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Introduction

The June 9-11, 2009 International Conference on the Environmental Implications and Applications of Nanotechnology at the University of Massachusetts Amherst convened leading nano researchers, nano policy and regulatory experts, practitioners, manufacturers and users to better understand the environmental aspects of nanotechnology -- from characterization, fate and transport, and environmental health and safety, to green nanotechnology and new nanotechnology applications for pollution control, remediation, and sustainability. Sessions addressed both new research findings and policy and regulatory issues in three concurrent tracks over the course of three days. The conference featured keynote presentations from leading nano researchers and regulatory experts, 78 platforms presentations, plenary sessions, 65 poster presentations, and special events. The Conference concluded with a special plenary panel of experts representing academia, industry, and government who drew upon the conference sessions and their various perspectives to discuss effective science-based decision making for the safe use of nanotechnology.

Please note that many presentations addressed new and unpublished research, therefore not all authors elected to submit their manuscripts for this proceedings publication.

This conference proceedings collection is comprised of 21 papers, 10 from oral presentations and 11 from poster presentations.

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Toxicological Effects of Nanocrystal Exposure in Teleosts

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Keywords: quantum dots, toxicity, Fundulus heteroclitus

Abstract
Quantum dots (QDs) are semi-conducting nanocrystals that emit tunable fluorescence which is both brighter and more stable than traditional fluorophores. The medicinal, manufacturing, telecommunications and life science research fields are harnessing the unique physical, chemical, optical, and electrical properties of these metallic clusters, while the environmental ramifications of increased production and usage are relatively unknown. To assess their teratogenic potential, Fundulus heteroclitus embryos were aqueously exposed to 0, 1, 5, 10, 50, and 100 ppm Lecithin encapsulated CdSe/ZnS QDs from the 4-cell stage until hatching. While QDs aggregated and adhered to the chorion (shell) of the teleosts in a dose-dependent manner, a significant increase in developmental abnormalities was noted only at the highest QD concentration. QDs were not acutely or sub-chronically toxic to adult Fundulus when fed 1 or 10 µg of QD /fish/day for 3 months. Fecundity and liver oxidative stress biomarkers (total glutathione and lipid peroxidation) were also unaltered by treatment.

Introduction
Engineered nanoparticles (NPs) are an exponentially growing market as these materials are being incorporated into personal care products, electronics, automobiles, clothing, toys, and paints; yet little is known about the fate of these particles after use and disposal. Recent reports suggest that these commercially available products will leach nanoparticles into aqueous environments (Kaegi, 2008; Benn, 2009) thus posing a risk to the organisms that inhabit these ecosystems. Our investigations focused on the developmental and toxicological effects of Lecithin encapsulated CdSe/ZnS QDs on adult and larval Fundulus heteroclitus, the mummichog, following dietary exposure in adult fish as well as water-borne and maternal exposures in embryos. Initial observations of QD behavior in natural estuarine waters (~20 ppt) showed considerable aggregation and precipitation to the sediments. Fundulus, which are found in tidal creeks and estuaries from Maine through the Gulf of Mexico, reside in the water column, but lay their eggs on the sediments. These fish are likely to be exposed to engineered NPs via the water column, sediments, and food items.

Methods
QD Preparation
CdSe/ZnS Evidots (ED-C11-TOL) were purchased from Evident Technologies (Troy, NY, USA). The QDs obtained had approximate diameters of 1.9, 2.4, 4.0, and 5.2 nm and emit at wavelengths of 490 (blue), 540 (green), 600 (orange), and 620 nm (red), respectively (Evident Technologies, 2008). Lecithin (#102147) was purchased from MP Biomedicals, Inc. (Solon, OH, USA). Suspensions of CdSe/ZnS quantum dots were prepared according to Dubertret et al. (2002). The QD to Lecithin ratio was 1:2 for the Mixed QD solution and 1:1 for the Maple Red QD solution. While large aggregates were formed in suspension (micron
sized aggregates), smaller aggregates exhibited Brownian diffusion suggesting they were still in the nano-size range.

