Journal of Advances in Environmental Health Research

Acute toxicity of titanium dioxide nanoparticles in Daphnia magna and Pontogammarus maeoticus

Seyed Ali Johari[1], Saba Asghari[1]

1 Department of Fisheries, School of Natural Resources, University of Kurdistan, Sanandaj, Iran

Original Article

Abstract

Titanium dioxide nanoparticles ($nTiO_2$) are the world's second most widely consumed nanomaterial and large quantities of this material enters the aquatic ecosystem annually. Therefore, understanding the effects of $nTiO_2$ on aquatic organisms is very important. The present study used Daphnia magna as a model freshwater organism and Pontogammarus maeoticus as a brackish water organism to evaluate short term toxicity of a well characterized $nTiO_2$ suspension. According to the results, acute exposure of D. magna and P. maeoticus to $nTiO_2$ concentrations ranging from 0.1 to 200 mg/l did not cause any mortality; therefore, lethal concentrations could not be calculated [lethal concentrations (LC) > 200 mg/l]. Observations showed that the TiO_2 nanoparticles were trapped on the surface of the body, under the carapace, and in the gut of the D. magna. Although the results of the present acute toxicity experiment did not show $nTiO_2$ to be toxic to the tested aquatic organisms in an environmentally relevant concentration, further studies are needed on the chronic effects of lower concentrations of this nanomaterial in simulated natural ecosystems.

KEYWORDS: Acute, Amphipoda, Cladocera, Nanoparticles, Titanium Dioxide, Toxicity

Date of submission: 14 Oct 2014, ***Date of acceptance:*** 12 Jan 2015

Citation: Johari SA, Asghari S. **Acute toxicity of titanium dioxide nanoparticles in Daphnia magna and Pontogammarus maeoticus.** J Adv Environ Health Res 2015; 3(2): 111-9.

Introduction

The increasing use of engineered nanomaterials in human life and industrial applications is currently showing inventory listings of 1628 nanotechnology-based consumer products.[1] According to estimations, the worth of nanoproducts will be about $3 trillion by 2020.[2] The 3 most common nanomaterial mentioned in consumer product inventories are silver, titanium, and carbon with 383, 179, and 87 products, respectively.[1]

Nanomaterials have many advantages in terms of life and livelihood improvement, but they may also cause risks to humans and the environment. That is why it is important to recognize the adverse effects of nanomaterials, an issue which is addressed in "nanotoxicology". In addition, part of the engineered nanomaterials produced globally will reach water bodies; 0.4–7% according to Keller et al.[3] Thus, understanding the effect of these substances on aquatic organisms is very important, an issue which is addressed in "aquatic nanotoxicology". Titanium dioxide nanoparticles ($nTiO_2$) is important because of its application in self-cleaning windows, coatings, paints, UV-absorbent cosmetics, and also antimicrobial and antifouling coatings.[4,5] According to estimates, it appears that the

Corresponding Author:
Seyed Ali Johari
Email: a.johari@uok.ac.ir

concentration of nano-TiO_2 in aquatic environment is between 0.7 to 16.8 micrograms per liter.[6,7] Moreover, an estimated 15600 tons of nano-TiO_2 enter aquatic ecosystems annually worldwide.[3] Therefore, understanding the effects of nano-TiO_2 on aquatic organisms is critical.

Daphnia magna is very sensitive to pollutants and is a suitable aquatic organism through which to evaluate the toxic effects of chemicals in freshwater environment. Therefore, standard toxicity test methods on this aquatic animal have been developed by organizations such as the Organization for Economic Cooperation and Development (OECD), US Environmental Protection Agency (EPA), and International Standards Organization (ISO).[8,9]

There are several recent publications about the toxic effects of some nanomaterials on amphipods.[10-20] Most of these studies show that these sediment-dwelling organisms are likely to have a high potential for exposure to nanomaterial, are highly susceptible to the effects of nanomaterial, and should be considered in the risk assessment of these substances. Gammaridae is a family of amphipods that lives in a wide range of salinities in sea coasts as well as brackish and freshwater environments and generally feed on detritus and herbal materials. Pontogammarus maeoticus is distributed in the Ponto-Caspian region including the Caspian, Azov, and Black Seas.[21] This benthic infauna specie can be found in abundance in the brackish water of Iranian coasts of the Caspian Sea, and usually feeds on detritus.[22] This aquatic organism itself is an important prey for important commercial fish of the Caspian Sea, including sturgeons. To our knowledge, only two studies on nano-TiO_2 toxicity in gammarids have been conducted on a freshwater gammarus (Gammarus fossarum) and there is no information on nano-TiO_2 toxicity in brackish water gammarids.[11,17]

Therefore, in the present study, we used D. magna as a standard freshwater organism and Pontogammarus maeoticus as a brackish water organism to evaluate the short term toxicity of nano-TiO_2 suspension.

