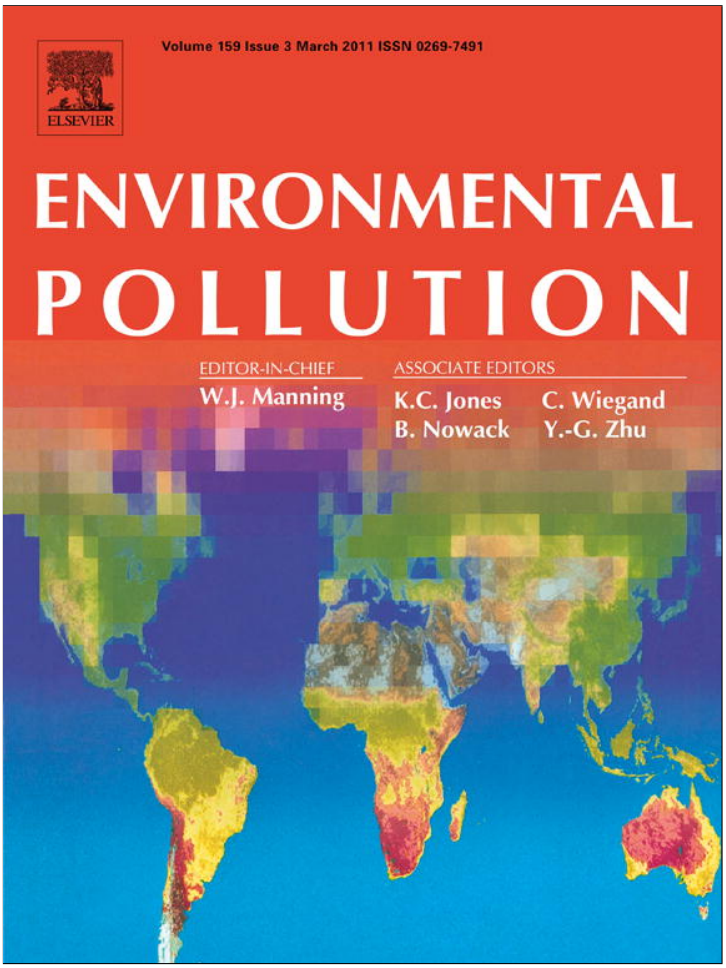

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Review

Ecotoxicity of nanosized TiO_2. Review of in vivo data

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Ecotoxicity of nanosized TiO_2.

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ABSTRACT

This report presents an exhaustive literature review of data on the effect of nanoparticulate TiO_2 on algae, higher plants, aquatic and terrestrial invertebrates and freshwater fish. The aim, to identify the biologically important characteristics of the nanoparticles that have most biological significance, was unsuccessful, no discernable correlation between primary particle size and toxic effect being apparent. Secondary particle size and particle surface area may be relevant to biological potential of nanoparticles, but insufficient confirmatory data exist. The nanotoxicity data from thirteen studies fail to reveal the characteristics actually responsible for their biological reactivity because reported nanotoxicity studies rarely carry information on the physicochemical characteristics of the nanoparticles tested. A number of practical measures are suggested which should support the generation of reliable QSAR models and so overcome this data inadequacy.

1. Introduction

The potential of nanoparticles (NPs) or nanomaterials to react with biological systems has been recognized in recent years and a number of (eco)toxicity studies of these emerging pollutants have appeared. Considerable attention was paid to fullerenes (C_{60}), carbon nanotubes, quantum dots, metal oxides such as TiO_2, ZnO, Fe_2O_3, Fe_3O_4, CuO, CeO_2, SiO_2 and Al_2O_3, and nanoparticulate metals such as Au, Ag, Co, and Ni. From the perspective of ecotoxicity, titanium dioxide (TiO_2) nanoparticles are by far the most extensively studied metal oxide nanoparticles (Cattaneo et al., 2009; Kahru and Dubourguier, 2010). One of the reasons for the large amount of (eco)toxicity data on nanosized TiO_2 is the adoption of this nanomaterial by a variety of industries; nanosized TiO_2 was among the first nanomaterials made readily commercially available to a wide variety of research activities.

TiO_2 is a naturally occurring mineral that can exist in three crystalline forms, known as rutile, anatase, and brookite, and in an amorphous form (Reyes-Coronado et al., 2008). The element titanium is also found in ilmenite ($FeTiO_3$) and other minerals and ores, processing of which can be produce TiO_2 (NRC, 1999). Rutile phase is the most common form of TiO_2 found in nature (EPA, 2009). Commercial production of nano-TiO_2 between 2006 and 2010 has been estimated at 5000 metric tons per year, more than 10 000 metric tons per year between 2011 and 2014 (UNEP, 2007) and approximately 2.5 million metric tons by 2025 (Robichaud et al., 2009).

Anatase phase exhibits the highest photocatalytic activity and because of that it is used in catalysis and photocatalysis applications. Rutile is known as white pigment providing opacity to paints, papers, inks, and consumer products such as toothpaste. In cosmetic products, rutile phase is used as a pigment and thickener and it is used in plastics and other applications for its ultraviolet (UV) light absorbing properties (Mueller and Nowack, 2008). Anatase and brookite are used as electrodes in dye-sensitized solar cells (Jiang et al., 2002). Such properties have led to use of nano-TiO_2 for a wide variety of applications, including self-cleaning surface coatings, light-emitting diodes, solar cells, disinfectant sprays, sporting goods, water treatment agents and topical sunscreens (EPA, 2009). Such widespread use of nanosized TiO_2 could lead to significant release of nano-TiO_2 into the environment leading to a potential for increased environmental exposure to TiO_2 nanoparticles (Hall et al., 2009).

