

## DAPHNIA MAGNA MORTALITY WHEN EXPOSED TO TITANIUM DIOXIDE AND FULLERENE (C<sub>60</sub>) NANOPARTICLES

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**Abstract**—Nanoparticles (1–100 nm) comprise the latest technological advances designed to do everything from absorb environmental toxins to deliver drugs to a target organ. Recently, however, they have come under scrutiny for the potential to cause environmental damage. Because compounds in this miniature size range have chemical properties that differ from those of their larger counterparts, nanoparticles deserve special attention. Our main objective was to assess the potential impact that nanoparticles may have on release into aquatic environments. We prepared titanium dioxide (TiO<sub>2</sub>) and fullerene (C<sub>60</sub>) nanoparticles by filtration in tetrahydrofuran or by sonication. *Daphnia magna* were exposed to the four solutions using U.S. Environmental Protection Agency 48-h acute toxicity tests. Images of the particle solutions were recorded using transmission-electron microscopy, and the median lethal concentration, lowest-observable-effect concentration, and no-observable-effect concentration were determined. Exposure to filtered C<sub>60</sub> and filtered TiO<sub>2</sub> caused an increase in mortality with an increase in concentration, whereas fullerenes show higher levels of toxicity at lower concentrations. Exposure to the sonicated solutions caused varied mortality. Understanding the potential impacts of nanoparticles will help to identify the most appropriate nanotechnology to preserve the aquatic environment while advancing medical and environmental technology.

**Keywords**—Fullerenes Titanium dioxide Nanoparticles *Daphnia magna* Mortality

### INTRODUCTION

Manufactured nanoparticles, ranging from 1 to 100 nm, have great promise as a tool for everything from drug delivery to environmental cleanup and computer construction [1]. Many nanoparticle compounds occur naturally and are used in many vital life processes [1]. The manufacture of particles on the nanoscale, however, began in 1985, after the discovery of carbon nanoparticles, or fullerenes, that had special properties [2]. Nanoparticles in general deserve special attention, because compounds in this miniature size range have chemical properties that differ from those of their larger counterparts [3]. As the size of the particle decreases, the number of atoms exposed on the surface and the amount of energy available for release increase [3]. Because of these unique characteristics, the interaction of the particle with its environment also changes. The change in reactivity is why the potential of nanoparticles for use in industry and pharmaceuticals is so immense [4], but this also is the reason for environmental concern.

In the past, nanoparticles have received comparatively less attention than compounds with the same composition but larger size [3]. Recently, however, they have come under scrutiny for their potential to cause environmental damage [1]. Of particular interest is the impact on aquatic organisms, because these particles may be released into freshwater systems as a result of their proposed use in sensor technologies and environmental cleanup of waste from industry and medicine.

Our main objective in the present study was to assess the potential impact that nanoparticles may have on release into aquatic environments. We used two types of particles that differ in their chemical composition, titanium dioxide (TiO<sub>2</sub>) and fullerene (C<sub>60</sub>), to determine the effects of exposure on survival

of an aquatic model species, *Daphnia magna*. Currently, TiO<sub>2</sub> is being used to develop self-cleaning surfaces [5] and solar energy cells [4] and as a photocatalyst in sterilization, air cleaning, and water purification. Fullerenes have many potential applications, including energy storage, sensors, and semiconductors [6]. Carbon nanotubes have been found to be threefold as effective as activated carbon for binding dioxin [1,7].

Both types of nanoparticles have shown some biological activity as well. Cai et al. [8] found that TiO<sub>2</sub> acted as an antitumor agent by inhibiting the growth of cancer cells [5,8]. Additionally, fullerenes have exhibited the ability to bind to lipids in the liver and brain, which may have beneficial implications for their use of drug delivery [9].

