

Ecotoxicology of Nano-TiO₂ – An Evaluation of its Toxicity to Organisms of Aquatic Ecosystems

Clemente, Z.^{1,2*}, Castro, V. L.², Jonsson, C. M.² and Fraceto, L. F.^{1,3}

¹ Department of Biochemistry, Institute of Biology, State University of Campinas – UNICAMP, Rua Monteiro Lobato, 255, CEP 13083-862 Campinas, SP, Brazil

² Laboratory of Ecotoxicology and Biosafety, Embrapa, Rodovia SP340 km 127.5, C.P. 69, CEP 13820-000 Jaguariúna, SP, Brazil

³ Department of Environmental Engineering, São Paulo State University – UNESP, Avenida Três de Março, 511, CEP 18087-180 Sorocaba, SP, Brazil

Received 9 March 2011;

Revised 12 Sep. 2011;

Accepted 19 Sep. 2011

ABSTRACT: The production and use of synthetic nanoparticles is growing rapidly, and therefore the presence of these materials in the environment seems inevitable. Titanium dioxide (TiO₂) presents various possible uses in industry, cosmetics, and even in the treatment of contaminated environments. Studies about the potential ecotoxicological risks of TiO₂ nanoparticles (nano-TiO₂) have been published but their results are still inconclusive. It should be noted that the properties of the diverse nano-TiO₂ must be considered in order to establish experimental models to study their toxicity to environmentally relevant species. Moreover, the lack of descriptions and characterization of nanoparticles, as well as differences in the experimental conditions employed, have been a compromising factor in the comparison of results obtained in various studies. Therefore, the purpose of this paper is to make a simple review of the principal properties of TiO₂, especially in nanoparticulate form, which should be considered in aquatic toxicology studies, and a compilation of the works that have been published on the subject.

Key words: Nano-TiO₂, Nanotechnology, Ecotoxicology, Water, Aquatic organisms

INTRODUCTION

Nanotechnology is a rapidly expanding area of research which already has a wide variety of commercially available products. The material most commonly utilized in nanoproducts is silver, followed by carbon, titanium, silicon, zinc and gold (Meyer *et al.*, 2009, Project on Emerging Nanotechnologies, 2009). An initial estimate indicates that nanotechnology may lead to a revolution in the development and fabrication of products that could contribute with up to one trillion dollars to the global economy by 2015 (Roco, 2001). Nanomaterials have dimensions of less than 100 nanometers (nm), while nano-objects have dimensions smaller than 100nm and nanoparticles (NPs) have three dimensions with less than 100 nm (Stone *et al.*, 2010). However, the literature often describes NPs as particles that possess at least one dimension in the order of 1 to 100 nanometers (nm). The Royal Society of Chemistry suggests that 100 nm is the cut-off point above which particles will not enter cells through receptor-mediated processes (RSCRAE, 2005), and some experimental evidence has emerged that corroborates this dimension

*Corresponding author E-mail: zairaclemente@hotmail.com

(Chithrani and Chan, 2007, Clift *et al.*, 2008). Another important cut-off dimension is particles smaller than 40 nm, which can enter the nucleus, while particles smaller than 35 nm can, potentially, cross protective barriers such as the hematoencephalic barrier (Oberdorster *et al.*, 2004). However, these values should serve as guidelines, since the real size to be considered depends on other factors of the material and on details of its surface.

Titanium dioxide (TiO₂) has been used commercially since 1900, particularly in coatings and pigments. In 2002, the production capacity of this oxide was estimated at 4.6 million tons (Winkler, 2003). A review published by the United States Environmental Protection Agency (USEPA) estimated the annual production of TiO₂ nanoparticles (nano-TiO₂) to be 2000 metric tons in around 2005, with 65% of this production used in products such as cosmetics and sunscreen lotions (USEPA, 2009). The growing use of NPs generates effluents or wastewaters, raising concerns about the environmental risks and impacts

of nanotechnology. Due to the wide utilization and promising uses that have emerged from nano-TiO₂, this material has been the target of several ecotoxicology studies. Based on a compilation of published works that evaluate the toxicity of nano-TiO₂ to aquatic organisms, the article reviews the main properties of TiO₂, especially in nanoparticulate form, which should be considered in aquatic toxicology studies.

In nature, TiO₂ occurs only in the form of oxide or oxides mixed with other elements. Mineral deposits are usually of volcanic origin, but are also found in beach sand (Winkler, 2003). TiO₂ can be found in three crystalline forms: anatase (tetragonal), rutile (tetragonal) and brookite (orthorhombic), and its main reserves are located in Canada, the US, Scandinavia, South Africa, the Mediterranean Sea, and Australia (Titaniumart, 2010). Titanium dioxide, also known as titanium oxide (IV) or titania (molecular weight 79.88), is insoluble in water, chloric acid, nitric acid and ethanol, but is soluble in concentrated and heated sulfuric, hydrogen fluoride and alkaline media (NRC, 1999).

TiO₂ is obtained mainly from ore containing ilmenite (FeTiO₃), natural rutile (TiO₂) and leucoselenite-like ilmenite. TiO₂ particles are referred to as primary, aggregates or agglomerates. Primary particles are individual crystals bound by crystal planes. Aggregates are sintered primary particles connected by their crystal faces. Agglomerates are multiple primary particles and aggregates that are joined together by van der Waal forces (IARC, 2010). Primary particles typically have a diameter of 0.2 to 0.3 μm, although larger aggregates are also formed (further details about bulk TiO₂ are given in Diebold, 2003). Several TiO₂ NPs are produced today (Xiaobo, 2009), with variations in particle size, surface area, purity (due to doping, coating or quality control), surface characteristics, crystalline shape, chemical reactivity and other properties. One of the main differences between bulk TiO₂ and nano-TiO₂ is the larger surface area of a given mass or volume of NPs compared to an equivalent mass or volume of bulk TiO₂ particles (Shao and Schlossman, 1999). Approximately 35-40% of atoms are located on the surface of a 10 nm NP compared with less than 20% on particles larger than 30 nm. This higher surface area reinforces several properties, such as photocatalytic activity and ultraviolet absorption at given wavelengths (Shao and Schlossman, 1999). Bulk TiO₂ absorbs ultraviolet radiation (<400nm). Because of its high refractive index, it is also very effective in dispersing radiation. Both dispersion and absorption are important in the attenuation of ultraviolet radiation (UV), making it an effective ingredient in sunscreen lotions (USEPA, 2009). Small primary particles are less able to disperse visible light and are more transparent, while larger size

particles are more opaque. Hence, sunscreen formulations containing nano-TiO₂ have become popular due to their greater transparency on the skin compared to the white appearance of formulations containing bulk TiO₂.

The theoretical calculations of Palmer *et al.* (1990) and experimental data of Sakamoto *et al.* (1995) showed that the UVB attenuation of submicrometric TiO₂ particles is predominantly due to their absorption, while UVA attenuation is essentially due to their dispersion. The findings of Shao and Schlossman (1999) contribute to the idea that smaller particle sizes, and hence larger specific surface areas, are better for UVB attenuation. In contrast, the intensity of UVA dispersion is greater the larger the particle size (Shao and Schlossman, 1999). TiO₂ is a semiconductor, i.e., a crystalline solid whose electrical conductivity is intermediate between that of conductors and insulators. Thus, an important application of this material is in the electronics industry and in processes of heterogeneous photocatalysis.

The principle of heterogeneous photocatalysis involves the activation of a semiconductor by solar or artificial radiation. A semiconductor is characterized by two energy regions: the region of lower energy is the valence band (E_v), where the electrons cannot move freely, and the higher region is the conduction band (E_c), where the electrons move freely through the crystal, producing electrical conductivity similar to that of metals. These two regions are divided by a "band-gap" zone. Fig. 1 shows a schematic representation of a semiconductor particle. The absorption of photons with energy higher than the band-gap energy (E_g) causes the promotion of an electron from the E_v to the E_c, with the concomitant generation of a gap (h+) in the E_v. In the absence of suitable scavengers species, the stored energy is dissipated within milliseconds by recombination, with the formation of an unpaired electron. If a suitable scavenger or a surface defect is available to contain the electron or gap, recombination is prevented and redox reactions occur subsequently. E_v gaps are potent oxidants (potential of +1.0 to +3.5 V, depending on the semiconductor and pH) that are able to generate radical species (HO•, O₂•, HO₂•, etc.) from water molecules adsorbed on the semiconductor surface, which can subsequently oxidize other molecules (Nogueira and Jardim, 1998, Gaya and Abdullah, 2008, Malato *et al.*, 2009). There are indications that the reaction occurs only in the adsorbed phase of the semiconducting particle, hence, organic molecules that can effectively adhere to the surface of the photocatalyst are more susceptible to direct oxidation (Gaya and Abdullah, 2008).

The minimum E_g required for a photon to cause the photogeneration of charged species in TiO₂

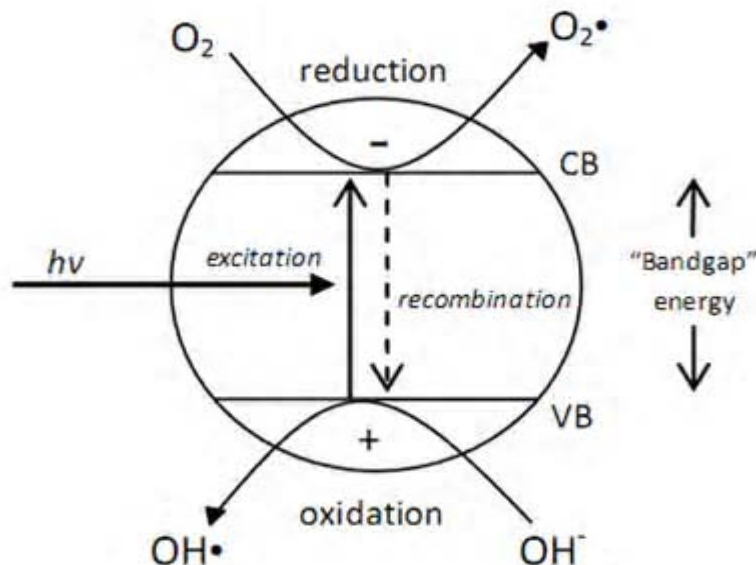


Fig. 1. Schematic representation of a TiO₂ particle, where E_v and E_c are the Valence Band and Conduction Band, respectively (adapted from Nogueira and Jardim, 1998)

(anatase form) is 3.2 eV, which corresponds to a wavelength of 388 nm. In fact, the photoactivation of TiO₂ occurs in the range of 300-388nm (Nogueira and Jardim, 1998, Gaya and Abdullah, 2008). Thus, the strong resistance of TiO₂ to decomposition and photocorrosion, its low cost, and the possibility of using solar UV radiation, makes it particularly interesting for processes of heterogeneous photocatalysis (Malato *et al.*, 2009).

