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Effects of titanium dioxide nanoparticles derived from consumer products on the marine diatom *Thalassiosira pseudonana*

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Abstract

Increased manufacture of TiO₂ nanoproducts has caused concern about the potential toxicity of these products to the environment and in public health. Identification and confirmation of the presence of TiO₂ nanoparticles derived from consumer products as opposed to industrial TiO₂ NPs warrant examination in exploring the significance of their release and resultant impacts on the environment. To this end, we examined the significance of the release of these particles and their toxic effect on the marine diatom algae *Thalassiosira pseudonana*. Our results indicate that nano-TiO₂ sunscreen and toothpaste exhibit more toxicity in comparison to industrial TiO₂ and inhibited the growth of the marine diatom *T. pseudonana*. This inhibition was proportional to the exposure time and concentrations of nano-TiO₂. Our findings indicate a significant effect, and therefore, further research is warranted in evaluation and assessment of the toxicity of modified nano-TiO₂ derived from consumer products and their physicochemical properties.

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Introduction

Among metal oxide nanoparticles, titanium dioxide (TiO₂) nanoparticles have been widely used in industry, agriculture, household goods, and consumer products including, but not limited to, sunscreens, cosmetics, electronics, food packaging, and food products. These products are produced in large quantities, primarily due to their whiteness and opacity (Popov et al. 2005; Aruoja et al. 2009; Pang et al. 2012). According to a survey of global quantities, the global production of nano-TiO₂ averages 3000 tons per year and is estimated within the range of 101 to 10,000 t/year, based on the response rate of 56 % (10 out of 18 answers) (Piccinho et al. 2012).

As predicted, cosmetics, including sunscreens, were among the top usage at 70–80 %, followed by plastics (20 %), paints (10–30 %), and cement (1 %) (Pang et al. 2012), indicating most of the TiO₂ released from cosmetics are likely to enter the environment. Other statistics indicate that nano-TiO₂ share of bulk TiO₂ world production is predicted to be converted completely into nanoscale TiO₂ by 2025 at 2.5 million MT growth rate (Robichaud et al. 2009).

The nano-TiO₂ widely used in sunscreens is reported as being primarily in the form of a rutile crystal form rather than in the crystal structure of anatase (found mainly in the industrial form). This mineral has a needle or near-spherically shaped smaller size (<20 nm) and often had silica and alumina as coating materials (Lewicka et al. 2011). Although the physicochemical properties of TiO₂, white powder, insoluble in water, and gravity of 3.9 (anatase); density of 4.23 g/cm³; and boiling point of 2500–3000 °C; melting point (1855 °C), suggests its benign nature, its release into the aquatic environment and its subsequent effects are likely to be inevitable from manufacturing and widespread consumption of nano-TiO₂ products.

The estimated release of nano-TiO₂ from the model products tested in this study suggests potential significant release of TiO₂ into the coastal seawater and sewage treatment plants. For example, the total amount of toothpaste used per brushing typically ranges from 0.2 to 5.0 g, depending on the age of the person (Levy et al. 1995). On average, 2.6 g of toothpaste containing from 0.1 to 0.5 % of TiO₂ (Weir et al. 2012) is used per person per brushing. Assuming brushing three times per day, four persons per household, and 120 million households in the USA, approximately 4100 metric tons of TiO₂ are released into wastewater treatment plants (WWTPs) per year.

Although more than 96 % of these influent nano-TiO₂ particles are treated by WWTPs (Westerhoff et al. 2011), approximately 164 metric tons/year (4 % of the influent nano-TiO₂ particles) may be still found in the treated effluent. Specifically, nano-TiO₂ particles smaller than 30 nm were found in the effluent of WWTPs (Kiser et al. 2009; Westerhoff et al. 2011). Significant amounts of nano-TiO₂ particles might be transported to surrounding rivers and coastal seawaters where nano-TiO₂ particles can impact living organisms. Similarly, 16,000 to 25,000 tons of sunscreens containing TiO₂ are expected to be used in tropical countries each year (Danovaro et al. 2008). The study revealed that at least 25 % of sunscreens applied to the skin is washed off and might be released into the ocean while swimming. Based on

this calculation, assuming sunscreen containing 4 % TiO₂, the potential release of TiO₂ into coastal seawaters is estimated to be 160 to 240 metric tons per year. Interestingly, sunscreens were reported to be a main concern due to directly washing off into the marine environment, which increases concern about effects on the marine ecosystem (Weir et al. 2012).

