadcasting, radar tracking etc. have both been classified as possible human carcinogens by IARC. This classification was predominantly driven by epidemiological evidence. However, since the 1950s some *in vivo* and *in vitro* studies have indicated a genotoxic and carcinogenic potential of these agents. Some conflicting findings could be explained but the bottom line was often: results are inconsistent. During the 1970s to the 1990s a view became prevalent that these fields cause acute effects only and at high levels. This view was soon broadly adopted and became the fundament of exposure guidelines almost worldwide. For both types of fields many animal carcinogenicity studies have been conducted that gave ambiguous results partly attributable to methodological differences but also to a fundamental problem that has rarely been recognized. Briefly, none of the 'known' carcinogens would be positive in animal experiments if conducted in analogy to most of those performed in these fields. If we focus on *in vitro* genotoxicity studies, there are also ambiguities in the results that are difficult to explain since at least some of those were well conducted. Recently, we have completed a series of experiments that could shed light on the reasons for diverging findings. We have shown that both types of fields are able to induce nucleotide excision repair. A consequence is that exposure on the edge of the equilibrium between DNA damage and repair must result in huge ambiguity. Furthermore, we have shown that after *in vivo* human experimental exposure to a RF-EMF during one week no genotoxic but clear cytotoxic effects occur. Since chronic exposure to a cytotoxic agent could also increase cancer risk, this possibly offers a new mechanistic.

Induction of DNA damage in smokers and chewers

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Numerous studies show that cytogenetic experiments can be used to assess the contribution of chromosomal damage caused by tobacco smoking and to identify factors which have an impact on the individual risks. Most micronucleus (MN) experiments with exfoliated cells showed that cigarette smoke causes chromosomal damage and acute cytotoxicity, but clear effects were only seen in heavy smokers. We showed that the introduction of inadequate (non-DNA-specific stains) leads to an overestimation of the effects, and that the MN rates increase with the amount of tar, while the nicotine content of the cigarettes was inversely related to chromosomal damage. Another relevant factor that increases the effects is the duration of cigarette consumption (pack-years). We have showed that smoking has severe effects on the cervical cells of women regardless of their hormonal status. Studies with water pipe smokers yielded generally clear positive results. In individuals consuming electronic cigarettes, evidence of the induction of chromosomal damage and cytotoxic effects was found in three studies. Also betel chewing leads to MN induction and to acute cytotoxic effects, regardless if the chewers consumed the nuts and leaves with or without tobacco. No data are currently available regarding the use of nicotine patches. We also conducted studies, with preparations of plant materials, except tobacco, and found higher MN rates in khat chewers in Ethiopia, while no adverse effects were detected in a study, which was conducted among coca chewers in Peru.

Periodontitis – DNA Damage and Cancer

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Periodontitis (PD) is a widespread, chronical, multifactorial and inflammatory disease of the oral cavity. About half of the population in Europe are affected and a severe stadium was diagnosed in approximately 7% (worldwide). Several studies indicate that PD patients have an (up to 5-fold) increased risk for oral cancer. Furthermore, it was reported that also the prevalence of other forms of cancer (colorectal cancer, prostate cancer, liver cancer, etc.) is significantly higher. Cytogenetic studies and micronucleus (MN) experiments with exfoliated buccal and gingival cells which reflect chromosomal damage (structural and numerical aberrations) indicate that these effects may be due to damage of the genetic material which plays a key role in neoplastic transformation. However, the overall results which have been published so far are controversial. Clear positive findings were reported 8 out of 14 studies. In some investigations additional nuclear anomalies were evaluated which reflect acute cytotoxic effects and an increase of these parameter was found in most investigations. It was postulated that the DNA damaging and acute toxic effect are due to release of bacterial toxins that cause inflammations and as a consequence release of reactive oxygen radicals. Notably higher levels of inflammatory cytokines and lipid peroxidation products were found in PD patients. Some studies indicate that MN rates in individuals with PD are associated with alcohol consumption and diabetes, which are known risk factors for the disease. The discrepancies of the results of MN studies may be due to methodological shortcomings, i.e. to the use of inadequate stains, and invalid diagnosis (lack of data concerning the severity of the disease). We are currently realizing comprehensive investigations to find out which stages of the disease lead to chromosomal damage and acute cytotoxicity and also if therapeutic measures can reduce the toxic effects.

