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## Collecting and characterizing industrial air pollution, towards an animal-free prediction of nanomaterial-induced adverse outcomes

**Objective:** New in-vitro models are currently being developed to predict in vivo adverse outcomes, including using advanced microscopy and spectroscopy techniques to track key events in vitro. The in vitro data will be used to develop in-silico models for quantitative prediction of adverse outcomes in vivo. In order to calibrate and validate the in-silico models, different types of industrial airborne material have been collected and characterized.

**Methods:** Nine different materials were identified and collected based on industrial relevance (both established high-production materials and advanced nanomaterials for emerging technologies), representing different stages of a material's life cycle. Particles smaller than 2.5  $\mu\text{m}$  were collected from three cement materials (airborne clinker dust, airborne cement dust, and airborne ambient particles outdoors at the cement facility), dental filling material, bimetallic engineered nanoparticles (CoNi and NiMo), micro- and nanoplastics (particles from recycled plastic materials and particles from degradation of 3D-printed plastics), and particles from recycling of waste from electric and electronic equipment (WEEE). All materials were collected at production/recycling plants, except for 3D-printed plastic, where printed items were grinded into micro- and nanosized pieces using a rotational, high-speed aluminum oxide abrasive stone grinding bit and collected, to simulate environmental degradation. The majority of these materials were characterized in terms of size distributions and size resolved chemical composition, more than half of the materials were also characterized in terms of particle morphology. Endotoxin levels of all materials were assessed.

**Results:** An initial finding highly relevant when it comes to human exposure is that for all the material types, except for the 3D printed particles, the airborne particle sizes were smaller than 4  $\mu\text{m}$ , a particle size range that is deposited primarily in the alveoli tract (the deep lung), upon inhalation, and several of the materials had size distribution peaks in the nano range (<100nm). Chemical composition (ICP-MS) of the three cement industry materials showed that much of the particles outdoors do come from the cement production process, but that there are additional chemical species in the outdoor particles indicating contribution from other sources too. The dental filling material had a major size mode at 3  $\mu\text{m}$ , but SEM revealed primary particles in the nano size range. The CoNi and NiMo bi-metallic particles (generated at a laboratory facility) were exclusively nanosized with a majority of the particles being below 20 nm. Analyses by XPS and TEM-XEDS showed a high level of purity with only minor oxygen and carbon contaminations. The particles collected at a plastic bottle recycling facility, showed almost no particles larger than 2  $\mu\text{m}$ , and a peak of particles around 150 nm, assessed by SMPS and APS. The WEEE particle size distributions show that even if the mass is dominated by the coarse particles, the vast majority of particles are in the sub micrometer range and 66–86% of the number concentration are in the nanoparticle size range. These particles were highly diverse regarding chemical content, both in the sense that each particle was composed by multiple elements, and that the chemical content of the different particles varied considerably, reflecting the varying composition of electronic waste. The size resolved chemical analyses (by PIXE) shows that Si, S, and P compose a larger fraction of the WEEE particles with decreasing particle size, and thus compose a higher fraction of nanoparticles compared to the coarse particles. Another strong trend is the relative abundance of Fe in the very coarse particle fraction.

**Conclusion:** By collecting and characterizing nine different materials, released from industrial processes or in industrial environments, at different stages of a material's lifecycle, we have been able to provide materials to the demonstration and validation of adverse outcome predictions in-vitro and in-silico. The airborne materials are different both in chemical composition and in stage of the lifecycle, but still constitute exposures (and thereby potential health effects) of high relevance for today's and tomorrow's industry. The multidisciplinary

approach, by combining skills from aerosol scientists, toxicologists and modelers, will bridge the gaps in nanotoxicology and nanosafety to enable work towards an animal-free prediction of adverse health outcomes.

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