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Nanosized TiO_2 is used extensively in sunscreen cosmetics as an inorganic UV absorbant that can allow a film transparent to visible light to be applied to human skin (Jaroenworaluck et al., 2005). A surface coating, for example silica and other compounds, can also be added to nanosized TiO_2 to decrease its photoreactivity so that nano-TiO_2 can be used to protect human skin, plastic, and other objects from UV radiation (EPA, 2009). Other surface modification of TiO_2 nanoparticles, for example with polyaniline also improves the applicability of nano-TiO_2 in conductive coating, charge storage, electrochromic activities, photovoltaic properties, electrocatalytic applications, and absorption materials of solar cell (Li et al., 2003).

Currently very few data exist regarding observed environmental concentrations of TiO_2 nanoparticles (Griffitt et al., 2008). Evidence that TiO_2 nanoparticles can leach from exterior facade paints and discharge into surface waters has been provided. The concentrations of metallic Ti found in the surface runoff were as high as 600 μg/L (Kaegi et al., 2008). Kiser et al. (2009) has measured the levels of titanium nanomaterial removal and release from wastewater treatment plants. They found out that raw sewage contains 100–3000 μg/L of Ti. Concentrations of Ti in effluents from wastewater treatment plants ranged from <5 to 15 μg/L. As Ti is removed, it accumulates in settled solids with concentrations ranging from 1 to 6 μg/mg. Two studies modelled the quantities of TiO_2 nanoparticles released into the environment (Mueller and Nowack, 2008; Gottschalk et al., 2009) and the predicted environmental concentrations are presented in Table 1. The predicted concentrations of nanosized TiO_2 arising from use in consumer products were 24.5 μg/L for water and 1030 μg/kg for soil (Boxall et al., 2007). It is estimated that once nanoparticles are introduced into water, they will most probably aggregate and partition to sediment and suspended particulate matter (Boxall et al., 2007). Aggregated particles are generally less mobile and can interact with filter feeders and sediment-dwelling organisms (Farré et al., 2009). The extent of aggregation is governed by pH, ionic strength, and the nature of the electrolytes (Navarro et al., 2008; Sharma, 2009). Humic acids have been shown to significantly influence the aggregation of nano-TiO_2 (Pettibone et al., 2008; Domingos et al., 2009).

Much knowledge already exists on the effects of TiO_2 nanoparticles on biological systems. Nano-TiO_2 is photoinducible, redox active and thus a generator of potential reactive oxygen species (ROS) at its surfaces. Nano-TiO_2 has been shown to generate ROS in the presence of UV light (Armelao et al., 2007) or in its absence (Reeves et al., 2008) of UV light. The precise mechanisms of toxicity of nanosized TiO_2 and other metal nanoparticles are largely unknown (Griffitt et al., 2008), but recent studies have shown that the toxicity of nanoparticles is generally governed by properties such as particle size, shape, and surface properties (Crane et al., 2008; Navarro et al., 2008).

There is an emerging literature on the ecotoxicity of nanosized TiO_2, with a majority of the studies dealing with aquatic organisms, *viz.* algae, freshwater invertebrates and fish. Of the algae, invertebrates and fish species tested, freshwater invertebrates, for which currently the most data exists, are the most studied group, followed by algae and finally, freshwater fish. Similar distributions of data between groups of organisms were also observed for other NPs (Cattaneo et al., 2009; Kahru and Dubourguier, 2010). However, almost no information on the toxic effects of TiO_2 nanoparticles on terrestrial, sedimentary, marine species and higher plants is available and further research in this field is needed.

The aim of this paper is to review the nano(eco)toxicity data of nanosized TiO_2 on algae, higher plants, aquatic and terrestrial invertebrates and freshwater fish and to link these data to the physicochemical characteristics of tested nano-TiO_2. We hypothesize that published in vivo studies on nano-TiO_2 would allow identification as to which physicochemical characteristics of nanoparticles are related to their biological effect. We consider the suitability of the existing ecotoxicity protocols to be used also for nanoparticles and discuss some possible future directions in nano (eco)toxicity.

Table 1
Modelled concentrations of TiO_2 nanoparticles released into environmental compartments in different countries.

Environmental compartment	Predicted environmental concentration		
	Switzerland	Europe	U.S.
Water	0.7–16 μg/L[a] 0.016–0.085 μg/L[b]	0.012–0.057 μg/L[b]	0.002–0.010 μg/L[b]
Soil	0.4–4.8 μg/kg[a] 0.21–1.04 μg/kg[b]	1.01–4.45 μg/kg[b]	0.43–2.3 μg/kg[b]
Sludge treated soil	/	70.6–310 μg/kg[b]	34.5–170 μg/kg[b]
Sediment	426–2382 μg/kg[b]	273–1409 μg/kg[b]	44–251 μg/kg[b]
Air	0.0015–0.042 μg/m^3[a] 0.0007–0.003 μg/m^3[b]	0.0005 μg/m^3[b]	0.0005 μg/m^3[b]
Sewage treatment plant effluent	3.50–16.3 μg/L[b]	2.50–10.8 μg/L[b]	1.37–6.70 μg/L[b]
Sewage treatment plant sludge	172–802 mg/kg[b]	100–433 mg/kg[b]	107–523 mg/kg[b]

[a] Mueller and Nowack, 2008.
[b] Gottschalk et al., 2009.

