At lower concentrations of 0.01 ug/ml and 0.1 ug/ml, mean SI values for T (CD3+) and B (CD19+) cells combined were <2.5, indicating lack of proliferation. By contrast, positive responses (SI>2.5) were observed among silicotic patients at higher silica concentrations (SI value for NM-200 at 1 ug/ml was only 2.27). Considering only B (CD19+) cells, positive SI values were found *in silico*tic patients across all concentrations.

Discussion: CFSE assay shows to be a useful technique as it allows assessment of cell subpopulation-specific SI and enables detection of reduced cell viability, thereby avoiding misinterpretation. We showed that exposure to nano-silica induced lymphocyte proliferation *in silico*sis patients unlike healthy controls.

P21-14
Occupational exposure to metals and PAHs: combining literature-based exposure and *in vitro* hazard data towards a mixture risk assessment

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The environment within industrial settings is commonly characterized by the existence of a complex mixture of chemicals from different raw materials and transformation processes. Occupational co-exposure to chromium (Cr), Nickel (Ni), and Polycyclic Aromatic Hydrocarbons (PAHs) may occur in diverse workplaces, such as the aeronautic and waste management, (e.g incineration) sectors. Such co-exposure raises concern in terms of occupational health, as these substances are recognized lung carcinogens and mainly act by genotoxic mechanisms, increasing the likelihood of interactive toxic effects. The fact that current regulatory practices are usually focused on single chemical substances, without integrating the possibility of combined or aggregated exposures and effects, may lead to a risk underestimation.

In this work, developed under the scope of HBM4EU Initiative (https://www.hbm4eu.eu), a literature-based mixture risk assessment (MRA) exercise for occupational exposure to metals and PAHs was performed. In addition, *in vitro* toxicity data was obtained for the same mixtures to provide support to its hazard assessment.

Human biomonitoring (HBM) data on Cr(VI), Ni and/or PAHs was extracted from occupational studies conducted in the European Union and searched in literature databases. Selected reference values were used to calculate risk quotients (RQ) for each substance based on the retrieved exposure data; the combined risk was given by the sum of the RQ, i.e., the Background Exposure Exceedance Score(BEES). In parallel, we evaluated the combined cyto- and genotoxicity of the same chemicals (assessed by the MTT and micronucleus assays) in the human alveolar A549 cell line.

In most of the analysed studies, we observed that BEES levels, estimated from the exposure to metals mixture or to metals and PAHs, exceeded RQ levels considered acceptable for the individual substances. Only two studies, conducted in hazard waste incinerator settings, presented urinary exposure levels for the three substances. They showed a value of BEES of concern (>1) for all exposure scenarios, even for workers performing activities considered of low exposure or no-exposure, such as laboratory and administrative workers. *In vitro* assays supported that A549 cells exposure to these substances resulted in interactive cytotoxic and genotoxic effects that may underlie health effects different from those predicted from single exposures.

Our findings show the limitations of applying occupational exposure reference values defined on a single substance basis to workplaces where exposure to chemical mixtures occur, highlighting the relevance of performing MRA as a more realistic approach to guide suitable risk management measures in occupational settings.

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P22 – Regulatory toxicology (REACH)

P22-01
Reproductive toxicity of Zenolide using analogues and comply with RAAF

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Zenolide is a macrocyclic musk, which needed REACH-Annex IX registration being marketed at 100-1000 tons. Reproductive toxicity was not anticipated based on its features. It consists of a C12-dicarboxylic acid (DDDA) connected with Ethylene glycol (EG) with two ester bonds. To fulfil the REACH requirements for reproductive toxicity, reproductive organ information from a 90-day study can be used in combination with developmental toxicity, when these are sufficiently similar to OECD TG testing. To prevent animal testing, analogues can be used when the requirements of Annex XI are fulfilled. The purpose of this presentation is to show that analogues can predict the reproductive toxicity of Zenolide. To begin with, a 28-day study of Zenolide is available, in which reproductive organs are assessed, showing no effect acids and alcohols. Therefore Zenolide is unlikely present in the systemic circulation. For the acid, DDDA, a Reproscreen study shows no effects >= 1000 mg/kg bw. EG has a 90-repeated dose study showing a NOAEL of 150 mg/kg bw and no effects on reproductive organs and for developmental toxicity the NOAEL is 1000 mg/kg bw via dietary testing. Literature data on metabolism show that esters breakdown into the respective acid and alcohol. Moreover, a similar macrocyclic musk (MCM) shows no developmental toxicity and other repeated dose and reproductive effects: all NOAELs >= 1000 mg/kg bw. Based on these data the conclusion was that Zenolide is not toxic for fertility and developmental effects, meeting the requirements and saving 1200 animals. However, according to ECHA RAAF guidance, Zenolide metabolism data is needed to support the read across to its metabolites. Also a bridging study is needed to support the read across with its analogue MCM. To meet these requirements experimental work on the breakdown of Zenolide was performed as well as a Reproscreen study. The metabolism study showed that the