In light of the extensive use of nanosized TiO₂ ingredients in products, accumulative hazards to the environment and human health may occur, although to the best of our knowledge, no consolidated data about any long-term effects are available.

Given the significant production and usage of products containing nano-TiO₂, and the subsequent release of TiO₂ into an aquatic media, an ecosystem may incur harmful effects, thereby having a detrimental impact on aquatic organisms with ultimate accumulation in drinking water (Aitken et al. 2009). Despite the likelihood of nanoparticles being released into surface waters and resultant drinking water sources, few studies have examined leaching of nanoparticles into drinking water and their health implications (Jain and Pradeep 2005; Lv et al. 2009).

TiO₂ is known to kill bacteria, viruses, and organic and inorganic contaminants via activation as a photocatalyst under light (Adesina 2004; Giraldo et al. 2010). In spite of the advantages of its application to treat contaminants and having antibacterial properties (Senjen 2009; Haghi et al. 2012), the exposure of nano-TiO₂ was shown to increase the mortality of green algae (*Desmodesmus subspicatus*) (Hund-Rinke and Simon 2006), carp (*Cyprinus carpio*) (Zhang et al. 2007; Linhua et al. 2009), *Daphnia magna* (Zhu et al. 2010), and marine organisms such as marine phytoplankton, *Phaeodactylum tricornutum* (Wang et al. 2016) and cyanobacteria, *Anabaena variabilis* (Cherchi and Gu 2010). Moreover, exposure of TiO₂ was shown to enhance the bioaccumulation of contaminants (e.g., perfluorooctanesulfonate) in zebrafish (Qiang et al. 2015). More recently, long-term exposure revealed adverse effects on zebrafish (e.g., concentration and time-dependent inhibition of growth with a decreased liver weight ratio in zebrafish) (Chen et al. 2011).

Additional concern is increasing, especially in environmental systems that are heterogeneous with the presence of other contaminants. As an example, cadmium was found to strongly adsorb onto nano-TiO₂ owing to the NPs' physicochemical properties (small size, large surface area, and strong electrostatic attraction). After being transported in the aquatic environment, the NPs that carried the contaminants could end up being accumulated in aquatic organisms (Hartmann et al. 2012).

A recent study by Minetto et al. (2014) indicates that only cell growth inhibition tests have been applied to assess the effect of TiO₂ on marine microalgae. Several studies showed influencing factors such as concentration, UV irradiation, pH, and ionic strength (tested target species: *T. pseudonana*, *S. costatum*, *I. galbana*, *D. teriolecta*, *E. coli*, and *P. subcapitata*) (Miller et al. 2012; Lin et al. 2014; Aruoja et al. 2009) for the toxicity of TiO₂ NPs. For example, toxicity was enhanced when there was an increase in ionic strength at or near isoelectric point (IEP) due to aggregation (French et al. 2009; Lin et al. 2014; Waalewijn-Kool et al. 2013), increased exposure time (Manzo et al. 2015; Ahmad and

Sardar 2013; Hartmann et al. 2012; Aruoja et al. 2009; French et al. 2009), or in the presence of UV irradiation (Brunet et al. 2009; Manke et al. 2013; Li et al. 2014).

While such studies have investigated the toxicity effects of nanomaterials, as well as how they are influenced by physicochemical properties of nanomaterials, the potential risks to marine environments from consumer product-derived metal oxide nanoparticles—including nano-TiO₂—have been neglected. Therefore, this study aims to investigate the potential impact of consumer product-derived nano-TiO₂ on the marine diatom algae.

Diatoms account for 40 % of total carbon fixation in the marine ecosystem and provide a base for the marine food chain (Falciatore and Bowler 2002). The marine diatom *Thalassiosira pseudonana* is often chosen as a model organism for marine toxicity studies (Yung et al. 2015; Peng et al. 2011), given their relevance in the overall balance of the ecosystem. Moreover, it is often considered as a reliable model organism for growth in seawater, which represents a global marine distribution (Brand 1984; Hasle and Heimdal 1970), and it is used to indicate marine pollution.