Embryonic Development Studies

Uncontaminated eggs and sperm obtained from a breeding colony of *Fundulus heteroclitus* maintained by our laboratory were subjected to in vitro fertilization. Eggs that successfully reached the 2- to 4-cell stage (Armstrong & Child, 1965) were randomly assigned to a treatment regime in 6-well plates. Embryos were grouped at 10-15 eggs per well with each well receiving 0.2 mL of treatment solution per embryo. The Mixed QD (containing blue, green, and orange QDs) and Lecithin stock solutions were diluted with sterile deionized water and filtered natural seawater (vol/vol) to obtain the appropriate treatment solution. Final nominal concentrations were 0, 1, 5, 10, 50, and 100 ppm QDs with a salinity of 17 ppt. A second round of testing was conducted to evaluate the effects of the Lecithin on embryo development; concentrations were 0, 100, and 200 ppm Lecithin (no QDs). Embryos were monitored for morphological variations and hatching.

Adult Dietary Toxicity Study

Five food diets were prepared: a standard diet (SD), SD + Lecithin (10 µg Lec./day), Low QD (1 µg QD, 10 µg Lec./day), High QD (10 µg QD, 10 µg Lec./day), and Cd (5.9 µg CdCl₂, 10 µg Lec./day). Each diet contained ground tropical fish flakes, ~2% gelatin, shrimp puree for flavoring, and sterilized deionized water. The appropriate amount of Maple Red QD solution, Lecithin solution, and/or CdCl₂ was added to each mixture and fashioned into discs. Thirty male-female pairs of sexually mature *Fundulus heteroclitus* were obtained from tidal creeks in Morehead City, NC. Each pair received SD food cubes during the observation period to ensure proper pairing and reproductive activity. At the end of the 1 month observation period, pairs were assigned to a diet which was administered daily for 3 months. Embryos were collected daily from egg traps and subsets of eggs were either: (1) reared in clean seawater and observed for morphological deformities; (2) measured for QD content via ICP-mass spectrometry; or (3) preserved in buffered formalin for microscopy. At the conclusion of the study, adult *Fundulus* were humanely euthanized. Liver tissues were partitioned and preserved for (1) ICP-mass spectrometry analysis, (2) histological examination, (3) lipid peroxidation assessment, and (4) total glutathione measurement (Cellular biomarkers, 2003).

Figure 1. Light micrographs of *F. heteroclitus* embryos exposed to 0, 1, or 50 ppm (mg/L) CdSe/ZnS QDs. The photographs show QDs affixed to the chorions in the 1 and 50 ppm treatments while only background fluorescence of the yolk sac is visible in the Control embryo.
**Results and Discussion**

*Embryonic Development Studies*

While the QDs attached to the chorion (shell) in a dose-dependent fashion (Fig. 1), QDs were not visible within the body of the fry suggesting that their propensity for aggregation in marine environments as well as their charge greatly affected their ability to traverse the chorion. Incidences of abnormal development (early mortality and/or morphological deformities) were increased in the 100 ppm Maple QD and 200 ppm Lecithin treatments, 10% in both treatments versus 2.5-5% in their respective Controls. Sub-lethal deformities were predominately blood pools and deformed limbs. Given the 1:2 QD to Lecithin ratio of the Mixed QD suspension, the toxicity was attributed to the Lecithin and not the NPs. Hatching rates were notably decreased from Controls (85.0%) at the 2 highest QD concentrations (77.5% and 72.5%). Lecithin exposures showed no effect on hatching success suggesting that the QDs were responsible for the decline. We hypothesize that the QD aggregates could be stabilizing the chorion and preventing the fry from emerging.

*Adult Dietary Toxicity Study*

The QDs were not acutely toxic to the fish; a 96-hour LC$_{50}$ could not be calculated. Fecundity rates were slightly depressed in the High QD and Cd treatments, however, the rates were not significantly different due to the high variation between individual mating pairs. Evaluation of spawn-to-spawn rates reveals that the Lecithin may be responsible for the decline in reproduction. Liver total glutathione and lipid peroxidation levels were not significantly different with regard to treatment suggesting that the QDs did not elicit an oxidative stress response at the time of sacrifice.