Because of their increased binding and reactivity, however, nanoparticles also can be detrimental. Titanium dioxide can have undesirable effects when inhaled and has a damaging impact on rodents. At the one-year mark, rats continued to exhibit adverse effects even with an initial exposure of only 13 weeks [10]. Warheit et al. [11] also found that exposure to carbon nanoparticles caused inflammation and cell injury in rats. Additionally, fullerenes were shown to be detrimental in aquatic environments. They caused oxidative damage in largemouth bass (*Micropterus salmoides*) [12] by acting through the same mechanism of action found to be beneficial for their use in drug delivery [13]. Fullerenes are able to travel to the brain, bind with lipids, and cause the production of oxidative stress compounds when fish are exposed to concentrations of only 1 ppm [12].

*Daphnia* organisms are bioindicators used by various organizations, including the U.S. Environmental Protection Agency (U.S. EPA). Because a single *D. magna* organism will filter an average of 16.6 ml/h [14], it interacts with large portions of the environment and, therefore, has a greater potential to be affected by ingestion of pollutant particulates compared

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with that of other aquatic organisms. Nanoparticles have the potential to be taken in during filtration and could interfere with the physiology or the feeding ability of the animal. Because *D. magna* are a vital connection in the food chain between the algae that they consume and the ecologically and economically important fish that consume them, it is imperative to understand the toxic response of *D. magna* to nanoparticles.

We exposed *D. magna* to varying concentrations of each nanoparticle to compare the similarities and differences of exposure. Although both C<sub>60</sub> and TiO<sub>2</sub> are nanoparticles, C<sub>60</sub> is hydrophobic, whereas TiO<sub>2</sub> is hydrophilic and can be superhydrophilic when exposed to ultraviolet light [4,5]. Because of these basic chemical differences, we hypothesized that the ability of *D. magna* to encounter and react to each nanoparticle would differ. The average diameter of TiO<sub>2</sub> particles is 10 to 20 nm [15], whereas the diameter of C<sub>60</sub> is only 0.72 nm [16]. Because of this size differential, we also hypothesized that the smaller C<sub>60</sub> particle would be more detrimental to *D. magna*.

The ultimate goal of the present research was to determine the best particles for environmental work (i.e., those with the least impact on aquatic organisms). These results could have implications for regulating the release of different types of particles as waste or in environmental remediation. Additionally, the information gained from the present study will suggest methods to test the toxicity of other types of nanoparticles.

## MATERIALS AND METHODS

### Nanoparticle preparation

Fullerenes are hydrophobic and do not readily go into solution; therefore, particles were prepared by two methods. First, particles were placed in deionized H<sub>2</sub>O and then a bath sonicator for at least 30 min in an attempt to break them into small, noncoagulating particles. For the other particle preparation, 20 mg of uncoated C<sub>60</sub> nanoparticles (Alfa Aesar, Ward Hill, MA, USA) were placed in 200 ml of tetrahydrofuran (THF) as described by Oberdorster [12] and Deguchi et al. [17]. This solution was sparged with nitrogen and left overnight in the dark on a stir plate. The solution was then filtered through a 0.22- $\mu$ m nylon filter (Gelman Sciences, Ann Arbor, MI, USA), and 200 ml of deionized water were added. The suspension was placed in a Büchi rotovapor (Büchi Labor-technik, Flawil, Switzerland) to evaporate THF, then removed and pipetted into an Erlenmeyer flask. This procedure was repeated twice. The final solution was then filtered through a 0.22- $\mu$ m nylon filter and stored until usage. To visualize the particle size and distribution and to determine concentration in the mixtures, transmission-electron microscopy (TEM) images of subsamples of each treatment solution were taken. Polystyrene latex spheres (average diameter, 93 nm) of a known concentration were added, as was phosphotungstic acid for negative staining to quantify the unknown number of C<sub>60</sub> nanoparticles. Using an airbrush, droplets of solution were sprayed onto the Formvar-coated (Electron Microscopy Sciences, Fort Washington, PA, USA) copper grids that previously had been placed in a DNA ultraviolet cross-linker in an effort to minimize hydrophobicity. The solution was allowed to dry, and the samples were then examined.