In line with the study's overall aim, two model products' nano-TiO₂ data (sunscreens and toothpastes) were compared to industrial-type nano-TiO₂ on the growth inhibition of the diatom, *T. pseudonana*, as a way to gain insights into nanopollution in a marine environment. The study's specific objective is to discover the extent of potential impact of nano-TiO₂ from consumer products on marine algae in comparison to industrial nano-TiO₂ by identifying the toxicity kinetics of different types of nano-TiO₂ (such as industrial and the model products derived TiO₂) as a function of exposure time and concentrations.

Materials and methods

Materials and nano-TiO₂ suspension

Commercial TiO₂ nanopowder (>99.7 % purity, <25 nm particle size, 45–55 m²/g surface area, anatase) was purchased from Sigma-Aldrich (St. Louis, MO). Sunscreen (Gardener's Armor™, Cincinnati, OH, 4 % TiO₂, 4 % colloidal oatmeal) and toothpaste (Colgate-Palmolive Company, New York, NY, primary ingredients: 0.24 % of sodium fluoride and TiO₂ as an inactive ingredient) were purchased from a local public store (Miami, FL) and nano-TiO₂ particles were extracted from these products. The zeta potential and hydrodynamic particle size (determined by dynamic light scattering) were measured at 25 °C using a Malvern Zetasizer Nano ZS90 analyzer (Malvern Instruments, Westborough, MA), as a function of pHs (Fig. 2: no buffer, industrial nano-TiO₂ at 5 mg/L, pH adjusted by either adding 1 M of HCl or NaOH), and after 72 h of exposure to *T. pseudonana* (Fig. 7: control: the diatom algae alone; TiO₂ concentration: 5 mg/L). Changes in pH were also monitored constantly with a pH meter (Orion™, 720A⁺, USA) employing a glass electrode (Orion™, 8156BNUWP, USA).

All suspensions were prepared by suspending nano-TiO₂ in artificial seawater containing f/2 medium (Guillard and Ryther 1962; Guillard 1975) to the desired concentrations of nano-TiO₂, both in the form of industrial and extracted particles from the two model products: sunscreen and toothpaste. Vortexing TiO₂ suspension at 3200 rpm was performed for a short

time (1 min) for homogeneity. In addition, the samples with algae were shaken to reduce sedimentation. Absorbance measurements using a Beckman Coulter DU 720 spectrophotometer were performed on a colloidal suspension of TiO_2 in seawater by progressively diluting the original sample within the following tested set of TiO_2 concentrations (100, 50, 20, 10, 5, 2, 1, 0.5, 0.25, 0.13 mg/L). The nano- TiO_2 concentrations in the water were determined by measuring the absorbance peak wavelength at 350 nm using a spectrophotometer. All of the standard curves involved the use of five concentrations and were all linear with regression coefficients greater than 0.995. The limit of detection (LOD) was 0.13 mg/L (Fig. S2).

Method for the synthesis of artificial seawater

As per artificial seawater mixed with an f/2 medium, the artificial seawater was prepared by dissolving 24.72 g of NaCl (>99.0 % purity, Fisher Scientific, Fair Lawn, NJ), 0.671 g of KCl (99.7 % purity, Sigma-Aldrich, St. Louis, MO), 1.029 g of CaCl_2 (>99.0 % purity, Sigma-Aldrich, St. Louis, MO), 4.656 g of MgCl_2 (>99.0 % purity, BDH Chemicals, Radnor, PA), 3.069 g of MgSO_4 (>99.5 % purity, Sigma-Aldrich, St. Louis, MO), and 0.18 g of NaHCO_3 (99.9 % purity, Mallinckrodt, Paris, KY) in 1.0 L of ultrapure water (18.2 M Ω) produced using a three-stage Millipore Milli-Q plus 185 purification system (Millipore, Billerica, MA). This artificial seawater was adjusted to a pH of 8.0 either by adding 1 M NaOH or HCl and monitored with a pH meter (OrionTM, 720A+, USA) employing a glass electrode (OrionTM, 8156BNUWP, 149 USA).