**Conclusions**

CdSe/ZnS QDs aggregate in marine environments and precipitate out of the water column, thus benthic dwellers and feeders will encounter these engineered NPs. Mummichog embryos appear to be protected from QD penetration because of the interaction between the QD’s surface charge and the embryo’s protective outer chorion. Adult fish ingesting QDs showed no effect on liver glutathione or lipid peroxidation levels. Moreover, fecundity and embryonic development were not significantly affected by QDs despite continued exposure for 3 months suggesting that these particles may be benign in the environment.

**Acknowledgements**

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**Literature Cited**


Aqueous Toxicity, Uptake and Transfer of QDots in Freshwater Algae and Ceriodaphnia dubia

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Keywords: Quantum Dots, Aqueous toxicity, Food chain transfer, Pseudokirchneriella subcapitata, Ceriodaphnia dubia

Abstract
Semiconductor nanocrystals are presently used for research and diagnostic techniques in biological testing and increased applications of these nanoparticles include electronics and communication. An outer layer or protective organic coating on these particles protects the inner core of these fluorescent nanocrystals. Cadmium and selenium present in the inner core are not immediately bioavailable due to the protective coating. US EPA test protocol and fluorescence microscopy were used to determine the fate and effect of Quantum Dots using standard aquatic test organisms. No lethality was measured following 48-h aqueous exposures of Ceriodaphnia dubia to suspensions as high as 110 ppb, but 96-h median lethality (LC50) to Pseudokirchneriella subcapitata was measured at 37.1 ppb QDs. QD-dosed algae transfer to C. dubia was verified with fluorescence microscopy. Outer organic layers present on the QDs provided protection from toxic exposures of the toxic metal core to C. dubia during laboratory exposures. The transfer of core metals from intact nanocrystals occurred at levels well above toxic threshold values, indicating potential exposure of higher trophic levels. Studies of the fate and effects of nanoparticles containing potentially toxic metals should include studies of trophic level transfer to determine potential metal exposure in higher trophic organisms.

Introduction
Metal-based nanoparticles include a wide range of materials such as TiO₂, gold and silver nanoparticles, and Quantum Dots (QDs). QDs are semiconductor nanocrystals used in medical research due to their stable fluorescence and more recently are being investigated for their use in photoelectronics and electrical applications. They consist of a Cd/Se or Cd/Te crystalline core (diameter ~4 nm), a shell of ZnS, and an outer organic polymer coating. The overall diameter of the resulting QD (Qdot® 545 ITK™ Carboxyl Quantum Dots Fisher Scientific, Fisher part no Q21391MP; Invitrogen Molecular Probes, Eugene, OR, USA) nanocrystal is approximately 10 to 25 nm.

Investigations have reported that the surface coatings of QDs are subject to photolysis or oxidation (1-3) and result in dissolution of the core so that toxic metals are available as hydrated ions. The results of one in vitro study suggest that this significantly increases the toxicity associated with QD exposure in cultures of primary rat hepatocytes (1). Intact QDs offer advantages as diagnostic tools for in vivo and in vitro testing due to their unique physical characteristics; however, their water miscible nature increases mobility as an aqueous contaminant and research is needed to help determine the uptake by aquatic organisms.

Aqueous phase exposures to test organisms of metal-based nanoparticles have incorporated most research (4,5) with most studies failing to include dietary intake of food-associated nanoparticles. Aquatic organisms may be exposed to both water and dietary metal ions the environment and both may invoke toxicity (6,7). Metal bioavailability and uptake routes remain unclear. Both water and dietary cadmium are important to C. dubia exposure but
dietary uptake of cadmium in *P. subcapitata* resulted in lower body burden than aqueous intake alone (7). Food source metals are taken up within the anterior midgut of the daphnia and subsequently distributed into internal soft tissues while the distribution of aqueous cadmium includes outer body surfaces (8). Studies of development of metal tolerance in *Daphnia magna* include aqueous and dietary uptake (9) and the induction of a metallothionein-like protein (10,11). However, properties of nonessential metals transferred within nanocrystals may differ from elemental cadmium in the above toxicological studies. The coatings on QDs may provide protection to organisms during exposure, but QDs exposed to environmental factors (photolysis or oxidation) have been shown to lose their protective organic coatings (1-3) leading to bioavailability of core metals to aqueous organisms. Additionally, the fate of intact QDs following organism uptake, via food or aqueous route, has yet to be established.

