Toxicity and Bioaccumulation of Nanomaterial in Aquatic Species

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ABSTRACT

With applications in consumer products, medicine, and the environment, nanotechnology is expected to grow into a one trillion dollar industry. Environmental exposure to nanoparticles, already used in 700 products ranging from stain-free clothing to sunscreens and cosmetics, is steadily increasing. Such a burgeoning technology, however, has many potential negative effects, as little is known about the toxicity and bioaccumulation of nanoparticles, especially in aquatic environments.

A better understanding of these particles can mitigate environmental devastation before this technology fully develops. This project investigated the effects of nano-scale zinc oxide (ZnO) and carbon fullerenes (C60) on *Chlamydomonas reinhardtii*, green alga, and *Daphnia magna*, water fleas. Both organisms are model organisms for a toxicology test; more importantly, *C. reinhardtii* is a natural food of *D. magna*, making a bioaccumulation study possible. Algae, in general, play an integral role in the ecological system, producing the biomass that forms the basic nourishment for food webs and much of the oxygen humans breathe. Thus large alga population changes due to nanoparticle toxicity will have negative effects on the entire environment.

Three different toxicity tests were conducted: two toxicology tests in which particles were directly introduced in the environments of *C. reinhardtii* and *D. magna* and a third test for bioaccumulation of nanoparticles from the alga to the Daphnia. The organisms were treated with nanoparticles, first sonicated to minimize aggregation, at 1, 5, and 10 parts per million (ppm), regular-ZnO or C12, or no particles at all. In the bioaccumulation test some Daphnia were fed nano-treated alga in fresh water while others fed fresh alga with nano-treated water to pinpoint the method of transfer.

The toxicity tests concluded that nano-ZnO and C60 were more toxic to the organisms than regular-sized particles. More alga death occurred at 1ppm of nano-ZnO and C60 as compared to 10ppm of regular-sized particles; similar results occurred with the Daphnia. The alga data also revealed that zinc oxide was more toxic than carbon, a factor probably due to the higher solubility of the former.

The most important result was that the effects of nanoparticles were greatest in the long-term. At 10ppm of nanoparticles, alga populations were never able to recover. Studies so far have only focused on the short-term toxicity of these particles, in accordance with the Environmental Protection Agency's (EPA's) acute toxicity tests. Yet, a long-term study may be more practical in expanding our understanding, as these particles are unlikely to leave the environment after exposure.

The data from the bioaccumulation test indicated that a transfer of nanomaterials from the alga to the Daphnia occurred, primarily through water but also through the alga, but the trend is not conclusive. Modifications in experimental design such as monitoring the Daphnia over a longer period of time may result in a better understanding of the bioaccumulation of nanoparticles and their chronic effects on organisms.

In addition to lengthening the experimental period, modifications, including utilizing new methods to evaluate Daphnia response to nanoparticles such as using DNA microarray as well as discovering a manner to keep the particles suspended in the medium, will provide a better understanding of the effects of bioaccumulation. It is also imperative to characterize the particles themselves and their aggregate state, as I have begun to do with dynamic light scattering. Such characterization will be useful in determining the mechanisms for nanotoxicity and the long-term properties of nanoparticles in the environment.

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1. INTRODUCTION

The Nanotechnology Revolution

Nanotechnology, concerning the creation and application of objects ranging between 1 and 100 nanometers, has evolved from an obscure technology to one applicable in many environments and products (Colvin, 2003). Today, nano-scale zinc oxides are used in sunscreen lotions and scratch-resistant glass while carbon nanotubes are incorporated into tennis rackets, and nano-engineered chemical treatments performed on fabrics to render them stain-resistant (Bergeson and Auerbach, 2004). The National Science Foundation has estimated that nanotechnology applications may be valued at more than 1 trillion dollars in the global economy by 2015 (Bergeson and Auerbach, 2004). Apart from consumer products, nanomaterials are being tested for medical and environmental uses. In 2004, the University of Texas Health Science Center at Houston joined with C Sixty, Inc., a pioneering biopharmaceutical company, to study the use of fullerenes in the delivery of anesthesia and contrast imaging dyes (Mervill, 2003)**.** Now, nanomaterials, superparamagnetic iron oxide particles, are believed to be able to allow for the detection of cancer earlier than traditional diagnostic tools (Medical and Pharmaceutical Applications for Nanomaterials and Nanoparticles). Furthermore, nanotechnology offers potential in the area of ecological forecasting through computerized sensors the size of dust particles that can relay information on the slightest pollutants (Bergeson and Auerbach, 2004).

Research on Nanotoxicity

The burgeoning of the nanotechnology industry has not proceeded, however, without efforts to understand the properties of these materials. The early studies conducted by D. B. Warheit for the Dupont Company demonstrated that nanoparticles (more specifically titanium dioxide, carbon black, and diesel particles) produced higher lung inflammation, fibrosis, and tumor responses in rats than fine-sized particles (Warheit, 2004). C.W. Lam and his associates conducted a similar experiment and found granulomas, aggregates of chronic inflammatory cells (Lam, et al., 2004). E. Oberdörster, from Duke University conducted a study of the acute toxicity of carbon fullerenes with juvenile largemouth bass, discovering that fullerenes caused lipid perioxidation in the brains of the bass (Oberdorster, 2004).

More recent research from Rice University yielded a possible method to mitigate the toxicity of nanomaterials. Researchers adorned the carbon spheres with simple chemicals, hydroxyl or carboxyl groups, and found that the more decorated the fullerenes, the less toxic they were. The concentration needed to kill half the cells was more than 10 million times that required with the naked Buckyballs (Goho, 2004). Researchers hypothesized that when they are naked, the fullerenes aggregate in a solution, generating reactive chemicals known as free radicals (Goho, 2004). Researchers at Lawrence Berkeley National Laboratory in conjunction with the University of Kentucky, Affymetrix, and the University of California, have delved further into the reasons for cell death. Primary researcher, F. F. Chen indicated that, “apoptosis,

cell-cycle delay, cellular transport, and inflammation are linked to the [nanoparticle] treatments" (Kalaugher, 2005).