Materials and Methods

Powdered TiO_2 nanoparticles ($nTiO_2$) were purchased from US Research Nanomaterials, Inc. (Houston, USA). According to information provided by the manufacturer, this white powder consists of more than 99% pure anatase TiO_2 nanoparticles with average size of 10-25 nm. A stock suspension of 400 mg/l was prepared by dispersing 40 mg of this powder in 100 ml distilled deionized water following vigorous vortex (Thermo Scientific M37610) for 30 minutes at room temperature, and then, sonication for 6 hours in a bath-type sonicator (Branson 8510EXT-0011). This suspension was not very stable; therefore, it was sonicated for a further 15 minutes before every use. The pH of the final suspension was 6.02.

Transmission electron microscopy (TEM) analyses of the dry $nTiO_2$ powder and its suspension were performed using an H-7100FA transmission electron microscope (Hitachi, Japan) with an acceleration voltage of 125 kV. For each type, the diameters of 80 randomly selected particles were measured at magnifications of 100,000 using Axio Vision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany). Energy dispersive X-ray (EDX) analyses of dry $nTiO_2$ powder and its suspension were performed using an EX200 energy-dispersive X-ray analyzer (Horiba, Japan). Absorption spectral measurements of $nTiO_2$ suspension were obtained using a Spectra-MAX-PLUS 384 UV–visible spectrophotometer (Molecular Devices, USA) in a range of 190-1000 nm.

Daphnia acute toxicity tests

Acute (48 hours) toxicity experiments were performed according to the "Daphnia Sp. acute immobilization test" (OECD test guideline number 202).[8] The M4 media was used as exposing media and all exposure solutions were prepared immediately before starting the tests through diluting the nano-TiO_2 stock in fully aerated M4 media. The concentrations used for daphnia acute toxicity tests were 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 150, and 200 mg/l nano-TiO_2 and M4 media without the addition of nanoparticles in the control groups. After adding appropriate amounts of stock to M4 media, the stock mixtures were continuously stirred with a magnetic stirrer to distribute the suspension at stable concentration to the extent possible. For each concentration, 10 randomly selected neonates, which were younger than 24 hours old, were placed in glass beakers containing 100 ml of exposing media in triplicate. The animals were not fed during the experiments, and all tests were conducted in a water bath system with a constant temperature (20 ± 2 °C) and 16-hour light/8-hour dark photoperiods. After 24 and 48 hours of exposure, any immobilization and mortality of the daphnids in test beakers were assessed according to Annex 1 of OECD 211 based upon which an animal can be taken as dead when it is immobile (i.e., when it is not able to swim) or there is no observed movement of post-abdomen or appendages within 15 seconds after gentle agitation of the test container.[23] It should be noted that, for greater certainty of the results, the tests were continued for 2 more days (a total of 96 hours). Furthermore, the visible adsorption and uptake of TiO_2 particles by D. magna were observed and recorded using a microscope (Olympus CX41) equipped with a digital camera (DIXI 3000 mega pixel, NEK Corp., Germany).

Gammarus acute toxicity tests

The samples of the gammarid specie of P. maeoticus were collected 1 week before the beginning of the experiment from the coast of the Caspian Sea near Noor city, Iran, far from any settlement and agricultural activity (36° 35' 1.8'' N, 52° 2' 32.8'' E). In the laboratory, gammarids were kept in aerated sea water with a salinity of 12 g/l (salinity of their living area in the South of the Caspian Sea) at a constant temperature of 20 ± 1 °C. They were fed lettuce leaves ad libitum until the beginning of the experiment. Only adults with a body length between 8 and 10 mm were used for toxicity experiments. This range was selected to minimize the effect of body size on the results of the experiments.