According to the manufacturer, the QD:cadmium is a 1:260 molar ratio (12) and Cd:Se is present at a 4:1 ratio (13) providing 65 ppb selenium per ppb QD. High core concentration of cadmium and largely unknown environmental fate of intact semiconductor nanocrystals reveals the potential for metal exposure to aquatic organisms. With an increasing demand and commercialization of these and other nanomaterials (14) the need exists to determine the environmental fate and effects to nontarget organisms. This study was to determine transfer and uptake of QDs through aqueous and dietary exposures and establish if a food chain transfer of intact particles exists. The freshwater algae, *P. subcapitata* and the cladoceran, *C. dubia*, were selected as established model species in standard toxicological studies and ecological risk assessments (15,16). These organisms provide a simple model for food chain transfer as well as published endpoints to nonessential metals for evaluation and comparison.

**Methods and materials**

Exposures utilized QD® 545 ITK™ Carboxyl Quantum Dots. Peak emission of these particles is between 541 nm and 549 nm and the organic polymer coating of these nanoparticles was treated so carboxyl groups were available on their surface. The QD concentration as stated by the manufacturer in purchased suspensions as 7.7 µM was used in all nominal calculations. All test organisms were cultured in the Ecotoxicology Research Facility at Arkansas State University (ASU ERF, Jonesboro, AR, USA), according to U.S. EPA (16) protocol. The ASU ERF enforces good laboratory practices and quality assurance guidelines as set forth by the facility.

Aqueous toxicity exposures – Acute 48-h toxicological endpoints were measured with *C. dubia* using U.S. EPA standardized methods (15) and 96-h chronic endpoints were measured with *P. subcapitata* (16). Serial dilutions for *C. dubia* used U.S. EPA (16) moderately hard water (hardness = 90 mg/L as CaCO³) and *P. subcapitata* dilutions were in Bold’s media (17). All exposures followed U.S. EPA protocol: algae were kept at constant rotation of 100 cpm and microscopic cell counts were used to obtain 96-h algal endpoints.

Organism sensitivity was examined using dilutions of CdCl₂ • 2.5 H₂O in moderately hard water for *C. dubia* 48-h acute endpoints and Bold’s media for *P. subcapitata* 96-h survival/growth endpoints. All results were analyzed using Toxcalc® (18) (α = 0.05) and Cd was measured with Inductively Coupled Plasma Mass Spectrophotometer for final calculations.

Food chain transfer – Algae exposed to QDs during 96-h toxicity tests were washed X3 in Bold’s media to remove unbound QDs (1000 rpm for 5 min). Algal suspensions were then reconstituted to 3.5 × 10⁷ cells/ml in Bold’s media. QD exposed algae were introduced to 12 adult *C. dubia* (24-h age cohort) and allowed to feed for 24 h (targeted algal concentration = 2.0 × 10⁶ cells/ml). Three to four organisms were randomly chosen and examined under brightfield and fluorescence microscopy to determine pixel intensity as described below.
Two controls accompanied food chain exposures: unfed and *C. dubia* exposed to control algae from toxicity test. Statistical endpoints were calculated as mean pixel intensity of organism for QD/algal concentration. One-way ANOVA determined significant differences ($\alpha = 0.05$) (19).

**Results & Discussion**

Reference exposures – 48-h LC50 to Cd for *C. dubia* was 31.9 $\mu$g/L (exposure range 23.8 to 82.1 $\mu$g/L) as compared to published endpoints of 54 to 59 $\mu$g/L (21-23). LC50 reference endpoints to *P. subcapitata* was 129 $\mu$g/L (exposure range 32.2 to 330 $\mu$g/L) as compared to a published endpoint of 50 $\mu$g/L (24).