Nano- TiO_2 extraction from sunscreen and toothpaste

A modified method from the literature (Samontha et al. 2011; Nischwitz and Goenaga-Infante 2012) was used to extract the TiO_2 NPs from sunscreen and toothpaste. To extract the TiO_2 NPs from the sunscreen and toothpaste, 2.0 to 3.0 g of these products (sunscreen/toothpaste) were measured and placed into a Falcon tube. Then, 30 mL of hexane (>99.9 % purity, Honeywell, Burdick & Jackson, Muskegon, MI) was added, sonicated for 1 min, and then centrifuged at 4400 rpm for 5 min. The hexane portion in the tube was discarded and 30 mL of ethanol (>95 % purity, Pharmco-Aaper, Shelbyville, KY) was added to the residue left at the bottom of the tube.

The mixture was sonicated for 1 min and centrifuged at 4400 rpm for 5 min. The organic phase (ethanol solution) in the tube was discarded and 30 mL of DI water was added, followed by manual shaking for 2 min and centrifugation at 3000 g for 10 min to decant the water. This step was repeated twice. Finally, the samples were dried in an oven at 100 °C for at least 12 h, and the samples were placed in a desiccator. Prior to use, and to obtain a finer TiO_2 powder, the dried samples were ground using a sterilized grinder. To verify if the extraction method would alter the composition of nanoparticles (e.g., surface coatings), energy dispersive spectroscopy (EDS) analysis (Supporting Information: Fig. S1) was carried out and the results confirmed the presence of a titanium element from both industrial- and consumer product-extracted materials. Moreover, the elemental compositions of these materials were identical to those from industrial TiO_2 , although the peak intensity varied. On the other hand, the composition analysis of the sample extracted from toothpaste showed an additional silicon element, possibly due to the hydrated silicon dioxide contained

in the toothpaste as an inactive ingredient. It is noted that toxic effects from the naturally occurring release of TiO₂ from these products could be different from our study as, depending on brand and manufacturers, various ingredients contained in such products could influence the release of TiO₂ and consequently toxicity on marine diatoms.

Culture methods for marine diatom algae, *T. pseudonana*

A total of 20 mL of the marine diatom algae was supplied by the University of Texas at Austin (Austin, TX). According to the information provided by the supplier, the algae *T. pseudonana* is characterized as unicellular and brackish, having centric cells and shells made of silica. The culture of *T. pseudonana* (VWR International, Radnor, PA) was then prepared by adding artificial seawater with f/2 medium to the originally purchased culture. The culture was then incubated at a constant temperature of 26 °C, with 12 h:12 h (dark:light) cycles maintained by a Verilux VT 10 (5000 lx, white light).

Detection of *T. pseudonana* peak absorbance wavelength and inhibition (%) as a function of exposure time

The growth inhibition of the marine diatom *T. pseudonana* was evaluated by measuring absorbance in the following sequences. Since the algae cell concentration purchased from the vendor was unknown, the pure samples from the homogenized culture (1:1 dilution—the absorbance of the algal cell *T. pseudonana* in artificial seawater) were measured and subsequent measurements were performed on half dilutions (1/2 D) of each concentration at each step until a 1:256 dilution level was reached, which corresponded to the detection limit of the spectrophotometer (i.e., 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256). The literature reported different peak absorbance wavelengths, with most ranging from 672 to 678 nm (Davis et al. 2006; Sobrino et al. 2008). Therefore, eight different concentrations were tested for absorbance in a range of 650–700 nm. The absorbance relationship with this concentration was found to be linear with $R^2 = 0.995$ at 674 nm, thereby determining the peak absorbance wavelength of *T. pseudonana* culture as 674 nm. The absorbance of *T. pseudonana* was measured at 674 nm in all measurements.

The NP suspension (15 mL) in artificial seawater was inoculated in 15 mL of algae mass culture (50 mL Petri dish) and gently mixed afterwards. Both for industrial-type TiO₂ NPs and for extracted NPs, the concentrations tested were 5 mg/L. Control samples were prepared by adding 15 mL of artificial seawater containing f/2 medium to 15 mL of algae mass culture (50 mL Petri dish). Absorbance was measured for all samples at $t = 0$ using a Beckman Coulter DU 720 spectrophotometer. All samples were then incubated (conditions were $t = 26$ °C, 12:12 light:dark cycle, 5000 lx white light) for the ideal growth conditions (James 1978), and these measurements were repeated at fixed time steps (0, 5, 12, 24, 48, 72, and 96 h).