Test organisms were placed in 200 ml glass beakers containing 10 organisms and 150 ml of freshly prepared test solution. All the tests were conducted in a water bath system with a constant temperature (20 ± 1 °C) and 16-hour light/8-hour dark cycles. Feeding of organisms was stopped 6 hours before the beginning of toxicity tests and the animals were not fed during the bioassays. In this study, fully aerated sea water was used as the exposure media and the test solutions were prepared immediately prior to use by diluting the nano-TiO_2 stock in the sea water. After adding appropriate amounts of the stock to the sea water, the stock mixtures were stirred using a magnetic stirrer to distribute the suspension at as stable a concentration as possible. The concentration used for gammarus acute toxicity tests were 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 150, and 200 mg/l nano-TiO_2. Each bioassay included a completely random design, consisting of treatments and controls in triplicate. To evaluate the toxicity of each chemical, the mortality of the gammarids in each test beaker were assessed after 24, 48, 72, and 96 hours of exposure.

Results and Discussion

Particle characterization

In the case of dry $nTiO_2$ powder observed by TEM, most of the particles were needle shaped (Figure 1. A). Moreover, 86.23% of the particles had diameters between 5 and 30 nm (Figure 2.), and only 5.07% of the particles had diameters of more than 50 nm with a maximum diameter of

81 nm. The count median diameter (CMD) for the particles was 13.90 nm (Figure 3). Furthermore, the geometric mean diameter (GMD) and geometric standard deviation (GSD) of dry $nTiO_2$ powder were 17.50 nm and 1.71, respectively. In the case of $nTiO_2$ suspension, TEM images showed that in an aqueous environment the nanoparticles clump and form large aggregates (Figure 1. B). About 28.47% of these clumps were 15 to 100 nm, 52.55% were 100 to 500 nm, and 18.98% were above 500 nm. As seen in figure 4, EDX analysis confirmed that only elemental titanium was presented in $nTiO_2$ powder. Spectral scans of the sonicated $nTiO_2$ suspension gave the typical profile expected with a distinct peak at about 330 nm (Figure 5) and was similar to previous reports for TiO_2 nanoparticles.[24,25]

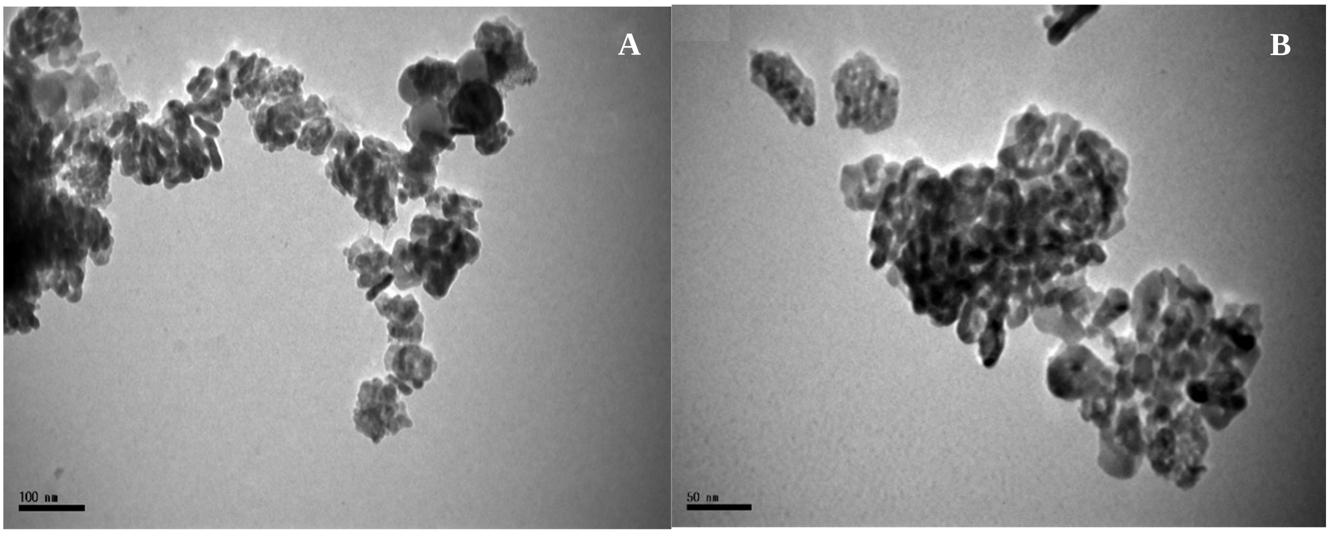

Figure 1. Transmission electron micrographs of dry titanium dioxide nanoparticles ($nTiO_2$) powder (A) and aqueous suspension of $nTiO_2$ (B)