Titanium dioxide mixtures were prepared using the same method described above (i.e., sonication or placement in THF and filtered). However, TiO<sub>2</sub> is easily suspended in water, so additional solutions were prepared by starting with deionized H<sub>2</sub>O and omitting THF to verify that THF was not causing

the effects seen in either experiment. Titanium dioxide concentration was determined using ultraviolet spectroscopy (range, 325–350 nm).

### Acute exposures of *D. magna* to nanoparticles

The 48-h acute toxicity tests using *D. magna* were conducted using U.S. EPA standard operating procedure 2024 [18]. *Daphnia magna* cultures were maintained in 500-ml beakers at 15°C and fed *Selenastrum capricornutum* and Alfalfa stock (General Nutrition, Pittsburgh, PA, USA). When an individual *D. magna* contained mature eggs, the female was placed in a 50-ml centrifuge until giving birth. Once this occurred, the female was placed in mass culture with other females that also had given birth. Subsequent offspring (second to sixth broods) were used for experimentation. Ten offspring less than 24 h old were harvested and placed together in 50 ml of test solution using a disposable plastic transfer pipette. Survival numbers were recorded and assessed at the 1-, 24-, and 48-h mark. Negative controls of *D. magna* in moderately hard reconstituted water (MHRW) [19] were run with each trial. Additional controls having MHRW with THF evaporated off were used to see if the THF caused additional mortality. *Daphnia magna* were not fed for the duration of the experiment.

Initially, trials were conducted at various concentrations to observe effects and to obtain the concentration that caused 100% mortality. After this range was achieved, trials were repeated so that at least five replicates were conducted at each concentration for both sonicated solutions. The filtered TiO<sub>2</sub> trials had at least four replicates at each concentration, whereas the filtered C<sub>60</sub> trials had at least six replicates at each concentration. Experiments were conducted over several days, according to offspring availability. If enough *D. magna* were present, then two control groups were used each day; if offspring production was low, then only one control group was used.

*Daphnia magna* were exposed at eight concentrations (40, 180, 260, 350, 440, 510, 700, and 880 ppb) of the filtered C<sub>60</sub> nanoparticles. Additionally, *D. magna* were exposed at eight concentrations (0.2, 0.45, 0.9, 2.25, 4.5, 5.4, 7.2, and 9 ppm) to sonicated C<sub>60</sub> that did not undergo the filtration process. Exposure to filtered TiO<sub>2</sub> used seven concentrations (0.2, 1, 2, 5, 6, 8, and 10 ppm), and exposure to the sonicated, unfiltered TiO<sub>2</sub> used six concentrations (50, 200, 250, 300, 400, and 500 ppm).

Mortality was analyzed regarding nanoparticle concentration using a probit method analysis (U.S. EPA Probit Analysis Program, Ver 1.5; Environmental Monitoring Systems Laboratory, Cincinnati, OH, USA). This allowed the median lethal concentration (LC50) to be calculated. Mean and variance also were calculated among treatments using analysis of variance (ANOVA; SigmaStat Software, Ver 3.10; Systat Software, Point Richmond, CA, USA). The lowest-observable-effect concentration (LOEC) and no-observable-effect concentration (NOEC) were then calculated using Dunnett's test (Sigma Plot, Ver 9.0; Systat Software).

## RESULTS

### TEM imaging

The solutions prepared for the experiment had mixtures of particles that varied greatly in size. The four images in Figure 1 show the general distribution of each nanoparticle in water.

