Title:	Toxicity screening and ranking of diverse engineered nanomaterials using established hierarchical testing approaches with a complementary in vivo zebrafish model.
Authors:	Sehrawat, Sharvan (/jspui/browse?type=author&value=Sehrawat%2C+Sharvan)
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Abstract:	The DF4nanoGrouping framework endorsed by the European Centre for Ecotoxicology and Toxicology of Chemicals employs acellular assays, in vitro and in vivo animal testing to perform hierarchical toxicity screening and classification of engineered nanomaterials (ENMs) for hazard prioritization with minimal animal testing. While independent in vitro and in vivo nanotoxicity models are widely available, models that maximize toxicological information while minimizing costs and animal testing are much preferred by frameworks such as DF4nanoGrouping due to ethical and financial constraints over rodent testing. Herein, we investigated the zebrafish embryo as an additional test system that can be employed within the hierarchical testing frameworks, such as the DF4nanoGrouping. Toxicity data for ten diverse and well-studied ENMs, namely zinc oxide (ZnO), copper oxide (CuO), titanium dioxide (anatase, TiO2-A; rutile, TiO2-R), cerium dioxide (CeO2), zinc ferrite (ZnFe2O4), zirconium dioxide (ZrO2), quantum dots (Qdots), nano gold (nAu), and respirable microscopic crystalline SiO2 (MIN-U-Sil 5), was compiled using the acellular FRAS assay, in vitro THP-1 cells and the in vivo zebrafish model, combined with state-of-the-art dosimetry models. ZnO was the most toxic of the tested ENMs in both models, yet significant changes in several descriptive endpoints, which were differentially affected by various ENMs, were noted only in the zebrafish model. The zebrafish model provided better resolution in toxicity endpoints and ranking of ENMs and was inclusive of the outcomes of the THP-1 model. Based on zebrafish toxicity endpoint data, CuO, CeO2 and crystalline SiO2 ranked moderate to highly toxic and comparable to ZnO. TiO2-A, TiO2-R and ZnFe2O4 caused moderate toxicity, whereas ZrO2, Qdots and Au ranked minimally toxic. Our comparative analysis suggests that the zebrafish model, due to its ability to offer comparable high throughput and costs to in vitro cell models, but richer organ-level toxicological data, provides an attractive testing platform for hazard testing and prioritization of ENMs, and can serve as a bridge between in vitro and in vivo rodent models. The zebrafish model deserves more consideration in nanotoxicology research for hazard screening, ranking and prioritization purposes.
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