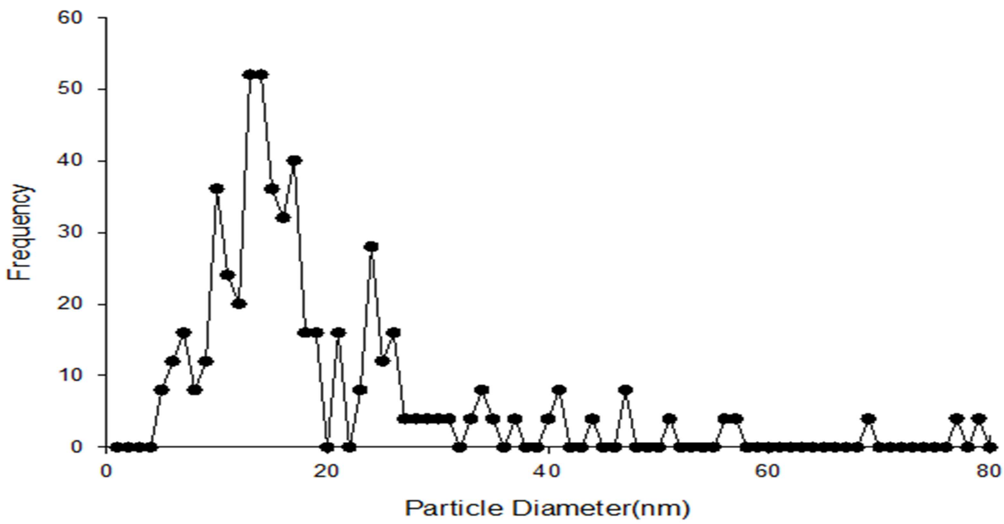

Figure 2. Size distribution of particles based on number frequency determined from transmission electron microscope data in dry titanium dioxide nanoparticles ($nTiO_2$) powder

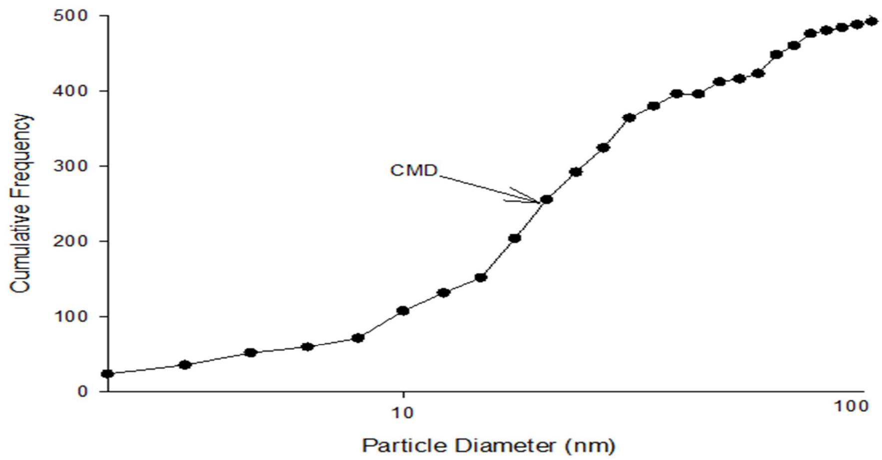

Figure 3. Size distribution of particles based on cumulative frequency determined from transmission electron microscope data in dry titanium dioxide nanoparticles ($nTiO_2$) powder

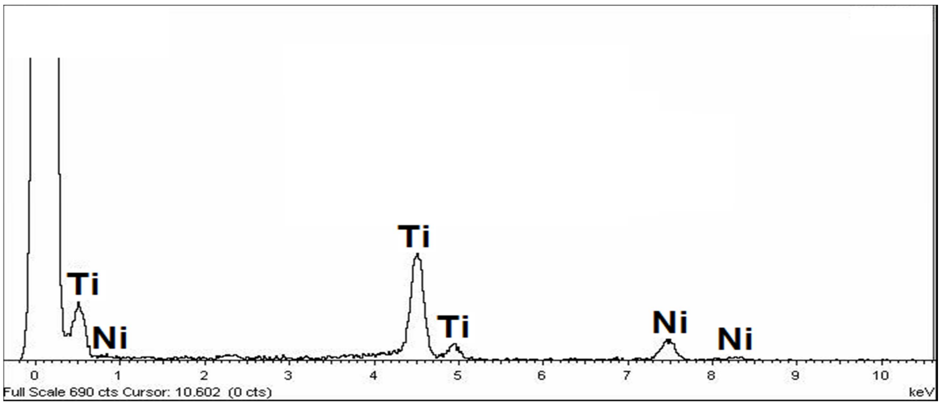

Figure 4. Energy dispersive X-ray (EDX) spectrometer patterns of dry titanium dioxide nanoparticles ($nTiO_2$) powder [Ni signals in EDX spectrometer are from transmission electron microscopy (TEM) grid]

