Environmental Pollution 158 (2010) 1748–1755

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Sublethal toxicity of nano-titanium dioxide and carbon nanotubes in a sediment dwelling marine polychaete

Tamara Galloway [a,*], Ceri Lewis [a], Ida Dolciotti [b], Blair D. Johnston [a], Julian Moger [c], Francesco Regoli [b]

[a] School of Biosciences, Hatherly Laboratories, University of Exeter, EX4 4PS, Exeter, Devon, UK
[b] Università Politecnica delle Marche, Institute of Biology and Genetics, Via Ranieri, Monte Dago,60121 Ancona, Italy
[c] School of Physics, Stocker Road, University of Exeter, Devon EX4 4QL, UK

This study explores the hypothesis that nano-TiO_2 and single walled nanotubes (SWNT) can cause sublethal impacts to Arenicola marina exposed through natural sediments.

A R T I C L E I N F O

Article history:
Received 25 August 2009
Received in revised form
6 November 2009
Accepted 12 November 2009

Keywords:
Ecotoxicology
Arenicola marina
Nanoparticle
Coherent anti-stokes Raman microscopy
Genotoxicity

A B S T R A C T

The ecotoxicology of manufactured nanoparticles (MNPs) in estuarine environments is not well understood. Here we explore the hypothesis that nanoTiO_2 and single walled nanotubes (SWNT) cause sublethal impacts to the infaunal species *Arenicola marina* (lugworm) exposed through natural sediments. Using a 10 day OECD/ASTM 1990 acute toxicity test, no significant effects were seen for SWNT up to 0.03 g/kg and no uptake of SWNTs into tissues was observed. A significant decrease in casting rate ($P = 0.018$), increase in cellular damage ($P = 0.04$) and DNA damage in coelomocytes ($P = 0.008$) was measured for nanoTiO_2, with a preliminary LOEC of 1 g/kg. Coherent anti-stokes Raman scattering microscopy (CARS) located aggregates of TiO_2 of $>$200 nm within the lumen of the gut and adhered to the outer epithelium of the worms, although no visible uptake of particles into tissues was detected.
© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Materials engineered at the nanoscale are increasingly used in a wide range of industries, and nanotechnology is a major scientific and economic growth area. Significant release of manufactured nanoparticles (MNPs) into the environment appears an inevitable consequence of their widespread use and varied applications (Owen and Depledge, 2005; Galloway, 2008). Despite this, the environmental fate and biotic impact of MNPs remains uncertain, with a paucity of ecotoxicological data available (RCEP, 2008). An inherent difficulty in assessing the impact of MNPs is that their diverse chemical properties and hence toxicity will change in the aquatic environment, including partitioning to sediment and suspended particulate matter, biological and abiotic degradation, agglomeration and aggregation. Aggregation decreases the interfacial free energy and hence reactivity of particles (He and Zhao, 2005) and could in addition have pronounced effects on MNP uptake and cellular localisation, particularly if aggregated MNPs fail to cross the membrane barrier (Adams and Rowland, 1993). It is therefore vital to consider the behaviour of MNPs in the environmental matrix of interest when evaluating their potential toxic responses.

Colloidal behaviour can help to predict the behaviour of nanoparticles released into the aquatic environment. Insoluble MNPs can form colloidal suspensions, the stability of which is determined by the interaction between attractive and repulsive forces between charged particle surfaces interacting through a liquid medium (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). Reducing or eliminating the charge, for example through an increase in ionic strength will cause colloids to agglomerate or form an interconnected matrix. In high cation environments such as marine aquatic systems and estuaries, colloids comprised of MNPs including titanium dioxide, fullerenes, iron and carbon nanotubes (Dunphy Guzman et al., 2006; Hyung et al., 2007) will aggregate to some extent and form large agglomerates which will tend to settle out of solution (Stolpe and Hassellov, 2007). The extent of aggregation will in turn depend on the physico-chemical characteristics of the particles themselves (including size and surface characteristics) and the local environment (pH, ionic strength and organic carbon content)(Dunphy Guzman et al., 2006; Hyung et al., 2007; Phenrat et al., 2007). Whilst information on the interaction of MNPs, either in free or aggregated form, with sediments and

* Corresponding author. Tel.: +44 1392 263436; fax: +44 1392 263700.
E-mail address: t.s.galloway@exeter.ac.uk (T. Galloway).