The Need for Bioaccumulation Studies

Though a panoply of studies have been conducted in just the last few years alone, a scientific understanding on the effects of nanomaterials on organisms and their environments has not been established, due to the large variety and differing characteristics of nanomaterials. And one especially lacking area in terms of scientific understanding is the possible bioaccumulation of nanoparticles throughout the various trophic levels. Because little is known about the biodegradation of nanomaterials, which may result in changes in their physical structure or surface characteristics, and the majority of nanomaterials are not expected to biodegrade, bioaccumulation is an essential characteristic of nanomaterials to study (Taku, 2006). Researchers at Rice University's Center for Biological and Environmental Nanotechnology, CBEN, have shown that nanomaterials can accumulate in living things over time, with ever-increasing concentrations in microbes, in the worms that eat the microbes, and in animals higher up the food chain (Weiss, 2007). Whether nanomaterials are this detrimental to an aquatic environment remains to be studied.

Objectives of this Project

This research project investigated the effects of carbon fullerenes (C60) and zinc oxide nanoparticles (ZnO) on *Chlamydomonas reinhardtii*, a species of green algae, and *Daphnia magna*, a freshwater crustacean. The first two objects of this experiment were to better understand the effects of two common types of nanomaterials on *C. reinhardtii* and *D. magna* by measuring the possible population changes in a controlled group of *C. reinhardtii* and *D. magna* over an initial period of 48 hours, which was then extended to 480 hours to gauge long-term effects. The third object was to test for bioaccumulation of nanomaterials from the alga to the Daphnia. A physiological evaluation of individual *D. magna* and a population evaluation were performed.

Aquatic species were chosen for these toxicology tests for a few reasons: (1) both *C. reinhardtii* and *D. magna* are model organisms with several advantages; *C. reinhardtii* grow exponentially, reaching their carrying capacity in 3 to 5 days, and respond to small changes in the environment while *D. magna* are valuable because of their sensitivity to toxic substances, ubiquitous distribution, and extensive use in toxicity testing (Clesceri, et al., 1998)], (2) *C. reinhardtii* is a natural food of *D. magna*, thus making a bioaccumulation and more specifically biomagnification study, possible (Clesceri, et al, 1998), and (3) algae, in general, play an integral role in the ecological system, producing the biomass that forms the basic nourishment for food webs in large bodies of water and producing much of the oxygen humans breathe. Thus, any population changes at the bottom of the aquatic food chain will heavily affect the marine and freshwater biomes and the health of water worldwide.

2. METHODS

Culturing the *C. reinhardtii*

The strain of *C. reinhardtii* used was a pure, nonpathogenic strain from the American Tissue Culture Collection, cultured in the Life Sciences Laboratory at Arizona State University.

- Place 1 mL of 3-day-old *C. reinhardtii* into a flask with 100 mL of sterile medium. The recipe for this medium is listed below.
- The alga grows optimally when exposed to a light intensity of 20 µmol.
- The recipe for *C. reinhardtii* medium

Stock Medium (5X)	g/L	Final Concentration (1X)
$NaAc.3H_2O$	5.0	7.5 mM
$NaCitrate.2H_2O$	1.5	1.0 mM
K_2HPO_4	2.62	3.0 mM
KH_2PO_4	4.75	7.0 mM
NH_4Cl	2.0	7.5 mM
$CaCl_2.2H_2O$	0.074	0.1 mM
$MgSO_4.7H_2O$	1.22	1.0 mM
$FeCl_3.6H_2O$	0.012	0.01 mM

- The stock medium was prepared by adding the chemicals in the order listed to water. The phosphates should be dissolved before adding divalent cations to avoid precipitation. Ferric chloride can be dissolved into a small amount of water before adding slowly to the stock medium. The final pH should be 6.3 ~ 6.5.
- The growth medium was prepared by diluting 200 mL of stock and 1 mL of trace metal solution to 1L of water. This medium was then poured in flasks and the entire units were autoclaved (to make sure no bacterial remnants are in the culture) to promote optimal alga growth.

Trace Metal Solution (1000X)	mg/100mL
H_3BO_3	100
$ZnSO_4.7H_2O$	100
$MnSO_4.4H_2O$	40
$CoCl_2.6H_2O$	20
$Na_2Mo_4.2H_2O$	20
$CuSO_4.5H_2O$	6

Adding Nanomaterials and Particulate Matter to the *C. reinhardtii* Environment

These nanoparticles were purchased either from Fisher Scientific or from Sigma Aldrich. The concentrations added, 1ppm (parts per million), 5ppm, 10ppm, were based on prior experiments such as the experiment with carbon fullerenes and largemouth bass conducted by Eva Oberdörster, who used a concentration of around 3.8ppm (Oberdorster, 2004).

- Carbon fullerenes (C60) are hydrophobic, and thus they must be treated prior to introduction into the environment. To become more soluble, the particles need to be placed in deionized H_2O and then a

sonicator for around 30 minutes to break them into small, non-coagulating particles (Lovern and Klaper, 2006). Because nano zinc oxide (ZnO) is more soluble, it is not necessary, but recommended that the particles are sonicated.

