Phototoxicity of titanium dioxide nanoparticles in the starlet anemone *Nematostella vectensis*

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Writer's Comment: The six weeks I spent at the Bodega Marine Lab taking ETX 127 (Environmental Stress and Development in Marine Organisms) comprised the most demanding, captivating, and rewarding class I took at UC Davis. As an Environmental Toxicology undergraduate, being given the opportunity to conduct my own research was invaluable, especially at the junction of subjects I intend to pursue: toxicology and ecology. Nanotoxicology is the branch of toxicology that attempts to investigate negative implications of nanotechnology, necessary considerations for an industry with such prodigious potential and seemingly few environmental consequences. The frontiers of nanoparticles and nanotechnology intrigue me immensely. This paper discusses the intentions and findings of my research project. I continue to be inspired by my summer in Bodega Bay; I could not imagine a more pristine location or supportive marine lab community. Tremendous gratitude goes out to both my brilliant and generous instructors, without whom I would remain daunted by the questions asked of ecotoxicologists.

Instructor's Comment: This was written as Malina's project paper in Environmental Toxicology/Nutrition 127, "Environmental Stress and Embryo Development in Marine Organisms" in Summer Session I at the Bodega Marine Laboratory. This is a unique upper-division course (10 units) that features a hands-on research experience at the marine laboratory along the beautiful Sonoma coast, in which students develop their own project related to stress on early life stages in the marine environment. Malina designed experiments to address her hypotheses regarding nanomaterials, analyzed her data, wrote a paper in a peer-review journal format, and presented her research in a formal symposium at the Bodega Marine Laboratory. Malina is a dedicated student, and it was a pleasure to watch her take full advantage of this research opportunity to become a talented and curious scientist. We were impressed by her writing and by her ability to see the bigger picture of her project and the implications of her work.

– Gary Cherr, Departments of Environmental Toxicology and Nutrition/ Bodega Marine Laboratory and Elise Fairbairn, Bodega Marine Laboratory

Abstract

Nanoparticles of titanium dioxide (nano-TiO2) are increasingly used in personal care, commercial, and industrial applications. Although considered mildly phototoxic, nano-TiO2 is frequently included in sunscreen as a UV protectant. Phototoxicity refers to the activation of an otherwise innocuous compound, converting it to a more hazardous form via UV radiation, which is naturally part of sunlight. Despite the tremendous potential for hazardous effects of polluted nanoparticles in the efflux of water through estuarine and marine ecosystems, there is a lack of understanding and regulation of nanoparticles' environmental fate. *Nematostella vectensis* is a well-suited estuarine model for establishing possible effects of nano-TiO2 phototoxicity as it is constantly exposed to sunlight in its natural environment. This study aims to determine what concentrations of nano-TiO2 are toxic, if nano-TiO2 is toxic without photoactivation, and how estuarine invertebrates are affected by nano-TiO2 toxicity. Both tentacle and whole-organism exposures were conducted in no-light and UV treatments, with varied concentrations of nano-TiO2 ranging from 0.1 to 1000 ppm. Water, alginate, and peroxide were used as controls. ROS production as result of photoactivation of nano-TiO2 was expected to produce quantifiable cell damage. Quantification of damage was performed with a live/dead cell stain and analyzed with a confocal microscope and accompanying software. Toxicity, indicated by propidium iodide fluorescence, increased with higher concentrations in no-light treatments. A similar trend was observed in the UV treatment, but to a lesser extent. Phototoxicity was not overwhelmingly observed in this experiment, although nano-TiO2 produced toxicity even without UV exposure.

Keywords: phototoxicity; anemone; nanoparticles; titanium dioxide; UV radiation; acute exposure.

1. Introduction

Nanomaterials, or nanoparticles, are a highly diverse group of substances that are emerging as a potential environmental hazard. The prefix "nano" denotes any substance whose particles range in the 0-100 nm size. Particles may be of any mother compound or any shape, but their minute size often allows them to behave dissimilarly to a bulk version of the mother compound (Shi et al, 2013; Keller et al, 2010).

Nanoparticles may be found in a wide range of products and are on the cusp of technology. As the scientific community learns about novel properties of compounds that are only associated with extremely tiny size, nanoparticles, including nanometals, are becoming commonly used.

However, many westernized countries, including the United States, have no regulations for nanomaterials as a class. The FDA may only regulate new materials and chemicals in use. Nanomaterials are generally not new, just a much smaller, finer form of existing materials. Disclosure of what kind or how much of any nanocompound is in a product, beyond the requirements of the existing bulk compound, is not required (Sharma, 2009; Shi et al., 2013). Thus, the void of knowledge concerning how much nanomaterial is circulated through commerce is expanding.

