Result: Oxidative stress (*i.e.*, superoxide generation and superoxide metabolic process) and mitochondrial dysfunction (*i.e.*, mitochondrial RNA processing, regulation of mitochondrial organization, and regulation of mitochondrial translation) were supported by GSEA ($p<0.05$). NRF2 signaling pathway activation (*i.e.*, GPX4, HO1, KEAP1, NRF2, PRDX1, SOD1, SOD2, TXN1, TXN2, TXNIP, and TXNRD1) was shown at gene and/or protein levels in the lungs of mice chronically exposed to UFPs ($p<0.05$). Changes of oxygen consumption rate, respiratory chain complexes (*i.e.*, I, II, III-core 2, IV, and V-alpha), gluconeogenesis (*i.e.*, PGK1, enolase 1, enolase 2, PC, and PGAM1), glycolysis (*i.e.*, GAPDFH, PKM2, PKM1/2, and PDH), and dynamics (*i.e.*, P(Ser616)-DRP1 and FIS1 *versus* MFN1 and MFN2) supported mitochondrial dysfunction in the lungs of mice chronically exposed to UFPs ($p<0.05$). Taken together, these results closely designated mitochondria as early critical lung targets of air pollution-derived UFPs.

OS01-10
Transcriptomics enhanced risk assessment of chemical mixtures

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Toxicological risk assessment has traditionally focused on the harmful effects of individual chemicals, despite humans being exposed to complex mixtures in real-life scenarios. However, recent studies have reported synergistic responses for pesticide mixtures, which is a cause for concern with regards to consumer safety. Thus, it is crucial to determine the modes of action (MoA) that underlie the toxicological outcomes for chemical mixtures.

Transcriptomics is a key technology for the development of new approach methodologies (NAMs) but has so far found limited use in the regulatory context. This is partly due to a lack of information on the correlation of the molecular effects on transcription with the resulting biochemical/physiological changes and the subsequent adverse effects.

Previous studies have examined hepatotoxic mixture effects, *in vitro* and *in vivo*, establishing a set of marker genes to predict the steatotic potential of pesticides in human HepaRG liver cells. A transcript signature was also developed to identify and classify genotoxic compounds.

This study aimed to characterize the quantitative relationships between biochemical measurements and transcriptome data using human HepaRG cells as a suitable model for the analysis of liver steatosis and genotoxicity *in vitro*. Triglyceride accumulation and DNA damage were measured for a series of seven concentrations of four single compounds (two steatotic and two genotoxic). Cells were also treated with mixtures consisting of a steatotic and a genotoxic compound in a dose-dependent manner. Furthermore, the transcriptomic response to all treatments was evaluated by 3' RNA-seq.

The bioinformatic analysis revealed significant dose-dependent changes in gene expression after 24 h treatment with the four individual substances in decreasing order: aflatoxin B1 > propiconazole > etoposide > thiacloprid. Enrichment analyses revealed up-regulation of genes related to DNA repair and cell cycle-related genes by genotoxic compounds. In contrast, treatment with steatosis inducers showed up-regulation of gene clusters related to lipid metabolism and PPAR signaling. The correlation analysis of gene expression changes with the biochemical endpoints also provided promising results.

Further bioinformatics analyses towards benchmark dose (BMD) and for single compounds and mixtures will be a key tool in next-generation risk assessment. Another aspect covered by the project is the investigation of the suitability of the transcript marker patterns in mixture exposure settings. In the future, the knowledge gained by these *in vitro* experiments will be complemented with quantitative relationships *in vivo*. Overall, this project aims to improve the usability of transcriptome data for risk assessment.

OS02 | Short Orals Session 2

OS02-01
Novel clinical phenotypes and chemical classifications in drug-induced cholestasis: analysis of a database of 390 patients developed by collaborative literature review and machine learning support

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Purpose: Approximately 35–45% of patients with drug-induced liver injury show cholestasis (either pure cholestasis or mixed hepatocellular-cholestasis). In current clinical practice, the level of serum transaminases, alkaline phosphatase and bilirubin are the most commonly used parameters to diagnose drug-induced cholestasis (DIC). However, for patient stratification, the potential value of other parameters such as those derived from examination (anamnesis) and histopathological analysis (when liver biopsy is performed) have not been investigated. Moreover, until now, the clinical focus has been put in patient classifications rather than in drug/chemical classifications. In the present study, we performed a large-scale literature review to collect demographic, clinical examination, biochemical and histopathological data from patients with DIC by using an innovative collaborative review system with machine-learning (ML) support. Subsequently, data analyses were performed to investigate DIC sub-phenotypes for patient stratification, as well as to determine drug clusters based on different clinical profiles, which could, in the long-term, help to predict the cholestatic potential of new chemicals.

Methods: DIC related articles were searched by keywords (including the names of 18 model cholestatic drugs) and boolean operators, in Medline, EMBASE, Web of Science Core Collection, Scopus, Epistemonikos and Cochrane Database of Systematic Reviews. Articles titles and abstracts were uploaded onto Sysrev, a web-based collaborative platform that facilitates article review (with the help of ML models) and data extraction. Once articles of interest were selected, demographic, clinical examination, biochemical and liver histopathological data were extracted, and statistical and multivariate analyses were performed.

Results: From 3921 unique articles found in several literature databases, 2112 were reviewed manually, and 1809 by ML once the trained model algorithm performed with high sensitivity and specificity. Following this strategy, 842 articles were labelled as included, from which 467 full texts were found and uploaded to Sysrev. Within this set of articles, we identified 203 clinical case reports, and 264 animal studies, reviews, and epidemiological studies. Data extracted from the 203 clinical case reports belonged to 390 patients with DIC. Data analyses of this cohort showed that drugs causing cholestasis cluster in three major groups characterized by different clinical phenotype. Furthermore, we uncover novel associations between clinical chemistry, examination and histopathological parameters that could help to better predict patient's outcome.

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