- Then, the C60s and nano-ZnOs need to be diluted to the concentrations mentioned above: 1ppm, 5ppm, and 10ppm. This was done by preparing a stock solution that consists of 10% of the particulate matter and adding 1µL for 1ppm, 5µL for 5ppm, and so on.
- Three experiments were conducted. The first two were toxicity tests using the two types of nanomaterials directly on *C. reinhardtii* and *D. magna*. Instead of introducing the nanomaterials after one day of growth, the materials were introduced when the alga reached a stable population. Then, the population changes were measured over a period of 480 hours.
- The third experiment was a test of bioaccumulation of nanomaterials through the early stages of an aquatic food chain. First, the nanomaterials were introduced into the alga medium; this was done after one day of alga growth as to provide the alga enough time to absorb the nanoparticles, if indeed the cells absorbed the materials. Then, the nanomaterials-treated alga was fed to *D. magna* and the physiological and population changes in the *D. magna* measured.

Counting *C. reinhardtii*

This method involved heating the alga in order to immobilize them for counting.

- Extract 1 mL of the alga sample from the 100 mL flask and place it into a tube. Heat this tube at around 60ºC - 70ºC for about 25 minutes.
- Load 40 µL of this solution from the tube into each of the chambers of the Newbauer Improved Counting Chamber.
- Cover the counting chamber with a plastic cover slip and place under the microscope and count the cells.
- This number needs to be multiplied by 10^4 to get the number of cells per mL.

Removing *C. reinhardtii* Cells from the Culture

This process took the alga remaining in the flask after 5 days of growth and changed it into a pellet form that can be preserved at -80ºC. One set of Daphnia was treated directly with the nano-treated alga, another population treated with the medium that had been exposed to nanomaterials and fresh alga, and another control population treated with fresh alga. This way, physiological or population changes in the Daphnia could be attributed to either bioaccumulation through the ingestion of alga, transmission of nanomaterials through the alga medium, or a combination of the two.

- Take 2mL of alga and place it into a tube. Centrifuge the tube for about a minute at 1000 rpm or for as along as needed so a very small pellet of alga forms at the bottom of the tube. This separates the medium, which will be the supernatant from the nanoparticles and the alga.
- Remove 1mL of the medium and re-centrifuge the tube until pellets of alga appear; this should take about two minutes of centrifuging at around 5,000 rpm.
- Remove the supernatant from the tubes of alga and medium not treated with nanoparticles.
- Remove the supernatant from the tubes of alga and medium treated with nanoparticles and place this medium in the tubes of alga that were not treated with nanoparticles (the supernatant for these tubes should have been removed in the previous step so this serves to resuspend those alga).
- Take 1mL of medium (not treated with any particles) and re-suspend the alga treated with nanoparticles.

Culturing *Daphnia Magna*

The *D. magna* were ordered from Carolina Biological, and a fresh culture was ordered for each experiment. The *D. magna* were maintained in synthetic reconstituted water and were cultured according to the recommendations of the *Standard Methods for the Examination of Water and Wastewater 20th Edition*.

Testing with *D. magna*

The *D. magna* were observed on both a physiological level and a population one. Both observations were made in accordance with those recommended by the *Standard Methods for the Examination of Water and Wastewater 20th Edition*. Each toxicity test consisted of 10 adult *D. magna* in a well (Clesceri, 1998). Physiological changes such as heartbeat were measured by placing the *D. magna* under a light microscope. Other indications of stress, such as the production of ephippia eggs, were also monitored.

Using Dynamic Light Scattering to Measure Actual Size of Nanoparticles – The Brookhaven Instruments – ZetaPals Particle Sizing Software was used to measure the size of the particles.

- Prior to measurement, some particles were filtered, meaning that they had been suspended in room temperature water for two weeks and sent through a 200nm filter, and sonicated.
- Each cycle of DLS was set at three minutes and the dust cut off at 50. The temperature was 25º C and the particles were dissolved in a solution of water. The wavelength was at 659.0nm and the refractive index for each particle inputted before the trials.

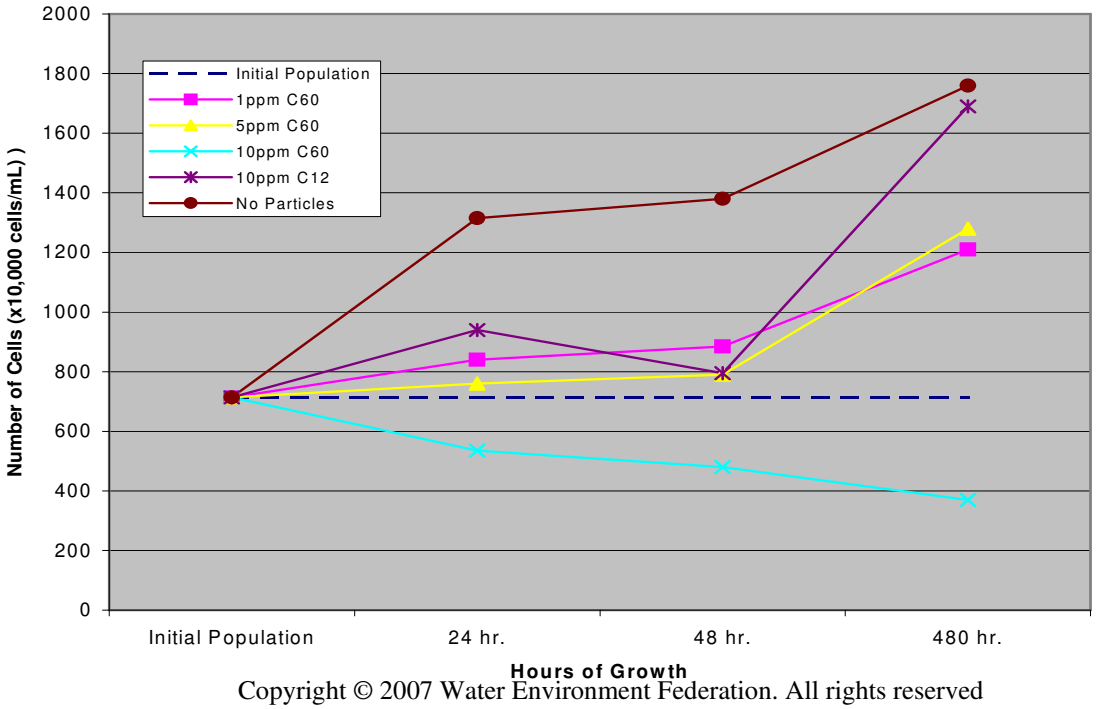
Data Analysis

The data was analyzed using a paired, one-tailed t-test to establish significance. A paired t-test was chosen because it compares a value in one group to that corresponding value in another group, instead of a value randomly selected from the second group. This way, the population of alga after 24 hours of exposure to nanoparticles was directly compared to the population of alga after 24 hours of exposure to regular particles and so on.