Nanometals have a diverse range of applications. Nanosilver is commercially popular for its anti-microbial properties. Nanocopper is often used in agricultural pesticides or antifouling agents. Carbon nanotubes are used in some laboratory and medical applications. Nanoparticles of titanium are most commonly used in sunscreens, but they can also be found in cosmetics, plastics, paints, industrial coatings, wastewater treatments, and many other products (Manke et al., 2013).

Use of titanium dioxide in sunscreens is widely known, but whether the compound is a nano- or fine-particle is often not differentiated, and there is no monitoring for how much sunscreen washes into the water system. Zinc oxide is considered a more effective UV protectant in sunscreens, but it has been proven to be moderately toxic in aquatic systems, and so titanium dioxide is used almost as prolifically, with supposed reduced toxicity (Lewicka et al., 2013). The multitude of uses provides many opportunities for titanium to enter water outflow to the environment, but due to the lack of regulation, real statistics on how much titanium reaches natural water systems are unavailable.

As much as new beneficial properties of nanoparticles are being discovered, reciprocal research on the potential toxicity of nanoparticles is not as forcefully pursued. Tinier particles possess different physiochemical properties; they may also present different hazards or bioavailability (Manke et al., 2013). Phototoxicity is an emerging concern in regards to many nanoparticles, and especially pertinent to aquatic toxicology, as myriad species of ecological and commercial interest spend time in the water column within light-penetrating depths (Manke et al., 2013).

Natural sunlight includes both ultraviolet and photosynthetically-active radiation. Phototoxicity, or photo-activation, refers to toxic compounds that are activated by UV radiation to become more harmful than either the parent compound or irradiation alone. UV light can harm organisms by being directly absorbed by proteins, including RNA and

DNA, and subsequently producing oxidative free radicals (Pelletier et al., 2006; McDonald and Chapman, 2002). The radicals or sub-structures produced by degradation of phototoxic pollutants have the potential to damage organisms at any life stage. It is widely accepted that titanium, while unable to pass through cell membranes, is moderately phototoxic (Sharma, 2009; Shi et al., 2013). Titanium's inability to traverse cell membranes prevents it from using an intracellular mechanism to harm cells, but it may still react to form oxygen radicals outside the cell (Brezova et al., 2004). Reactive oxygen species have the potential to oxidize cells or tissues, leading to impairment of cell function, necrosis, or induced apoptosis.

Titanium dioxide toxicity has been studied in several model organisms, but studies that examine aquatic toxicity, especially in marine or estuarine systems, are scant. Nanomaterial reactivity can be affected by salinity change, and saltwater ecosystem health is just as important to environmental stewardship as are the freshwater ecosystems that usually host toxicity studies (Keller et al., 2010). It is important to understand how anthropogenic pollutants can affect environmental health, for both the wellbeing of the global commons and human health. Estuaries provide valuable natural benefits; in the realm of pollutant exposure and toxicity, they are particularly concerning due to their huge influx of watershed outflow and their enormous impact on ecosystem services, including metal retention and pollutant sinks (Jacob and Otte, 2002). High fluctuation of conditions within estuaries and wetlands, such as temperature and salinity, may potentially elevate levels of toxicity, which in turn compound toxic effects.

The model organism of this study, the starlet anemone *Nematostella vectensis*, is a widely utilized model in genetics and biological studies. Unlike most other anemones, it lives in brackish water and has no symbiotic zooxanthellae, making it an ideal model for examining estuarine toxicants. The lack of zooxanthellae eliminates the need to scrutinize how endosymbionts may mitigate or alter the sole effects of a pollutant.

In this study, the effects of nano-TiO2 paired with UV radiation exposure were examined in the starlet anemone. The goal of this study was to illuminate the extent to which nano-TiO2 can cause cellular damage in a marine invertebrate organism.

2. Materials and Methods

2.1 Equipment and Chemicals

Model organisms *Nematostella vectensis* were obtained from Bodega Marine Laboratory and the Tucker Lab in the Department of Cell Biology and Human Anatomy at UC Davis. All anemones were kept in pans of filtered seawater (FSW, 1:3 dilution in de-ionized water) maintained at room temperature, approximately 22º Celsius. Adults were kept in a dark incubator; juveniles were kept on a desktop subjected to approximately natural day/night light cycle. All anemones were fed brine shrimp nauplii on a daily basis and water was changed every 3-4 days.