Effect of TiO₂ concentrations at fixed exposure times

The NP suspension (15 mL) was inoculated in 15 mL of diatom culture (50 mL Petri dish) and gently mixed. Both the industrial-type TiO₂ NPs and extracted NPs were utilized at three different concentrations of TiO₂ NPs (1, 2.5, and 5 mg/L) to investigate the toxic effect of TiO₂ concentration on algae. The concentrations of nano-TiO₂ were measured using a

spectrometer as described in the sub-section under “materials and nano-TiO₂ suspension.” The same concentrations of both industrial and product-derived nano-TiO₂ were exposed to algae after preparation. The control samples were prepared by adding 15 mL of mixed artificial seawater and f/2 medium to 15 mL of algae mass culture (50 mL Petri dish). Absorbance was measured for all samples at $t = 0$ using a Beckman Coulter DU 720 spectrophotometer. All of the samples were incubated (incubation conditions $t = 26\text{ }^{\circ}\text{C}$, 12:12 light:dark cycle, 5000 lx white light), and measurements were repeated after 72 h of incubation. All experiments were run in triplicate with a standard error of mean of $\pm 10\%$.

Statistical analysis

Statistical analysis was carried out using the Student's t test and confirmed the statistical significance at $p < 0.05$ of the toxicity effects of exposure time and types of nano-TiO₂ on the diatom. However, the statistical significance at $p > 0.05$ was obtained on the toxicity effect as a function of nano-TiO₂ concentrations. While the difference in inhibition (%) (inhibition) does not appear to depend significantly on concentration, the Pearson correlation coefficient shows that a correlation exists with $r > 0.93$ in all types of nano-TiO₂.

X-ray diffraction analysis of solids

A subsample of pristine TiO₂ powder solids (about 20 mg) was taken to fill up the cavity (7 mm diameter) on an elemental silicon slide sample holder. The sample in the cavity was pressed to form a smooth surface using a stainless spatula. For TiO₂ samples reacting with algae, a suspension of TiO₂ and algae was filtered out on a 45-mm diameter and 45- μm pore size Whatman membrane filter paper in a vacuum filter holder and dried. The filter paper was quarterly cut and a quarter was taped to a flat zero-background quartz slide. The silicon or quartz slide was scanned with a Rigaku Miniflex X-ray diffractometer at a scan rate of $0.5^{\circ} 2\theta \text{ min}^{-1}$ and sampling width of $0.05^{\circ} 2\theta$ (Fe K α radiation, $\lambda = 1.9373\text{ \AA}$; operated at 30 keV and 15 mA). The mean crystallite dimension was estimated using the Scherrer equation.

Scanning electron microscopy analysis

Control (algae only) and experimental samples of algae exposed to different types of nano-TiO₂ were analyzed using scanning electron microscopy (SEM). All samples were fixed by 2 % glutaraldehyde in phosphate buffered saline (PBS) for at least 1 h. After fixation, the samples were centrifuged and then washed three times in a buffer. The prepared samples were dehydrated by a serial protocol using graded ethanol (three times for 20, 50, 70, 95, and 100 %) and dried with hexamethyldisilazane (HMDS) on glass coverslips. Finally, the samples were coated and imaged in a Philips XL-30 Field Emission SEM equipped with energy dispersive spectroscopy (EDS) to visualize the morphology change of *T. pseudonana*.

Results and discussion

Toxicity effects as a function of exposure time and the types of nano-TiO₂

To assess the potential toxicity of nano-TiO₂, the growth inhibition of a marine diatom was examined as a function of nano-TiO₂ exposure time and the correspondent types of nano-TiO₂. The growth inhibition (%) was estimated based on the literature (as shown in Eq. 1)

(Cao et al. 2011). Percentage growth inhibition plotted as a function of exposure time is shown in Fig. 1.

$$\%GI(t) = \frac{abs(t)_{control} - abs(t)_{sample}}{abs(t)_{control}} \cdot 100 \quad (1)$$

As shown in Fig. 1, after a lag phase of 24 h, increasing growth inhibition was observed in all samples. The most toxic effect in the first hours observed from industrial nano-TiO₂ was in accordance with a recent study on the diverse toxic effect of SiO₂ and TiO₂ nanoparticles on *Dunaliella tertiolecta* (Manzo et al. 2015). As indicated in the data, the release of both sunscreen- and toothpaste-derived nano-TiO₂ was more significant than that for industrial nano-TiO₂ and increased as time elapsed at a constant nano-TiO₂ concentration (5 mg/L).

