Science of the Total Environment 493 (2014) 891–897

Contents lists available at ScienceDirect

Science of the Total Environment

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

Size-, surface- and crystalline structure composition-related effects of titanium dioxide nanoparticles during their aquatic life cycle

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HIGHLIGHTS

- $nTiO_2$ toxicity is triggered inter alia by its initial particle size and surface area.
- Crystalline structure composition of $nTiO_2$ product affects its ecotoxicological potential.
- Toxic potential of $nTiO_2$ decreases during its aquatic life cycle (=after sedimentation).
- $nTiO_2$ toxicity differs among representatives of different spatial and ecological niches.

ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 19 June 2014
Accepted 20 June 2014
Available online xxxx

Editor: D. Barcelo

Keywords:
Daphnia magna
Gammarus fossarum
Crystallinity
Toxicity
Crustacea

ABSTRACT

Nanoparticle toxicity depends amongst others on particle characteristics and nanoparticle behavior during their aquatic life cycle. Aquatic organisms may be exposed to nanoparticle agglomerates of varying size, while lager agglomerates after settling rather affect benthic organisms. In this context, the present study systematically examined the role of particle characteristics, i.e. crystalline structure composition (anatase as well as mixture of anatase-rutile), initial particle size (55-, 100-, and 140-nm) and surface area, in the toxicity of titanium dioxide nanoparticles ($nTiO_2$) to the pelagic filter feeder *Daphnia magna* (n = 4) and the benthic amphipod *Gammarus fossarum* (n = 30). Smaller initial particle sizes (i.e. 55-nm) and anatase based particles showed an approximately 90% lower *Daphnia* EC_{50}-value compared to its respective counterpart. Most importantly, particle surface normalized EC_{50}-values significantly differed for nanoparticles equal to or below 100 nm in size from 140-nm sized particles. Hence, these data suggest that the reactive initial surface area may explain the ecotoxicological potential of different particle size classes only if their size is smaller or around 100 nm. In contrast to *Daphnia*, *Gammarus* was not affected by $nTiO_2$ concentrations of up to 5.00 mg/L, irrespective of their characteristics. This indicates fundamental differences in the toxicity of $nTiO_2$ during its aquatic life cycle mediated by alterations in their characteristics over time.

1. Introduction

The utilization of engineered nanoparticles is still increasing and expected to reach a $2.4 trillion contribution to the global economy by 2015 (Pearce, 2012). Amongst others titanium dioxide nanoparticles ($nTiO_2$) are heavily used as they have multiple advantageous properties (Fujishima et al., 2000; Schulz et al., 2002), making them a desirable additive for care-, remediation- and self-cleaning products (Di Paola et al., 2012; Kaegi et al., 2008; Sun et al., 2007). This frequent application at high quantities (Scheringer, 2008) inevitably results in $nTiO_2$-release into aquatic ecosystems for example through wastewater treatment plant effluents (Klaine et al., 2011; Westerhoff et al., 2011), wash-off from facades (Kaegi et al., 2008) or major accidents during transport (Nowack et al., 2014).

In this context, scientists investigated the acute and chronic ecotoxicological potential of $nTiO_2$ on aquatic organisms mainly employing the standard test organism *Daphnia magna* (e.g. Dabrunz et al., 2011; Dalai et al., 2013). These studies exhibited median effective and lethal concentrations ranging from low mg/L to g/L levels (cf. Dabrunz et al., 2011; Heinlaan et al., 2008). This broad range of $nTiO_2$ concentrations causing adverse effects among different studies is frequently attributed to varying particle properties such as initial particle size, surface area and crystalline structure composition, but was not yet empirically underpinned (cf. Dabrunz et al., 2011; Seitz et al., 2013). Moreover, once introduced into the aquatic environment, $nTiO_2$ start their aquatic life cycle being subjected to transformation processes that may have substantial implications on their fate and toxicity (Fig. 1). In this regard,

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http://dx.doi.org/10.1016/j.scitotenv.2014.06.092
0048-9697/

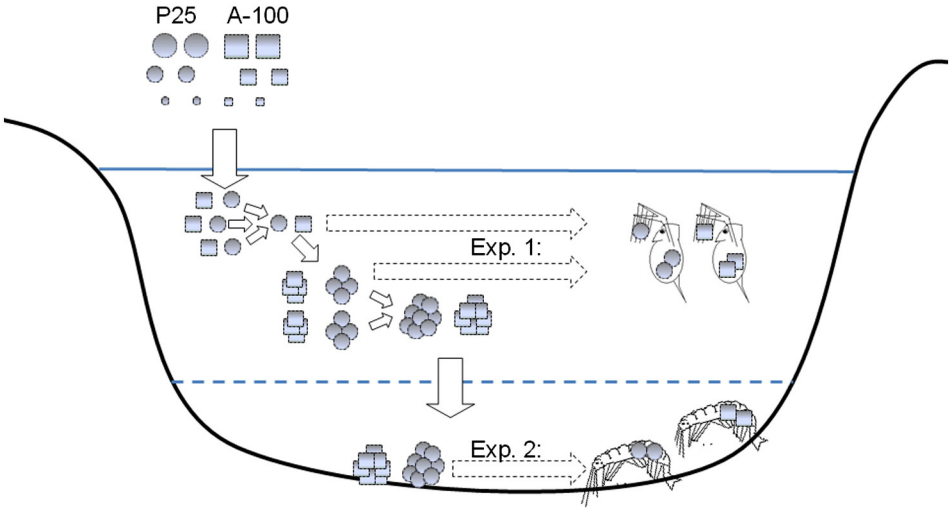

Fig. 1. Experimental derivation based on the aquatic life cycle of $nTiO_2$ varying in initial size and crystallinestructure composition (P25 and A-100). Experiment 1 covers potential particle characteristic and small agglomerate related effects of $nTiO_2$ towards pelagic living organisms at an early stage of nanoparticle life cycle. Experiment 2 focuses a later stage of the latter named and hence potential toxic effects on benthic organisms after nanoparticle agglomeration and sedimentation.