0269-7491/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2009.11.013

suspended solids is limited, it can be predicted that they will associate with bed sediments, with uncertain toxicological consequences (Boxall, 2007; Brant et al., 2007).

Here we study two MNPs, chosen for their widespread use and toxicological concern. Titanium dioxide (TiO_2) is of global importance as a sunscreen and pigment and its physico-chemical properties are widely documented. Micron TiO_2 is considered inert, but nanoTiO_2 is highly photoactive. In aqueous media this facilitates the transformation of water molecules adsorbed to the particle surface, yielding hydroxyl radicals capable of causing oxidative damage to cellular components and to DNA (Wang et al., 2007). NanoTiO_2 has been reported to cause oxidative damage in both mammalian and fish systems, including inflammation, cellular and genetic damage, both with and without exposure to ultraviolet A (UVA) radiation (Reeves et al., 2008). Available ecotoxicology data suggests sublethal toxicity in the µg mg/l range, although little is known of the toxicity of particles when exposed through sediments (Boxall, 2007).

Carbon nanotubes have wide-ranging industrial and commercial applications. They are amongst the least biodegradable of man-made materials (Lam et al., 2004), insoluble in water and lipophilic by nature (Wu et al., 2006). High dispersion rates and rapid formation of micrometer range aggregates are reported for single walled carbon nanotubes (SWCNTs) in aqueous media (Cheng and Cheng, 2005). Some studies have reported uptake of SWCNTs into cells where they may induce cellular damage. Sublethal toxic effects to aquatic organisms have been reported for fish and invertebrates in the mg/L range (Smith et al., 2007; Roberts et al., 2007; Templeton et al., 2006). Little is known of the behaviour of SWCNTs in marine sediments. Petersen et al. (2008) report uptake and depuration of SWCNTs from sediment to the oligochaete *Lumbriculus variegatus*, and that the SWCNTs were not readily taken up into the organism's tissues.

The traditional sentinel species for sediment toxicity testing is the polychaete worm *Arenicola marina* (lugworm). Polychaetes form the dominant infaunal biomass of many mud flats and estuaries, and are important vectors for the transfer of sediment-associated contaminants to higher trophic levels, since they form the primary food source for many commercial fish and crustaceans. *A. marina* is a detritus feeder which ingests massive volumes of sediment to extract organic matter from digestible detritus, microbes and benthic micro-organisms. There is therefore potential for large scale exposure of *A. marina* to any nanoparticles residing in sediments (Lewis and Galloway, 2008).

Here we investigate the potential for manufactured nanoparticles in marine sediments to cause sublethal toxicity to the infaunal polychaete *A. marina*, using the 10-day acute toxicity test according to OECD/ASTM 1990 guidelines.

2. Materials and methods

2.1. Chemicals

Single walled nanotubes SWNT (90%, cat no: 652512-250 MG) and nanoTiO_2 (99.9%, cat. No. 634662-100G, mixed anatase and rutile) were from Sigma–Aldrich (UK). Elemental analysis was provided by the manufacturer, size and surface area was determined in house. SWCNTs had C 96.32%, Al 0.08%, Cl 0.40%, Co 2.90%, S 0.29%, outer diameter 1–2 nm, length 0.5–2.0 µm (TEM), mean surface area 420 m^2/g (XRD). TiO_2 had K 82.3 ppm, Zn 9.7 ppm, Na 6.0 ppm, Fe 3.1 ppm, Li 0.4 ppm, primary crystallite size 23.2 nm, equivalent spherical diameter 32.4 nm, specific surface area 46.3 m^2/g.

2.2. Collection and maintenance of animals

Adult *A. marina* were collected from Mothercombe beach, England (50°18″41 N, 3°56″45 W), during April 2008 and maintained in well-aerated artificial seawater (salinity 36 ppt) at 12 °C for 48 h.

2.3. Preparation of sediment

Natural sediment collected from the same site was used. Sediment was sieved (2 mm) and incubated at 12 ± 1 °C, 30 ppt salinity for 24 h. Aliquots ($n = 3$) of sediment were dried overnight at 150 °C then assessed for grain size using a sieve shaker (Fritsch Analysette 03.502; amplitude 10, 15 min). Samples for total organic carbon analysis were freeze dried for 4 days, incubated at 450 °C, 24 h and loss of mass on ignition used to determine the organic carbon content (Heiri et al., 2001).