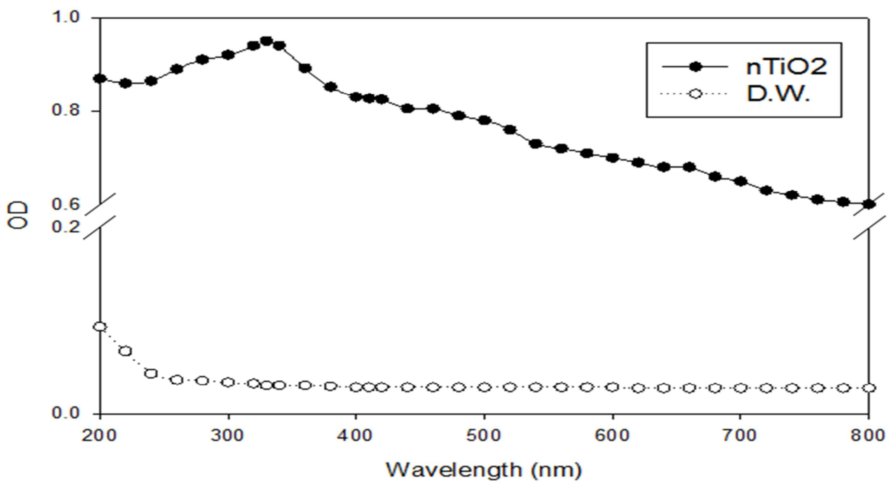

Figure 5. UV–VIS absorption spectra of suspensions of titanium dioxide nanoparticles (nTiO2) and distilled water (D.W.)

Effects of TiO2 nanoparticles on daphnids and gammarids

During the experiments, the mean ± SD of the water pH and dissolved oxygen in the exposure vessels of daphnids were 7.81 ± 0.13 and 7.44 ± 0.19 mg/l, respectively. Moreover, the mean ± SD of the water pH and dissolved oxygen in the exposure vessels of gammarids were 8.3 ± 0.1 and 8.4 ± 0.3 mg/l, respectively. Both in the freshwater (daphnia medium) and brackish water (gammarus medium), sediments of aggregated TiO_2 nanoparticles became gradually visible at the bottom of the test beakers with time lapse. During the exposure period, the rate of mortalities in the control groups of daphnids and gammarids was less than 5% and 10%, respectively. According to the results of this study, short term exposure of D. magna to $nTiO_2$ concentrations ranging from 0.1 to 200 mg/l did not cause any mortality even after a longer testing time of up to 96 hour. Thus, we could not calculate lethal concentration of $nTiO_2$ for daphnids [lethal concentrations (LC) > 200 mg/l]. In addition, similar results were obtained in toxicity experiments on P. maeoticus within 96 hours of exposure to $nTiO_2$. Therefore, $nTiO_2$ suspension prepared by sonication in this study did not cause any mortality in D. magna and P. maeoticus during 96 hours of exposure, and therefore, median lethal concentrations (LC_{50}s) were estimated to be greater than 200 mg/l. Generally, LC_{50} data provides a good baseline for toxicity tests. According to the US EPA toxicity categories, European Union legislation, and European Union Council Directive 67/548/EEC of 27 June 1967, any substance with a short term LC_{50} of greater than 100 mg/l must be classified as “practically non-toxic” to aquatic organisms.[26,27] Furthermore, according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), any substance with a short term LC_{50} of greater than 100 mg/l must be classified as “category acute 4” to aquatic organisms and may have long lasting harmful effects on aquatic life.[28] The results of several studies on the acute toxicity of different $nTiO_2$ in Daphnia magna are summarized in table 1. As can be seen, most of these studies found acute effect levels of $nTiO_2$ at greater than 100 mg/l, and this is in agreement with our results. The only studies in which the calculated LC_{50} values were less than 100 mg/l were those by Garcia et al.[29] who had used a laboratory synthesized nano-TiO_2 which stabilized using tetramethylammonium hydroxide (TMAOH), and Lovern and Klaper [24] who examined the toxicity of nano-TiO_2 suspension after filtration. However, in another study by Wiench et al.,[30] the calculated LC_{50} of $nTiO_2$ was greater than 100 mg/l even after filtration. As mentioned before, no information is available on the acute toxicity of $nTiO_2$ on gammarids. Nevertheless, in the case of amphipods, acute toxicity of nano-TiO_2 was evaluated in Hyalella azteca by Li et al.[18] According to their results, under a laboratory light, nano-TiO_2 showed low toxicity in H. azteca (LC_{50} = 631 mg/l). However, in the presence of simulated solar radiation, nano-TiO_2 toxicity in this amphipod showed a 21-fold increase compared to a standard laboratory light (LC_{50} = 29.9 mg/l). Although this increase did not make nano-TiO_2 toxic to H. azteca in an environmentally relevant concentration, but this shows the importance of conducting toxicity experiments in natural conditions.

