

# Assessment of Crystal Morphology on Uptake, Particle Dissolution, and Toxicity of Nanoscale Titanium Dioxide on *Artemia Salina*

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## ABSTRACT

Knowledge of nanomaterial toxicity is critical to avoid adverse effects on human and environment health. In this study, the influences of crystal morphology on physico-chemical and toxic properties of nanoscale TiO<sub>2</sub> (*n*-TiO<sub>2</sub>) were investigated. *Artemia salina* were exposed to anatase, rutile and mixture polymorphs of *n*-TiO<sub>2</sub> in seawater. Short-term (24 h) and long-term (96 h) exposures were conducted in 1, 10 and 100 mg/L suspensions of *n*-TiO<sub>2</sub> in the presence and absence of food. Anatase form had highest accumulation followed by mixture and rutile. Presence of food greatly reduced accumulation. *n*-TiO<sub>2</sub> dissolution was not significant in seawater ( $p < 0.05$ ) nor was influenced from crystal structure. Highest toxic effects occurred in 96h exposure in the order of anatase>mixture>rutile. Mortality and oxidative stress levels increased with increasing *n*-TiO<sub>2</sub> concentration and exposure time ( $p < 0.05$ ). Presence of food in the exposure medium alleviated the oxidative stress, indicating deprivation from food could promote toxic effects of *n*-TiO<sub>2</sub> under long-term exposure.

## KEYWORDS

Anatase, Artemia Salina, Crystal Morphology, Nanoscale Titanium Oxide, Particle Dissolution, Rutile, Toxicity

## INTRODUCTION

Our understanding about the environmental and human health effects of nanoscale materials is still in its infancy despite growing research exploring the environmental and biological fate and toxicity of nanomaterials (Scown, van Aerle & Tyler, 2010). This is mainly because the physico-chemical and toxicological properties of materials at nanoscale are a complex phenomenon governed by numerous parameters. Nanomaterials possess very large surfaces (e.g., more atoms per unit area) owing to their extremely small size (1-100 nm), which evidently results in larger contact surfaces and liberation of more toxic elements and ions upon degradation, respectively. Particle size, shape/morphology, surface charge and methods of synthesis also impart significant differences in toxicological properties of nanomaterials (Hu et. al, 2009; Huang, Wu & Aronstam, 2010). Moreover, the physiochemical conditions of test environment (e.g., salinity, pH, temperature, light etc.) and the resilience of model organism may alter observed effects.

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Titanium dioxide nanoparticles ( $n\text{-TiO}_2$ ) exhibit photocatalytic and antibacterial properties under UV-light (Lai et al., 2008). In recent years,  $n\text{-TiO}_2$  have been extensively used in various consumer products, including sunscreens, toothpaste, food additives, paints, surface coatings, water disinfection and degradation of pollutants in air and soil (Nowack & Bucheli, 2007; Battin et al., 2009; Weir et al., 2012; Jovanovi, 2015).  $n\text{-TiO}_2$  is considered an aquatic pollutant due to numerous exterior uses and applications in water treatment. Nevertheless, the information about safety of  $n\text{-TiO}_2$  to aquatic environments and species is controversial though it is among the earliest nanoscale compounds investigated extensively so far (Jovanovi, 2015). Some groups did not observe any adverse effects on different biological species (Lovern & Klaper, 2006; Lai et al., 2008; Aruoja et al., 2009; Zhu et al., 2011; Fang et al., 2015), while some others reported dose dependent toxic effects (Heinlaan et al., 2008; Zhu et al., 2010; Ates et al., 2013a; Mansfield et al., 2015). For instance,  $n\text{-TiO}_2$  was reported to be more toxic to microalgae *Pseudokirchneriella subcapitata* ( $\text{LC}_{50} = 5.83 \text{ mg L}^{-1}$ ) than bulk  $\text{TiO}_2$  ( $\text{LC}_{50} = 35.9 \text{ mg L}^{-1}$ ) (Aruoja et al., 2009). In contrast, no apparent toxicity was observed from  $n\text{-TiO}_2$  to crustaceans *Daphnia magna* and *Thamnocephalus paltryrus*, and the bacteria *Vibrio fischeri* (Heinlaan et al., 2008), but previously exposed *Daphnia magna* offsprings were found more sensitive to  $n\text{-TiO}_2$  (Bundschuh et al., 2012). Similarly, aqueous suspensions of  $n\text{-TiO}_2$  were not acutely toxic but induced oxidative stress on marine abalone (*Haliotis diversicolor supertexta*) (Zhu et al., 2011). Japanese medaka (*Oryzias latipes*) embryos showed premature hatching and high mortality from chronic exposure to  $n\text{-TiO}_2$  (Paterson et al., 2011). Toxicity to rainbow trout (*Oncorhynchus mykiss*) varied with the route of exposure (Handy et al., 2008). Direct exposure to  $n\text{-TiO}_2$  colloids caused severe adverse effects whereas no acute toxicity was detected from dietary exposure.

Titanium dioxide ( $\text{TiO}_2$ ) is found in nature in three major polymorphs; anatase, rutile and brookite that exhibit varying degrees of photo-catalytic properties (Liu et al., 2012). Rutile and anatase are most abundant. Of these, rutile is also known as the most stable and benign crystalline form of  $\text{TiO}_2$ . Anatase is a less dense, softer form of  $\text{TiO}_2$  that possess higher catalytic activity owing relatively larger band gap energy (about 3.0 eV for rutile and 3.2 eV for anatase) which imparts more oxidation power and deeper surface activity of the electrons of anatase than rutile (Liu et al., 20012; Luttrell et al., 2014). The literature regarding the toxicological effects of  $n\text{-TiO}_2$  clearly show that observed effects vary substantially with concentration, species or test models, and duration of exposure. In addition, in most studies the  $n\text{-TiO}_2$  utilized was either purely anatase (Paterson et al., 2011; Mansfield et al., 2015), purely rutile (Ates et al., 2013a), or a mixture of rutile and anatase (Zhu et al., 2010; Kim et al., 2010). It is, however, difficult to pinpoint the influence of crystal morphology (e.g., crystal phase) on the overall toxicity of  $n\text{-TiO}_2$  among totally different test environments and organisms/species. Anatase form was found more toxic than anatase/rutile mixture to human lung epithelial cells (Hsiao & Huang, 2011). DeMatteis et al. (2016) also reported that anatase polymorph could be more prone to degradation and physicochemical stresses (pH, light exposure) to cause higher toxic effects. Therefore, there is a need to investigate different crystal polymorphs  $n\text{-TiO}_2$  systematically and simultaneously to elucidate their physicochemical and toxicological properties.

In this study, we performed exposures on brine shrimp (*Artemia salina*) with anatase, rutile polymorphs of  $n\text{-TiO}_2$  and their mixture (anatase/rutile) in an attempt to determine the influences of crystal polymorph on particle uptake, degradation (ion release), agglomeration, and toxicity. In an acute exposure, *Artemia salina* larvae were exposed to 1, 10 and 100 mg/L aqueous suspensions of anatase, rutile, and the anatase/rutile mixture of  $n\text{-TiO}_2$  in seawater for 24 and 96 h under 16 h light and 8 h dark regime. Accumulation (e.g., total body burden) and free Ti ion levels were determined by inductively coupled plasma mass spectrometry (ICP-MS) analyses of exposed artemia and exposure medium (seawater) samples, respectively. Toxic effects were examined by determining mortality and malondialdehyde (MDA) levels as a lipid peroxidation biomarker in tissues of exposed artemia.

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