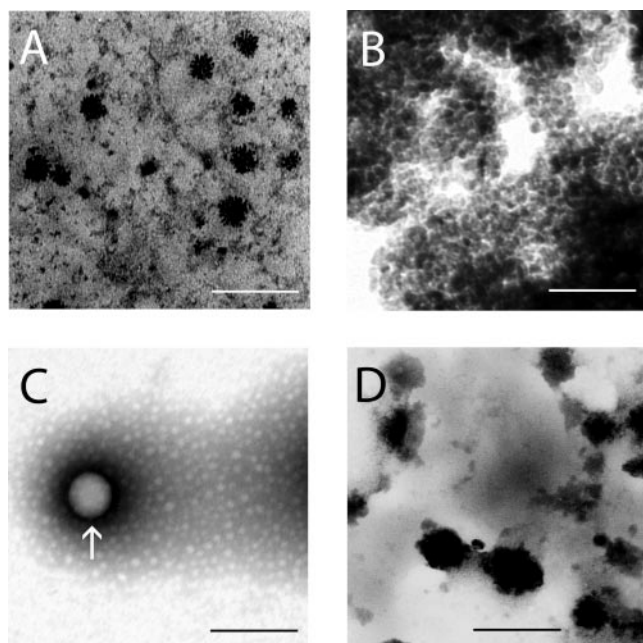


Fig. 1. Transmission-electron micrographs of titanium dioxide and fullerene nanoparticles. (A) Buchi-filtered titanium dioxide. (B) Sonicated, unfiltered titanium dioxide. (C) Buchi-filtered fullerenes. The arrow denotes a polystyrene latex sphere used for determining particle concentration. (D) Sonicated, unfiltered fullerenes. Bar = 200 nm.

Figure 1A and B shows  $\text{TiO}_2$ ; Figure 1C and D shows  $\text{C}_{60}$ . Figure 1A and C shows the dispersed and smaller particles that are characteristic of the filtered solution. Figure 1B and D shows the general clumping exhibited in the sonicated solution. Filtered  $\text{C}_{60}$  averaged 10 to 20 nm in diameter, whereas sonicated  $\text{C}_{60}$  ranged from 20- to 100-nm clumps. Titanium dioxide had a similar pattern, with an average diameter of 30 nm for the filtered  $\text{TiO}_2$ , but the sonicated solution had large clumps, ranging from 100 to 500 nm. The variation in toxicity for each solution is shown below.

#### Filtered $\text{TiO}_2$

Exposure to filtered  $\text{TiO}_2$  concentrations ranging from 0 to 10 ppm caused an increase in mortality with an increase in concentration (Fig. 2A). The controls experienced minimal fatalities (<2%). Concentrations of 0.2 ppm produced no fatalities and was indistinguishable from the control replicates. A concentration of 1 ppm exhibited 6% mortality, whereas a concentration of 2 ppm showed 9% mortality. Concentrations of 5 and 6 ppm showed 47 and 50% mortality, respectively. A concentration of 8 ppm caused 94% mortality, whereas a concentration of 10 ppm caused 100% mortality. The LC50 for filtered  $\text{TiO}_2$  was calculated at 5.5 ppm, with the LOEC being 2.0 ppm and the NOEC 1.0 ppm.

#### Sonicated $\text{TiO}_2$

No experiments involving sonicated  $\text{TiO}_2$  exhibited mortality greater than 9% (Fig. 2B). The control showed 2% mortality. The highest mortality occurred at a concentration of 350 ppm. The highest and lowest concentrations (500 and 50 ppm, respectively) both had 9% mortality. The lowest mortality was seen at a concentration of 300 ppm, at which fatalities were similar to those in the control (2%). Because mortality varied across concentrations and never exceeded 11%, the LC50 for the sonicated  $\text{TiO}_2$  solution was zero. Additionally, the slope