After exposing daphnia to $nTiO_2$ suspensions, some pigmentation was visible in parts of the brood chamber which was not observable in controls (Figure 6). These pigments can be a sign of nanoparticle accumulation under the carapace. Moreover, at higher concentrations, aggregates of nanoparticles were attached to the external surface and appendages of D. magna (Figure 6). In addition, large amounts of dark material were found in the gut tract of daphnia after nanoparticle exposure.

Consequently, the $nTiO_2$ tested in this study can clearly be ingested by D. magna, and therefore, an accumulation could occur in the gut. In some cases, the ingestion of particles was enough to prevent movement of the daphnia through the water column and caused them to sink to the bottom of the beakers. Similar results were observed in our previous study on toxicity of silver nanoparticles in D. magna.[31] These results suggest that exposure of aquatic organisms to such nanoparticles could pose a risk of bioaccumulation, especially for filter-feeding copepods such as D. magna.

Table 1. A summary of the results of several studies on acute toxicity of titanium dioxide nanoparticles ($nTiO_2$) in Daphnia magna

Average size of TiO_2 particles in exposure media	Source of nanoparticles	Suspension/preparation method	48-hour LC_{50} (mg/l)	Reference
15 to 100 nm (28.47%) 100 to 500 nm (52.55%) > 500 nm (18.98%)	Commercial powder	Sonication	> 200	Present study
10 nm	Synthesized in the lab	Stabilizer (TMAOH)	16	29
> 580	Commercial powder	Sonication	> 100	32
n.d.	Commercial powder	Shaking	143.4	33
n.d.	Commercial powder	Sonication	> 250	34
< 100 nm to clumps larger than 200 µm	Commercial powder	Stirring, sonication, or filtration	> 100	30
140 nm	n.d.	Not specified	> 100	35
30 nm	Commercial powder	Filtration	5.5	24
Larger clumps (100 to 500 nm)	Commercial powder	Sonication	> 500	24

n.d. = Not determined; TMAOH: Tetramethylammonium hydroxide; LC_{50}: Lethal concentrations