3. RESULTS

Graph 1

Acute Toxicity of C60 Test on *C. reinhardtii*



Graph 2

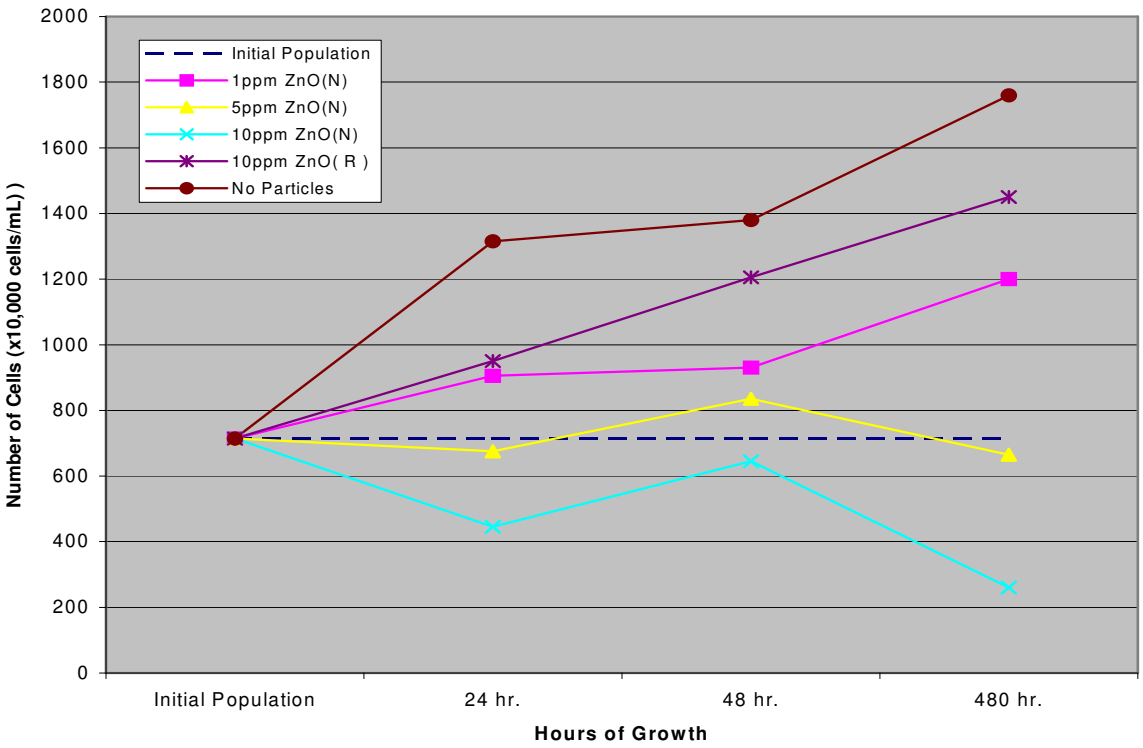
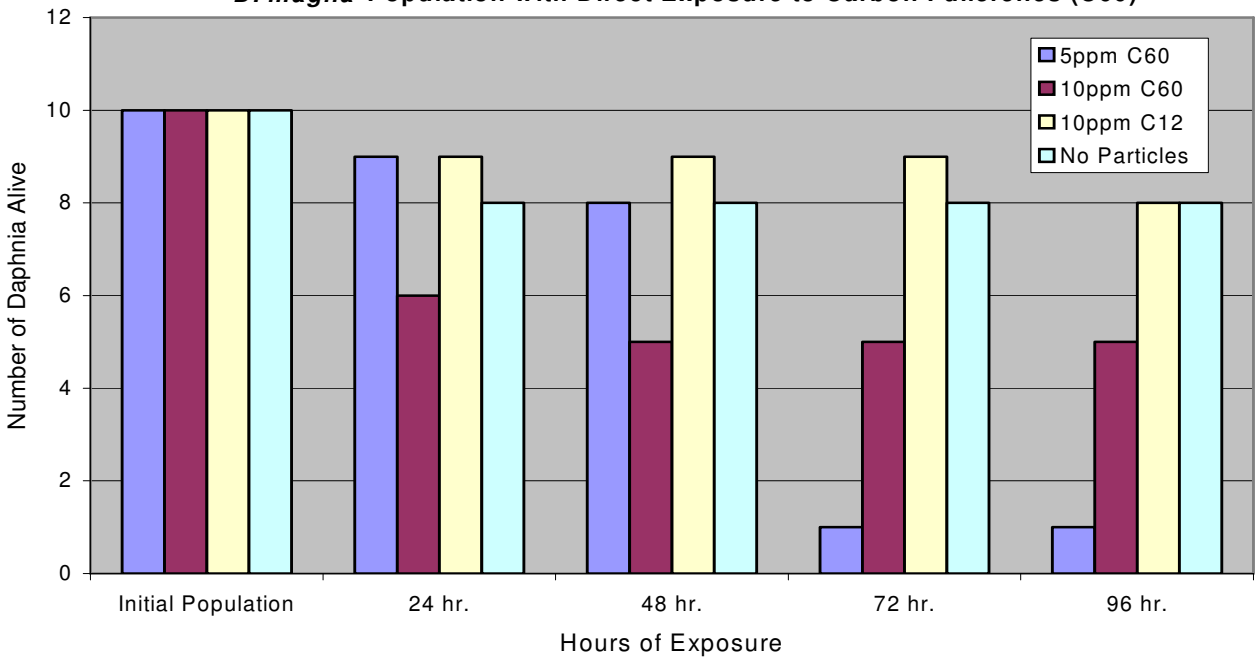