2. Toxicity of nanosized TiO_2 to freshwater algae

The toxicological effects of nano-TiO_2 on algae have been summarised in several papers in recent years (Klaine et al., 2008; Kahru and Dubourguier, 2010). Toxicity to algae has been assessed with three species: *Pseudokirchneriella subcapitata*, *Desmodesmus subspicatus* and *Chlamydomonas reinhardtii* (Available as Table S1 in Supporting Information – SI). Growth inhibition was generally assessed after 72 h (Hund-Rinke and Simon, 2006; Warheit et al., 2007; Blaise et al., 2008; Wang et al., 2008; Aruoja et al., 2009; Hartmann et al., 2010) or 96 h (Griffitt et al., 2008; Hall et al., 2009) and very diverse 72-h EC_{50} values were reported for TiO_2 nanoparticles. For *P. subcapitata* for example, these ranged from 5.83 mg/L of Ti (Aruoja et al., 2009) to 241 mg/L of TiO_2 (Hartmann et al., 2010). The 72-h EC_{50} determined for *D. subspicatus* was 32 mg/L of TiO_2 (particle size of 25 nm, mainly anatase), but when another TiO_2 product (particle size of 100 nm, 100% anatase) was tested, no effect was observed at levels below 50 mg/L of TiO_2 (Hund-Rinke and Simon, 2006).

Extremely variable 72-h EC_{50} and LC_{50} values were reported for TiO_2 nanoparticles tested with *P. subcapitata* (Table 2). No clear relationship between the primary size of particles and effects on algae *P. subcapitata* could be discerned. For example, particles described as less than 100 nm in diameter after filtration were not at all toxic to these algae (Blaise et al., 2008), while sonicated particles with diameters between 25 and 70 nm in diameter were very toxic (Aruoja et al., 2009). In a study where the toxicity of two different sizes (<10 nm and 30 nm) of TiO_2 nanoparticles were compared, no clear relationship between the size and effect was observed (Hartmann et al., 2010). A correlation between specific surface area of the particles and effect concentrations was found (Fig. 1(A)). The toxicity of nano-TiO_2 to algae *P. subcapitata* decreases with increasing specific surface area. For example, the particles with a specific surface area of 5.8 m^2/g are much more toxic to algae *P. subcapitata* than particles with a specific surface area of 288 m^2/g. As can be seen from Fig. 1(B) the effect concentrations as reported by Warheit et al. (2007) could not be linked to the median values of particle size in media. The 380 nm diameter particles in media and particles 140 nm in diameter in media have similar toxicities to algae *P. subcapitata* with 72-h EC_{50} of 16 mg/L

Table 2
Physicochemical properties of nano-TiO_2 and toxicity values for algae *Pseudokirchneriella subcapitata*.

Species	Particle size (nm)[a]	Crystal phase	BET (m^2/g)[b]	DLS (nm)[c]	ZP (mV)[d]	Toxicity value (mg/L)	Ref
P. subcapitata	/	~99% TiO_2 core with ~1% Al surface coating	5.8	~380		EC_{50} = 16 (12–22) (conc. not measured) EC_{50} = 61 (52–72) (nominal conc.)	1
P. subcapitata	/	79% rutile/21% anatase; 90 wt% TiO_2, 7% alumina, 1% amorphous silica	38.5	140	/ /	EC_{50} = 21 (16–26) (conc. not measured) EC_{50} = 87 (83–91) (nominal conc.)	1
P. subcapitata	<10	67.2% anatase/32.8% amorphous	288	1261	−23	EC_{50} = 241 (95.6–609)	2
P. subcapitata	25–70	/	/	/	/	EC_{50} = 5.83 (3.75–7.58)	3
P. subcapitata	30	72.6% anatase/18.4% rutile/9% amorphous	47	416	−21	EC_{50} = 71.1 (59.4–85.1)	2
P. subcapitata	<100	99.9% TiO_2	/	/	/	IC_{25} > 100	4

References: (1) Warheit et al., 2007; (2) Hartmann et al., 2010; (3) Aruoja et al., 2009; (4) Blaise et al., 2008.
Abbreviations/Explanations: / – no data available; EC_{50} = median effective concentration; IC_{25} = 25% inhibition concentration; full species name: *Pseudokirchneriella subcapitata*; () – indicates the 95% confidence intervals.
Particles whose primary particle size was larger than 100 nm were omitted.
[a] Particle size reported by the manufacturer.
[b] Specific surface area measured with Brunauer, Emmett and Teller method (BET).
[c] Median values for particle size in media determined with dynamic light scattering (DLS).
[d] Zeta potential.

and 21 mg/L. The 380 nm particles in media are much more toxic to algae *P. subcapitata* than those of 416 nm and 1261 nm (Fig. 1(B)). No correlation between crystalline form of particles used in the studies and toxicity values for algae could be found (Table 2).