Exposures concentrations were based on previous literature reports and were in 1 kg sediment per 2 L glass beaker (approx, depth of 10 cm sediment, 6 cm water). Each sediment was prepared with either SWNT (0.003–0.03 g/kg), nanoTiO_2 (1–3 g/kg, verified to ± 80% by ICP–OES), seawater alone or bulk (micron scale) TiO_2 (3 g/kg). Nine animals for each treatment, replicated ×3, were exposed in individual beakers. Nanoparticles were sonicated (Sonicor TS9045 high voltage ultrasonic bath) in 200 mL Milli Q water for 30 min and added to beakers to give the required final concentrations. The contents of each beaker were stirred by hand for 15 min and artificial seawater (at higher salinity) added to a final volume of 1.5 L, salinity 36 ppt, maintained at 15 °C for 24 h prior to the addition of animals.

2.4. Chemical analysis

Sediment samples were oven dried and 500 mg samples digested with 3 ml HNO_3 at room temperature for 3 weeks, after which 1 ml H_2O_2 and 2 ml HNO_3 was added. Samples were digested for 5 h at 70 °C, centrifuged at 3000× g for 5 min, dried at 105 °C overnight and the residue made up to 10 ml in dH_2O prior to analysis by inductively coupled plasma–optical emission spectrometry (ICP–OES). For analysis of tissues, animals were placed for 24 h in constantly aerated clean seawater, rinsed with dH_2O and dried at 70 °C for 48 h, before being weighed and transferred to pyrex tubes for acid digestion and analysis, as above. Water samples were treated as for sediments except the second digestion was for 24 h at 190 °C. Biota-sediment accumulation factors were calculated as the ratio of the concentration in tissues normalised to lipid content (assuming an average lipid content of 5% across the course of the exposure) to the concentration in the sediment normalised by its organic carbon content.

2.5. In vivo exposures

Animals were exposed for 10 days at 15 °C, 8 h light: 16 h dark. Animals were fed every other day with 1% *Isochyrsis* sp. solution and feeding behaviour monitored every 48 h. Casts were collected, dried overnight and weighed. Seawater was changed every 48 h following cast collection.

2.6. Behavioural assay

The OECD/ICES *A. marine* burrowing bioassay was followed (Adams and Rowland, 1993). After 10 days, animals were removed from exposure sediments and their ability to re-bury into clean sediment assessed. Animals were then transferred to clean seawater, and held for 24 h to void any sediment in their guts (Lewis and Galloway, 2008).

2.7. Lysosomal membrane stability assay

The neutral red retention (NRR) assay was adapted from Marchi et al. (2004). Coelomic cells were collected by syringe into chilled anticoagulant, pH 7.3 (Lewis and Galloway, 2008). Cell suspension, 40 µL, was placed onto poly-L-lysine coated slides, transferred to a light-proof humidity chamber for 30 min, after which excess fluid was removed to leave a monolayer of cells and 40 µL of 0.33% neutral red solution (in PBS) was pipetted onto the cells. After 15 min, slides were examined under a light microscope every 30 min. The time taken for 50% of the cells to shows signs of dye leakage was recorded for each sample.

2.8. Comet assay

Coelomocytes were collected as above and checked for viability with Eosin Y (all > 90% viability). The Comet assay measures DNA damage as single and double strand breaks and was performed using alkaline conditions at 5 °C (Lewis and Galloway, 2008). Briefly: 1 h lysis, followed by 45 min denaturation in electrophoresis buffer (0.3 M NaOH and 1 mM EDTA), electrophoresis 30 min at 25 V and 300 mA followed by neutralisation. Slides were stained with 20 mgL^{-1} ethidium bromide and examined using a fluorescent microscope (excitation: 420–490 nm; emission: 520 nm). The percent DNA in the comet tail in 100 cells per preparation was quantified using Kinetic V COMET Software.

2.9. Gut histology

A. marina from the seawater control, the highest SWNT exposure (0.03 mg/g) and highest TiO_2 exposure (3 mg/g) were narcotized by immersion in 7% $MgCl_2$ in seawater. Animals were cut longitudinally with fine scissors and their guts carefully dissected, washed with 0.1 M phosphate buffer (pH 7.4) and fixed in 3%

Download English Version:

https://daneshyari.com/en/article/4426164

Download Persian Version:

https://daneshyari.com/article/4426164

Daneshyari.com