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Epigenomics
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Germ Cell and Heritable Effects
DNA Repair and Mutagenic Mechanisms
Genotoxicity Risk Assessment and Public Health

In this issue: Abstracts from the 13th International Conference on Environmental Mutagens and 53rd Annual Meeting of the Environmental Mutagenesis and Genomics Society, August 27 - September 1, 2022 - Ottawa, Canada, Maintaining Genomic Health in a Changing World
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chromothripsis. We conducted a long-term live cell imaging study in HeLa-H2B-GFP cells after treatment with different genotoxic agents to identify the most relevant fates for micronuclei. Different doses of these agents were used to clarify, if different treatments result in different fates for micronuclei or micronucleated cells. Cell death, persistence and mitosis of the cell as well as the formation of micronuclei were analyzed for the duration of 96 h. Timing and duration of these events were also monitored. First results after treatment with tert-Butyl hydroperoxide show, that the most relevant fates for micronuclei are persistence and reincorporation, whereas other fates like extrusion and degradation seem to occur only rarely or never. Furthermore, also the results for other genotoxic agents and experimental conditions will be presented. These outcomes can contribute to the question, if micronuclei and micronucleated cells are consequence or cause of carcinogenesis and chromothripsis.

P4

In Vitro Genotoxicity Evaluation of PAHs in Mixtures Using Experimental Design

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Because they crawl, play on the ground, bring hands and objects to their mouths, children below 6 years old ingest up to 100 mg of dust per day[1]. Dust acts as a sink for environmental pollutants. Specifically, Poly Aromatic Hydrocarbons (PAHs) are of concern as they are ubiquitous in the environment, persistent, bio-accumulative, and genotoxic. Toxicity effects are generally assessed for single PAH, whereas PAHs are present as mixtures in environmental conditions. The toxic equivalent factors (TEF) are routinely used in risk assessments to account for additive effects of PAHs in mixtures[2]. Yet, several studies have hinted that the binary PAH mixtures effects were beyond additive effects[3].

In this study, we aimed to (1) investigate in vitro genotoxic effect interactions for 6 PAHs in mixtures; (2) examine whether these effects of PAH mixtures can be roughly predicted by using TEFs. An experimental design study was carried out. Two levels of concentration were chosen for each of the 6 PAHs. In vitro genotoxic effects were assessed on gastric cells by the alkaline comet assay and the cytokinesis-block micronucleus assay.

Results showed that the toxic equivalent quantity (TEQ) can help to predict the toxicity of the mixture, but only for extreme TEQs. For intermediary TEQs, the toxic effects were rather linked to the concentrations of benzo(a)pyrene. A few interactions were observed on each endpoint of both assays, except for cytostasis where no interactions were observed.

This research helps advancing the challenging issue of contaminant mixtures' effects on human health.

P5

Investigation of Potential Respiratory Adverse Effects of Micro/nanofibrillated Cellulose and Cellulose Nanocrystals Using Human Lung Cell Lines

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Micro/nanofibrillated (CMF/CNF) and nanocrystalline (CNC) celluloses are innovative materials with enormous potential for industrial and biomedical applications. Their expanding production/application urges the investigation of their safety for human health.

This study aimed at investigating the potential respiratory outcomes of two CMF/CNF and one CNC produced from bleached Eucalyptus globulus kraft pulp using human alveolar epithelial (A549) cells grown in monoculture or co-cultured with THP-1 monocyte-derived macrophages, by assessing their cellular

uptake, cytotoxic, immunotoxic, genotoxic, and epigenetic effects.

The nanocelluloses were characterized for their physicochemical properties: CMF displays a low percentage of nanofibrils while CNF comprises 100% fibrils with a diameter (D) circa 11 nm; CNC consists of nanorods with D of 4-5 nm and aspect ratio around 42. TEM analysis evidenced that CMF and CNF were internalised into A549 cells whereas CNC were not. Neither cytotoxicity (colorimetric and clonogenic assays) nor ROS induction was observed for any of the nanocelluloses. CMF caused chromosomal alterations (*in vitro* micronucleus assay) in A549 cells while negative results were obtained in co-culture and for the other micro/nanocelluloses in mono- or co-culture. Results in progress of DNA damage and gene mutation analyses will complement mutagenesis assessment. Additionally, potential inflammatory and epigenetic effects are being evaluated.

These results contribute to the weight of evidence of nanocelluloses biological effects and knowledge of the underlying molecular mechanisms. Such information will drive the synthesis of the safest nanocelluloses, thus minimising potential negative impacts of their use on human and environmental health.

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P6

Development and Validation of Fourteen Human TK6-Derived Cell Lines That Individually Express a Human Cytochrome P450 for Genotoxicity Testing

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In vitro genotoxicity testing has played a key role in identifying DNA-reactive chemicals for hazard identification. However, most mammalian cells used for in vitro genotoxicity testing lack effective metabolizing enzymes. We recently developed a battery of TK6-derived cell lines that individually express one of fourteen cytochrome P450s (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7). The increased expression and metabolic function of each CYP in the corresponding cell line were confirmed using real-time PCR, Western blotting, and mass spectrometry analysis; the parental TK6 cells and empty vector (EV) transduced cells had negligible CYP levels. Subsequently, we evaluated these cell lines using two prototypical polyaromatic hydrocarbon mutagens, DMBA and B[a]P, that require metabolic activation to exert their genotoxicity. When compared to EV controls, DMBA-induced cytotoxicity, phosphorylation of histone H2A.X, and micronucleus (MN) formation were significantly increased in TK6 cells with CYP1A1, 1B1, 2B6, and 2C19 expression, while B[a]P increased cytotoxicity, DNA damage, and chromosomal damage in TK6 cells expressing CYP1A1 and 1B1. In addition, we identified specific CYPs responsible for bioactivating three pyrrolizidine alkaloids (PAs). Among the fourteen cell lines, cells expressing CYP3A4 showed significant increases in PA-induced cytotoxicity and genotoxicity. Three PAs induced concentration-dependent increases in %MN in three CYP3A variant-expressing TK6 cell lines (CYP3A4, 3A5, and 3A7). These results indicate that our TK6 cell system holds promise for genotoxicity screening of compounds requiring metabolic activation, identifying specific CYPs involved in bioactivation, and discriminating the genotoxic compounds that have different chemical structures.

P7

A Multi-Biomarker Micronucleus Assay Using Imaging Flow Cytometry

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The micronucleus (MN) assay, incorporating additional DNA-damage biomarkers, is well established by traditional flow cytometry and slide-scanning microscopy platforms. The multi-endpoint data obtained is important in genetic toxicology as it enables chemical mode-of-action (MoA) determinations. Here we use imaging flow cytometry (IFC) to combine the high-throughput advantages of flow cytometry with the image-based, spatial context of fluorescence microscopy. This enables per-cell assessments of MN-induction with no need for cell lysis. The Amnis®ImageStream platform allows capture of 7+ channels of data, enabling simultaneous analysis of multiple parameters such as cell cycle position and MN-induction alongside DNA damage biomarkers such as ɣH2AX, P53 and pH3 via immunofluorescence