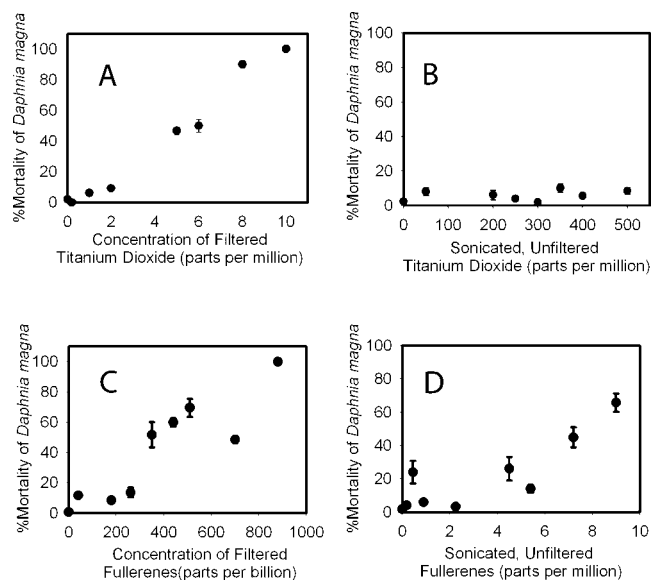


Fig. 2. (A) Mortality of *Daphnia magna* on exposure to filtered titanium dioxide. Concentrations ranging from 0 to 10 ppm correspond with their mortalities (median lethal concentration [LC50], 5.5 ppm). (B) Mortality of *Daphnia magna* on exposure to sonicated, unfiltered titanium dioxide. Concentrations ranging from 0 to 500 ppm correspond with their mortalities. (Because of the lack of mortality, the LC50 was zero). (C) Mortality of *D. magna* on exposure to filtered fullerenes. Concentrations ranging from 0 to 880 ppb correspond with their mortalities (LC50, 460 ppb). (D) Mortality of *D. magna* on exposure to sonicated, unfiltered fullerenes. Concentrations ranging from 0 to 9 ppm correspond with their mortalities (LC50, 7.9 ppm).

did not differ significantly from zero; therefore, the LOEC and NOEC were incalculable.

#### Filtered $\text{C}_{60}$ in THF

Exposure to filtered  $\text{C}_{60}$  caused an increase in mortality with increasing concentration greater than that from exposure to filtered  $\text{TiO}_2$  (Fig. 2C). The concentration range of filtered  $\text{C}_{60}$  nanoparticles was 0 to 880 ppb, because 100% mortality was realized at 880 ppb. The controls experienced minimal fatalities (0.72%). A concentration of 40 ppb showed 12% mortality. Concentrations of 180 and 260 ppb showed similar numbers of fatalities. A concentration of 180 ppb had 8% mortality, and a concentration of 260 ppb had 14% mortality. Increases in mortality were exhibited at 350 and 440 ppb (52 and 60%, respectively). The increasing trend continued, with 70% mortality at 510 ppb. However, at 700 ppb, mortality showed a slight drop, with only 48% fatalities, with one replicate showing no death and three replicates showing 10% mortality. Nevertheless, 100% mortality was always exhibited at 880 ppb, the maximum concentration tested. The LC50 for exposure to filtered  $\text{C}_{60}$  was 460 ppb, and the LOEC was 260 ppb and the NOEC 180 ppb.

#### Sonicated $\text{C}_{60}$

Concentrations of sonicated  $\text{C}_{60}$  ranging from approximately 0 to 9 ppm varied greatly in effect (Fig. 2D). The highest mortality rates occurred at 9 ppm, whereas the second-highest rates occurred at 7.2 ppm. A concentration of 0.45 ppm had 24% mortality, and a concentration of 4.5 ppm had 26% mortality. At 5.4 ppm, however, only 13% mortality was found. Mortality rates of less than 10% were seen at 0.9, 0.18, and 2.3 ppm. The control exhibited 2% fatalities. The sonicated

*Daphnia* mortality to TiO<sub>2</sub> and C<sub>60</sub> nanoparticles

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Table 1. Median lethal concentration (LC50), lowest-observable-effect concentration (LOEC), and no-observable-effect concentration (NOEC) for all solutions as well as the 100% mortality level

	LC50	100% Mortality	LOEC	NOEC
Sonicated titanium dioxide	NA <sup>a</sup>	NA	NA	NA
Filtered fullerenes	460 ppb	880 ppb	260 ppb	180 ppb
Filtered titanium dioxide	5.5 ppm	10 ppm	2.0 ppm	1.0 ppm
Sonicated fullerenes	7.9 ppm	NA	0.5 ppm	0.2 ppm

<sup>a</sup> NA = not applicable.