Interestingly, the extent of the significant release of nano-TiO₂ was in the order of sunscreen, toothpaste, and industrial. Significant differences among the three types of nano-TiO₂ (sunscreen, toothpaste, and industrial) appeared obvious, especially at 72 h. The pHs were measured as neutral at each sampling step. Given the known isoelectric point of TiO₂ (5.6) (Joo et al. 2009) and our experimental results as shown in Fig. 2 (industrial nano-TiO₂), the neutral pH may not be attributable to the aggregation and the associated toxicity. However, the results indicate that the aggregation of TiO₂ enhances toxicity in biological organisms as the exposure time increases, which is consistent with a recent study that demonstrated the contributing factors of toxicity as exposure duration, aggregation, and concentrations (Clément et al. 2013).

Assessing toxicity as a function of nano-TiO₂ concentrations

Studies have shown that toxicity could be associated with aggregation, size, crystal structure, and concentrations of nano-TiO₂ (Manzo et al. 2015; Clément et al. 2013; Hund-Rinke et al. 2010). Higher concentrations may agglomerate, which could then be linked to the extent of toxicity. As shown in Fig. 1, the toxicity of nano-TiO₂ on the marine diatom algae increased over time, especially from the nano-TiO₂ extracted from commercial products. Similarly, as nano-TiO₂ concentration increased, higher toxicity (increased growth inhibition) was observed. The greatest growth inhibition was in the culture grown with the sunscreen-extracted TiO₂ NPs, followed by toothpaste TiO₂ NPs and industrial-type nano-TiO₂. As shown in Fig. 3, the results highlight that toxicity depends on the types of nano-TiO₂ in the order of sunscreen, toothpaste, and industrial. However, the dosages of nano-TiO₂ also had an effect, indicating that aggregation may exhibit more toxicity than nanosized TiO₂.

The toxicity dependency on the concentrations of industrial or synthesized nano-TiO₂ was reported with increased toxicity in biological organisms when nano-TiO₂ concentrations increased (Hund-Rinke et al. 2010; Lin et al. 2014), though the studies showed antibacterial properties at very high concentrations. Nevertheless, no studies have investigated the correlation between possible toxicity released from nano-TiO₂ products and nanoparticles' concentrations. As observed in Fig. 3, the extent of toxicity was influenced by nano-TiO₂ concentrations and the types. These results indicate that aggregation forms and particle sizes may have an effect, though it was not significant as shown in Fig. 3.

Based on the aforementioned results, it was discovered that nano-TiO₂ extracted from the two model products not only significantly contributed to toxicity as measured by growth inhibition but also increased the concentrations affected by the toxic effects the same during the exposure time. Interestingly, the inhibiting effects on algal growth appear within 24 h, and the extent of toxicity becomes most significant in 72 h for the three tested samples (i.e., sunscreen, toothpaste, and industrial). Regardless of the exposure time and concentrations of nano-TiO₂, the product-derived nano-TiO₂ showed significant release and resultant toxicity, which was found to differ from the industrial-type nano-TiO₂.

Figure 4 shows X-ray diffractograms of the initial and after-test TiO₂ nanomaterials. The toothpaste TiO₂ sample contained anatase (pdf# 01–071-1166) and small amounts of quartz (pdf# 01–079-1906). The toothpaste TiO₂ sample collected after the algae toxicity test also contained calcite (pdf# 01–072-1650) that may have been derived from the precipitation of adding calcium ions and bicarbonate ions. The sunscreen TiO₂ sample also contained anatase before the algae test. After the toxicity test, the sample exhibited additional X-ray diffraction (XRD) peaks, with the highest peaks matching those of halite (NaCl, pdf# 01–070-2509) as a residual salt. The industrial TiO₂ also contained anatase before the toxicity test. After the test, additional XRD peaks matched those of calcite and halite. Particle size estimation using the Scherrer equation was only qualitative for samples collected after the algae toxicity test due to the limited number of samples harvested on the filter membrane (Table 1). For toothpaste and sunscreen TiO₂ NPs, the particle size was increased after the algae test, indicating that the TiO₂ NPs may have adsorbed biomolecules from the algae. On the other hand, the size of industrial TiO₂ decreased after contact with the algae, which could be a result of the leaching of surface coatings from the industrial TiO₂ (Liu et al. 2013a, b; Liu et al. 2014).