Nanotitanium dioxide (nano-TiO2) and hydrogen peroxide (H2O2) were provided laboratory stocks originally purchased from Sigma-Aldrich. All nanoparticle solutions were prepared by adding nanomaterial solid to artificial seawater (ASW) with alginate, vortexing, and sonicating for same-day use (Fairbairn; et al, 2011).

All experimental exposures used clear plastic 12-well plates for specimen treatments. For 'no light' (NL) treatments, well plates were wrapped in heavy-duty, nonstick aluminum foil. Transparent plexiglass covers were used in all light exposures. Photosynthetic active radiation (PAR) treatments used UV-blocking plexiglass, which blocks about 85% of UV radiation but allows PAR, or visible light, to pass through unobstructed (Incardona; et al, 2012). UV radiation (UV) treatments used UV-transparent plexiglass, which allows all light radiation to pass through.

All tissue and organismal samples were fixed with 2% paraformaldehyde in filtered seawater (1:3 dilution).

2.2 Probing for reactive oxygen species in solution with luminol

To establish a response curve for reactive oxygen species (ROS) generation by nano-TiO2, a Costar 96-well plate was loaded with concentrations of nano-TiO2 from 0.1-100 ppm in FSW (1:3 dilution). FSW and FSW with alginate were used as negative controls. H2O2 was used as a positive control for ROS, in concentrations of 0.1µM-1mM, to emulate the positive controls seen in other ROS studies (Lewicka et al, 2013). Half of the well plate was covered in foil for an NL treatment; the other half was left exposed for UV radiation. All concentration and light treatments were duplicated. Each well contained 50 µL of the treatment solution and 140 µL of ASW (1:3 dilution).

The well plate was left outdoors in full sun for up to one hour. Upon termination of light exposure, 10 µL of luminol was immediately added to each well and the plate loaded into a TECAN plate reader for fluorescence reading.

2.3 Acute toxicity of nano-TiO2 in brine shrimp nauplii

To establish an acute toxicity dose-response curve for nano-TiO2, brine shrimp nauplii were exposed to nano-TiO2, bulk-TiO2, nano-CuO, and CuSO4 in concentrations from 0.1-1000 ppm in FSW and subjected to NL or UV exposure treatments. Copper treatments were used as a comparison due to the amount of existing data on copper effects and behavior in aquatic environments. Bulk titanium was used to compare the effects between nano- and bulk-size particles. All concentrations and light treatments of contaminants were repeated in duplicate.

100 µL of concentrated nauplii in FSW were added to glass shell vials containing 7.5 mL treatment solution in FSW. Vials were set up in plastic well plates for stability. Outdoor exposure duration in full afternoon sun was one hour.

Post-exposure, nauplii were qualitatively assessed for abnormality. Abnormality was defined as physical malformation, mortality, or inability to swim. Percentage abnormality was calculated at 0, 22, 48, 72, and 96 hours post-exposure.

2.4 Phototoxicity of nano-TiO2 in anemone tentacles

Phototoxicity of nano-TiO2 was examined first in Nematostella tentacles. Clear plastic 12-well plates were loaded with 1 mL of treatment solution. Concentrations of 0.1, 1, 10, 50, 100, and 1000 ppm nano-TiO2 were used. Negative controls were ASW (1:3 dilution) and ASW with alginate. Positive controls were H2O2 concentrations of 0.1 µM, 1 µM, 1 mM, and 0.98 M. All solution concentrations had NL, PAR only, and UV exposures. All treatments had a duplicate.

Anemone tentacles were harvested from organisms by submerging adult and large juveniles in MgSO4 (7.5% weight/volume) to anesthetize them. After 1-4 minutes of submersion, anemones relaxed sufficiently to allow trimming of tentacles at base of stalk with surgical metal scissors.

NL treatments were covered in foil. PAR only treatments were cov-

ered with UV-blocking plexiglass. UV-exposure treatments were covered by UV-transparent plexiglass. Light treatment duration was 30 minutes. Temperature was maintained between 30-32º Celsius by keeping well plates in a tray of cool water during outdoor exposure.

Immediately after light exposure, tentacles were transferred to 1mL of diluted ASW for rinsing and 5 µL propidium iodide was added per well, according to Molecular Probes recommended protocol for propidium iodide staining. Samples were incubated with propidium iodide for 10 minutes, then rinsed and fixed. After 2 hours of fixation, tentacles were mounted on glass slides for fluorescence imaging.