Many studies have demonstrated the potential use of heterogeneous photocatalysis with TiO₂ for the degradation of organic and inorganic compounds (Chatterjee and Dasgupta, 2005, Fujishima and Zhang, 2006). For the most part, photodegradation leads to the total mineralization of pollutants, generating CO₂, H₂O and inorganic acids (Malato *et al.*, 2009). This property is applicable in the production of self-cleaning surfaces, cleaning products, in the remediation of contaminated soil and water, or even the deodorization of environments and the destruction of gas-phase volatile compounds. The hydroxyl radicals generated during TiO₂ irradiation are also able to react with most biological molecules, resulting in bactericidal and virucidal activity (Nogueira and Jardim, 1998, Li *et al.*, 2008).

Studies suggest that anatase and rutile have different photocatalytic properties, with anatase possessing the better combination of photoactivity and photostability (Gaya and Abdullah, 2008, USEPA, 2009). The rutile form is inactive for the photodegradation of organic compounds, although the reason for this is not completely clear (Nogueira and Jardim, 1998, Malato *et al.*, 2009). However, the low

adsorption capacity of O₂ on its surface is pointed out as one of the possible factors.

Among the different titanium oxide products, TiO₂ P25 fabricated by Evonik Degussa Corp. (Germany) is the one most commonly used because of its reasonably well defined nature (typically a mixture of 70:30 anatase:rutile, nonporous, surface area of about 50 m²/g, and average particle size of 30 nm) and its high photoactivity when compared to that of other sources (Nogueira and Jardim, 1998, Malato *et al.*, 2009).

Surface treatment of nano-TiO₂ can alter its light absorption and photocatalytic activity. In applications such as paints, coatings and cosmetics, which require chemical stability, the photocatalytic properties of TiO₂ are generally suppressed by coating it with silica and aluminum layers (Diebold, 2003, Li *et al.*, 2008). Doping of nanostructured TiO₂ materials has also often been employed to modify its band-gap energy and increase its photocatalytic activity. TiO₂ is generally used in suspension (also called slurry), but can also be used immobilized in an inert matrix coating surfaces (Gelover *et al.*, 2006, Gaya and Abdullah, 2008, Malato *et al.*, 2009).

Immobilized TiO₂ has been reported to have low catalytic activity when compared to systems in suspension (Gaya and Abdullah, 2008, Malato *et al.*, 2009). The mineralization rate generally increases with the concentration of the catalyst up to a limit of high concentration. Wei *et al.* (1994) used P25 for the disinfection of *E. coli* in water and reported that the disinfection rate depended mainly on two variables: the intensity of incident light and the TiO₂ dose.

In general, for any photocatalytic application, the optimal concentration should be determined in order to avoid an excess of catalyst and to ensure the total absorption of photons, i.e., to ensure the entire exposed surface of the particles is illuminated. When the concentration of TiO₂ is too high, the turbidity prevents radiation from penetrating and reaching all the particles (Herrmann, 1999). In photocatalysis studies, the optimal of TiO₂ have been a temperature of 20 to 80°C, a concentration of 200-500 mg/L, oxygen concentration of pO₂ ≥ 0.21 atm and pH preventing pHzpc (Malato *et al.*, 2009).

NPs tend to aggregate in the environment and can therefore be eliminated or captured by sedimentation. NP aggregates are generally less mobile and can interact with filtering organisms and with organisms that feed on sediment, or even with suspended organic matter. It is therefore important to understand the behavior of TiO₂ NPs in aquatic environments in order to understand their toxicology. The pH, ionic concentration and nature of the electrolytes in aqueous suspensions have been reported as important parameters in the aggregation of nano-TiO₂ (Sharma, 2009).

The pH of aqueous solutions significantly affects TiO₂, including the particle charge, the size of aggregates and the position of the E_c and E_v. The pH at which the surface of an oxide has no electrical charge is defined as the zero point charge (pHzpc). The pHzpc of nano-TiO₂ varies from 4.5 to 7, depending on the particle's size and crystal shape, with smaller particles presenting lower pHzpc (Kosmulski, 2002 cited by Sharma, 2009). Finnegan *et al.* (2007) reports pHzpc values of ~5.9 for rutile and of ~6.3 for anatase. A pHzpc of 6.3 has been reported for Degussa P25 (Kosmulski, 2009).

The surface of titanium will remain positively charged in an acid medium and negatively charged in an alkaline medium (Gaya and Abdullah, 2008). The lack of surface charge renders an electrostatic potential null, because it does not produce the repulsive interaction needed to separate the particles in the liquid. Therefore, TiO₂ particles tend to aggregate close to the pHzpc.

Particle aggregation interferes in the ability of the suspension to transmit or absorb radiation. However, this variation in particle size may be an advantage when the objective is to separate TiO₂ from water (by sedimentation and/or filtration) at the end of a photocatalytic treatment (Malato *et al.*, 2009).

Like other NPs, nano-TiO₂ can bind to organic matter, thus modifying its properties and behavior. The adsorption of acid fulvic and humic acid on nano-TiO₂ has proved to be pH-dependent and favors the

dispersion and suspension of these particles in aquatic environments (Domingos *et al.*, 2008, Yang *et al.*, 2009). On the other hand, the adsorption of oxalic acid appears to destabilize nano-TiO₂ suspensions, increasing the sedimentation rate at pH 2, although no change in the sedimentation rate has been observed at pH 6.5 (Pettibone, 2008).

The adsorption of organic matter on nano-TiO₂ may also alter the adsorption of toxic compounds (Sharma, 2009). Nano-TiO₂ has been reported to show adsorption behavior towards metals such as Cu(II), Cr(III), Mn(II), Ni(II), Zn(II), Cd(II), Mo(VI) (Kaur and Gupta, 2009). When an aqueous suspension of bacteria and other microorganisms is in the presence of TiO₂ in the dark, a slight reduction in the concentration of colonies can be observed due to the possible agglomeration of TiO₂ with the bacterial cells and subsequent sedimentation (Malato *et al.*, 2009).

STUDIES OF THE AQUATIC ECOTOXICOLOGY OF TiO₂

NPs differ from bulk particles in terms of their heterogeneous size distribution, surface charge, composition, degree of dispersion, etc. Therefore, in a toxicology study, it is important to determine not only their exposure concentration but also other measures (Hasselov *et al.*, 2008). At the NanoImpactNet Workshop held in 2008, a list was proposed of the six principal characteristics of nanomaterials to be discriminated in environmental studies: size, dissolution/solubility, surface area, surface charge and surface chemical composition. Information such as size distribution, crystal structure, morphology, agglomeration/dispersion, etc. may also be important (Stone *et al.*, 2010). Nonetheless, the authors recognize that the characterization of nanomaterials may be time-consuming and costly, as well as complex, and therefore its application should depend on the objectives of the study (Stone *et al.*, 2010). It was also agreed that the properties should be characterized in test systems and not in the "bottles" that are supplied, and that certain properties such as agglomeration and dissolution should be listed as "rates" rather than "states" in view of the dynamic nature of nanoparticulate systems.

Unfortunately, methods to measure all the properties are not available. For example, there is still no method available to measure the surface area in an aqueous dispersion of NPs. Moreover, there is still a paucity of information about the extent to which the limitations of the different methods may influence the correct interpretation of results. The bias of a technique can be reduced by using multiple techniques, although this is difficult due to time and cost constraints (Stone

et al., 2010). Hasselöv et al.'s paper (2008) presents information about the main methods available for the characterization of NPs.

The fact that TiO₂ is highly insoluble, non-reactive with other materials, thermally stable, and non-flammable enabled it to be declared innocuous to the organism (WHO, 1969). However, studies have demonstrated an apparently species specificity in the generation of lung tumors in rats that inhaled TiO₂ for long periods (Hext et al., 2005). In addition, other significant data in the literature confirm the occurrence of lung inflammation, oxidative stress and involvement of other organs after respiratory and oral exposure to nano-TiO₂ (Ferin et al., 1992, Wang et al., 2007, Warheit et al., 2007a). Recently, the International Agency for Research on Cancer (IARC) classified TiO₂ as "possibly carcinogenic for humans" (IARC, 2010).

The various possible sources of contamination of water bodies by nano-TiO₂ make it essential to assess its effects on ecosystems, i.e., its ecological, public health and economic consequences. There is still a paucity of studies about the presence of nano-TiO₂ in the environment. Natural TiO₂ NPs have been found in river water (Wigginton et al., 2007). In Switzerland, due to the climatic conditions, researchers reported nano-TiO₂ particles peeling off painted façades and being carried into surface waters, Ti concentrations of about 16 µg/L were found in urban runoff (Kaegi et al., 2008).

Nanoecotoxicology studies are relatively recent, the first publication involving an assay with fishes dated 2004 (Orberdorster, 2004). Tables 1 to 3 summarize published works about the effects of TiO₂ NPs on aquatic organisms.

With regard to the bioavailability of nano-TiO₂ to aquatic organisms, the literature is still inconclusive. In a recent paper, Johnston et al. (2010) did not observe significant absorption of nano-TiO₂ in *Oncorhynchus mykiss* exposed for 10 days to concentrations of up to 5 mg/L. Federici et al. (2007) also did not find accumulation of nano-TiO₂ in *O. mykiss* exposed for 14 days to concentrations of up to 1 mg/L. On the other hand, some studies report that the nano-TiO₂ present in water may accumulate in *Cyprinus carpio*, *Danio rerio* and *Daphnia magna*, even at concentrations of 0.1 and 1 mg/L, although low factors of bioconcentration were determined (Zhang et al., 2006, Zhu et al., 2010a, b). Zhu et al. (2010a) report the occurrence of trophic transfer of nano-TiO₂ in *D. rerio* fed with contaminated daphnids, but discard the possibility of biomagnification. Other studies have shown that the presence of nano-TiO₂ may elevate the absorption of other contaminants in fishes, such as As and Cd (Sun et al., 2007, 2009, Zhang et al., 2007).