Table 1. T-Test Results for Nanotoxicity Test with *C. reinhardtii*

T- Test Results (Paired Two Sample for Means - One Tailed)								
	C60 Group I		C60 Group II		ZnO Group I		ZnO Group II	
	p- value	Significance	p-value	Significance	p- value	Significance	p-value	Significance
No Particles v. 1ppm	0.003103	99.69%	0.008245	99.18%	0.004462	99.55%	0.004671	99.53%
No Particles v. 5ppm	0.007810	99.22%	0.000193	99.98%	0.024428	97.56%	0.022521	97.75%
No Particles v. 10ppm	0.019429	98.06%	0.012892	98.71%	0.029880	97.01%	0.021696	97.83%
No Particles v. 10ppm Regular	0.047422	95.26%	0.110123	88.99%	0.111997	88.80%	0.044412	95.56%

From graphs 1 and 2 as well as table 1, it is evident that nanoparticles C60 and nano-ZnO are more toxic to alga populations than regular sized particles. Even at 1ppm, the nanoparticles were more toxic than 10ppm of regular-sized particles. The t-test proves that the data is significant. The only two points where the data is not 95% significant also happens to be when no particles were compared with 10ppm of regular particles. The difference between the control alga, or alga grown without any particles, and the alga exposed to 10ppm C12 or regular-ZnO was only 88.99% and 88.80% significant respectively. This indicates that the toxicity of C12 and ZnO particles were not as great.

Graph 3
***D. magna* Population with Direct Exposure to Carbon Fullerenes (C60)**



Graph 4
***D. magna* Population with Direct Exposure to Nano-ZnO**

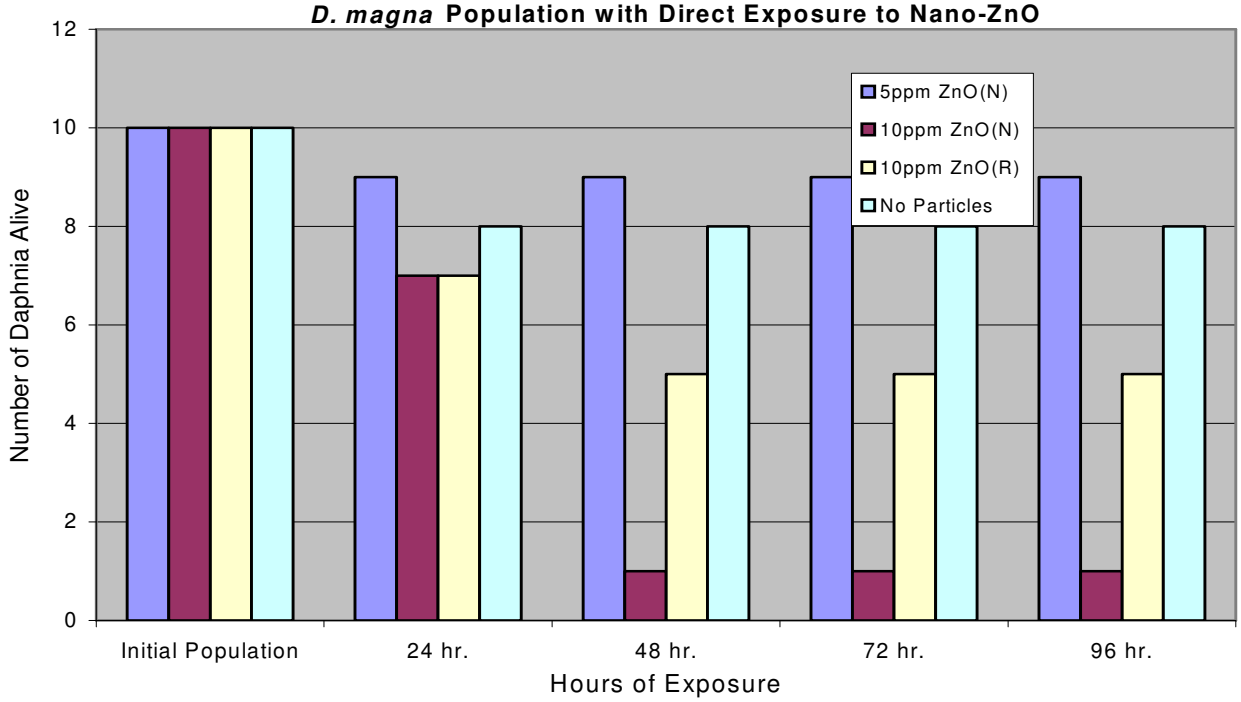
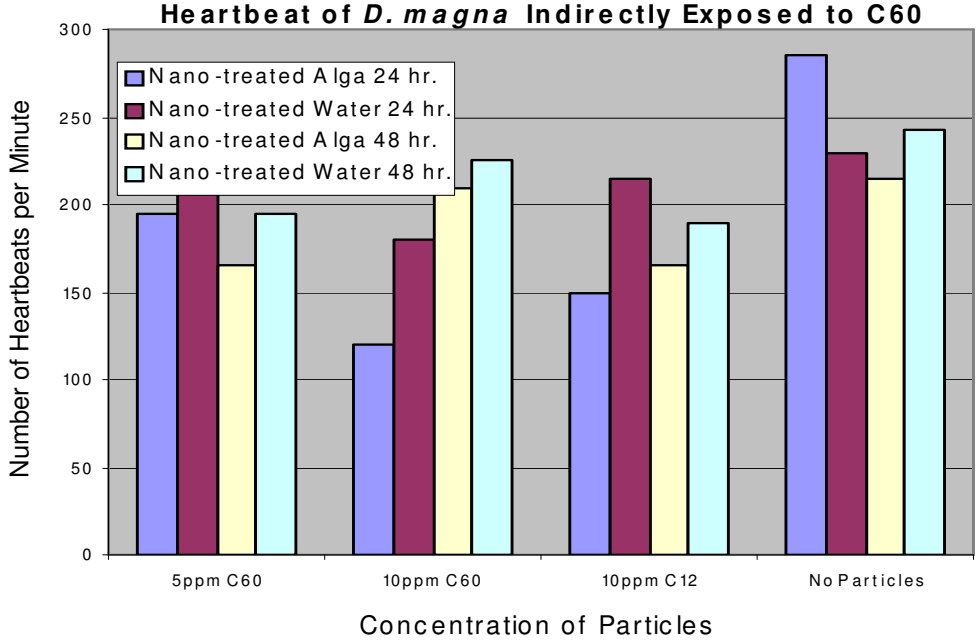


