Abstract

Background: Although engineered nanomaterials (ENM) are currently regulated either in the context of a new chemical, or as a new use of an existing chemical, hazard assessment is still to a large extent reliant on information from historical toxicity studies of the parent compound, and may not take into account special properties related to the small size and high surface area of ENM. While it is important to properly screen and predict the potential toxicity of ENM, there is also concern that current toxicity tests will require even heavier use of experimental animals, and reliable alternatives should be developed and validated. Here we assessed the comparative respiratory toxicity of ENM in three different methods which employed *in vivo*, *in vitro* and *ex vivo* toxicity testing approaches.

Methods: Toxicity of five ENM (SiO_2 (10), CeO_2 (23), CeO_2 (88), TiO_2 (10), and TiO_2 (200); parentheses indicate average ENM diameter in nm) were tested in this study. CD-1 mice were exposed to the ENM by oropharyngeal aspiration at a dose of 100 μg. Mouse lung tissue slices and alveolar macrophages were also exposed to the ENM at concentrations of 22-132 and 3.1-100 μg/mL, respectively. Biomarkers of lung injury and inflammation were assessed at 4 and/or 24 hr post-exposure.

Results: Small-sized ENM (SiO_2 (10), CeO_2 (23), but not TiO_2 (10)) significantly elicited pro-inflammatory responses in mice (*in vivo*), suggesting that the observed toxicity in the lungs was dependent on size and chemical composition. Similarly, SiO_2 (10) and CeO_2 (23) were also more toxic in the lung tissue slices (*ex vivo*) and alveolar macrophages (*in vitro*) compared to other ENM. A similar pattern of inflammatory response (e.g., interleukin-6) was observed in both *ex vivo* and *in vitro* when a dose metric based on cell surface area (μg/cm^2), but not culture medium volume (μg/mL) was employed.