The results of toxicity tests have usually been expressed as lethal (LC₅₀), effective or inhibitory (EC₅₀) concentrations that cause, respectively, mortality, abnormality of inhibition to 50% of the exposed organisms. A wide variability has been found in the results reported in the literature with regard to toxicity tests. This variability may be due to the different characteristics of nano-TiO₂ and treatments applied, as well as to experimental designs. Thus, exhaustive discussion has focused on the need for the proper characterization of NPs under study, and for the standardization of nanoecotoxicological evaluation methods. The lack of information in some works makes it difficult to compare results (Warheit et al., 2008). Discussions have also focused on the lack of analytical techniques for the characterization of NPs in the media utilized for ecotoxicological assays.

Lovern and Kapler (2006) reported an LC₅₀ of 5.5 ppm in *D. magna* exposed for 48 h to filtered nano-TiO₂, but did not observe mortality or behavioral abnormalities after exposure for the same period to concentrations of up to 500 ppm of the same nano-TiO₂, although the suspension was sonicated. Although several authors considered acute exposure to nano-TiO₂ of low toxicity to *Daphnia* (Warheit et al., 2007b, Griffith et al., 2008, Heinlaan et al., 2008, Lee et al., 2009, Strigul et al., 2009, Wiench et al., 2009, Kim et al., 2010, Rosenkranz, 2010), prolonged exposure has presented varied results. The exposure of *D. magna* to Degussa P25 (sonicated) for 21 days showed a LC₅₀ of 2.62 mg/L and alteration of the reproduction and growth rates (EC₅₀ 0.46 mg/L) (Zhu et al., 2010b), while exposure for the same period to different types of BASF nano-TiO₂ (sonicated) did not cause mortality but reduced the reproductive capacity (EC₅₀ 26.6 mg/L) (Wiench et al., 2009). Kim et al. (2010) did not find reproductive impairment but reported a 70% mortality rate in *D. magna* exposed for 21 days to 5 mg/L of Sigma Aldrich nano-TiO₂.

Some studies appear to suggest that nano-TiO₂ has low acute toxicity for fishes, and LC₅₀ is indicated as 124,5 mg/L for *D. rerio* (Xiong et al., 2011) and >100 mg/L for *O. mykiss* (Warheit et al., 2007b). Similarly, the exposure of *D. rerio* eggs to nano-TiO₂ for 96 hours at concentrations of up to 500 mg/L did not cause alterations in the survival and hatching rates, or malformations (Zhu et al., 2008). The exposure of embryos of *Pimephales promelas* to concentrations of up to 1 mg/L for 7 days also caused no significant mortality or observable malformations (Jovanovic et al., 2011). On the other hand, some studies have shown that the prolonged exposure of fish to concentrations of 1 to 200 mg/L did not cause mortality, but observed dose-dependent elevation of the respiratory rate and

swimming behavior, as well as increased production of mucus (Federici *et al.*, 2007, Hao *et al.*, 2009).

Evidence of adverse effects of a given contaminant at sublethal concentrations is extremely important in environmental risk assessment, since it may generate a cascade effect with consequences at the level of individuals, communities and the ecosystem. Thus, the use of biomarkers in risk assessments offers the advantage of allowing for the detection of potentially toxic exposure well before real adverse effects occur (Nascimento *et al.*, 2008, Prospéri and Nascimento, 2008).

Studies have shown that the toxicity of some nanomaterials such as TiO₂ may be implicated in the generation of reactive oxygen species (ROS) (Kahru and Dubourguier, 2009, Pelka *et al.*, 2009, Sharma *et al.*, 2009). ROS can react with the majority of biomolecules and damage lipids, proteins and nucleic acids (Valavanidis *et al.*, 2006).

Exposure in aqueous media appears to be more severe than via the diet for *O. mykiss* (Handy *et al.*, 2008). The prolonged exposure of fish to nano-TiO₂ induced biochemical and histopathological alterations in their gills, liver and intestines (Federici *et al.*, 2007, Hao *et al.*, 2009, Johnston *et al.*, 2010, Palaniappan and Pramod, 2010). Exposure to nano-TiO₂ can trigger oxidative stress in *D. magna*, fishes and mollusks (Federici *et al.*, 2007, Hao *et al.*, 2009, Canesi *et al.*, 2010a, Kim *et al.*, 2010, Xiong *et al.*, 2011). Lysosomal instability has also been reported in polychaetes and mollusks exposed to nano-TiO₂ (Canesi *et al.*, 2010a, Galloway *et al.*, 2010). The intravenous administration of high doses of nano-TiO₂ in fish has shown that it accumulated in the kidneys, with slow depuration, but no significant alterations were observed in the function of this organ (Scown *et al.*, 2009). An experiment with *D. magna* showed that even after a period of 72 hours in clean water, the depuration of adsorbed TiO₂ was not complete (Zhu *et al.*, 2010b).

With regard to genotoxicity in aquatic organisms, nano-TiO₂ presents controversial results. Nano-TiO₂ has presented genotoxicity in some studies (Griffith *et al.*, 2009, Galloway *et al.*, 2010, Jovanovic *et al.*, 2011) but not in others (Lee *et al.*, 2009). Griffith *et al.* (2009) reported that exposure to nano-TiO₂ altered the expression of 171 genes in *D. rerio* involved mainly in ribosome structure and activities, but not in the regulation of oxidative stress. Jovanovic *et al.* (2011) also observed upregulation of genes involved in inflammatory response (especially in phagocytic processes), and suppression of neutrophil function in fish that received an intraperitoneal dose of nano-TiO₂. The immune system also appears to be an important target of TiO₂ NPs in bivalves (Canesi *et al.*, 2010b).

In bioassays with aquatic organisms, the circadian cycle is usually established using fluorescent lamps. These lamps emit basically visible light, while in natural conditions these organisms are exposed to solar radiation (infrared, visible and ultraviolet light). There is ample evidence of the formation of reactive oxygen species when TiO₂ is exposed to UV radiation (Brezová *et al.*, 2005). Several studies have reported the phototoxic effects of TiO₂ normal or NPs), and its consequent use in the disinfection of water (Wei *et al.*, 1994, Carp *et al.*, 2004, Adams *et al.*, 2006). The photocatalytic properties of nano-TiO₂ can augment its toxic effects in aquatic organisms under environmental conditions, but few studies so far have taken this into consideration. *In vitro* studies have shown that co-exposure to nano-TiO₂ and ultraviolet radiation increases cyto- and genotoxicity in fish cells (Reeves *et al.*, 2008, Vevers and Jha, 2008). The pre- and co-illumination of nano-TiO₂ has also been shown to elevate its toxicity in daphnids (Hund and Rinke, 2006, Marcone *et al.*, 2010).

There are still uncertainties about the characterization of exposure to nanoparticles in the testing systems of all ecotoxicity assays except those that involve the oral administration of nanoparticles. These uncertainties include how the substance is dosed and maintained in the test medium, the measurement and characterization of NPs in the test system, the understanding of the abiotic factors that influence the behavior of NPs in the test system, and a consensus about the dosimetry (Crane *et al.*, 2008).

Today there are several guidelines for conducting ecotoxicological assays (OECD, USEPA, DIN Standards, etc.). However, their use for nanoecotoxicological assays is still under question (Stone *et al.*, 2010). The use of these methodologies must be evaluated for each type of nanoparticle. Testing with nano-TiO₂ presents various particularities, such as its photocatalytic properties and absorption of UV radiation, its aggregation and sedimentation behavior in water and its interaction with organic matter. Performing assays to determine lethal and effective concentrations in the proposed ranges of concentration is particularly difficult. The OECD, for example, suggests finding the CL₅₀ up to the concentration of 100mg/L, however, nano-TiO₂ forms a whitish suspension when dissolved in water, and in concentrations equal to or higher than 10mg/L, it precipitates rapidly if no dispersion method is used. Wiench *et al.* (2009) found that TiO₂ does not disperse well at 10-100 mg/L and that sedimentation occurs within 24-48 hours. For uncoated TiO₂ (BASF, >99%, 70% anatase, 30% rutile, 20-30nm, 48.6m²/g), the

Table 1. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on microcrustaceans (Continues)

| Test species | Product tested | Treatment of the product | Physicochemical characterization | Bioassay | Results |
|--|---|---|---|---|--|
| <i>D. magna</i> (Kim et al., 2010) | Sigma A dirch nano-TiO ₂ (40 nm, 30% rutile, 70% anatase) | 10% solution in water with pH 2 (without sonication) ? stock solution (1mg/L) in moderately hard synthetic water (MHV). | N4 and DL5 ultramicro particle analyzer. | Acute assay 48h. Without feeding during the test. USEPA 1993. Chronic assay, semi-static, 21 days. Renewal of medium and daily feeding. Concentrations tested 0, 1, 2, 5, 10 mg/L. Evaluations were made of SOD, GPX, CAT and GST activity in groups exposed for 5 days to 0, 0.5, 1, 2.5, 5, and 10 mg/L of TiO ₂ . GPX and GST were also tested after fractionation of the nanoparticles (<200, <400, and <800 nm) | Acute assay: mortality did not reach 50% even at 10mg/L so the LC ₅₀ could not be determined. Chronic assay: highest mortality at 5 and 10mg/L (70 and 80%, respectively). No reproductive impairment observed. Increase in CAT at 10mg/L, no difference in SOD. GPX higher at 5mg/L. GST increased at 5 and 10 mg/L. TiO ₂ was found in the intestines of daphnids and glued to their antennae and external surface. |
| <i>D. magna</i> (Rossekranz, 2011) | Degussa P25 nano-TiO ₂ | 100mg/L solution was prepared in culture medium for daphnids ? sonication (30 min). The remaining solutions were made from serial dilutions of 1:10. | INA | Acute assay 48h. No food during the test. 100, 10, 1 and 0.1 mg/L. Chronic assay 21 days. Medium changed daily. Daily feeding. Concentrations: 0.001, 0.1 and 1 mg/L | Acute assay 48h: 10% mortality at 100mg/L. High molt frequency, dose-dependent. Chronic assay: high molt frequency only on the first day of exposure, at 1mg/L. |
| <i>D. magna</i> (Zhu et al., 2010b) | Degussa P25 nano-TiO ₂ (21 nm, 50% rutile, 20% anatase) Size of aggregates in culture medium In - 580.5 nm 12h - 2349.0 nm 24h - 5328.6 nm | Stock solution (1g/l) in ultrapure water ? sonication (10 min, 50 W/L, 40kHz) ? new sonication (10 min, 50W/L, 40kHz) prior to dilution in culture medium for daphnids. | SEM, DLS ICP-OES (concentration of Ti in the solution and in daphnids). | Acute assay 72h semi-static. OECD 202. Medium renewed daily. No food during the test. Concentrations tested 0, 1, 0.5, 1, 5, 10, 50 and 100 mg/L. Chronic assay 21 days semi-static. OECD 211. Daily renewal of medium and daily feeding. Concentrations tested 0.1, 0.5, 1 and 5 mg/L. Bioaccumulation and depuration test 24h of accumulation (samples were collected at 0, 2, 6, 12 and 24h) and 72h of depuration (samples were collected at 6, 12, 24, 48 and 72h). Concentrations tested 0.1 and 1 mg/L with and without daily feeding. | Acute assay In 48h: NOEC <50mg/L, EC ₅₀ > 100mg/L, LC ₅₀ > 100mg/L. In 72h: NOEC <0.1 mg/L; EC ₅₀ = 1.62 mg/L; LC ₅₀ = 2.02 mg/L. Chronic assay At 0.1 mg/L reproduction declined. At 0.5 mg/L reproduction and growth were inhibited. Mortality was recorded in groups 1 and 5 mg/L after 8 days of exposure. LC ₅₀ = 2.62mg/L. The feeding rate decreased as the exposure concentration increased (exposure of 5h). Bioaccumulation test Group 0.1 mg/L: Concentration plateau in 12 h, BCF= 5.66x 10 ⁴ , time elapsed to accumulate 50% of the depuration = 26.76h. Group 1mg/L: Plateau in 24h; BCF= 1.18x10 ⁵ , time elapsed to accumulate 50% of the depuration level = 3.72h time to reach 50% depuration = 74.52h. Depuration was not complete, 20% of the saturation concentration remained in the daphnids at the end of the experiment. Feeding during exposure to TiO ₂ increased the accumulation time and reduced the depuration time. |