2.5 Whole-organism phototoxicity of nano-TiO2 in starlet anemones

Phototoxicity testing for whole organism samples followed the same protocol as tentacle exposure, with the following exceptions: no tentacle harvest was required, so entire organisms were put into 3 mL of treatment solution; a 4.9 M concentration of peroxide was included as an ultimate positive control; no PAR treatment was done. Anemones were anesthetized before staining and were stained for 35 minutes. Fixation occurred overnight before mounting slides. All treatments were done in duplicate.

2.6 Microscopy and fluorescent imaging

Samples were removed after fixing and mounted onto glass microscope slides with mounting medium. An Olympus Confocal Laser Scanning Microscope paired with FLUOVIEW software was used to view samples' fluorescence. To comparatively scale all subsequent fluorescence intensities, NL water controls were used to 'zero' the fluorescence readings with the photomultiplier tube (PMT) values. Qualitative assessment occurred only for the fluorescing tentacle samples; quantitative results were gathered for the whole-organism exposure to gauge relative fluorescent intensity.

Two images of each whole-organism sample were taken, one of the tentacles and one of the basal body. All four fluorescence images for each treatment were subsequently used for analysis of fluorescence intensity relative to controls.

3. Results

3.1 Reactive oxygen species in solution

Despite multiple trials with light exposure durations ranging from 20 minutes to 1 hour, no significant trends in fluorescence intensity were observed using the TECAN plate reader (data not shown).

3.2 Acute toxicity of nano-TiO2 in brine shrimp nauplii

No trends significant from the control groups were observed in nauplii abnormality or lethality for any treatment (data not shown).

3.3 Phototoxicity of nano-TiO2 in anemone tentacles

Phototoxic damage was assessed by the amount of fluorescence from damaged cells using propidium iodide (PI). Phototoxic damage was assumed to include compromised cell membranes, making it possible for PI to elucidate damage by binding to newly-unprotected DNA-containing organelles, namely nuclei. All tentacles were imaged at the same intensity levels as the NL water control. All water controls regardless of light treatment exhibited zero fluorescence (Figure 1). Higher fluorescence was exhibited only in the treatments of combined higher concentration and UV exposure.

Tentacles trimmed from anemones shriveled to a large degree. Juvenile tentacles were difficult to track in treatment solutions.

3.4 Whole-organism phototoxicity of nano-TiO2 in starlet anemones

NL controls of peroxide for whole-organism exposures exhibited a mild trend of toxicity (Figure 3). NL exposure of nano-TiO2 exhibited a strong trend of higher fluorescence intensity with higher concentrations (Figure 4). UV exposures of peroxide similarly showed a mild trend of toxicity (Figure 5). UV exposure of nano-TiO2 exhibited only a slight trend in increasing fluorescence with increasing concentration (Figure 6).

Fluorescence images of tentacles consistently showed higher fluorescence readings than basal body images. Across all UV treatments streaks of cells fluoresced in a pattern around the muscular tentacle ring.

Statistical analysis of fluorescence intensities was superficially conducted; standard deviations of intensity for all treatment groups were calculated with the four fluorescence measurements of each treatment,

despite there being only two true duplicates (Table 1). Assessment of discretely fluorescing damaged nuclei did not occur due to the relative few samples that exhibited them.

Anemones put into treatment were mostly closed due to the physical disturbance of transferring bodies from holding dishes to well plates, but quickly opened. At the end of exposure, the water and alginate controls, peroxide treatments up to 1mM, and nano-TiO2 treatments up to 50 ppm all had organisms visibly alive. Bodies were partially extended and most had tentacles out-stretched. All other treatments had anemones closed and retracted. 100 ppm and 1000 ppm treatments of nano-TiO2 had visibly settled-out titanium aggregates, some of which appeared to coat the mucus film of the anemone.

4. Discussion

Initial dose-response curve experiments with nano-TiO2 and luminol in solution yielded no discernable trends. The lack of usable data could be a result of nano-TiO2 aggregating due to its relative insolubility. This would have produced unrealistic values during fluorescence detection in the plate-reader.

The reason for lack of a discernable trend from the attempted acute toxicity experiment in brine shrimp nauplii is not clear. It is possible that the inconsistency in nauplii number per vial, despite the consistent volume addition, impeded their survival in the controls. Dissolved oxygen content or stress from transport may have decreased their viability, or possibly this intermediate developmental stage of the invertebrate is an especially tolerant organism in regards to the tested compounds.

