

Environmental impact of metal-bearing nanoparticles and graphene family nanomaterials

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The development of Nanotechnology and Nanoscience has resulted in the synthesis of innovative nanomaterials (NMs) with unique properties and the appearance of new consumer products useful for a variety of biomedical, textile, cosmetic, food and other industrial applications. However, the increased use of NMs could pose a risk for the environment and human health. NMs can interact with biological systems but there is still limited information on their fate, distribution and toxicity to living organisms. Among NMs, metal and metal oxide nanoparticles (NPs) are already present in thousands of consumer products. Similarly, carbon-based NMs such as graphene and its derivatives graphene oxide (GO) and reduced GO (rGO) show outstanding structural, electrical, optical, catalytic and mechanical properties and are thus increasingly used in many applications, which could lead to their release into the environment. Further, graphene NMs can adsorb organic pollutants, hence they could act as carriers of these pollutants to organisms.

In this work, we investigated the bioavailability and adverse effects of water-borne metal-bearing NPs (Ag, Au, CdS, CuO, SiO₂, TiO₂ and ZnO) in mussels *Mytilus galloprovincialis* and zebrafish *Danio rerio*, as sentinel and model aquatic organisms, in comparison to bulk and ionic forms of the same metals. For this, both *in vitro* and *in vivo* approaches were applied. *In vitro* cell-based assays and embryo toxicity tests provide quick and reproducible high throughput tools for the screening of NP toxicity and mechanisms of action. *In vivo* experiments allow assessing adverse effects using a battery of molecular, cellular and tissue-level biomarkers. Results allowed to classify studied NPs based on their toxicity to mussel cells *in vitro* and to zebrafish embryos. Overall, NP forms were less toxic than ionic forms but more toxic than the bulk forms, suggesting that observed responses were partly due to dissolved metals. NP toxicity depended on their physico-chemical properties and their behaviour in exposure media (aggregation, dissolution). Ag, CdS and CuO NPs were the most toxic NPs tested and SiO₂ NPs the least toxic. Size, mode of synthesis and the presence of additives influenced NPs toxicity. Common mechanisms of action of Ag, CdS and CuO NPs were ROS production and oxidative stress, DNA damage and activation of lysosomal activity and MXR transport activity in mussel cells. Effects on hemocyte phagocytic activity were particle specific on exposure to CdS and Ag NPs. In zebrafish, Ag, CdS and CuO NPs were internalized and caused an array of sublethal effects from changes in liver transcriptome to gill histopathologies. In a separate set of experiments of dietary exposure to 5 nm Ag NPs, food web transfer of Ag NPs was demonstrated, from the microalgae *Isochrysis galbana* to mussels and from the crustacean *Artemia* brine shrimp to zebrafish, with subsequent deleterious effects on exposed organisms and also on their offspring (embryo malformations). Finally, a similar *in vitro* and *in vivo* approach is being adopted to assess the internalization and toxicity of graphene derivatives. GO, GO-PVP and rGO-PVP showed low and dose-dependent cytotoxicity to mussel hemocytes, increased ROS production and caused a significant decrease in plasma membrane (PM) integrity. Graphene nanoplatelets were internalized by disrupting hemocytes' PM and by endocytic mechanisms. In exposures of hemocytes to graphene with adsorbed oil compounds, these increased nanoplatelets toxicity indicating that graphene nanoplatelets may act as "Trojan horse" carriers of oil compounds. Overall, obtained data may contribute to the risk assessment of nanomaterials in the environment and to the development of safe-by-design approaches to produce safer environmentally sustainable nanomaterials.

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