Table 1. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on microcrustaceans (Continues)

| | | | | | |
|---|---|---|---|--|---|
| <p><i>D. magna</i> and <i>Chironomus riparius</i> (larvae) (Lee et al., 2009)</p> | <p>Sigma Aldrich nano-TiO₂: 7nm (300.84nm²/g) and 20nm (6.604nm²/g)</p> | <p>Acute assay 96h. OECD 1984, 1998. Concentration tested: 1mg/l</p> | <p>TEM, BEI</p> | <p>Solution (1mg/L) in culture medium ? sonication (15 min).</p> | <p>No genotoxicity (comet assay), alteration in growth, mortality or reproduction were observed in any group.</p> |
| <p><i>D. magna</i> (Strgul et al., 2009)</p> | <p>nano-TiO₂ prepared by hydrolysis of the titanium sulfate solution (6 nm agglomerates 0.5-2 nm)</p> | <p>Acute assay 24h and 48h OECD 202. Concentrations tested: 2.5; 8; 25; 80; 250 mg/L</p> | <p>DLS</p> | <p>Stock solution ? sonication (30min).</p> | <p>TiO₂ presented low toxicity and LC₅₀ could not be calculated. Animals expts at 80 and 250mg/L for 24h were slower.</p> |
| <p><i>Daphnia magna</i> (Wrench et al., 2009)</p> | <p>Bulk TiO₂: BASF nano-TiO₂: non-coated (>99%: 70/30 anatase/rutile: 20-30nm: 48.6m²/g) - FLITE-SF (80%: 50nm: 100m²/g: rutile) - FLITE-SFS - FLITE-SF-MAX</p> <p>It was found that 10-100 mg/L did not disperse well and sedimentation occurred in 24-48h. For non-coated TiO₂, after 16h in bidistilled water, the concentration in the supernatant went from 100 to 83mg/L and to 33mg/L in surface water. For coated TiO₂, agglomeration and sedimentation were slow.</p> | <p>Acute assay 48h. OECD 202. Chronic assay 21 days. OECD 211. Only with T-Lite SF-S - semi-static assay (medium changed 3 times per week). Daily feeding. Concentrations tested: 0.01 a 100 mg/L.</p> | <p>TEM, ultracentrifugation.</p> | <p>Stock solution (100mg/L) in demineralized water ? sonication (5min) or magnetic agitation (10 min) or both methods ? dispersion in M4 or SW medium (natural surface water) ? sonication and filtration (20mm) ? UV irradiation (30 min 200W/m²).</p> | <p>Acute assay EC₅₀>100mg/L in all the treatments EC₁₀ non-coated nano-TiO₂ sonicated in M4 = 85.1mg/L. In SW = 3.7mg/L. Bulk TiO₂ sonicated in M4 = 9 mg/L; in SW = 13.8mg/L. Chronic assay There was no mortality, but reproductive effects were observed. NOEC = 3mg/L LOEC = 10mg/L EC₅₀ = 2.6 mg/L EC₁₀ = 5.02 mg/L</p> |
| <p><i>D. pulex</i> and <i>Ceriodaphnia dubia</i> (Griffith et al., 2008)</p> | <p>Deagussa P25 nano-TiO₂ (30% rutile, 80% anatase, 45.41m²/g, 30.5±6.7 nm; ZP-25.1; poly-disperse 0.197, largest particle diameter observed in suspension was 687.5nm)</p> | <p>Acute assay 48h static. American Society for testing and materials guidelines. No food given during the test.</p> | <p>BEI; Coulter LS 13320; polydispersity; Zeta reader Mk 21-II; scanning micrographs.</p> | <p>Stock solution in ultrapure water (1mg/ml) ? sonication (6 W, 225 Hz, 6 one-half second pulses).</p> | <p>LC₅₀ >10mg/L for both tested organisms.</p> |
| <p><i>D. magna</i> and <i>Thamnocephalus platyurus</i> (Hehnen et al., 2008)</p> | <p>Sigma Aldrich nano-TiO₂ (25-70nm). Riedel-de Haen bulk TiO₂</p> | <p>Acute assay 48h. in the dark. Standard Operational Procedures of Daphtoxkit FTM magna (1996). <i>T. Thamnocephalus</i> (larvae): Acute assay 24h. in the dark. Thannotoxkit FTM (1995). Concentrations tested: 0.01 to 20000 mg/L.</p> | <p>INA</p> | <p>Stock solution in ultrapure water (40g/L) ? sonication (30 min) ? storage at 4°C ? vortex ? exposure dosage.</p> | <p><i>D. magna</i>: LC₅₀ of nano-TiO₂ ~ 20000mg/L. (Bulk TiO₂ and other values of toxicity were not tested). <i>T. Thamnocephalus</i>: LC₅₀, LC₂₀ and NOEC of nano- and bulk TiO₂ > 20,000 mg/L.</p> |

Table 1. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on microcrustaceans

| | | | | | |
|---|---|---|--|--|--|
| <i>D. magna</i> (Lovern et al., 2007) | Nano-TiO ₂ : 30nm (in suspension) | THF was used to ensure dispersion. The THF was eliminated by evaporation and filtration and confirmed by spectrophotometry. | TEM characterization according to Lovén and Kapler (2006). | Acute assay 60 min. USEPA 23. Concentration tested: 2ppm (LOEC calculated in a previous experiment). | TiO ₂ did not significantly alter the heat rate, jump, movement of appendices, and curvature of the abdominal claw. |
| <i>D. magna</i> (Wärheit et al., 2007b) | DuPont Haskell TiO ₂ : fine TiO ₂ (380nm in water, 5.8m ² /g, 100% rutile, 99% TiO ₂ and 1% aluminum) afC (140 ± 44nm in water; 38.5m ² /g; 79% rutile 21% anatase; 90% TiO ₂ ; 1% amorphous silica; 7% aluminum). | INA | DLS, BET, X-ray fluorescence, X-ray diffraction. | Acute assay 48h, static. OECD 202. Concentrations tested: 0.1, 1, 10 and 100 ng/L. | LC ₅₀ 48h > 100ng/L for both types of TiO ₂ . There was 10% of immobility at concentrations of 10 and 100ng/L at the end of 48h for both compounds tested. |
| <i>D. magna</i> (Adams et al., 2006) | Sigma Aldrich nano-TiO ₂ : 65 nm, 950 nm and 44 µm. Smaller particles (65 nm) appeared larger (on average 320nm) and larger ones (950nm and 44 µm) appeared smaller (320nm and 44 µm), respectively, when in suspension | Solution in ultrapure water (µg/L); agitation? exposure dosage | DLS optical microscopy. | Prolonged assay 8 days. Concentrations tested: 1, 10 and 20 ppm. | 20 ppm of nano-TiO ₂ was lethal for 40% of the organisms. |
| <i>D. magna</i> (Hund Rieke and Simon, 2006) | Product 1: 25nm, mainly anatase. Product 2: 10nm, 100% anatase. | The TiO ₂ suspension was agitated and pre-illuminated in SUNTEST CPS. Particles were washed following the manufacturer's instructions? mother's dilution? sonication? continuous agitation and irradiation in a solar light simulation system (300-800 nm 250W, 30 min)? samples were transferred and incubated for 72h with visible light | INA | Acute assay 48h. ISSO 634I, OECD 202 and DIN 3812-30. Concentrations tested: 1, 1.5, 2, 2.5, 3 ng/L. | There was no concentration-effect curve, so the EC ₅₀ could not be determined for any group. Pre-illumination increased the toxicity of the two nano-TiO ₂ products. E.g.: at 1 and 2.5 ng/L of product 1, immobilization went from 0 to 20% and from 28 to 73%, respectively, when there was pre-illumination. |
| <i>D. magna</i> (Lovén and Kapler, 2006) | INA. Mean diameter of filtered TiO ₂ : 30nm; in sonicated solution: 100 to 500 nm | Solutions were prepared in three ways: 1) Dilution in distilled water? 2) 20mg were placed in 200 ml sonication for 30 min. THF? pulverization with nitrogen? over-night on moving plate? filtration? dilution in deionized water? evaporation of the THF? filtration. 3) Same as 2, but without THF. | TEM spectroscopy | Acute assay 48h. USEPA 2024. No food given during the test. Groups: 1) control, 2) THF group, 3) filtered TiO ₂ (0.2, 1, 2, 5, 6, 8, and 10 ppm), and 4) sonicated and non-filtered TiO ₂ (50, 200, 250, 300, 400, and 500 ppm). | Filtered TiO ₂ : there was no mortality at 0.2 ppm, but 1% mortality at 1 ppm. LC50=5.5ppm LOEC= 2 ppm NOEC= 1 ppm. Sonicated TiO ₂ : no group suffered mortality > 9%. NOEC, LOEC and LC ₅₀ not applicable. When there was no mortality, no immobility or swimming abnormalities were observed in any group. |

BCF = bioconcentration factor
BET= Brunauer, Emmett, Teller method for surface area calculation
CAT = catalase activity
DLS = dynamic light scattering
INA = information not available