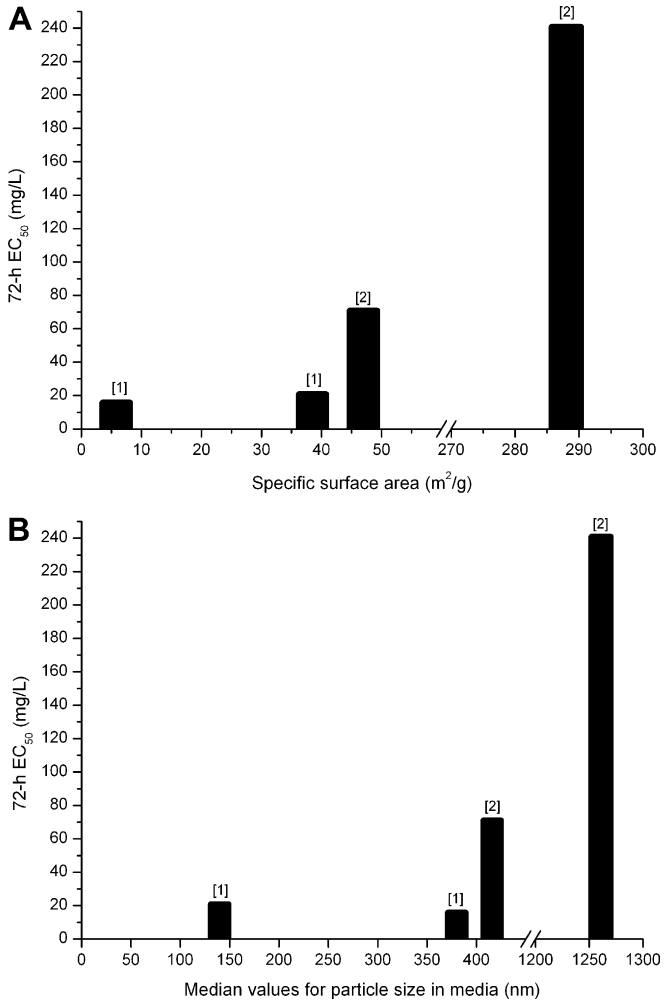

Fig. 1. Relation between 72-h EC_{50} values and specific surface area (measured with Brunauer, Emmett and Teller method (BET)) (A) and median values for particle size in media (determined with dynamic light scattering (DLS)) (B) for nano-TiO_2 for algae *Pseudokirchneriella subcapitata*. References: [1] Warheit et al., 2007; [2] Hartmann et al., 2010.

Studies by Hund-Rinke and Simon (2006), Wang et al. (2008) and Aruoja et al. (2009) in which particles of quite similar sizes were studied with the three different species of algae suggest that *P. subcapitata* is more sensitive to nanosized TiO_2 than *C. reinhardtii*, with *D. subspicatus* the least sensitive. However, in view of the fact that very variable 72-h EC_{50} values, some of them very high, were obtained for *P. subcapitata*, this cannot be confirmed. It will be possible to assess species-specific sensitivity to nanosized TiO_2 only after more data are generated in tests on all three species with nanoparticles prepared similarly.

Effects of nanosized TiO_2 (21 nm, surface area 50 m^2/g) on the unicellular green alga *C. reinhardtii* have been observed. Above 10 mg/L lipid peroxidation was induced and growth was inhibited. The transcriptional expression profiling of stress response genes (*sod1*, *gpx*, *cat*, and *ptox2*) revealed that transient up-regulation occurred in cultures containing as little as 1.0 mg/L of TiO_2 but no major effect on the function of the chloroplast was found (Wang et al., 2008).

Chronic exposure experiments were also performed with algae *P. subcapitata*. No changes in growth were observed after 96 h (Griffitt et al., 2008), but a significant decrease of 25% at 1 mg/L of TiO_2 was reported in a study by Hall et al. (2009) (Table S1 in the SI).

3. Toxicity of nanosized TiO_2 to plants

There is a limited number of studies of plants available. Seeger et al. (2008) tested the toxicity of two types of TiO_2 nanoparticles (Degussa P25, 25 nm in diameter; Hombikat UV100, diameter <10 nm) on willow trees with the short-term test endpoints including: transpiration, growth, and water use efficiency. No significant toxic effects to willow cuttings were found for TiO_2 nanoparticles at concentrations below 100 mg/L. Extensive research on the effects of nanoanatase TiO_2 on the spinach, *Spinacia oleracea* has been performed (Hong et al., 2005; Zheng et al., 2005; Lei et al., 2007). Nanoanatase TiO_2 was shown to promote photosynthesis, improve spinach growth, promote the vigor of aged seeds, and chlorophyll biosynthesis in spinach. The purpose of this work was not to assess the toxic effect, but rather to explore the advantages of such treatment with special emphasis on the activity of the plant's photosynthetic apparatus. No EC_x/LC_x values were derived from these experiments. Toxic effects of nanosized TiO_2 (100 nm) in two plant systems, *Allium cepa* and *Nicotiana tabacum* have been also observed. It was found that nano-TiO_2 induced DNA damage, inhibition of the growth and increased lipid peroxidation in *A. cepa* root at concentration 4 mM (calculated 319 mg/L) and induced DNA damage in *N. tabacum* leaf at 2 mM (calculated

157 mg/L) (Ghosh et al., 2010). In addition, Klančnik et al. (2010) reported that TiO_2 nanoparticles (particle size of 15 nm, anatase) had no effect on macroscopic parameters (numbers roots per bulb, the average length of roots and total length of the root system of each bulb) and microscopic parameters (shares/portions of mitotic phases, chromosome aberrations and micronuclei) of onion *Allium cepa* up to 1 mg/L, but affected the mitotic index in root tips.

4. Toxicity of nanosized TiO_2 to freshwater invertebrates

The (eco)toxicological effects of nano-TiO_2 on freshwater invertebrates have been summarised in several papers in recent years (Baun et al., 2008; Handy et al., 2008; Klaine et al., 2008; Cattaneo et al., 2009; Farré et al., 2009; Kahru and Dubourguier, 2010). There are a lot of data available for freshwater invertebrates (Klaine et al., 2008; Cattaneo et al., 2009) especially for crustaceans *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Chydorus sphaericus*, *Thamnocephalus platyurus*, cnidaria *Hydra attenuata* and midge *Chironomus riparius* (Table S2 in SI).