Use of human genotoxicity methods to assess the radiosensitivity of cancer patients

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A variety of different methods have been used to investigate the radiosensitivity of cancer patients treated with radiotherapy in order to predict the risk for developing adverse effects, improve the therapeutic success, and patient's quality-of-life. The most investigations were realised with peripheral blood cells from head and neck cancer, breast cancer, and prostate cancer patients. The irradiation response of patient-derived lymphocytes has been associated with acute and late normal tissue reactions to radiotherapy. The approaches which were used include measurements of cytotoxic effects (including apoptosis and cell cycle delay), identification and analyses of specific genes and use of methods which reflect genotoxicity and DNA repair. The most frequently employed approaches that detect damage of the genotoxic material which is induced by ionizing radiation (double strand breaks and oxidative damage) are chromosomal aberration (CA) analyses of metaphase cells, micronucleus (MN) experiments detecting structural and numerical CA, γH2AX assays which can be used to measure double strand breaks, and single cell gel electrophoresis (SCGE) assays reflecting single and double DNA strand breaks. Individual differences in the repair capacity were mainly studied in SCGE experiments in which the time kinetics of the disappearance of "comets" was measured. Overall the results of these studies are highly controversial, possibly due to inconsistent experimental designs and methodological shortcomings (including small study groups). However, the possible correlations between repair kinetics of radiation-induced DNA damage in cancer patient lymphocytes after irradiation and the severity of radiotherapy adverse effects should be further explored in larger cohorts. In the last years standardised/validated protocols have been pushed for MN, SCGE, γH2AX, and DNA repair (BER and NER) experiments with humans that will be used in an ongoing Horizon Europe Twinning project (RadExIORSBoost 101158832) to identify biological parameters which reflect the individual normal tissue radiosensitivity of patients with prostate cancer undergoing radiotherapy.

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Use of telomere length in human biomonitoring studies

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The chromosome ends (i.e. telomeres) consists of a repetitive non-coding nucleotide sequence (TTAGGG in humans), which forms a loop, supported by shelterin proteins. This serves to avoid it from being detected as a double strand break. The telomeric sequence is added to DNA by telomerase, which is active in proliferating cells and silenced in somatic cells. While telomeric DNA functions to avoid loss of vital information during replication of DNA (i.e. end-replication problem), replication of cells with low telomerase activity will lead to a gradual loss of telomere length. *In vitro* studies have shown that cells with too short telomeres go into replicative senescence (or apoptosis). Alternatively, cells may go through a crisis and become immortalized, typically with reactivated telomerase activity. Studies from numerous studies have shown an age-associated shortening in telomere length in human leukocytes. Observations from cross-sectional studies indicate a large inter-individual variation in telomere length, which depends on genetics, age, sex, life-style factors, diet and external exposures. A number of studies have assessed the role of external exposures on telomere length in human leukocytes, although without showing consistent results on the same type of agents such as air pollution, heavy metals and persistent organic pollutants. Lastly, studies on associations between leukocyte telomere length and diseases suggest that some diseases (e.g. cardiovascular diseases) is associated with short telomeres, whereas at least cancers are associated with long telomeres. In summary, research is still needed to link environmental/occupations exposures, telomere length, and risk of disease and mortality in humans.

Use of micronucleus experiments with exfoliated cells for the detection of bladder and cervical cancer

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Micronucleus (MN) experiments with exfoliated urothelial-derived (UDC) and cervical cells (CC) may be useful tools for the prediction and detection of cancer in these organs. UDC can be collected from urine by centrifugation, while CC can be scored in smears sampled in routine health checks. Increased frequencies of MN were found in UDC in populations consuming contaminated (e.g. As) drinking water, in occupationally exposed workers and also in individuals with bladder infections. These exposures are associated with increased cancer risks. Some investigations indicate that this approach can be also used the recurrence of bladder cancer after therapeutic measures. MN in CC were scored in few studies with smokers while a larger number of studies have investigated MN in patients with preneoplastic lesions and cancer. The results of a meta-analysis showed that the MN rates increased with the degree of neoplastic transformation. The highest frequencies were found in patients that were diagnosed cancer. Taken together, these results indicate that the MN test which can be performed in combination with routine screening (Pap-test) may be useful for the detection/prevention of cervical cancer.

Advancing Standardization in Occupational Monitoring Through Automated Microscopy

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The continuous evaluation of workplace risks to employees' genetic material is a fundamental aspect of occupational medicine. Genetic toxicology employs various assays that quantify biological markers, such as chromosomal aberrations and micronuclei, primarily through microscopy. Although numerous studies have validated the reliability of these assays, and several are incorporated into OECD guidelines, standardization remains a challenge due to the predominance of manual evaluation. Automated microscopy presents a promising approach to improving the standardization of test evaluation in genetic toxicology. While the concept itself is not new, and automated systems for evaluating established genetic toxicology assays are already utilized in fields such as preclinical pharmaceutical testing, their application in occupational exposure monitoring has yet to be established. Recent advancements, including the integration of artificial intelligence through Deep Neural Networks (DNN), offer new perspectives on the potential of automated microscopy in this domain. Using the Metafer slide scanning platform software from MetaSystems as an example, we will provide an overview of the feasibility of these technologies in occupational monitoring. Where possible, preliminary results from ongoing studies employing the cytokinesis-block micronucleus assay will be presented. We propose that the automation of microscopy-based evaluations, guided by well-defined assessment parameters, can substantially contribute to the standardization of genetic toxicology assays recommended for occupational monitoring. This hypothesis is supported by evidence from related fields facing similar standardization challenges, such as preclinical pharmaceutical testing and radiation protection, where automation has already demonstrated significant benefits, as extensively documented in the literature.