Propidium iodide fluorescence in the tentacle exposure treatments showed a visible increase with combined higher concentration and UV exposure. The lack of fluorescence in all water controls regardless of light treatment is brief confirmation that UV light itself was not toxic, and it was only in the treatments with TiO2 that higher fluorescence was exhibited. This is consistent with previous studies that have found nano-TiO2 to be phototoxic (Brezova et al., 2004). Qualitative assessment is helpful but not as useful as quantitative data. Quantification of fluorescence could have been conducted for this part of the experiment using FLUOVIEW but was not for this preliminary phase. Quantification of whole-organism propidium iodide fluorescence, however, proved interesting.

Overall, fluorescence intensity trends seen in the whole-organism experiment were somewhat unexpected. NL controls of peroxide for whole-organism exposures exhibited a mildly toxic trend (Figure 3). A stronger trend in increasing fluorescence was anticipated but is not completely unusual since there was no UV irradiation.

Intriguingly, the NL exposure of nano-TiO2 exhibited a very strong trend of high fluorescence intensity with higher concentrations (Figure 4). This was unexpected in that titanium is not presumed to elicit toxicity without photoactivation (Lewicka et al., 2013). The toxicity seen in this exposure may be indicative of higher generic toxicity of titanium dioxide than previously thought, or that nano-TiO2 may act via a mechanism of toxicity alternate from that of bulk-TiO2, which has been studied far more extensively.

UV exposures of peroxide similarly showed a mild trend of toxicity (Figure 5). A more severe increasing trend was expected for peroxide, which is commonly used as a positive control in ROS studies (Lewicka et al., 2013). It was not until the highest concentrations of peroxide, 0.98 and 4.9 M respectively, that fluorescence of high magnitude was seen. These levels of peroxide are not very realistic in terms of environmental relevance but were included to ensure that a response could be observed.

UV exposure of nano-TiO2 exhibited only a slight trend in increasing fluorescence with increasing concentration (Figure 6). Higher levels of fluorescence were expected for this treatment than for the NL nano-TiO2 exposure, but both a more obvious trend and comparable individual fluorescence values were seen in the NL exposure. Only in the 1000 ppm UV treatment for nano-TiO2 was extreme fluorescence observed, which would likely only be an environmentally relevant concentration in an anomalous event of point pollution. Milder concentrations of nano-TiO2 did not produce a visible trend. From this data alone, it would seem that titanium is not phototoxic except at extreme concentrations. This does not match the findings of other phototoxicity studies: non-production of ROS from nano-TiO2 has typically been seen only in tandem with inert oxide coatings, an ingredient used in sunscreens for human protection but not included in this study (Lewicka et al., 2013; Brezova et al., 2004). If the findings of this experiment are accurate, this may indicate that nano-TiO2 is not very toxic, at least in the cnidarian organism, until high concentrations.

The statistical analysis of fluorescence intensities performed with

treatment groups were calculated with the four fluorescence measurements of each treatment, despite there being only two true duplicates (Table 1). The subsequent results appeared to exhibit the same trends, to the same degree, as when graphs were constructed only with averages. The relative fluorescence of tentacle images was consistently much more intense than basal body images; most of the trend seen in intensity appears to be from the tentacle fluorescence. The fluorescence observed in the tentacle ring may inspire further research; it is possible that cells in this muscular section are simply more dense and exhibit a more concentrated area of cell damage, or it is possible the tentacles contain a compound that renders them more sensitive to either the treatment or staining. Basal body fluorescence numbers were included for their value as a background assessment; these contributed much to the high variation in standard deviation numbers.

Assessment of discretely fluorescing damaged nuclei did not occur judging by the relatively few samples that exhibited them. Discretely fluorescing nuclei were expected across UV treatments and were expected to be especially visible in higher concentration treatments. Longer or more concentrated staining may have been needed, or it is possible that the toxicity observed was accurate and that not much damage occurred.

Sources of error in this study include shortcuts taken in the interest of time: lack of extensive man-hours for analytical processing reduced the number of replicates that could be produced. A limited number of specimens also created a pressure to downscale the experiment, thus yielding fewer data points than optimal. If repeated, inclusion of more replicates would likely have an outstanding effect of improving trends seen and would allow for statistically significant values.