Extremely variable 48-h EC_{50} and LC_{50} values were reported for TiO_2 nanoparticles in *D. magna*, for which the most data are available (Table 3) as has been reviewed by Kahru and Dubourguier (2010). The values ranged from 5.5 mg/L (Lovern and Klaper, 2006) up to 20 000 mg/L (Heinlaan et al., 2008). No clear conclusions concerning a correlation between the primary size of particles and observed effect could be drawn (Fig. 2(A)). No effects of nano-TiO_2 at levels up to 100 mg/L were observed in the case of coated 10 nm and uncoated 20–30 nm particles (primary size) (Wiench et al., 2009), 25–70 nm (Heinlaan et al., 2008) and 21 nm (Zhu et al., 2010) and no effects up to 10 mg/L for particles less than 40 nm in size (Kim et al., 2010). In contrast, Zhu et al. (2009) observed effects on mortality (48-h $LC_{50} = 143$ mg/L) and immobility (48-h $EC_{50} = 35$ mg/L) in the case of particles of ≤20 nm. Lovern and Klaper (2006) observed significant differences in toxicity to daphnids when filtration of the TiO_2 suspension permitted testing of smaller 30 nm particles. When the suspension of particles was no filtered, aggregates of size 100–500 nm were present and no toxicity to daphnids was observed. The same 48-h EC_{50} values (>100 mg/L) for daphnids were reported for particles of different size and different specific surface areas (Fig. 2(B)). These data fail to show a relation between 48-h EC_{50} values and specific surface area. Below 100 mg/L, no toxicity to daphnids was observed with particles of three different sizes in media. The 48-h EC_{50} values could not be linked to the median values of particle size in media (Fig. 2(C)), but this conclusion, reached on the basis of only two studies, could not be confirmed. No correlation between crystalline form of particles used in the studies and toxicity values for water flea *D. magna* was found (Table 3).

Very high variability of 48-h EC_{50} values was also observed for other crustaceans and no conclusions on the relative sensitivity of certain freshwater invertebrates could be made (Blaise et al., 2008; Griffitt et al., 2008; Heinlaan et al., 2008; Velzeboer et al., 2008; Hall et al., 2009).

Notwithstanding the variable EC_{50} values, it cannot be overlooked that in some cases TiO_2 nanoparticles have proved to be very toxic to freshwater invertebrates. In some studies, low values of toxicity data were found for *Daphnia pulex* (48-h $LC_{50} = 9.2$ mg/L), *Ceriodaphnia dubia* (48-h $LC_{50} = 7.6$ mg/L) (Hall et al., 2009), and *Hydra attenuata* (96-h $EC_{50} = 10$–100 mg/L) (Blaise et al., 2008).

Sublethal effects of nanosized TiO_2 (<25 nm, anatase form) on *Daphnia pulex* after exposure of 24 h have been observed. It was found that nano-TiO_2 at 500 mg/L elevated the activity of the antioxidant enzymes catalase and glutathione-S-transferase and at

Table 3
Physicochemical properties of nano-TiO_2 and toxicity values for the water flea *Daphnia magna*.

Species	Particle size (nm)	Crystal phase	BET (m^2/g)[a]	DLS (nm)[b]	TEM (nm)[c]	Toxicity value (mg/L)	Ref
D. magna	/	/	/	/	30	$LC_{50} = 5.5$	1
D. magna	/	/	/	/	100–500	Could not be assessed	1
D. magna	/	~99% TiO_2 core with ~1% Al surface coating	5.8	~380	/	$EC_{50} > 100$	2
D. magna	/	79% rutile/21% anatase; 90 wt% TiO_2, 7% alumina, 1% amorphous silica,	38.5	140	/	$EC_{50} > 100$	2
D. magna	7[d]	/	300.81	/	/	No effects	3
D. magna	≤20[d]	>99.5% anatase	/	/	/	$LC_{50} = 143.387$ (106.466–202.818) $EC_{50} = 35.306$ (25.627–48.928)	4
D. magna	20[d]	/	66.604	/	/	No effects	3
D. magna	20–30[d]	>99.5% TiO_2; 70% anatase/30% rutile; uncoated	48.6	/	/	$EC_{50} > 100$	5
D. magna	21[d]	20% rutile/80% anatase	50	580.5, 2349.0,3528.6	/	$EC_{50} > 100$ $LC_{50} > 100$	6
D. magna	25[d]	Mainly anatase	/	/	/	Could not be assessed	7
D. magna	25–70[d]	/	/	/	/	LC_{50} ~ 20 000	8
D. magna	<40[d]	30% rutile/100% anatase	/	/	/	$LC_{50} > 10$	9
D. magna	100[d]	100% anatase	/	/	/	Could not be assessed	7
D. magna	Length 50, width 10d	79–89% rutile; coated TiO_2 T-Lite™ SF	100	/	/	$EC_{50} > 100$	5
D. magna	Length 50, width 10d	73–83% rutile; coated TiO_2 T-Lite™ SF-S;	100	/	/	$EC_{50} > 100$	5
D. magna	Length 50, width 10d	69–73% rutile; coated TiO_2 T-Lite™ MAX	100	/	/	$EC_{50} > 100$	5
D. magna	6[e]	/	/	/	/	Effect; data not given	10

References: (1) Lovern and Klaper, 2006; (2) Warheit et al., 2007; (3) Lee et al., 2009; (4) Zhu et al., 2009; (5) Wiench et al., 2009; (6) Zhu et al., 2010; (7) Hund-Rinke and Simon, 2006; (8) Heinlaan et al., 2008; (9) Kim et al., 2010; (10) Strigul et al., 2009.
Abbreviations/Explanations: EC_{50} = median effective concentration; LC_{50} = medial lethal concentration; full species name: *Daphnia magna*;() – indicates the 95% confidence intervals; / – no data available.
Particles whose primary particle size was larger than 100 nm were omitted.
[a] Specific surface area measured with Brunauer, Emmett, and Teller method (BET).
[b] Median values for particle size in media determined with dynamic light scattering (DLS).
[c] Average particle size in test solution determined with transmission-electron microscopy (TEM).
[d] Particle size reported by the manufacturers.
[e] Delivered in agglomerates of 0.5–2.0 mm.