EC₁₀ = effective concentration for 10% of exposed organisms
EC₅₀ = effective concentration for 50% of exposed organisms
GPX = glutathione peroxidase activity
GST = glutathione S-transferase activity

ICP-OES = inductively coupled plasma optical emission spectroscopy
LC₅₀ = lethal concentration for 50% of exposed organisms
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
ZP = zeta potential

SEM = scanning electron microscopy
SOD = superoxide dismutase activity
TEM= transmission electron microscopy
THF = tetrahydrofuran

Table 2. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on fishes (Continues)

| Test species | Product tested | Treatment of the product | Physicochemical characterization | Bioassay | Results |
|--|---|---|---|--|--|
| <i>D. rerio</i> adults (Xiong et al., 2011) | nano-TiO ₂ from Nanjing University of Technology (anatase, purity 99%, diameter 20-70nm, hydrodynamic diameter 251-630nm, ZP-13.1mV) bulk TiO ₂ from Tianjin Guaiqiang Chemical Reagent Co. (anatase, purity 99%, diameter 128-949nm, hydrodynamic diameter 272-597, ZP -27.8mV) | test suspension in aerated single-distilled water? sonication (15 L, 100W, 40kHz for 20 min). | TEM, DLS | Acute assay 96h, semi-static (solution changed every 24h). No food given during the test. Concentrations tested: 0, 10, 50, 100, 500, 200 and 300 mg/L. From biomarkers analysis, fish were exposed to 50mg/L under light or dark conditions. | nano-TiO ₂ : LC50 = 124.5 mg/L SOD activity decreased in liver tissues and increased in gut tissues, in both groups (under light or dark conditions). CAT activity in liver tissue was observed to be reduced in both groups. There was elevated protein carbonyl levels. Lipid peroxides were also found in the gills and gut tissues. GSH content increased in gut tissue, and (under dark conditions) decreased in liver. MDA concentrations increased in gills and gut tissues. Morphological changes in gill cells (cell membrane damage, irregular cell outlines, pyknotic nuclei and trend of complete disruption of gill cells). bulk TiO ₂ : LC50 > 300mg/L no changes in SOD and CAT activities and in MDA content. There was an increase in GSH in gut tissue. |
| <i>O. mykiss</i> (Johnson et al., 2010) | Nano-TiO ₂ (34.2±1.73nm, ZP-9), bulk TiO ₂ and ionic titanium (titanium metal standard solution, Fisher Scientific). | Stocks solution (250µg/L) in ultrapure water? sonication (30min)? exposure dosage. | TEM, ICP-MS, DLS, particle sizer, CARS, multiphoton microscopy. | Prolonged assay 10 days, semi-static (change of 50% of the water every 2 days). Concentrations tested: 500 (nano-TiO ₂) and 5000 µg/L (nano- and bulk TiO ₂ and ionic Ti). Test exposure via de-lit 21 days. Concentrations tested: 0.01 and 0.1% nano-TiO ₂ in food. | No significant absorption of Ti was detected in any group. The Ti concentration in the gills increased in the group exposed to ionic Ti. High levels of Ti were found in the stomach of fish fed with medium and high doses of TiO ₂ . TiO ₂ aggregates were found on the surface of the gill epithelium after 24 and 96h of exposure and inside lamellae after 14 days of exposure. |
| <i>D. rerio</i> adult (Palaniappan et al., 2010) | Sigma Aldrich nano-TiO ₂ (purity 99.7%, anatase, 20nm, 200±20nm ²). Particle size: 14.1±1.52nm. Nice Chemicals bulk TiO ₂ (99.7% purity, anatase). | Stocks solution (10 ppm) in ultrapure water? sonication (6h)? storage at -20°C? sonication (30 min)? exposure dosage. | TEM. | Prolonged assay 14 days. Concentrations tested: 0ppm of nano-TiO ₂ or 100ppm of bulk TiO ₂ . | Mortality was not observed during the experiment. The biochemical constituents of the gills showed alterations. These alterations were greater in the group exposed to nano-TiO ₂ than in the one exposed to bulk TiO ₂ . Example: alterations in the amide I bands. |
| <i>D. rerio</i> (Zhu et al., 2010b) | Degussa P25 nano-TiO ₂ (21nm). | Stocks solution (1µg/L in ultrapure water? sonication (10 min, 50W/L, 40kHz). | SEM, DLS. | Trophic transfer test. Daphnids were exposed to 0.1 or 1mg/L of TiO ₂ for 24h, after which they were collected and washed in culture medium and supplied to <i>D. rerio</i> as food. The test involved 14 days of absorption followed by 7 days of depuration (feeding with non-contaminated daphnids). The TiO ₂ concentration in the daphnids was determined as follows: 4.52 ± 0.36 mg/g (in the group exposed to 0.1mg/L) and 61.09 ± 3.24 mg/g (in the group exposed to 1mg/L). The fish were sampled on days 0, 1, 3, 5, 7, 10, 14, 15, 17, 19 and 21. Prolonged exposure test. 14 days, followed by 7 days of depuration. Semi-static (water changed daily). Concentrations tested: 0.1 and 1mg/L. | No mortality or abnormalities were observed. Trophic transfer of TiO ₂ occurred. There was no apparent bioaccumulation. Trophic transfer test Concentration of Ti in the fish group fed with daphnids 0.1 mg/l = 106.57 ± 14.89 mg/kg and group fed with daphnids 1 mg/l = 522.02 ± 12.94 mg/kg. BCF < 1. Prolonged exposure test Fish accumulated TiO ₂ , reaching a plateau of about 1.5mg/kg on day 3 (group 0.1mg/L) and of 100mg/kg on day 10 (group 1mg/L). BCF = 25.38 and 181.38 (at equilibrium for groups 0.1 and 1mg/L respectively). During the depuration phase, the concentration of TiO ₂ in the entire body was found to decline. |

Table 2. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on fishes (Continues)

| | | | | | |
|---|--|--|---|--|---|
| <i>D. rerio</i> female adults (Griffith et al., 2009) | Degussa P25 nano-TiO ₂ (45.41m ² /g; ZP -25, 1mV). Aggregates in powder: 20.5±6.7nm; in suspension: 220.8 to 687.5nm. | Stock solution in ultrapure water ? sonication (6s, 6W, 22kHz) ? exposure dosage. | BET, SEM, scanning micrographs. | Acute assay 48h static. Concentration tested: 1000µg/L. | Significant difference in the expression of 171 genes (microarray) - 60 up-regulated and 111 down-regulated (53 of these genes were affected by exposure to nano-copper and nanosilver). The affected genes were involved in ribosome structure and activity. No alteration was observed in genes related to regulation of oxidative stress. No histopathological differences were observed in the gills compared with the control group. |
| <i>Gyrinus carpio</i> juveniles (Hao et al., 2009) | Hongsheng Material nano-TiO ₂ (50nm, 30±10m ² /g rutile 98%). | Solution ? sonication (30min, 100W, 40kHz). | INA | Probnged assay 8 days semi-static (solution changed daily). No food given during the test. Animals were collected on days 1, 2, 4 and 6 for biochemical analyses. For histopathology the animals were exposed for 20 days. Concentrations tested 10, 50, 100 and 200mg/L. | No mortality occurred, but after 1h of exposure the respiratory rate and swimming rates increased, as well as the production of mucus, in a concentration-dependent way. The biomarkers of oxidative stress varied with the concentration and exposure time. At 100 and 200mg/L there was an increase in LPO and decrease in the gills and brain. Histopathological alterations were observed mainly at the highest concentrations. The liver showed vacuolization of cytoplasm and autosomes, including necrotic cell bodies and nuclear fragments that looked like apoptotic bodies and some foci of lipidosis. The gills show ed thickening, edema, fusion and hypertrophy of the lamellae and filaments. |
| <i>Oncorhynchus mykiss</i> juveniles (Scown et al., 2009) | Sigma A lrich nano-TiO ₂ (32.4nm, 46.3m ² /g, purity >99.9%, anatase and rutile). Particle size: 34.2nm, 18.6nm ² /g (in powder). 400-1100nm (in ring and water). ZP: 0.4 -0.6mV. | Solution (10mg/L) in ring ? sonication (30min). | BET | Intravenous administration (1.3mg/kg). Fish and blood samples collected 6h and 90 days post-injection. | 10 to 19% of injected Ti accumulated in the kidneys (up to 2.3µg/g). The concentration in the kidneys did not change significantly from 6h to 21 days post-injection, but after 90 days the concentration in the kidneys was significantly lower. The Ti level in the liver was approximately 1.5-fold lower. Preliminary studies showed that Ti did not accumulate in the brain, gills or spleen. No significant difference was found in blood TBARS at any time compared with the control. The histopathological analysis showed no alteration in the kidneys, but the TEM showed small aggregates apparently encapsulated around the tubules. Greatinine levels fluctuated in both the controls and the injected animals, but no effect was found in the plasma protein concentration. |
| <i>C. carpio</i> (Sun et al., 2009) | Degussa P25 nano-TiO ₂ (50m ² /g, 25nm) Arsenite (As III) prepared from As ₂ O ₃ . | Stock solution of nano-TiO ₂ (1g/l) ? sonication (10min, 50W/l, 40kHz) ? exposure dosage. | INA | Chronic assay. Groups: 1) control, 2) only As III (20µg/L ± 10.2); 3) As III + TiO ₂ (10mg/L ± 1.3). Animals were placed in the aquariums 2h after the addition of As and TiO ₂ . Semi-static test (water changed daily). Animals were collected on days 2, 3, 10, 15, 20 and 25. Food was given once a day during the test. Speciation was evaluated of As in water, in the presence of TiO ₂ , with and without sunlight. | The concentration of As in the carps increased from 42% (3 days) to 185% (second day) in the presence of nano-TiO ₂ . The order of accumulation of As and TiO ₂ in the different tissues was: viscera > gills > skin and scales > muscle. In the absence of sunlight, only a small amount of As III moved to As V (loaded, and therefore with less capacity to pass through biological membranes). With sunlight, about 75% of the As III moved to AsV in 1h. |
| <i>Danio rerio</i> adults and juveniles (Griffith et al., 2008) | Degussa P25 nano-TiO ₂ (20% rutile, 80% anatase, 45.41m ² /g, 20.5±6.7 nm, ZP -25, 1; polydispersion 0.197, largest particle diameter observed in suspension = 687.5nm). | Stock solution (1mg/ml) in ultrapure water ? sonication (6W, 22.5kHz, 6 half-second pulse s). | BET, Coulter LS 13 320 polydispersity; Zeta reader Mk 2-II; scanning micrographs. | Acute assay 48h static. | LC ₅₀ > 10mg/L of nanoparticles |