QD exposures to *C. dubia* ranged from 11 to 110 ppb (molar concentration). No LC50 could be measured to *C. dubia* (LC50 > 110 ppb QD). QD exposures to *P. subcapitata* ranged from 11.1 to 55.0 ppb with measured LC50 value = 37.1 ppb QDs. As calculated by the manufacturer’s stated 260:1 molar ratio of cadmium to QDs, organisms were exposure to cadmium levels within the intact QD cores orders of magnitude higher than reported free ion cadmium levels without eliciting a toxic response (17, 21-23). The organic polymer coating on water miscible QDs provided protection from core metals allowing non-lethal exposures to greater concentrations of intact core Cd as determined in the reference testing.

Algal QD uptake – Significant differences in pixel intensity in QD exposed algal cells during 96-h toxicity testing were measured among treatments and control (Fig.1). Intensity ranged from 0.47 ± 0.13 for controls to 11.14 ± 17.48 for cells exposed to 55.0 ppb QD (17).

It is also interesting to note that significant differences were measured among polygon areas in QD exposed algae and control algae. Altering of structural integrity was suggested as the typical sickle or crescent shape of *P. subcapitata* cells changed to a spherical shape. This was most apparent in the 55.0 ppb QD exposure as was noted microscopically. In algal cells free metal binding occurs rapidly by physical adsorption followed with slower uptake into the cell (25,26). Although the metals in the QD core were not exposed, we suspect that QDs similarly entered algal cells, possibly changing cell integrity and structure. Sublethal endpoints to single-cell structures may present new toxicological endpoints to nanostructures such as QDs.

Food chain transfer – All *C. dubia* exposed to the 24-h feeding regime of QD-exposed algae survived. Unfed and control algae-fed *C. dubia* exhibited mean pixel intensity (± SD) of 9.45 ± 1.69 and 9.97 ± 0.89, respectively (Fig.2). Organisms fed 11.1 ppb QD-algae had similar calculated pixel intensity as the fed and unfed control organisms (9.71 ± 0.91) and those fed the 55.0 QD-algae had significantly higher measured 24-h pixel intensity of 13.27 ± 0.53. Bioconcentration of QDs by *C. dubia* has been reported by previous studies (20) and this study provided evidence of uptake from a food source. The fluorescence intensity at 24-h following food uptake (55.0 ppb QD algae) (17) was comparable to measured fluorescence.
following 24-h aqueous exposure to 400 ppt QDs (pixel intensity = 12 ± 6.4) (20).

![Graph](image1.png)

**Fig 1.** Measured pixel intensity (mean ± SD) of algal cells following 96-h Qdot® 545 ITK™ Carboxyl Quantum Dots (Fisher Scientific, Fisher part no Q21391MP; Invitrogen Molecular Probes, Eugene, OR, USA) exposure. Sample size included control ( ), 11.1 ppb ( ), 55.0 ppb ( ) (n = 176). Asterisk denotes significant difference from control. (Figure reproduced from Bouldin et al., 2008 (17))

![Graph](image2.png)

**Fig 2.** Measured pixel intensity (mean ± SD) of Ceriodaphnia dubia following 24-h exposure to Qdot® 545 ITK™ Carboxyl Quantum Dots (Fisher Scientific, Fisher part no Q21391MP; Invitrogen Molecular Probes, Eugene, OR, USA) dosed algae. Asterisk denotes significant difference from control. Unfed ( ), Unexposed ( ), 11.1 ppb ( ), 55.0 ppb ( ) (Figure reproduced from Bouldin et al., 2008 (17))

**Conclusion**

Aquatic organisms exposed to aqueous concentrations of metals exhibit a higher total body burden than with food source exposure (7), however dietary Cd can be a potential source of toxicity. Since the diverticula in the anterior midgut of *C. dubia* is responsible for the uptake and subsequent redistribution of metals into the soft tissues (8), it is reasonable to suspect the digestive tract of these organisms may facilitate the eventual release of toxic core metals. Acute QD exposures to *C. dubia* not eliciting a toxic response does not eliminate the possibility of impact to these organisms. Chronic effects that are yet to be tested and measured food chain transfers may disrupt population dynamics in aquatic systems.

