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BEAM INTERACTIONS WITH MATERIALS AND ATOMS

Nuclear microscopy as a tool in TiO_2 nanoparticles bioaccumulation studies in aquatic species

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ABSTRACT

Engineered Titanium nanoparticles are used for a wide range of applications from coatings, sunscreen cosmetic additives to solar cells or water treatment agents. Inevitably environmental exposure can be expected and data on the ecotoxicological evaluation of nanoparticles are still scarce.

The potential effects of nanoparticles of titanium dioxide (TiO_2) on two model organisms, the water flea, *Daphnia magna* and the duckweed *Lemna minor*, were examined in semichronic toxicity tests.

Daphnia and *Lemna* were exposed to TiO_2 nanoparticles (average particle size value of 28 ± 11 nm ($n = 42$); concentration range, 1.4–25 mg/L) by dietary route and growth in medium containing the nanoparticles of TiO_2, respectively. Both morphology and microdistribution of Ti in the individuals were examined by nuclear microscopy techniques. A significant amount of TiO_2 was found accumulated in *Daphnia* exposed to nanoparticles. Nuclear microscopy imaging revealed that Ti was localized only in the digestive tract of the *Daphnia*, which displayed difficulty in eliminating the nanoparticles from their body. *Daphnia* showed higher mortality when exposed to higher concentrations of TiO_2 (>10 mg/L). The exposure to TiO_2 nanoparticles above 25 mg/L caused morphological alterations in *Lemna*. The roots became stiff and fronds colorless. The Ti mapping of cross-sections of roots and fronds showed that Ti was mainly deposited in the epidermis of the fronds and roots, with minor internalization.

In summary, exposure of aquatic organisms to TiO_2 nanoparticles may alter the physiology of these organisms at individual and population levels, posing risks to aquatic ecosystems.

1. Introduction

Nanotechnology industry is still in an exponential growth phase and panoply of applications is foreseen while information on the new properties and on potential benefits of engineered nanoparticles and nanomaterials has been spread. But the sustainable development of nanotechnology needs understanding on the environmental effects of nanomaterials. The new properties are mainly particle size-dependent and despite the good news, no doubt about the need for new knowledge on the safety, fate, behavior and biological effects of the engineered nanoparticles on organisms and ecosystems. Titanium dioxide nanoparticles (TiO_2-NP) have been used extensively for a wide range of applications [1] namely in self-cleaning surface coatings, light emitting diodes, solar cells, disinfectant sprays, sporting goods, water treatment agents and topical sunscreens. Due to the widespread use, increased environmental exposure to TiO_2-NP can be expected. The stability of TiO_2-NP aqueous suspensions, through sedimentation and aggregation kinetics, have been investigated [2] and is a critical step when evaluating engineered nanoparticles and nanomaterials toxicity to aquatic species. The effects of TiO_2-NP to freshwater aquatic organisms have been reported on bacteria, algae, crustaceans and fish [3–15]). Anyway consensual data on the ecotoxicological evaluation of nanoparticles are still scarce.

Bioassays are regularly used to assess effects of toxicants on living organisms and to determine levels of the substance in tissue samples. *Daphnia* and *Lemna* are widely used in ecotoxicity bioassays. *Daphnia magna* is the first used model organism in aquatic assays. *D. magna*, the freshwater water flea, is a cladoceran reaching a size of 5 mm, with a relatively short life span of about 2 months. *Lemna minor*, the duckweed, is being used as a model for superior plants. *L. minor* is a floating aquatic plant with an aerial part, which consists of fronds floating on the water and a hanging root that may grow to 2–3 cm long.

Nuclear microprobe techniques became a valuable tool in Biology as they provide detailed morpho-physiological information about cells and tissues and enable 2D elemental mapping of elements [16–18]. These techniques have been used previously to study the physiological elemental distribution in plants [19–21]

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