C<sub>60</sub> solution had a predicted LC50 of 7.9 ppm. Because mortality at 0.45 ppm was significantly different from zero, this was the LOEC. The NOEC was 0.18 ppm; however, the value of 5.4 ppm also was not significantly different from the control.

Table 1 shows the LC50, LOEC, and NOEC for all solutions and includes the 100% mortality level, if applicable.

*TiO<sub>2</sub> in THF*

Test solutions containing TiO<sub>2</sub> originating in THF that was evaporated from water and test solutions containing only TiO<sub>2</sub> in water showed no statistical difference in survivability ( $t = 0.904$ ,  $p = 0.400$ ) (Fig. 3). Additionally, the linear regressions of the two types of preparation were calculated and compared. Differences in the regression were statistically insignificant.

Additionally, the role of THF alone in solution was examined. Tetrahydrofuran was added to MHRW and evaporated out of solution. *Daphnia magna* were then exposed, and survival was not significantly affected ( $t = 22.045$ ,  $p < 0.0001$ ).

## DISCUSSION

Exposure to both filtered TiO<sub>2</sub> and filtered C<sub>60</sub> caused mortality. However, filtered C<sub>60</sub> caused toxicity at much lower concentrations than filtered TiO<sub>2</sub>. The LC50 for filtered C<sub>60</sub> was only 460 ppb, whereas filtered TiO<sub>2</sub> had a LC50 of 5.5 ppm. Complete mortality was experienced at 880 ppb for filtered C<sub>60</sub> and 10 ppm for filtered TiO<sub>2</sub>. This agrees with our hypothesis that the smaller particles would be more detrimental to *D. magna* survival. The difference in toxicity could be caused by the differences in reactivity of the particles or by

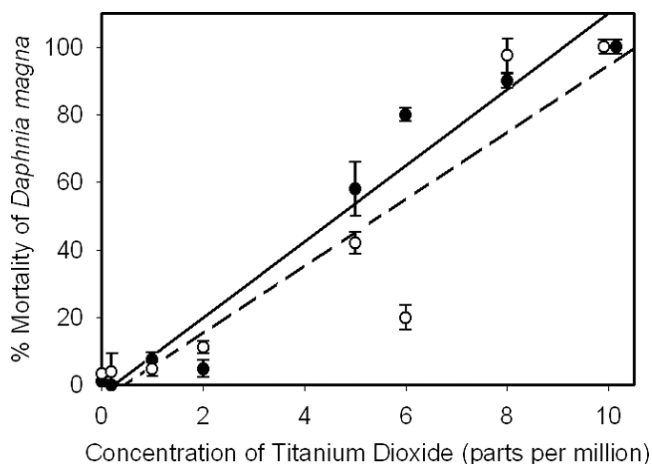


Fig. 3. Comparison of the two treatment methods for titanium dioxide. The regression equation for the filtered and tetrahydrofuran-treated nanoparticles was (●)  $y = 11.29x - 2.76$  (solid line). The regression equation for the filtered and deionized H<sub>2</sub>O-treated nanoparticles (○) was  $y = 9.93x - 4.51$  (dashed line).

size alone, being that fullerenes are smaller than TiO<sub>2</sub> (Fig. 1).