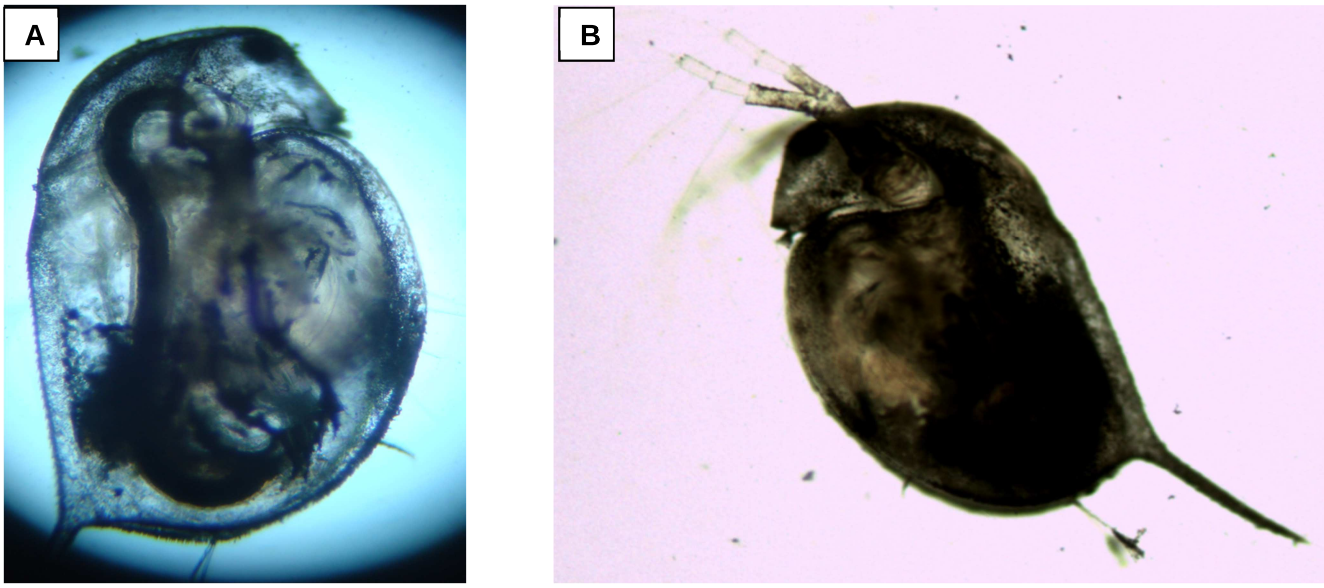

Figure 6. The uptake and adsorption of titanium dioxide (TiO_2) particles by Daphnia magna 24 hours after exposure to aqueous suspension of Titanium dioxide nanoparticles ($nTiO_2$) (100 mg/l) (Particles of $nTiO_2$ are observable on the whole body surface, in the brood chamber, and carapace. In addition, the dark appearance of the gut tract shows that $nTiO_2$ can be ingested by daphnia.)

Conclusion

As mentioned in the introduction, the estimated concentration of nano-TiO_2 in aquatic environments is currently less than 0.02 mg/l. Therefore, according to the results of the present lab scale study, it seems that this nanomaterial is not an acute hazard for aquatic ecosystems. However, it should be noted that this nanomaterial may have chronic toxic effects on aquatic organisms at low concentrations as it is currently present in the aquatic environment. Therefore, more studies are needed to evaluate the chronic aquatic toxicity of $nTiO_2$ in environmentally relevant concentrations. In addition, toxicity evaluation of this nanomaterial in a simulated natural ecosystem is required.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

We gratefully acknowledge the support of Prof. Il Je Yu from Hoseo University for TEM imaging.

References

1. Wilson Database. Consumer Products Inventory: An inventory of nanotechnology-based consumer products introduced on the market [Online]. [cited 2015]; Available from: URL: http://www.nanotechproject.org/cpi/
2. Roco MC. The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. Journal of Nanoparticle Research 2011; 13(2): 427-45.
3. Keller AA, McFerran S, Lazareva A, Suh S. Global life cycle releases of engineered nanomaterials. Journal of Nanoparticle Research 2013; 15: 1692.
4. Parkin IP, Palgrave RG. Self-cleaning coatings. J Mater Chem 2005; 15: 1689-95.
5. Huang Z, Maness PC, Blake DM, Wolfrum EJ, Smolinski SL, Jacoby WA. Bactericidal mode of titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology A: Chemistry 2000; 130(2-3): 163-70.
6. Mueller NC, Nowack B. Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 2008; 42(12): 4447-53.
7. O'Brien N, Cummins E. Ranking initial environmental and human health risk resulting from environmentally relevant nanomaterials. J Environ Sci Health A Tox Hazard Subst Environ Eng 2010; 45(8): 992-1007.
8. OECD. OECD Guidelines for the Testing of Chemicals OECD Guidelines for the Testing of Chemicals Fifteenth Addendum No: 202. Paris, France: OECD Publishing; 2004.
9. Baun A, Hartmann NB, Grieger K, Kusk KO. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 2008; 17(5): 387-95.
10. Kennedy AJ, Hull MS, Steevens JA, Dontsova KM, Chappell MA, Gunter JC, et al. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. Environ Toxicol Chem 2008; 27(9): 1932-41.
11. Bundschuh M, Zubrod JP, Englert D, Seitz F, Rosenfeldt RR, Schulz R. Effects of nano-TiO(2) in combination with ambient UV-irradiation on a leaf shredding amphipod. Chemosphere 2011; 85(10): 1563-7.
12. Mwangi JN, Wang N, Ritts A, Kunz JL, Ingersoll CG, Li H, et al. Toxicity of silicon carbide nanowires to sediment-dwelling invertebrates in water or sediment exposures. Environ Toxicol Chem 2011; 30(4): 981-7.
13. Fabrega J, Tantra R, Amer A, Stolpe B, Tomkins J, Fry T, et al. Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod Corophium volutator. Environ Sci Technol 2012; 46(2): 1128-35.
14. Jackson BP, Bugge D, Ranville JF, Chen CY. Bioavailability, toxicity, and bioaccumulation of quantum dot nanoparticles to the amphipod Leptocheirus plumulosus. Environ Sci Technol 2012; 46(10): 5550-6.
15. Hanna SK, Miller RJ, Zhou D, Keller AA, Lenihan HS. Accumulation and toxicity of metal oxide nanoparticles in a soft-sediment estuarine amphipod. Aquat Toxicol 2013; 142-143: 441-6.
16. Poynton HC, Lazorchak JM, Impellitteri CA, Blalock B, Smith ME, Struewing K, et al. Toxicity and transcriptomic analysis in Hyalella azteca suggests increased exposure and susceptibility of epibenthic organisms to zinc oxide nanoparticles. Environ Sci Technol 2013; 47(16): 9453-60.
17. Kalcikova G, Englert D, Rosenfeldt RR, Seitz F, Schulz R, Bundschuh M. Combined effect of UV-irradiation and TiO(2)-nanoparticles on the predator-