Table 2. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on fishes

| | | | | | |
|--|---|---|--|--|---|
| <i>O. mykiss</i> juveniles (Felder et al., 2007) | Degasat P25 nano-TiO ₂ (21nm 50±15nm ² /g 7.5% rutile, 25% anatase, purity 99%). Particle sizes were close to those specified by the manufacturer (24.2±2.8nm). The concentration of TiO ₂ (spectrometry) in the tank reached 95-98% of the target value 10 min after dosing. The concentration in water was measured before changing the solution to confirm that the concentration remained unchanged in 12h. | Stock solution (10g/L) in ultrapure water ? sonication (6h, 35kHz) ? sonication (30 min) ? exposure dosage. | TEM, spectral scans. | Prolonged assay 14 days, semi-static (80% of the water changed every 12h). Concentration tested: 0.1; 0.5 and 1 mg/L. Food was withheld 24h prior to and during the test (except on day 10). Fish were sampled on days 7 and 14. | There was no mortality. The fish did not accumulate Ti. Changes in behavior and mucus secretion were observed at the highest concentration. The gills showed increased occurrence of edema in secondary lamellae, morphological changes in mucocyte, hyperplasia of primary lamellae, and aneurysms. Vacuolization and erosion of villousities in the intestines was observed, as well as loss of sinusoidal space, some foci of lipidosis, occasional neutrophils and apoptotic bodies in the liver. No changes were observed in the brain. There was no clear effect of the treatment or of time on the Ti levels in the gills, liver or muscle. No hematological change was found. There was alteration of the levels of tissue Zn and Cu. A concentration-dependent reduction was found in the Na-K ATPase activity in the gills, intestines and brain at the end of the experiment (significant differences only among some groups). In general, there was an increase in TBARS at the end of the experiment in gills, intestines and brain, but not in liver. Concentration-dependent glutathione depletion occurred only in liver on day 14. |
| <i>C. carpio</i> (Zhang et al., 2007) | Degasat P25 nano-TiO ₂ (50nm ² /g; 21nm). | Stock solution in ultrapure water. | Laser particle analyzer, zeta potential analyzer, ICP-OES, atomic fluorescence spectroscopy. | Chronic assay. Adsorption of Cd on TiO ₂ and natural sediment particles (SP) were evaluated. Cd was added to the water (97.3 ± 69µg/L) first, followed by TiO ₂ (10mg/L) or SP (10mg/L). The animals were placed in the water 2 hours later. Food was given twice a day during the test. Fish were transferred to new solutions every day. The animals were sampled on days 2, 5, 10, 15, 20 and 25. | TiO ₂ showed higher capacity to adsorb Cd than SP. SP did not have a significant influence on Cd in fish. The presence of TiO ₂ elevated the accumulation of Cd. After 25 days of exposure, the concentration of Cd increased by 146%, and was 22µg/g. There was a positive correlation between the concentration of TiO ₂ and Cd. TiO ₂ and Cd accumulated mainly in the viscera and gills. |
| <i>C. carpio</i> (Sun et al., 2007) | Degasat P25 nano-TiO ₂ (50nm ² /g; 25nm, aggregates of 50-400nm in water). Arsenate (As V) (prepared from Na ₂ AsO ₄ •2H ₂ O). | Stock solution of nano-TiO ₂ (1g/l) ? sonication (10min, 50W/l, 40kHz) ? exposure dosage. | TEM | Chronic assay, semi-static (water changed daily). Groups 1) control, 2) only As V (200µg/l ± 102); 3) As V + TiO ₂ (10mg/l ± 13). Animals were placed in the aquariums 2h after the addition of As and TiO ₂ . Animals were collected on days 2, 5, 10, 15, 20 and 25. Food given once a day during the test. | |
| <i>O. mykiss</i> juveniles (Warheit et al., 2007b) | DuPont Haskal; Fine TiO ₂ (380nm in water, 5.8m ² /g, 100% rutile, 99% TiO ₂ and 1% aluminum). aC (1.4) = 44 nm in water; 38.5m ² /g, 79% rutile, 21% anatase, 90% TiO ₂ ; 1% amorphous silica; 7% aluminum. | INA | DLS, BET, X-ray fluorescence, X-ray diffraction. | Aacute assay 96h static, OECD 203. Concentrations tested: 0.1, 1, 10 and 100 mg/L. | LC ₅₀ 94h = 100mg/L for both types of TiO ₂ . There was 10% of immobility at the concentrations of 10 and 100mg/L at end of 96h in both groups exposed to fine TiO ₂ . |

BCF = bioconcentration factor
 BET = Brunauer, Emmett, Teller method for surface area calculation
 CARS = coherent anti-Stokes Raman scattering
 CAT = catalase activity
 DLS = dynamic light scattering
 ICP-OES = inductively coupled plasma optical emission spectroscopy
 ICP-MS = inductively coupled plasma mass spectroscopy
 LC₅₀ = lethal concentration for 50% of exposed organisms
 LPO = lipid peroxidation
 NOEC = no observed effect concentration
 POD = peroxidase
 ZP = zeta potential
 SEM = scanning electron microscopy
 SOD = superoxide dismutase activity
 TBARS = thiobarbituric acid reactive substance assay
 TEM = transmission electron microscopy
 THF = tetrahydrofuran
 INA = information not available

Table 3. Summary of papers published about the effects of nano-TiO₂ used in toxicology studies on other aquatic organisms

| Test species | Product tested | Treatment of the product | Physicochemical characterization | Bioassay | Results |
|---|--|--|----------------------------------|--|--|
| polychaete <i>Arenicola marina</i> (Calloway et al., 2010) | Sigma-Aldrich nano-TiO ₂ , cat. no. 684662-1 (23.2 nm, equivalent spherical diameter 32.4 nm, 46.3 m ² /g, 99.9% mixture of anatase and rutile; K 82.3 ppm, Zn 9.7 ppm, Na 6.0 ppm, Fe 3.1 ppm, Li 0.4 ppm). bulk TiO ₂ | Stock solution in ultrapure water? sonication (30 min)? mixed with natural treated sediment (collected at the same site where the animals were collected). | TEM, X-ray diffraction ICP-OES | Prologed assay 10 days, OECD/ASTM 1990. Exposure in seawater. Semi-static test (water changed every 48h). Feeding during the test. Concentrations tested: 1 to 3 g/kg of sediment. | The organic content of the sediment was 0.33±0.4%. No behavioral alterations were detected. A change was observed in the feeding rate of the group exposed to 2 g/kg of nano-TiO ₂ but not in the group exposed to 1 g/kg. No effect of exposure time was found. At 2 and 3 g/kg of nano-TiO ₂ , an impact was detected in the liposome stability (neutral red retention) and an increase in genetic impairment (comet assay). Bulk TiO ₂ did not alter the rate of genetic damage compared to the control. Microscopy revealed TiO ₂ aggregates of >200nm surrounding intestinal microvillousities, but no absorption by the intestinal epithelium, although TiO ₂ remained in the lumen. BCF = 0.156± 0.075 (group lg/kg) and 0.19±0.038 (group 3g/kg). |
| mollusk <i>Mytilus galloprovincialis</i> (Gamsi et al., 2010) | Degussa P25 nano-TiO ₂ (purity >99.5%) | Stock suspension (100µg/ml) in artificial seawater? sonication (15min, 100W, in a cold bath)? storage? sonication? exposure dosage | TEM, BET, DLS | Acute assay 24h, No feeding during the test. Concentrations tested: 0.05; 0.2; 1; 5 mg/L | No mortality was found in any exposure condition. There was destabilization of the lysosomal membrane in hemocytes at 1 and 5 mg/L and in the digestive glands at 0.2, 1 and 5 mg/L, as well as accumulation of lipofuscin and lysosomal neutral lipids in the digestive glands at 1 and 5 mg/L, and an increase in CAT at 1 and 5 mg/L and in GST at 0.2, 1 and 5 mg/L in the digestive glands. |

BCF = bioconcentration factor
 BET = Brunauer, Emmett, Teller method for surface area calculation
 CAT = catalase activity
 DLS = dynamic light scattering
 GST = glutathione S-transferase activity
 TEM = transmission electron microscopy

concentration in supernatant after 16 hours went from 100 to 83 mg/L in bidistilled water and to 33 mg/L in surface water, while agglomeration and sedimentation of coated TiO₂ were slow. Some studies have involved semi-static aquatic bioassays, changing the exposure medium every 24–48 hours (Tables 1, 2 and 3), while others have performed static assays involving mainly acute exposure.

The literature reports nano-TiO₂ aggregates of about 500 nm in water, but this number varies greatly as a function of products and treatments used. Most aquatic tests have been performed starting from the sonication of a stock solution, while few have involved only agitation or filtration of the solution (Tables 1, 2 and 3). Adams *et al.* (2006) employed only agitation of Sigma Aldrich nano-TiO₂ in water and observed that 65nm sized particles formed aggregates of 320 nm, while larger particles of 950 nm and 44 μm formed aggregates of 320 nm and 1 μm, respectively. Zhu *et al.* (2010b) report that in a culture medium for daphnids, even with sonication, P25 formed aggregates that increased over time: 580 nm (1h), 2349 nm (12h) and 3528.6 nm (24h). The aggregation state of NPs inevitably changes with dilution, but there is a growing discussion about the use of dispersants or sonication processes to increase the dispersion of NPs in suspension in aquatic toxicology studies, in view of their environmental applicability (Baveye and Laba, 2008, Crane *et al.*, 2008). One argument is that the study of non-dispersive materials would be of greater relevance to what actually takes place in the environment. Moreover, sonication may cause structural changes in nanomaterials, in fact, when performed in natural waters or in the presence of any electron donor, it may result in the generation of reactive oxygen species. The sonication time required changes according to the total concentration of the nanomaterial, and once sonication or agitation has stopped, the material does not remain dispersed for very long. On the other hand, the existence of natural dispersants in the environment, such as organic matter, would validate such studies (Crane *et al.*, 2008). However, one should not assume that aggregate materials will necessarily not be bioavailable. They may simply change the mode of respiratory exposure on the water column to exposure via diet through sediment (Handy *et al.*, 2008). Benthic organisms may be more exposed to NPs aggregates than to the material in the liquid phase. Similarly, the high concentration of ions in hard or marine waters will tend to cause aggregation of NPs, modifying the mode of exposure or organisms in these ecosystems (Handy *et al.*, 2008).