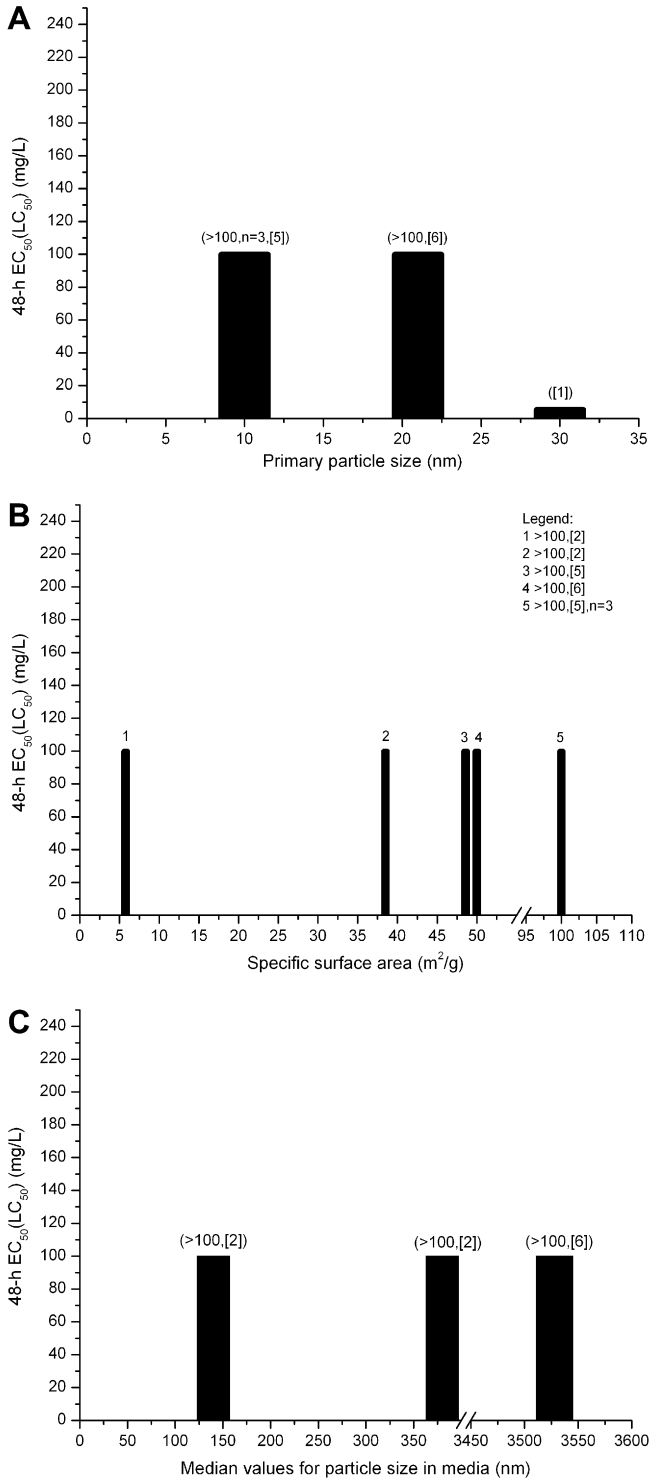

Fig. 2. Relation between 48-h $EC_{50}(LC_{50})$ values and particle size (reported by the manufacturer) (A), specific surface area (measured with Brunauer, Emmett and Teller method (BET)) (B) and median values for particle size in media (determined with dynamic light scattering (DLS)) (C) for nano-TiO_2 for the water flea *Daphnia magna*. References: [1] Lovern and Klaper, 2006; [2] Warheit et al., 2007; [4] Zhu et al., 2009; [6] Wiench et al., 2009; [7] Zhu et al., 2010; [9] Heinlaan et al., 2008; [10] Kim et al., 2010. Abbreviations/Explanations: > indicates higher $EC_{50}(LC_{50})$, (n) indicates the number of studies that have used same size of the particles where more than 1 author has used same size of the particles. The [4] reported the 48-h EC_{50} to be 35.3 mg/L for particles less than 20 nm, the [6] reported the 48-h EC_{50} to be >100 mg/L for particles between 20 and 30 nm, the [9] reported the 48-h LC_{50} to be >20 000 mg/L for particles between 25 and 70 and the [10] reported the 48-h EC_{50} to be >10 mg/L for particles less than 40 nm.

100 mg/L, decreased the protein content (Klaper et al., 2009). TiO_2 nanoparticles 30 nm in size had no effect on the hopping rate, heart rate, feeding appendage movement and postabdominal claw curling of *D. magna* at 2 mg/L (Lovern et al., 2007) and no genotoxic effects or significant changes in mortality, growth or reproduction of *D. magna* and *C. riparius* were found at 1 mg/L (Lee et al., 2009).