The possibility of titanium having more severe toxic effects than previously thought may warrant further research. TiO2 in both nano- and fine-particle is currently the subject of several studies investigating mammalian toxicity, as the minute size of nano-particles could mean they are able to travel through biological systems along unexpected routes, or, disturbingly, potentially pass through the blood-brain barrier (Long et al, 2007). Determining the toxic effects of commonly used compounds such as TiO2 is important for both human and environmental health.

The toxicity seen in this study in an estuarine cnidarian model may be helpful for guiding future studies concerned with wetland and estuary health. The Nematostella model is useful in that it uses an intermediately saline environment and may help link existing freshwater studies for nano-particles to marine studies. More work on model indicator organisms for marine ecosystems is necessary, as the subsequent outflow of potential nano-TiO2 inevitably extends to the oceans. Additionally, realistic monitoring of existent titanium concentrations and imminent outflow hazards are needed. If future studies on nano-TiO2, titanium, and other nanoparticles show positive toxicity effects on a reasonable scale, nanoparticles may merit monitoring.

Appendix

Figure 1. Relative fluorescence of anemone tentacles exposed to concentrations of 0-1000ppm of nano-TiO2. Propidium iodide (PI) fluorescent image indicating cell damage (left) is accompanied by the light image overlay (right). From top to bottom rows are the water controls increasing to the highest concentration. Left to right are the NL, PAR, and UV treatment comparisons.

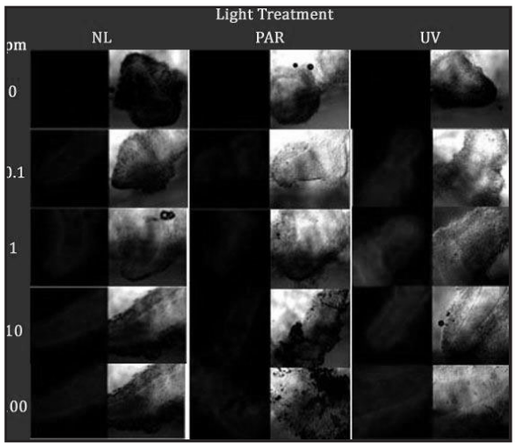

Figure 2. PI fluorescence showing cell damage in anemone tentacle ring. Left frame is PI fluorescence only, and right frame is an overlay of fluorescence on top of transmitted light image.

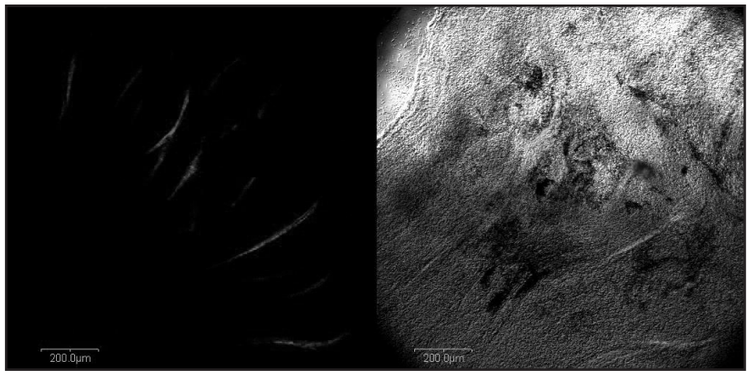

Figure 3. Total intensity for NL treatment peroxide concentrations, indicating total cell death and damage. Peroxide concentrations are along the x-axis, and fluorescence intensity is along the y-axis. A mildly increasing trend is seen with increasing concentration.

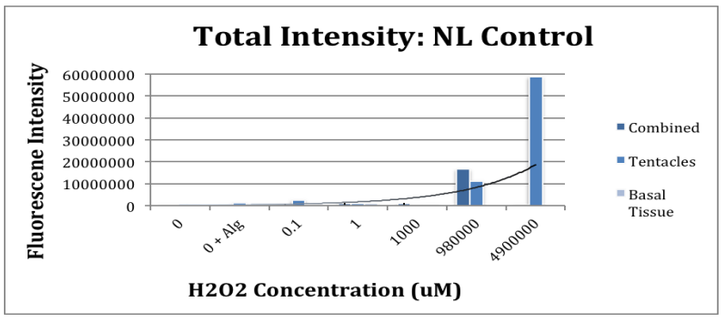

Figure 4. Total intensity for NL treatment nano-titanium dioxide concentrations, indicating total cell death and damage. Water and alginate controls and nano-TiO2 concentrations are along the x-axis, and fluorescence intensity is along the y-axis. While statistical significance cannot be calculated, the increasing trend of fluorescence with increased concentration is notable.