Table 2. T-Test Results for Nanotoxicity Test with *D. magna*

T- Test Results (Paired Two Sample for Means - One Tailed)				
	C60		ZnO	
	p- value	Significance	p- value	Significance
No Particles v. 5ppm	0.009777	99.02%	0.008065	99.19%
No Particles v. 10ppm	0.111642	88.83%	0.025687	97.43%
No Particles v. 10ppm Regular	0.035242	96.48%	0.017055	98.29%

Graphs 3 and 4 and table 2 demonstrate that nanoparticles are generally more toxic to *D. magna* than regular sized particles. Details are furthered addressed underneath the discussion section.

Graph 5



Graph 6

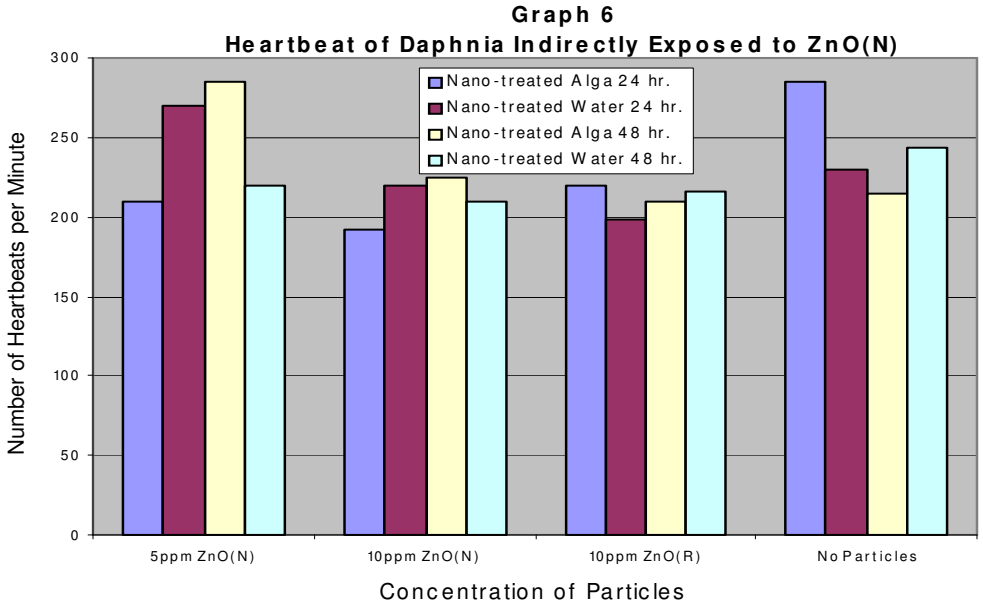
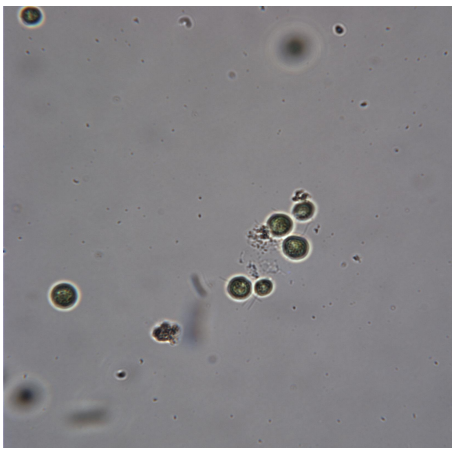


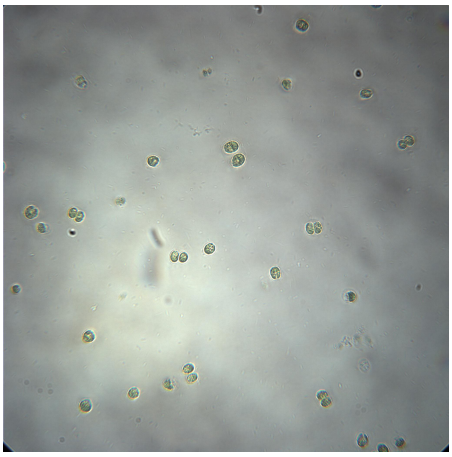
Table 3. T-Test Results for the Bioaccumulation Test

T- Test Results (Paired Two Sample for Means - One Tailed)				
	C60		ZnO	
	p- value	Significance	p- value	Significance
No Particles v. 5ppm	0.018235	98.18%	0.466060	53.39%
No Particles v. 10ppm	0.100392	89.96%	0.126351	87.36%
No Particles v. 10ppm Regular	0.044329	95.57%	0.040102	95.99%