Chronic studies performed with daphnids reveal that the lowest observed concentration of nano-TiO_2 effecting reproduction of *Daphnia magna* was 2 mg/L (Lovern and Klaper, 2006) and 10 mg/L after 21 days (Wiench et al., 2009) with a 25% inhibition of reproduction of *Ceriodaphnia dubia* observed after 7 days exposure to 8.5 mg/L TiO_2 nanoparticles (Hall et al., 2009) (Table S2 in SI).

5. Toxicity of nanosized TiO_2 to terrestrial and marine invertebrates

The toxicity of nanosized TiO_2 has been studied in three terrestrial invertebrates, one of which is the isopod *Parcellio scaber* (Crustacea, Isopoda) (Jemec et al., 2008; Drobne et al., 2009), and in two soil-dwelling organisms, the free-living nematode *Caenorhabditis elegans* (Wang et al., 2009) and the earthworm *Eisenia fetida* (Hu et al., 2010). In study with terrestrial isopods, various formulations of TiO_2 (primary size 15 nm, <25 nm, <50 nm) were spread over the surface of dry leaf and offered as the only food source. Here, no effect on mortality, weight change or feeding behaviour was found when the organisms were exposed to 3000 μg/g dry food for 3 days or 1000 μg/g TiO_2 in the diet for 14 days. However, some sublethal effects on antioxidant enzyme activities were found when animals were exposed under the same conditions as already described (Jemec et al., 2008; Drobne et al., 2009). Five days of dietary exposure of juvenile nematode *C. elegans* to nanosized TiO_2 (primary size 50 nm, 338–917 nm hydrodynamic diameter) for example, reduced its growth (calculated based on raw data EC_{50} of 34.3 ± 3.62 mg/L), the number of eggs inside the worm (calculated based on raw data EC_{50} = 45.3 ± 8.99 mg/L) and offspring per worm (calculated based on raw data EC_{50} = 31.1 ± 1.44 mg/L). After 24-h dietary exposure, an LC_{50} of 80 mg/L was determined (Wang et al., 2009). After 7 days exposure of *E. fetida* to 10–20 nm TiO_2 the antioxidant activity and lipid peroxidation were increased. At doses >1 g/kg DNA damage to the coelomocytes occurred and >5 g/kg some of the mitochondria showed abnormalities such as fracture, disorganization and reduction, or complete loss of the cristae. Significant accumulation of Ti in earthworms was reported (Hu et al., 2010). These studies suggest the toxic potential of nanosized TiO_2 to terrestrial invertebrates, but more data of this type are needed for a definitive hazard estimation.

There appears to be only one study concerning marine invertebrates. It was found that TiO_2 nanoparticles of primary size 23 nm or 32 nm have no effect on burrowing behavior of polychaete *Arenicola marina* lugworm, but have a significant impact on feeding behavior as well as on lysosomal stability and DNA damage of coelomocytes. This study also suggested a preliminary LOEC for nano-TiO_2 of 1 g/kg (Galloway et al., 2010).

6. Toxicity of nanosized TiO_2 to marine bivalves

To date, only one in vivo test with a bivalve, *Mytilus galloprovincialis* has been reported. The 24-h test with mussels showed that nano-TiO_2 (22 nm average particle size, 51 m^2/g) induced lysosomal membrane destabilisation in the hemocytes and digestive gland. It was also found that nano-TiO_2 induced increases in lysosomal lipofuscin and lysosomal accumulation of neutral lipids, and enhancement of the activity of catalase and glutathione transferase in the digestive glands. No effect on catalase and glutathione

transferase activities in the gills and no mortality was observed (Canesi et al., 2010).

7. Toxicity of nanosized TiO_2 to freshwater vertebrates (fish)

The (eco)toxicological effects of nano-TiO_2 on fish have been summarised in several papers in recent years (Handy et al., 2008; Klaine et al., 2008; Farré et al., 2009; Kahru and Dubourguier, 2010). Fewer studies are available for fish as opposed to algae and freshwater invertebrates, but toxicity data are available for zebrafish *Danio rerio* (Griffitt et al., 2008; Zhu et al., 2008), fathead minnow *Pimephales promelas* (Hall et al., 2009), rainbow trout *Oncorhynchus mykiss* (Federici et al., 2007; Warheit et al., 2007) and carp *Cyprinus carpio* (Hao et al., 2009). Comparisons of data published for certain species in different reports are not possible because the durations of exposure differed (Table S3 in the SI). Very high LC_{50} values such as the 48-h LC_{50} for *P. promelas* of >500 mg/L (Hall et al., 2009), the 96-h LC_{50} for *O. mykiss* > 100 mg/L (Warheit et al., 2007) were found, and no effects on *D. rerio* were observed below 500 mg/L (Zhu et al., 2008).