The way the particles were prepared, however, impacted their toxicity. Exposure to concentrations of unfiltered, sonicated TiO<sub>2</sub> powder that contained both micro- and nanoparticles did not cause mortality like that of the filtered nanoparticle solutions. Instead, solutions of sonicated TiO<sub>2</sub> caused very few fatalities. Concentrations of TiO<sub>2</sub> that were 25- and 30-fold greater than the concentrations that caused mortality in trials with filtered particles showed no difference in mortality from that of the control. Even concentrations as high as 500 ppm caused only 18% mortality. The TEM images of the solutions show that the particles in the filtered solutions are not clumped like those in the sonicated solutions. In the filtered TiO<sub>2</sub> solution, only the smaller particles were retained in the aqueous solution (filter size, 200 nm), and the average particle diameter was 30 nm. In the sonicated TiO<sub>2</sub> solution, however, all sizes of clumped particles remained, ranging from 100 to 500 nm in diameter. If *D. magna* encounter an individual nanoparticle, the reactivity may be greater than that of a nanoparticle reacting with many others in larger clumps.

Likewise, the sonicated C<sub>60</sub> mortality fluctuated from 5 to 66% and did not follow the trend of increasing mortality with increasing concentration. The highest mortality was seen at 9 ppm, but the concentration of only 0.45 ppm had 24% fatalities. Most likely, this also is caused by clumping of the nanoparticles with one another. On initial placement of the C<sub>60</sub> in water, the nanoparticles, because of the hydrophobic nature of these spheres, floated on top of the surface. After initial sonication, fewer particles were seen at the surface. Sonication was continued until most of the particles were dispersed. Clumping was observed in TEM imaging of the sonicated C<sub>60</sub>, causing the solution to be unequal throughout. This uneven distribution of nanoparticles would cause some trials to have more nanoparticles than expected and others to have less. Additionally, the lack of filtration in this preparation process yields an unequal particle size distribution. This might explain why one of the 1-ppm trials had 80% mortality yet another only 30%, and why concentrations that were supposed to contain only 9 ppm (much higher than the 880-ppb filtered solution that caused 100% mortality) showed only 66% mortality.

So that C<sub>60</sub> would go into solution easily, THF was added as a surfactant and then evaporated out of solution. Because TiO<sub>2</sub> will go into solution without THF, filtered TiO<sub>2</sub> solutions initially containing THF were compared to those prepared with no THF added. No significant differences occurred between *D. magna* exposed to filtered TiO<sub>2</sub> prepared with THF versus the filtered TiO<sub>2</sub> prepared with only deionized H<sub>2</sub>O. If a difference in mortality had been exhibited, it might have been attributed to THF possibly remaining in the solution. Because no statistical difference was found, however, this shows that the THF in the original solution was not the cause of mortality (Fig. 3).

Mortality is not the only indicator for the impact of nanoparticles on *D. magna*. Sublethal responses also were exhibited by behavioral changes in the filtered C<sub>60</sub> trials. Immobility was the most commonly seen change, with the *D. magna* appearing to be unable to swim down from the surface. Small-scale behavioral changes play an important role in predation risks [20], and immobility is a standard used by many organizations to determine water quality [21]. For most of the C<sub>60</sub> concentrations tested, little mortality was seen at the end of 1 h, but most of the *D. magna* often were immobilized at this time.

Swimming in small circles near the surface also was observed. After 24 h, some of the *D. magna* were dead, whereas others continued to swim abnormally near the surface. Some of the survivors often would swim normally at this point. However, by 48 h, the *D. magna* that survived usually reverted to the initial state after exposure and swam sporadically in small circles. Exposure to TiO<sub>2</sub> was much less severe, causing no immobility at any time or concentration. Even at concentrations as high as 500 ppm of sonicated TiO<sub>2</sub>, no sporadic swimming behavior was exhibited.