To gain insight into the interaction between algae and different types of nano-TiO₂, SEM analysis was conducted. As shown in Fig. 5a, the morphology of algae without any exposure to nano-TiO₂ (as a control) was shown to be a normal frustule structure with an intact cell surface. However, when the algae was exposed to nano-TiO₂, significant morphological changes occurred with fractures of the intact cell surface and irregular cell outlines as shown from the exposure to both industrial- and nanoparticle-driven TiO₂ (Fig. 5b–d). Interestingly, the aggregated form of TiO₂ was detected on the algae exposed to nano-TiO₂ (Fig. 6a, b). These microscopic analyses suggest that a significant alternation of the aquatic species (e.g., algae) occurs through bio-uptake upon exposure to nanomaterials contained in products. Moreover, significant changes in the physicochemical properties of TiO₂ appear as an aggregated form on the surface of the diatom algae (Fig. 6), which contributes to the toxicity.

Similar observations were recently reported (Wang et al. 2016; Li et al. 2015), indicating that the aggregation of nano-TiO₂ was found to destroy cell membranes and cell walls in the algae. According to the study (Wang et al. 2016), nano-TiO₂ exposed to the marine phytoplankton, *Phaeodactylum tricornutum*, showed toxic effects caused by aggregates, which are responsible to entrap algae cells, causing cell wall damage. While their study compared other possible toxic effects such as indirect effects (e.g., shading, oxidative stress), the dominant toxic effect was found to be physical effects (e.g., aggregates) of nano-TiO₂.

Changes in pH were monitored to assess if such a change affected toxicity (Fig. 7). Results showed a negligible pH effect, given the little change regardless of the types of nano-TiO₂ (e.g., control: 8.5→8.7; industrial TiO₂: 8.5→8.7; sunscreen TiO₂: 8.5→8.8; toothpaste TiO₂: 8.5→8.6). Interestingly, while little change in the hydrodynamic particle sizes were observed from industrial nano-TiO₂ and control (the diatom algae only), consumer products-derived nano-TiO₂ showed increased hydrodynamic particle sizes [toothpaste: 1310→1423 nm; sunscreen: 1289→1697 nm], and an especially significant increase from sunscreen-extracted nano-TiO₂ after exposure to the marine diatom algae for 72 h (Fig. 7). The surface charge of the diatom algae was approximately −16 mV, but after exposure to TiO₂ suspension, the absolute values of zeta potential decreased in the order of industrial TiO₂ (−10 mV), toothpaste TiO₂ (−7.7 mV), and sunscreen TiO₂ (−5.6 mV), and, as time passed, the zeta potential values changed little. The significant decrease of zeta potential and the increase of hydrodynamic particle size observed from sunscreen-extracted TiO₂ may have contributed more to the effects of toxicity as shown in Fig. 3. The zeta potential of the diatom alone decreased in 72 h. Such instability, due to the adsorption of cations present in seawater (e.g., Ca²⁺, Mg²⁺) on the surfaces of the diatom, may cause more of the nano-TiO₂ particles to be adsorbed onto the surfaces of the diatom.

This study shows that the toxicity of TiO₂ is dependent on the type of TiO₂, exposure time, and aggregation. However, it should be noted that the way of synthesis of TiO₂ could also influence the toxicity because, depending on brand and manufacturer, different ingredients (e.g., coating materials) are used.

Environmental significance

While there are no published results available for the potential toxicity effects of nano-TiO₂ derived from consumer products on marine organisms and their release behavior in comparison to industrial-type nano-TiO₂, our results show a significant release from the two model products—derived nano-TiO₂ and enhanced aggregation on the biological entity—which is attributable more to toxicity (increased toxicity as a function of nano-TiO₂ concentrations), and more time-dependent toxicity of consumer products-derived nano-TiO₂ compared to industrial-type nano-TiO₂. Based on this study, if commercial TiO₂ is to be released and washed off along with coating materials, naked TiO₂ would cause significant aggregation that contributed more to the toxicity of marine environments. These findings are the first in this area and contribute to our understanding of nanomaterial ecotoxicity in the marine environment.