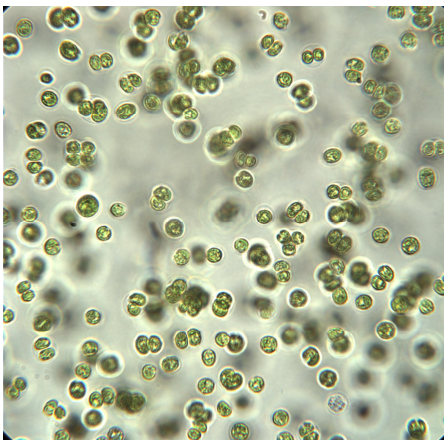
The nanoparticles appeared to slow down the heartbeat of some of the *D. magna* (Graph 5 and 6), but this trend was not very clear or conclusive. The t-tests (table 3) further proved that only a very weak correlation between heartbeat of Daphnia and the introduction of various particles into its environment exists.



Picture 1 – *C. reinhardtii* with 10ppm nano-ZnO (Notice the particle and cell aggregate)



Picture 2 – *C. reinhardtii* treated with 10ppm C60 (after 480 hours or 20 days of exposure to particles), similar results with nano-ZnO



Picture 3 – *C. reinhardtii* treated with 10ppm C12 (after 480 hours or 20 days of exposure to particles), similar results with ZnO

4. DISCUSSION

Toxicity of Nanoparticles on *C. reinhardtii*

Looking at the alga data (graphs 1 and 2), nanoparticles (C60 and nano-ZnO) are more detrimental to populations in the long-term rather than in an acute toxicity test. These particles are still toxic in the short term but more so after a longer period of time after an initial exposure. Populations treated with either 10ppm of C60 or nano-ZnO particles were unable to recover even within a 20-day period. The population of alga in the flask treated with 10ppm C60 was less than one half of that originally in the flask, and the population of alga treated with 10ppm nano-ZnO was around one third of what was originally present in the flask. Moreover, the coloration of the alga changed from a deep green to more of a yellow or yellow-green. In addition, many of the remaining cells appeared to have lysed or were undergoing difficulties when reproducing (pictures 2 and 3). The t-tests (table 1) further supports this conclusion, as the only two tests that came up with a p-value of higher than 0.05, thus a significance less than 95%, were the tests comparing the population differences from alga treated with C12 and regular-ZnO and those not treated with any particles. This means that the long-term effects of populations treated with 10ppm of either C12 of regular-ZnO were not very great, as the populations were able to rebound. The alga data also indicates that ZnO, in any form,

tends to be more toxic than carbon particles. This can be explained by the higher solubility of zinc oxide in water.

Toxicity of Nanoparticles on *D. magna*

The direct introduction of particles into the environment of the *D. magna* yielded similar results to the alga results (graphs 3 and 4). Generally, nanoparticles are more toxic than regular-sized particles. The order of toxicity levels, however, differs slightly, with regular-ZnO and C12 particles more toxic to *D. magna* than they were to *C. reinhardtii*. With zinc oxide, regular particles at 10ppm were more toxic than nanoparticles at 5ppm. This disparity can be largely attributed to the different sizes of the organisms. Because *D. magna* are much larger than *C. reinhardtii*, it may have a very different interaction with the regular-sized particles. Whereas the regular-sized particles are too large to affect *C. reinhardtii*, they may be of the right size to affect *D. magna*. Furthermore, previous toxicity tests have revealed zinc oxide to be naturally toxic to *D. magna*, which may be another reason for the disparity between the two organisms. It is also important to note that the deaths which occurred in the control wells, in which no particles were added, all occurred within the first 24 hours, when there appeared to be a lack of food in the *D. magna* environment. Photographs under the light microscope revealed the absence of alga in the deceased *D. magna* not treated with any particles. Conversely, alga was present in the deceased *D. magna* that had been exposed to nanoparticles. In addition, the total *D. magna* mortality rates were much lower than those found in previous studies conducted by S. Lovern at the University of Wisconsin-Milwaukee. This can be explained by the different methods of nanoparticle treatment. Lovern and her colleagues not only sonicated the particles but also filtered them with tetrahydrofuran (THF) prior to introduction into the *D. magna* environment. I chose not to use THF, as recent studies have indicated that total removal of THF may not be possible, implying that Lovern studied the effects of nanoparticles coupled with trapped remains of THF (Stone, et al. 2006).

Bioaccumulation of Nanoparticles from *C. reinhardtii* to *D. magna*

The bioaccumulation of nanoparticles into the environment of *D. magna* did not yield results as conclusive as the alga experiment (graphs 5 and 6). The nanoparticles appear to slow down the heartbeat of some of the *D. magna*, but this trend is not very clear (table 3). This could have occurred for a couple of reasons. First, bioaccumulation tests need to be run for a much more extended period of time; the effects may not be very evident in the parental generation but perhaps much more salient in the second or third generation of *D. magna*. Second, the concentration of nanoparticles introduced in the environment of the *D. magna* was incredibly small. The concentrations introduced into the alga were 5ppm and 10ppm of C60 or nano-ZnO as well as 10ppm of C12 or regular-ZnO. Only 2mL of this solution was centrifuged, and only 1mL of it fed to the *D. magna*. Therefore, the concentration of nanoparticles may have been too low or almost non-existent in the *D. magna* environment. Third, even if the particles were present in larger amounts, the distribution of these particles in the reconstituted water was uneven due to the absence of a source that continually resuspended them in the water. In the alga experiment, the shaking platform constantly kept the particles suspended in the medium; there was no such source present in the *D. magna* experiment, as *D. magna* generally prefer stagnant water.