In addition to observing juveniles during the toxicity assay, we exposed adult *D. magna* to C<sub>60</sub>, which caused sporadic swimming and disorientation. The adults swam into the vessel wall and continued to impact the wall several times. Normal behavior on colliding with a wall is to turn and swim in another direction, so this persistent ramming can be considered to be irregular. Understanding both the minute and the large-scale behavioral changes exhibited on exposure to these nanoparticles may be crucial in understanding the impact they will have on invertebrates. Therefore, the behavioral observations exhibited by *D. magna* in the present study should be further examined and quantified to compare against normal behavior with less risk of predation.

The results of the present study are important, because it is one of the first to examine the impact of nanoparticles on invertebrates. *Daphnia magna* are extremely important in the food web and will be impacted greatly by release of nanoparticles into aquatic systems because of their high levels of interaction with the environment through filter feeding. This experiment also contributes to our limited knowledge regarding the overall implications of these particles on freshwater ecosystems.

To our knowledge, only one scientific study has been published concerning the effect of nanoparticles on aquatic organisms [12]. Additionally, that study only addressed one type of nanoparticle, the fullerene. The present study, to our knowledge, is the only one to date that has examined the nanoparticle form of TiO<sub>2</sub> and a critical environmental organism. The sonicated TiO<sub>2</sub> solution in this experiment showed little detriment, as did a suspension of TiO<sub>2</sub> in the macroparticle form during a study by Johnson et al. [22] (median effective concentration, >1,000,000 µg/L). Because several experiments have found similar results in various organisms, TiO<sub>2</sub> is considered to have no health risks, and this is one of the reasons it is considered to be a suitable particle for use in photocatalysis and other applications [5]. Classification of TiO<sub>2</sub> as nontoxic may need to be re-evaluated at the nanoparticle level, however, because of its toxicity to *D. magna* as reported in the present research.

We have determined that both TiO<sub>2</sub> and C<sub>60</sub> negatively impact the survival of *D. magna*, whereas C<sub>60</sub> show higher levels of toxicity at lower concentrations. This project has shown that standard toxicity testing can be used to examine nanoparticle toxicity. It also has shown that particle preparation can greatly impact toxicity and that special care should be taken to determine the best methods of preparation for proper disposal of nanoparticle waste. How the nanoparticles are treated and the experiments are set up must be known, because this might have a large impact on the results when these nanoparticles are tested. Future classifications may need to group particles at larger sizes by chemical and physical properties and then compare those against the properties exhibited at smaller sizes to examine changes as a result of size.

The present work can be a foundation for research into

other nanoparticles. We are in the process of examining behavior to see the small-scale effects on organisms. The effects on reproduction and physiological factors, such as heart rate, swimming, and filtration, could greatly impact aquatic environments. Likewise, we are looking at the biochemical mechanism for toxicity of nanoparticles by examining gene expression on exposure. If a comparison of the particles from the present study with other nanoparticles can be performed, the negative side effects of using nanoparticles can be determined. Then, use of the best nanoparticles for maintaining aquatic ecosystems can be implemented.

## CONCLUSION

Understanding the potential impacts of these particles can help in identifying the most appropriate nanotechnology that will preserve the aquatic environment while also advancing medical and environmental technology. With the increased use of nanoparticles, the negative consequences of releasing these particles into the environment, either as by-products, medical waste, or ecological tools, need to be investigated. Contamination of waterways and public drinking water can occur as a side effect of manufacturing nanoparticles or from release of the particles themselves [1]. Before the present study, the impact of nanoparticles on aquatic organisms was virtually unknown. By using the U.S. EPA standard tests, our results are reliable and easily translated to other investigators and policymakers. Because no current regulations cover the release of nanoparticles into the environment, such regulations should be established, based not only on chemical components of larger sizes but also on size, because chemical properties change at the smaller scale. Further research will show if this type of classification is valid.

Because we know of so many positive potential uses of nanoparticles, we must find a way to employ them with the least environmental effect. When possible, the least toxic particles must be chosen. When more toxic particles must be used, limits and regulations on the mechanism of their release should be created.

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