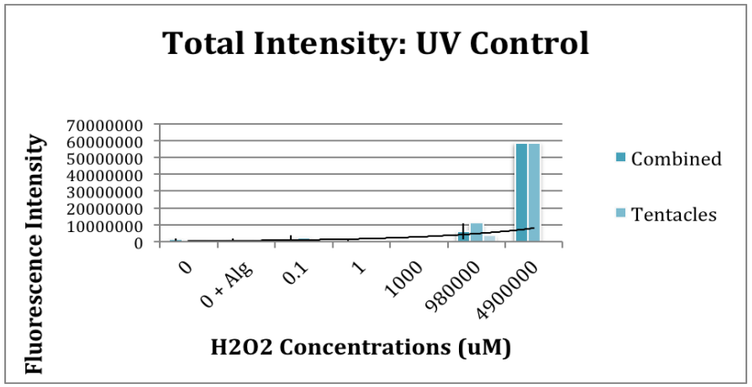

Figure 5. Total intensity for UV treatment peroxide concentrations, indicating total cell death and damage. Peroxide concentrations are along the x-axis, and fluorescence intensity is along the y-axis. A mildly increasing trend is seen with increasing concentration, similar to the NL.

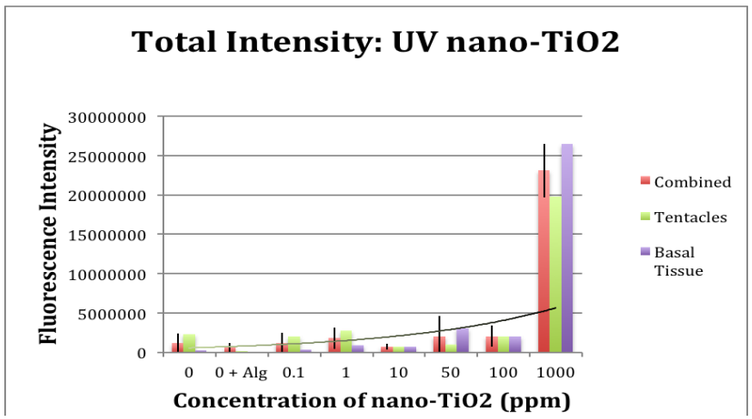

Figure 6. Total intensity for UV treatment nano-titanium dioxide concentrations, indicating total cell death and damage. Water and alginate controls and nano-TiO2 concentrations are along the x-axis, and fluorescence intensity is along the y-axis. A fluorescence trend is not very apparent until the highest concentration of nano-TiO2, but fluorescence is also seen in the controls.

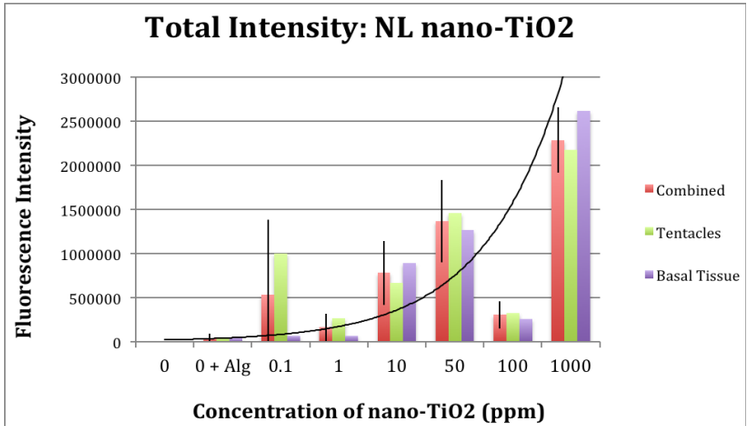

Table 1. Tabulated average fluorescence intensities for whole-organism exposure. Organized by treatment. Standard deviations are not true deviations due to duplicate treatments.