Sublethal effects of nanosized TiO_2 (21 nm, 25% rutile/75% anatase, surface area 50 m^2/g) have been reported on juvenile rainbow trout (*Oncorhynchus mykiss*) after 14 days waterborne exposure (Federici et al., 2007) and 8 weeks exposure through fish food (Ramsden et al., 2009). Different effects on fish were found and depend on the source and duration of exposure. The Na^+K^+-ATPase activity in the gills, brain and intestine decreased after 14 days; after 8 weeks this effect was observed only in the brain. Total glutathione in the gills and liver was unaffected by nano-TiO_2 in the diet. In contrast, waterborne exposure was found to cause increases in the total glutathione levels in the gills and depletion of hepatic glutathione. Lipid peroxidation increased in the gill, intestine and brain after 14 days, but decreased after 8 weeks. Other observed effects included gill pathologies such as edema and thickening of the lamellae, and a few foci of lipidosis and condensed nuclear bodies in liver cells (Federici et al., 2007). Sublethal effects were observed in a study with carp, *Cyprinus carpio*, exposed to nano-TiO_2 (50 nm particle size, surface area 30 m^2/g, rutile form in crystal structure, >98.0% purity). After 8 days of exposure, nano-TiO_2 caused oxidative stress, depletion of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) activities and increased lipid peroxidation level in liver, gill and brain tissues. After 20 days of exposure, cellular pathologies in the liver (necrotic and apoptosis hepatocytes), and damage to gill lamellae and gill filaments were observed. After 1 h exposure the breathing and swinging frequency of the exposed fish elevated gradually and behavioural changes were observed (Hao et al., 2009).

Seven days exposure at 542 mg/L of the fish *P. promelas* led to 25% inhibition of growth (Hall et al., 2009) (Table S3 in the SI). It is clear that further chronic studies with other organisms are necessary.

8. Relation between physicochemical characteristics of nano-TiO_2 and biological effect

The TiO_2 nanoparticles tested in ecotoxicity studies published to date have very varied physicochemical properties, such as diameter, crystalline form, coating, surface area, zeta potential, and purity (Tables S1–S3 in the SI). The variability of the tested material was further enhanced as a result of preparation and suspension of the nanoparticles in test media, for example by sonication, filtration, and use of different solvents and surfactants.

We hypothesized that physicochemical characteristics of nano-TiO_2 can explain the high variability in the toxicity data for nano-TiO_2 obtained by different authors conducting similar experiments

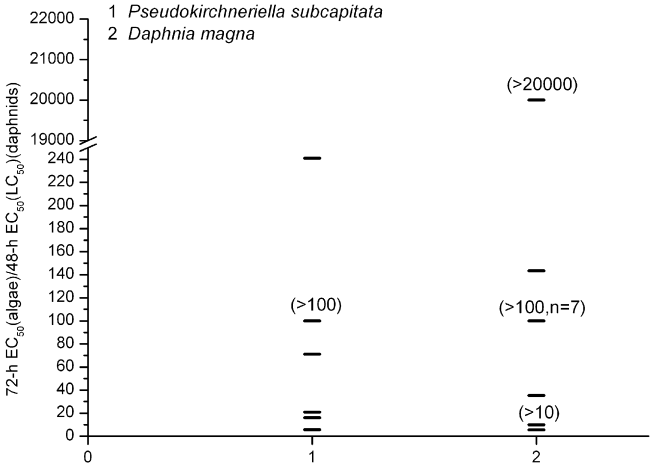

Fig. 3. The variability of 72-h EC_{50} values for TiO_2 nanoparticles obtained for algae *Pseudokirchneriella subcapitata* and 48-h $EC_{50}(LC_{50})$ for *Daphnia magna* presented by different authors (Tables 2 and 3). The number (n) of studies reporting certain values is noted where more than 1 author has reported the value (> indicates higher $EC_{50}(LC_{50})$).

with the same species. To test this hypothesis, only data collected with the same species, exposure duration and biomarker tested were compared. Two most investigated species are the algae *P. subcapitata* and crustaceans *D. magna*. Here, the reported EC_{50} values ranged from 5.8 – 241 mg/L for algae and 5.5 to >20 000 mg/L for daphnids (Fig. 3). We compared physicochemical properties of tested nanoparticles with toxicity data for these two species (Figs. 1 and 2) and found a correlation between toxicity data for algae and specific surface area and particle size in media (72-h EC_{50} values; Fig. 1(A) and (B)). For daphnids not appropriate data on effect concentrations related to particle surface area is provided (Fig. 2(B)). Evidence that secondary particle size may be most relevant for toxic response has been suggested (Warheit et al., 2007), but too few studies report the secondary particle sizes to permit firm conclusions in this regard.

9. Conclusions

The literature that has been reviewed on the effects of nano-TiO_2 in vivo supports three conclusions. First, the effect of nanoparticles is not correlated with primary particle size. There are indications that the effect may be related to secondary particle size and/or specific surface area but currently, there are insufficient published data with the same test species to allow comparison of data obtained on particles with different characteristics. Second, the conclusion from this review of the literature is that toxicity data for nanoparticles must be considered in relation to the biologically relevant characteristics, but are not limited to particle size and concentration of a suspension. If the material being studied is not thoroughly characterised for their physicochemical properties, the reported effect will be of a substance of unknown characteristics. The importance of characterising nanoparticles before testing is stressed by many researchers and international authorities (Warheit et al., 2007) but not fully respected. The nanotoxicity data obtained from heterogeneous sources with limited data concerning the physical chemistry of the nanomaterials are not suited for in silico technologies, such the development of quantitative structure–activity relationship (QSAR) models. When seeking predictive power, we should consider biologically relevant characteristics of nanoparticles. The third conclusion is that there is a need for tests with a battery of endpoints which include also the expose duration variables. The duration of exposure to

nanoparticles may be an important parameter when their toxicity potential is assessed. According to Baun et al. (2008) invertebrate tests are well suited to generate reproducible and reliable nano-toxicity data. Nano(eco)toxicology may take advantage of the fact that use of invertebrates as experimental animals is not subject to legal restrictions.

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Appendix. Supplementary information

Supplementary information associated with this article can be found in the online version at doi:10.1016/j.envpol.2010.11.027.

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