Error Analysis

Because the experiment occurred over the period of a few weeks and required multiple steps and evaluations, some errors may have occurred. To keep the *C. reinhardtii* population uncontaminated, all work was done under a fume hood and all materials were either disposable or autoclaved prior to experimentation. Yet, differing populations of alga still resulted. Some of these discrepancies can be explained by the inability to keep the light source exactly at 20 µmol in all areas of the shaking platform. It is also worthy to note that

even under the most auspicious of conditions, some populations simply grow at a different rate and to a different capacity than others.

Many of the discrepancies in the *D. magna* data resulted from the variability of *D. magna*. In future experiments, the experimental procedures for the *D. magna* can be improved significantly to reduce discrepancy in the data. First, the bioaccumulation tests need to be run for an extended period of time, as the effects of bioaccumulation are more salient after longer periods of exposure. Second, the concentration of nanoparticles needs to be increased in the original alga exposure, so that when this alga is fed to the *D. magna*, enough of the particles are transferred, either through the medium, the alga, or both into the medium of the *D. magna*. There must also be a mechanism to keep the particles suspended in the environment of the alga. This can be difficult, as *D. magna* prefer stagnant water.

Future Research

From this experiment, one can conclude nanoparticles are generally more harmful to *C. reinhardtii* and *D. magna* when exposure is long term. The mechanisms of the toxicity, whether the particles interfere with reproduction or other cell functions, are still unknown and that is an area for future studies. Prior research has indicated that nanoparticle toxicity is largely induced by oxidative stress, which results from the aggregation of nanoparticles. Yet, other explanations such as lipid perioxidation need to be studied further. From picture 1, it is possible for nanoparticles to attach to the glycolipids or glycoproteins on the membrane of the alga, and thus destroy the membrane. The pictures taken under the microscope indicate some reproduction problems in the alga but more specific physiological responses in the alga and Daphnia need to be known to determine reasons for toxicity.

A modified bioaccumulation study will also make for interesting research, especially when the *D. magna* treated with nanoparticles are introduced into the environment of *Danio rerio*, zebrafish. Any such bioaccumulation study will be long term, and the effects of the nanomaterials possibly measured in the second or third generation of the organism.

The different levels of toxicity of C60 and nano-ZnO also indicate that nano-toxicity cannot be generalized but will vary depending on the characteristics of the molecules. Thus, characterizing the particles, their size, shape, aggregate state will help in understanding the various mechanisms of toxicity. The preliminary results of dynamic light scattering indicate that none of the particles, once introduced in an aquatic environment, remained in nano-state. How this translates to toxicity is still unknown and serves even more as an impetus for future research into the area.

Thus far, research on nanoparticles does not condemn the further development of nanotechnology. This development, however, should proceed cautiously, taking every measure to understand and characterize nanoparticles. At the very least, Material Safety Data Sheets need to be developed separately for nanoparticles and a standardized testing method established. Only with this caution can the health and safety of consumers, in addition to the environment, be safeguarded

5. CONCLUSION

1. Both nano-ZnO and C60 are more toxic to *C. reinhardtii* than regular-ZnO and C12. The alga, when exposed to 1ppm of either nano-ZnO or C60, exhibited more cell death than when exposed to 10ppm of either regular-sized particle.
2. Nanoparticles have more toxic effects long-term than they do within the first 48 hours of exposure. Thus, studies on toxicity should go beyond the EPA's recommended 48-hour acute toxicity test in order to gain a better understanding of the nature of these particles.

3. Both nano-ZnO and C60 are more toxic to *D. magna* than regular-sized particles. Regular-sized particles are more toxic towards *D. magna* than they are towards *C. reinhardtii*, an occurrence probably attributed towards the size and nature of the *D. magna*.
4. Zinc-oxide particles are more toxic than carbon particles, probably due to the higher solubility of the former, thus demonstrating the wide array of nanoparticles and their differing characteristics.
5. Bioaccumulation does appear to occur from *C. reinhardtii* to *D. magna*; modifications in experimental design, including expanding the time period of testing, utilizing new mechanisms to evaluate *D. magna* response like DNA microarray, and discovering methods on suspending the particles in the *D. magna* environment, will yield a more accurate understanding of the process. I hope to also include *D. rerio* into future bioaccumulation studies so as to be able to study a longer food chain.

6. ABBREVIATIONS AND ACRONYMS

EPA: Environmental Protection Agency (United States)
ppm: parts per million, the equivalent of mg/kg
CBEN: Center for Biological and Environmental Nanotechnology (Rice University)
MSDS: Materials Safety Data Sheet
THF: Tetrahydrofuran

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Author

I am a senior at Hamilton High School in Chandler, Arizona, with interests in both the sciences and humanities. My parents have always encouraged me to follow my passions and have been supportive every step of the way. Thus, I have pursued Speech and Debate, Model United Nations, Science Research, and We the People during my high school career. These activities have cultured my patience, my ability to eloquently express myself, my leadership skills, and my openness to new experiences. Some of my accomplishments include being an AP Scholar with Distinction, National Merit Scholarship Winner, Arizona Governor's Young Innovator of the Year (2006), 2nd Place Grand Award Winner at Intel ISEF 2006, and 1st Place Grand Award Winner at Intel ISEF 2007.

Outside of academics and school, I volunteer at the Carl T. Hayden Veterans Affairs Medical Center. My time spent there has really taught me to appreciate my life and the strength of the veterans of this nation. I have organized fundraisers for the VA Medical Center, as well as a holiday card drive to show our veterans appreciation. Participating in these activities has been extremely rewarding and has led me to discover that my goal in life is to make a positive difference in the world.

I will be attending Rice University in the fall to pursue a major in both the biological sciences, possibly with a focus on environmental sciences and international affairs. I hope to find a career that will combine both of these interests in the future. Although I do have a plan of studies, I am open to new experiences and hope to make the most of college.

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