While more research is encouraged for further analysis and the development of the tools to model and assess the long-term toxicity effects, these results showcase the predicted significant impact on a marine environment because *T. pseudonana* inhabiting marine water globally is a critical marine organism responsible for about 40 % of the total carbon fixation in oceans (Armbrust 2009). Therefore, considering that inhibition of *T. pseudonana* increases 50 % from sunscreen and 35 % from toothpaste after 72 h of exposure to the marine diatom algae, uncontrolled and improper disposal of nano-TiO₂ products will raise issues about the need to develop safe nanoproducts and disposal methods as well as control of both intentional and unintentional releases of nano-TiO₂ particles.

The study by Mueller and Nowack (2008) indicated that the predicted environmental concentrations of nano-TiO₂ in water were less than 20 µg/L, which are still higher concentrations than the predicted no effect concentrations of less than 1 µg/L. Nonetheless, our study showcases that the long-term exposure of nano-TiO₂ could have detrimental effects on marine environments, while various environmental factors could influence the ultimate fate of TiO₂. According to recent studies, the concentrations of nano-TiO₂ tested for toxic effects on algae ranged from 5 to 30 mg/L (Li et al. 2015), from 5 to 100 mg/L (Xia et al. 2015), and from 5 to 200 mg/L nano-TiO₂ (Wang et al. 2016). Considering the lack of data on toxicity after long-term exposure, additional study is strongly encouraged to investigate the potential effects of long-term exposure of TiO₂ on marine environments under various environmental parameters.

In addition, prior to investigating algae growth inhibition in detail, the genotoxic and cell viability effects of TiO₂ NPs were encouraged as a precedent in identifying concurrent toxicity effects and their mechanisms; a recent study indicates that the most significant genotoxicity toward marine microalgae *Dunaliella tertiolecta* was observed from TiO₂ at algae NOEC (No Observed Effect Concentration), followed by SiO₂ and ZnO (Schiavo et al. 2016). In addition, at the concentration for algae growth, reactive oxygen species (ROS) production inside the algae cell was shown to generate free radicals that induce indirect genotoxicity (Schiavo et al. 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
*Growth inhibition (%) of *T. pseudonana* as a function of exposure time*

Figure 2.
The isoelectric point (IEP) of industrial nano-TiO₂ (concentration: 5 mg/L)

Figure 3.
Growth inhibition (%) of *T. pseudonana* as a function of nano-TiO₂ concentrations

Figure 4. X-ray diffractograms (Fe $K\alpha$ radiation, $\lambda = 1.9373 \text{ \AA}$) of industrial TiO_2 , sunscreen TiO_2 , toothpaste TiO_2 , and counterparts after algae toxicity test (A: anatase (TiO_2), C: calcite (CaCO_3), H: halite (NaCl), Q: quartz (SiO_2))

Figure 5. SEM images (×5000) of the marine diatoms *Thalassiosira pseudonana*: **a** algae, **b** algae exposed to industrial nano-TiO₂, **c** algae exposed to sunscreen, and **d** algae exposed to toothpaste (nano-TiO₂ suspension: 5 mg/L)

Figure 6.
SEM images (**a** $\times 10,000$; **b** $\times 160,000$) of aggregated nano-TiO₂ (concentration: 2.5 mg/L; industrial TiO₂ exposed to algae) [**a** 10,000 magnification and **b** 160,000 magnification]

Figure 7. Hydrodynamic particle size, zeta potential, and pH of TiO_2 after 72 h of exposure to *T. pseudonana* [control: only the diatom algae and TiO_2 concentration: 5 mg/L]

Table 1.

TiO₂ particle size estimated from XRD data using the Scherrer equation

Samples	Particle diameter (nm)
Initial toothpaste TiO ₂	6.1
Initial sunscreen TiO ₂	37.3
Initial industrial TiO ₂	10.5
Toothpaste TiO ₂ exposed to algae	37.5
Sunscreen TiO ₂ exposed to algae	46.8
Industrial TiO ₂ exposed to algae	6.2