A large part of acute exposure studies have been performed by withholding food from animals on the

day prior to and during the bioassay. In the case of prolonged exposure, daily feeding has generally been maintained, with a few exceptions (Federici *et al.*, 2007, Hao *et al.*, 2009). However, it should be noted that this is also a point to be evaluated carefully and standardized, in view of the capacity of organic matter to adsorb TiO₂.

The diversity of manufactured TiO₂ NPs, the quality of the medium, the aquatic species tested, and the objectives of each research, require that exposure conditions be evaluated separately.

CONCLUSION

Evaluating the potential biological impact of nanomaterials has become increasingly important in recent years. This is particularly relevant because the rapid pace of nanotechnology development has not been accompanied by a complete investigation of its safety or by the development of suitable methodologies for this investigation.

Concern about the environmental consequences of nanotechnology has been growing and has reached public opinion. Nano-TiO₂ is a nanoproduct with applications in a variety of areas, and is also promising for the remediation of contaminated environments. However, its potentially harmful effects should be investigated in depth to ensure its sustainable use. Because water bodies are the final destination of contaminants, the evaluation of the effects of nano-TiO₂ on aquatic organisms is extremely necessary. Several groups have started research in this area, however, their results are still not conclusive and the need remains to continue researching. In fact, the results vary considerably, probably due to differences in the experimental models and products tested. Therefore, we agree with the recommendation that nanoecotoxicology studies focus on the characterization of NPs and that the best exposure conditions for the different NPs be analyzed (considering their particular properties), in the attempt to standardize bioassays and facilitate the comparison of results. In addition, the standardization of nanoecotoxicological methodologies is useful for the construction of protocols to underpin and guide public policies.

ACKNOWLEDGMENTS

The authors thank CAPES and Rede Nanobiotec for awarding a doctoral grant to Zaira Clemente, as well as the Brazilian research funding agencies FAPESP, CNPq and FUNDUNESP for their financial support of this work.

REFERENCES

- Adams, L. K., Lyon, D. Y., McIntosh, A. and Alvarez, P. J. J. (2006). Comparative toxicity of nano-scale TiO₂, SiO and ZnO water suspensions. *Water Science & Technology*, **54** (11-12), 327-334.
- Baveye, P. and Laba, M. (2005). Aggregation and toxicology of titanium dioxide nanoparticles. *Environmental Health Perspectives*, **116** (4), 152.
- Brezová, V., Gabcová, S., Dvoranová, D. and Stasko, A. (2005). Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study). *Journal of Photochemistry and Photobiology B: Biology*, **79**, 121-134.
- Canesi, L., Fabbri, R., Gallo, G., Vallotto, D., Marcomini A. and Pojana, G. (2010a). Biomarkers in *Mytilus galloprovincialis* exposed to suspensions of selected nanoparticles (Nano carbon black, C60 fullerene, Nano-TiO₂, Nano-SiO₂). *Aquatic Toxicology*, **100** (2), 168-177.
- Canesi, L., Ciacci, C., Vallotto, D., Gallo, G., Marcomini, A. and Pojana, G. (2010b). In vitro effects of suspensions of selected nanoparticles (C60 fullerene, TiO₂, SiO₂) on *Mytilus hemocytes*. *Aquatic Toxicology*, **96**, 151-158.
- Carp, O., Huisman, C. L. and Reller, A. (2004). Photoinduced reactivity of titanium dioxide. *Progress in Solid State Chemistry*, **32**, 33-177.
- Chatterjee, D. and Dasgupta, S. (2005). Visible light induced photocatalytic degradation of organic pollutants. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, **6**, 186-205.
- Chithrani, B. D. and Chan, W. C. (2007). Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Letters*, **7**, 1542-1550.
- Clift, M. J., Rothen-Rutishauser, B., Brown, D. M., Duffin, R., Donaldson, K., Proudfoot, L., Guy, K. and Stone, V. (2008). The impact of different nanoparticle surface chemistry and size on uptake and toxicity in a murine macrophage cell line. *Toxicology Applied Pharmacology*, **232**, 418-427.
- Crane, M., Handy, R. D., Garrod, J. and Owen, R. (2008). Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. *Ecotoxicology*, **17**, 421-437.
- Diebold, U., 2003. The surface science of titanium dioxide. *Surface Science Reports*, **48**, 53-229.
- Domingos, R., Tufenkji, N. and Wilkinson, K. J. (2009). Aggregation of titanium dioxide nanoparticles: role of a fulvic acid. *Environmental Science & Technology*, **43** (5), 128-1286.
- Federici, G., Shaw, B. J. and Handy, R. D. (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout (*Onchorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. *Aquatic Toxicology*, **84** (4), 415-430.
- Ferin, J., Oberdorster, G. and Penney, D. P. (1992). Pulmonary retention of ultrafine and fine particles in rats. *American Journal of Respiratory Cell and Molecular Biology*, **6**, 535-542.
- Finnegan, M. P., Zhang, H. and Banfield, J. F. (2007). Phase stability and transformation in titania nanoparticles in aqueous solutions dominated by surface energy. *The Journal Physical Chemistry C*, **111**, 1962-1968.
- Fujishima, A. and Zhang, X. (2006). Titanium dioxide photocatalysis: present situation and future approaches. *Comptes Rendus Chimie*, **9**, 750-760.
- Galloway, T., Lewis, C., Dolciotti, I., Johnston, B. D., Moger, J. and Regoli, F. (2010). Sublethal toxicity of nano-titanium dioxide and carbon nanotubes in a sediment dwelling marine polychaete. *Environmental Pollution*, **158**, 1748 - 1755.
- Gaya, U. I. and Abdullah, A. H. (2008). Heterogeneous photocatalytic degradation of organic contaminants over titanium dioxide: a review of fundamentals, progress and problems. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, **9**, 1-12.
- Gelover, S., Gómez, L. A., Reyes, K. and Leal, M. T. (2006). A practical demonstration of water disinfection using TiO₂ films and sunlight. *Water Research*, **40**, 3274-3280.
- Griffitt, R. J., Luo, J., Gao, J., Bonzongo, J. C. and Barber, D. S. (2008). Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environmental Toxicology and Chemistry*, **27** (9), 1972-1978.
- Griffitt, R. J., Hyndman, K., Denslow, N. D. and Barber, D. S. (2009). Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicological Science*, **17** (2), 404-415.
- Handy, R. D., Henry, T. B., Scown, T. M., Johnston, B. D. and Tyler, C. R. (2008). Manufactured nanoparticles: their uptake and effects on fish - a mechanistic analysis. *Ecotoxicology*, **17**, 396-409.
- Hao, L., Wang, Z. and Xing, B. (2009). Effect of sub-acute exposure to TiO₂ nanoparticles on oxidative stress and histopathological changes in juvenile carp (*Cyprinus carpio*). *Journal of Environmental Sciences*, **21** (10), 1459-1466.
- Hasselov, M., Readman, J. W., Ranville, J. F. and Tiede, K. (2008). Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology*, **17**, 344-361.
- Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H. C. and Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere*, **71**, 1308-1316.
- Herrmann, J. M. (1999). Heterogeneous photocatalysis: fundamentals and applications to the removal of various types of aqueous pollutants. *Catalysis Today*, **53**, 115-129.
- Hext, P. M., Tomenson, J. A. and Thompson, P. (2005). Titanium dioxide: inhalation toxicology and epidemiology. *Annals of Occupational Hygiene*, **49** (6), 461-472.

- Hund-Rinke, K. and Simon, M. (2006). Ecotoxic effect of photocatalytic active nanoparticles (TiO₂) on Algae and Daphnids. *Environmental Science and Pollution Research*, **13**, 225-232.
- IARC (International Agency for Research on Cancer) (2010). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. V. 93. Carbon Black, Titanium Dioxide, and Talc. Retrieved December 12, 2010, from <http://monographs.iarc.fr/ENG/Monographs/vol93/mono93-7.pdf>.
- Johnston, B. D., Scown, T. M., Moger, J., Cumberland, S. A., Baalousha, M., Linge, K., Jarvis, K., Van Aerle, R., Lead, J. R. and Tyler, C. R. (2010). Bioavailability of nanoscale metal oxides TiO, CeO₂, and ZnO to fish. *Environmental Science Technology*, **44**, 1144–1151.
- Jovanovic, B., Anastasova, L., Rowe, E. W., Zhang, Y., Clapp, A. R. and Palic, D. (2011). Effects of nanosized titanium dioxide on innate immune system of fathead minnow (*Pimephales promelas* Raùnesque, 1820), *Ecotoxicology and Environmental Safety*, **74** (4), 675-683.
- Kaegi, R., Ulrich, A., Sinnet, B., Vonbank, R., Wichser, A., Zuleeg, S., Simmler, H., Brunner, S., Vonmont, H., Burkhardt, M. and Bollner, M. (2008). Synthetic TiO₂ nanoparticle emission from exterior facades into the aquatic environment. *Environmental Pollution*, **156**, 233–239.
- Kahru, A. and Dubourguier, H. C. (2009). From ecotoxicology to nanoecotoxicology. *Toxicology*, **269** (2-3), 105-119.
- Kaur, A. and Gupta, U. (2009). A review on applications of nanoparticles for the pre concentration of environmental pollutants. *Journal of Materials Chemistry*, **19**, 8279–8289.
- Kim, K. T., Klaine, S. J., Cho, J., Kim, S. H. and Kim, S. D. (2010). Oxidative stress responses of *Daphnia magna* exposed to TiO₂ nanoparticles according to size fraction. *Science of the Total Environment*, **408**, 2268 – 2272.
- Kosmulski, M. (2009). pH-dependent surface charging and points of zero charge. IV. Update and new approach. *Journal of Colloid and Interface Science*, **337**, 439–448.
- Lee, S. W., Kim, S. M. and Choi, J. (2009). Genotoxicity and ecotoxicity assays using the freshwater crustacean *Daphnia magna* and the larva of the aquatic midge *Chironomus riparius* to screen the ecological risks of nanoparticle exposure. *Environmental Toxicology and Pharmacology*, **28**, 86–91.
- Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., Li, D. and Alvarez, P. J. J. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Research*, **42**, 4591–4602.
- Lovern, S. B. and Klaper, R. (2006). *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environmental Toxicology and Chemistry*, **25** (4), 1132–1137.
- Lovern, S. B., Strickler, J. R. and Klaper, R. (2007). Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC70Hx). *Environmental Science & Technology*, **41**, 4465-4470.
- Malato, S., Fernández-Ibáñez, P., Maldonado, M. I., Blanco, J. and Gernjak, W. (2009). Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends. *Catalysis Today*, **147**, 1–59.
- Marcone, G. P. S., Oliveira, A. C., Almeida, G., Umbuzeiro, G. A. and Jardim, W. F. (2010). Influencia da fotoatividade de nanoparticulas de TiO₂ em testes de ecotoxicidade com *Daphnia similis*. V Encontro Nacional de Química Ambiental, São Pedro. Abstract book: p. 10.
- Meyer, D. E., Curran, M. A. and Gonzalez, M. A. (2009). An examination of existing data for the industrial manufacture and use of nanocomponents and their role in the life cycle impact of nanoproducts. *Environmental Science & Technology*, **43** (5), 1256-1263.
- Nascimento, I. A., Pereira, S. A. and Leite, M. B. N. L. (2008). Biomarcadores como instrumentos preventivos de poluição. (In P. A. Zagatto, and E. Bertolotti (Eds.), *Ecotoxicologia aquática: princípios e aplicações*, 2nd ed (p. 413-432). São Carlos: Rima).
- NRC, (1999). National Research Council, Toxicity of Military Smokes and Obscurants. National Academy Press, v. 2. Retrieved December 23, 2010 from http://www.nap.edu/openbook.php?record_id=9621&page=1.
- Nogueira, R. F. P. and Jardim, W. F. (1998). A fotocatalise heterogênea e sua aplicação ambiental. *Química Nova*, **21** (1), 69-72.
- Oberdörster, E. (2004). Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environmental Health Perspective*, **112**, 1058–1062.
- Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W. and Cox, C. (2004). Translocation of inhaled ultrafine particles to the brain. *Inhalation Toxicology*, **16**, 437–445.
- Palaniappan, P. L. R. M. and Pramod, K. S. (2010). FTIR study of the effect of nTiO₂ on the biochemical constituents of gill tissues of zebrafish (*Danio rerio*). *Food and Chemical Toxicology*, **48** (8-9), 2337-2343.
- Palmer, B. R., Stamatakis, P., Salzman, Bohren, C. F. and Allen, T. B. (1990). Optimum particle size of titanium dioxide and zinc oxide for attenuation of ultraviolet radiation. *Journal of Coatings Technology*, **62** (789), 95-98.
- Pelka, J., Gerhke, H., Esselen, M., Turk, M., Crone, M., Brase, S., Muller, T., Blank, H., Send, W., Zibat, V., Brenner, P., Scheneider, R., Gerthsen, D. and Marko, D. (2009). Cellular uptake of platinum nanoparticles in human colon carcinoma cells and their impact on cellular redox systems and DNA integrity. *Chemical Research in Toxicology*, **22**, 649–659.