Average Intensity: NL H_2O_2 Exposure				
Treatment (uM)	Total Avg.	Tentacle Avg.	Basal Avg.	Std Dev (Total)
0	1699.75	72746	1992.5	459.73
0 + Alg	42287	1054795	18146	46606.96
0.1	265220.5	2431875	295986	278684.04
1	803510	767815	913720	473708.34
1000	782312.5	310920	664125	459568.7
980000	16550000	11097000		
4900000	275050	58717000		

Average Intensity: NL nano TiO_2 Exposure				
Treatment (uM)	Total Avg.	Tentacle Avg.	Basal Avg.	Std Dev (Total)
0	1699.75	1699.75	1699.75	459.73
0 + Alg	42287	42287	42287	46606.96
0.1	533842	1003885	63799	846577.7753
1	166252.75	263530	68975.5	153874.3875
10	780862.5	668735	892990	364721.2724
50	1363542.5	1459250	1267835	466169.0236
100	305393.33	328450	259280	155756.6439
1000	2286475	2175900	2618200	372006.0875
Averaged Intensity: UV H_2O_2 exposure				
Treatment (uM)	Total Avg.	Tentacle Avg.	Basal Avg.	Std Dev (Total)
0	1123423	72746	217410	601182.1495
0 + Alg	574417	1054795	94039	1054565.564
0.1	1493688	2431875	555501	2057447.151
1	415794.25	767815	63773.5	465062.5543
1000	233450	310920	155980	105117.5694
980000	6071200	11097000	3558300	4699171.361
4900000	58717000	58717000		
Averaged Intensity: UV nano TiO_2 exposure				
Treatment (uM)	Total Avg.	Tentacle Avg.	Basal Avg.	Std Dev (Total)
0	1123423	2260463.5	217410	1297772.162
0 + Alg	574417	155980	94039	562176.9212
0.1	1169695	2034900	304490	1291938.292
1	1795922.5	2726250	865595	1342387.881
10	685442.5	701415	669470	370649.8891
50	2024090	990760	3057420	2546103.697
100	1986750	1965350	2008150	1368646.203
1000	23111500	19791500	26431500	3400000

References

Brezova, Vlasta; Gabcova, Sona; Dvoranova, Dana; Stasko, Andrej. 2004. Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study). Journal of Photochemistry and Photobiology B: Biology 79. 121-134.

Fairbairn, Elise A.; Keller, Arturo A.; Madler, Lutz; Zhou, Dongxu; Pokhrel, Suman; Cherr, Gary N. 2011. Metal oxide nanoparticles in seawater: Linking physicochemical characteristics with biological response in sea urchin development. Journal of Hazardous Materials 192. 1565-1571.

Incardona, John; Vines, C.; Linbo, T.; Myers, M.; Sloan, C.; Anulacion, B.; Boyd, D.; Collier, T.; Morgan, S.; Cherr, G.; Scholz, N. 2012. Potent phototoxicity of marine bunker oil to translucent herring embryos after prolonged weathering. PLoS ONE 7(2).

Jacob, Donna L.; Otte, Marinus L. 2002. Conflicting processes in the wetland plant rhizosphere: metal retention or mobilization? Water, Air, and Soil Pollution 3. 91-104.

Keller, Arturo A.; Wang, Hongtao; Zhou, Dongxu; Lenihan, Hunter S.; Cherr, Gary; Cardinale, Bradley J.; Miller, Robert; Ji, Zhaoxia. 2010. Stability and Aggregation of Metal Oxide Nanoparticles in Natural Aqueous Matrices. Environ. Sci Technol. 44. 1962-1967.

Lewicka, Suzanna A.; Yu, William W.; Oliva, Brittany L.; Contreras, Elizabeth Quevedo; Colvin, Vicki L. 2013. Photochemical behavior of nanoscale TiO2 and ZnO sunscreen ingredients. Journal of Photochemistry and Photobiology A: Chemistry 263. 24-33.

Long, Thomas C.; Tajuba, Julianne; Sama, Preethi; Saleh, Navid; Swartz, Carol; Parker, Joel; Hester, Susan; Lowry, Gregory V.; Veronesi, Bellina. 2007. Nanosize Titanium Dioxide Stimulates Reactive Oxygen Species in Brain Microglia and Damages Neurons in Vitro. Environmental Health Perspectives 115: 11. 1631-1637.

Manke, Amruta; Wang, Liying; Rojanasakul, Yon. 2013. Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity. BioMed Research International 2013. 1-15.

McDonald, Blair G.; Chapman, Peter M., 2002. PAH phototoxicity – an ecologically irrelevant phenomenon? Marine Pollution Bulletin 44. 1321-1326.

Pelletier, E.; Sargian, P.; Payet, J.; Demers, S. 2006. Ecotoxicological Effects of Combined UVB and Organic Contaminants in Coastal Waters: A Review. Photochemistry and Photobiology 82, 981-993.

Sharma, Virender K. 2009. Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment – A Review. Journal of Environmental Science and Health Part A: 44. 1485 – 1495.

Shi, H., R. Magaye, V. Castranova, and J. Zhao. 2013. Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicol 10:15.