prey interaction of gammarids and mayfly nymphs. Environ Pollut 2014; 186: 136-40.
18. Li S, Wallis LK, Ma H, Diamond SA. Phototoxicity of TiO(2) nanoparticles to a freshwater benthic amphipod: are benthic systems at risk? Sci Total Environ 2014; 466-467: 800-8.
19. Li S, Wallis LK, Diamond SA, Ma H, Hoff DJ. Species sensitivity and dependence on exposure conditions impacting the phototoxicity of TiO(2) nanoparticles to benthic organisms. Environ Toxicol Chem 2014; 33(7): 1563-9.
20. Park S, Woodhall J, Ma G, Veinot JG, Cresser MS, Boxall AB. Regulatory ecotoxicity testing of engineered nanoparticles: are the results relevant to the natural environment? Nanotoxicology 2014; 8(5): 583-92.
21. Barnard JL, Barnard CM. Freshwater Amphipoda of the World: Handbook and bibliography, Volume 2. Vernon, VA: Hayfield Associates; 1983.
22. Mirzajania AR. A study on the population biology of Pontogammarus maeoticus (Sowinsky, 1894) in Bandar Anzali, southwest Caspian Sea. Zoology in the Middle East 2003; 30(1): 61-8.
23. OECD. OECD Guidelines for the Testing of Chemicals / Section 2: Effects on Biotic Systems Test No. 211: Daphnia magna Reproduction Test. Paris, France: OECD Publishing; 2008.
24. Lovern SB, Klaper R. Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environ Toxicol Chem 2006; 25(4): 1132-7.
25. Federici G, Shaw BJ, Handy RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol 2007; 84(4): 415-30.
26. Directive 1999/45/EC. General classification and labeling requirements for dangerous substances and preparations (Annex VI) [Online]. [cited 1999]; Available from: URL: ec.europa.eu/environment/archives/dansub/pdfs/annex6_en.pdf
27. EUR-Lex. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 [Online]. [cited 2008 Dec 12]; Available from: URL: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32008R1272
28. UNECE. Globally Harmonized System of Classification and Labelling of Chemicals (GHS, Rev.4). New York, NY: United Nations; 2011.
29. Garcia A, Espinosa R, Delgado L, Casals E, Gonzalez E, Puntes V, et al. Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. Desalination 2011; 269(1-3): 136-41.
30. Wiench K, Wohlleben W, Hisgen V, Radke K, Salinas E, Zok S, et al. Acute and chronic effects of nano- and non-nano-scale TiO(2) and ZnO particles on mobility and reproduction of the freshwater invertebrate Daphnia magna. Chemosphere 2009; 76(10): 1356-65.
31. Asghari S, Johari SA, Lee JH, Kim YS, Jeon YB, Choi HJ, et al. Toxicity of various silver nanoparticles compared to silver ions in Daphnia magna. J Nanobiotechnology 2012; 10: 14.
32. Zhu X, Chang Y, Chen Y. Toxicity and bioaccumulation of TiO2 nanoparticle aggregates in Daphnia magna. Chemosphere 2010; 78(3): 209-15.
33. Zhu X, Zhu L, Chen Y, Tian S. Acute toxicities of six manufactured nanomaterial suspensions to Daphnia magna. Journal of Nanoparticle Research 2008; 11(1): 67-75.
34. Strigul N, Vaccari L, Galdun C, Wazne M, Liu X, Christodoulatos C, et al. Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to Daphnia magna and Vibrio fischeri. Desalination 2009; 248(1-3): 771-82.
35. Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicol Lett 2007; 171(3): 99-110.