- Pettibone, J. M., Cwiertny, D. M., Scherer, M. and Grassian, V. H. (2008). Adsorption of organic acids on TiO₂ nanoparticles: effects of pH, nanoparticle size, and nanoparticle aggregation. *Langmuir*, **24**, 6659-6667.
- PEN, (2009). Project on Emerging Nanotechnologies, Retrieved November 20, 2009 from <http://www.nanotechproject.org/inventories/consumer/>.
- Prósperi, V. A. and Nascimento, I. A. (2008). Avaliação ecotoxicológica de ambientes marinhos e estuarinos. (In P. A. Zagatto and E. Bertoletti (Eds.), *Ecotoxicologia aquática: princípios e aplicações*. 2nd ed. (pp. 270-292). São Carlos: Rima).
- Reeves, J. F., Davies, S. J., Dodd, N. J. F. and Jha, A. N. (2008). Hydroxyl radicals (OH) are associated with titanium dioxide (TiO₂) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. *Mutation Research*, **640** (1-2), 113-122.
- Roco, M. C. (2001). International strategy for nanotechnology research and development. *Journal of Nanoparticle Research*, **3**, 353-360.
- Rozenkranz, P. W. (2010). The ecotoxicology of nanoparticles in *Daphnia magna*. PhD Thesis, Edinburgh Napier University.
- RSCRAE, (2005). Royal Society of Chemistry and Royal Academy of Engineering. Nanoscience and Nanotechnologies: opportunities and uncertainties. (London: Royal Society).
- Sakamoto, M., Okuda, H., Futamata, H., Sakai, A. and Lida, M. (1995). Influence of particles size of titanium dioxide on UV-ray shielding property. *Journal of the Society of Materials Science*, **68** (4), 203- 210.
- Scown, T. M., Aerle, R., Johnston, B. D., Cumberland, S., Lead, J. R., Owen, R. and Tyler, C. R. (2009). High doses of intravenously administered titanium dioxide nanoparticles accumulate in the kidney of rainbow trout but with no observable impairment of renal function. *Toxicology Sciences*, **109** (2), 372-380.
- Shao, Y. and Schlossman, D. (1999). Effect of particle size on performance of physical sunscreen formulas. Presentation at PCIA Conference - Shanghai, China R.P. Retrieved April 29, 2010 from <http://www.koboproductsinc.com/Downloads/PCIA99-Sunscreen.pdf>.
- Sharma, V., Shukla, R. K., Saxena, N., Parmar, D., Das, M. and Dhawa, A. (2009). A. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicology Letters*, **18** (5), 211-218.
- Stone, V., Nowack, B., Baun, A., Brink, N., Kammer, F., Dusinska, M., Handy, R., Hankin, S., Hasselov, M., Joner, E. and Fernandes, T. F. (2010). Nanomaterials for environmental studies: Classification, reference material issues and strategies for physico-chemical characterisation. *Science of the Total Environment*, **408**, 1745-1754.
- Strigul, N., Vaccaria, L., Galduna, C., Waznea, M., Liua, X., Christodoulota, C. and Jasinkiewicz, K. (2009). Acute toxicity of boron, titanium dioxide and nanoparticles to *Daphnia magna* and *Vibrio fischeri*. *Desalination*, **248** (1-3), 771-782.
- Sun, H., Zhang, X., Niu, Q., Chen, Y. and Crittenden, J. C. (2007). Enhanced accumulation of arsenate in carp in the presence of titanium dioxide nanoparticles. *Water Air Soil Pollution*, **178**, 245- 254.
- Sun, H., Zhang, X., Zhang, Z., Chen, Y. and Crittenden, J. C. (2009). Influence of titanium dioxide nanoparticles on speciation and bioavailability of arsenite. *Environmental Pollution*, **157**, 1165-1170.
- Titaniumart, (2010). Photocatalysis applications of titanium dioxide TiO₂. Retrieved April 29, from <http://www.titaniumart.com/photocatalysis-ti02.html>.
- USEPA, (2009). United States Environmental Protection Agency, Nanomaterial Case Studies: Nanoscale titanium dioxide in water treatment and in topical sunscreen. EPA/600/R-09/057. Washington, DC.
- Valavanidis, A., Vlahogianni, T., Dassenakis, M. and Scoullou, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*, **64**, 178- 189.
- Vevers, W. F. and Jha, A. N. (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO₂) nanoparticles on fish cells in vitro. *Ecotoxicology*, **17**, 410-420.
- Xiaobo, C. (2009). Titanium dioxide nanomaterials and their energy applications. *Chinese Journal of Catalysis*, **30** (8), 839-851.
- Xiong, D., Fang, T., Yu, L., Sima, X. and Zhu, W. (2011). Effects of nano-scale TiO₂, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. *Science of the Total Environment*, **409**, 1444 - 1452.
- Yang, K., Lin, D. and Xing, B. (2009). Interactions of humic acid with nanosized inorganic oxides. *Langmuir*, **25**, 3571-3576.
- Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., Jia, G., Gao, Y., Li, B., Sun, J., Li, Y., Jiao, F., Zhao, Y. and Chai, Z. (2007). Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. *Toxicology Letters*, **168**, 176-185.
- Warheit, D. B. (2008). How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? *Toxicology Science*, **101**, 183-185.
- Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L. and Sayes, C. M. (2007a). Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. *Toxicology Science*, **95**, 270-280.
- Warheit, D. B., Hoke, R. A., Finlay, C., Donner, E. M., Reed, K. L., Sayes, C. M. and Finlay, C. (2007b). Development of a base set of toxicity tests using ultrafine TiO₂ particles as a component of nanoparticle risk management. *Toxicology Letters*, **171**, 99-110.

- Wei, C., Lin, W. Y., Zaina, Z., Williams, N. E., Zhu, K., Kruric, A. P., Smith, R. L. and Rajeshwar, K. (1994). Bactericidal activity of TiO₂ photocatalyst in aqueous media: toward a solar-assisted water disinfection system. *Environmental Science Technology*, **28**, 934-938.
- Wiench, K., Wohlleben, W., Hisgen, V., Radke, K., Salinas, E., Zok, S. and Landsiedel, R. (2009). Acute and chronic effects of nano- and non-nano-scale TiO₂ and ZnO particles on mobility and reproduction of the freshwater invertebrate *Daphnia magna*. *Chemosphere*, **76** (10), 1356-1365.
- Wigginton, N. S., Haus, K. L. and Hochella, M. F. (2007). Aquatic environmental nanoparticles. *Journal of Environmental Monitoring*, **9**, 1306-1316.
- Winkler, J. (2003). Titanium Dioxide. *European Coatings Literature*. (Hannover: Vincentz).
- WHO, (1969). World Health Organization, FAO Nutrition Meetings Report Series No. 46A: Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-caking Agents and Certain Other Substances. WHO/FOOD ADD/70.36.
- Zhang, X. Z., Sun, H. W. and Zhang, T. Z. Y. (2006). Bioaccumulation of titanium dioxide nanoparticles in carp. *Huan Jing Ke Xue-Chinese Journal of Environmental Science*, **27**, 1631-1635.
- Zhang, X., Sun, H., Niu, Q., Chen, Y. and Crittenden, J. C. (2007). Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere*, **67**, 160-166.
- Zhu, X., Zhu, L., Duan, Z., Qi, R., Li, Y. and Lang, Y. (2008). Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebrafish (*Danio rerio*) early developmental stage. *Journal of Environmental Science and Health, Part A*, **43**, 278-284.
- Zhu, X., Wang, J., Zhang, X., Chang, Y. and Chen, Y. (2010a). Trophic transfer of TiO₂ nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. *Chemosphere*, **79**, 928-933.
- Zhu, X., Chang, Y. and Chen, Y. (2010b). Toxicity and bioaccumulation of TiO₂ nanoparticle aggregates in *Daphnia magna*. *Chemosphere*, **78**, 209-215.