the agglomeration of particles (triggered for instance by the ionic strength in the surface water (Petosa et al., 2010)) affects their sedimentation as previously shown for instance by Dabrunz et al. (2011). This suggests that $nTiO_2$ pose initially, and hence directly following their release into the aquatic ecosystem, a risk for pelagic organisms such as daphnids (Dabrunz et al., 2011; Li et al., 2014a). At the later stages of their aquatic life cycle, $nTiO_2$ will settle down as a result of agglomeration processes potentially threatening benthic life (e.g. leaf shredding amphipods) (Bundschuh et al., 2011b; Li et al., 2014b; Li et al., 2014c). However, virtually nothing is known on how nanoparticles, in particular $nTiO_2$, with differing initial characteristics (e.g. initial particle size, surface area and crystalline structure composition) alter their ecotoxicological potential in the course of this aquatic life cycle.

Therefore, the present study assessed the role of $nTiO_2$'s initial size, total initial surface area, and crystalline structure composition systematically on its toxicity to the pelagic filter feeder *D. magna* and the benthic leaf shredding amphipod *Gammarus fossarum*. The scenarios were achieved by applying the $nTiO_2$ products P25 and A-100, which differed in their crystalline structure composition either containing a mixture of anatase (70%) and rutile (30%) or exclusively anatase (99%), respectively, at three initial particle size classes each (55-, 100-, 140-nm), which were chosen based on published studies (Dabrunz et al., 2011). Both test species experienced similar static $nTiO_2$ exposure conditions. While daphnids were checked after 96 h for immobilization, for gammarids, a sublethal response in the species' feeding rate on leaf material was chosen as endpoint since it is robust, sensitive and ecologically meaningful (Maltby et al., 2002).

2. Materials and methods

2.1. Nanoparticle characterization

Both titanium dioxide products were purchased as powders, either from Evonik (P25, Germany) or Crenox GmbH (A-100, Germany), featuring an advertised primary particle size of 21 nm and 6 nm, for P25 (~70% anatase and ~30% rutile) and A-100 (99% anatase), respectively. Their advertised surface area is approximately 50 (P25) and 230 m^2/g (A-100). In order to compensate for the differences in the respective advertised primary particle sizes among both materials for each product, dispersant and additive free, size homogenized, stable suspensions of three particle size classes (namely 55-, 100- and 140-nm) were obtained by stirred media milling (PML 2, Bühler AG, Switzerland). Subsequently centrifugation was accomplished in order to remove residual coarse material. Prior to their application each stock suspension was analyzed for its particle size distribution (intensity weighted) as well as its average initial particle surface area per volume (cf. Treuel et al., 2010) using dynamic light scattering (DelsaNano C, Beckman Coulter, Germany) and nanoparticle tracking analysis (LM20, NanoSight Ltd., United Kingdom), respectively (Table 1). Additionally, scanning electron microscope analyses were performed to verify the initial particle size of each applied $nTiO_2$ product (Fig. S-1 A–F). Moreover, the average particle size in the test medium was monitored after 24 h and at test termination of all bioassays (Table 1). However for the particle size monitoring, 3-mL samples were taken 2 cm beneath the water surface (= middle of the water column) from the center of one randomly selected replicate of a 2 mg $nTiO_2$/L concentration (enabling a sufficient intensity) ensuring a reliable monitoring of $nTiO_2$ over the whole study duration.

2.2. Test organisms

D. magna (Eurofins-GAB, Germany) were kept in permanent culture within climate controlled chambers (Weiss Environmental Technology Inc., Germany) with a 16:8 h (light:dark) photoperiod at 20 ± 1 °C. Organisms were cultured in groups of 25 animals using 1.5 L reconstituted hard freshwater (= ASTM-M) according to the ASTM International standard guide E729 (ASTM, 2007) enriched with selenium, vitamins (thiamine hydrochloride, cyanocobalamine, biotine) and seaweed extract (Marinure®, Glenside, Scotland; cf. Seitz et al., 2013). The medium was renewed three times a week, while daphnids were fed with the green algae *Desmodesmus* sp. on a daily basis with an equivalent of 200 μg C per organism.

G. fossarum were obtained from the Hainbach (a near natural stream close to Landau, Germany; 49° 14′ 19″ N, 8° 02′ 59″ E) and stepwise acclimatized to reconstituted water (SAM-5S Borgmann et al., 1998) as well as given laboratory conditions. For toxicity testing only male gammarids (identified by their position in the precopular pair) with a cephalothorax diameter between 1.6 and 2.0 mm were used, whereas organisms were sorted using a passive underwater separation technique (Franke, 1977). During their acclimatization, gammarids were fed ad libitum with preconditioned black alder leafs (*Alnus glutinosa* L. Gaertn.).

2.3. Preparation of leaf disks

Leaf disks, which served as food during the feeding activity tests with *Gammarus*, were prepared similar to the method described by