**Literature cited**

Incorporation and Detection of Nanoparticles in Marine Snow

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Keywords: polystyrene nanoparticles, marine snow, dialysis, percent incorporation

Abstract
Recently, nanoparticles have been added to many consumer goods as protective coatings, antimicrobials, and to improve material durability, increasing the likelihood that nanoparticles will be released into ecosystems adjacent to populated, industrialized regions. Despite the increased demand for nanomaterials, little research has addressed the potential toxicological effects on terrestrial and marine environments. In coastal systems, suspension-feeding bivalves are ubiquitous in the benthos, and are capable of filtering particles from large quantities of water over short periods of time. The profusion of bivalves in benthic environs, in addition to their ability to remove particles suspended in the overlying water column, could make bivalves more susceptible to contacting nanoparticles in coastal waters. Previous work by Ward and Kach (2009) demonstrated that 100-nm particles incorporated into marine snow can be efficiently captured by suspension-feeding bivalves. The purpose of the present work was to examine the rate at which 22-nm fluorescent polystyrene particles could be integrated into marine snow, and demonstrate some effective methods to detect their presence. To accomplish this, the nanoparticles were washed in Milli-Q water and dialyzed using dextran as a counter-dialysis medium. Known concentrations of nanoparticles were then added to sieved seawater and placed on a roller table for a period of 96 hours to form marine snow. Samples were analyzed with a fluorescent spectrophotometer to obtain an estimate of particle concentration. Results showed that dialysis could be utilized to wash the polystyrene nanoparticles, and direct and indirect sampling methods were equally effective in calculating the percent incorporation of nanoparticles in marine snow. These preliminary experiments will establish the protocols necessary to incorporate polystyrene and metal nanoparticles into marine snow, and ultimately use the marine snow to carry nanoparticles into suspension-feeding bivalves.

Introduction
Nanoparticles have applications in a range of products including medications, detergents, cosmetics, paint, sunscreen, and electronics (Handy et al. 2008). Coastal ecosystems in close proximity to densely populated, industrialized regions are particularly vulnerable to the infiltration of anthropogenic materials such as nanoparticles (Moore 2006). Concern is growing that the unique characteristics of nanoparticles such as their infinitesimal size and surface architecture are potentially hazardous to aquatic organisms (Moore 2006). In coastal marine and estuarine environs, suspension-feeding organisms are pervasive throughout the benthos, playing a critical role in nutrient cycling between the sediments and the overlying water column (Dame 1993). One predominant group of suspension feeders is the bivalve mollusks, which are sessile organisms capable of clearing particulate matter from significant volumes of water (Dame 1996). Their abundance in coastal habitats, as well as their predilection for suspension feeding, could make bivalves more susceptible to nanoparticles entering coastal waters (Gagne et al. 2008). Marine snow is an aggregation of inorganic and organic matter that occurs in natural waters primarily as a result of shear and differential settling (Figure 1; Simon et al. 2002).
Aggregation is further enhanced by the presence of biologically secreted transparent exopolymeric particles (Allerdige et al. 1993, Li et al. 2008). In a study conducted by Ward and Kach (2009), blue mussels (*Mytilus edulis*) and oysters (*Crassostrea virginica*) had extremely low capture efficiencies of freely suspended 100-nm fluorescent polystyrene particles. However, a significant increase in capture efficiency was observed when nanoparticles were incorporated into marine snow, indicating that the form of delivery of the nanoparticles is critical to uptake and ingestion by the bivalve (Figure 2). My studies will utilize smaller, 22-nm fluorescent polystyrene particles to formulate the methods, techniques, and experimental design necessary to feed polystyrene and metallic nanoparticles to commercially relevant bivalves. Developing such techniques will be important for future research on the interactions between nanoparticles and suspension-feeding bivalves, which may transfer nanoparticles to humans during consumption.

**Methods**

In preliminary trials, a white precipitate formed when 22-nm polystyrene particles were added to sieved seawater. A dialysis rinsing method was adapted from Vauthier et al. (2008) to address the contamination. Three milliliters of 22-nm polystyrene particles were combined with 3 mL of Milli-Q (MQ) water in 12,000 molecular weight cutoff dialysis tubing. The
dialysis tubing containing the nanoparticles was submerged in a glass cylinder containing a counter-dialysis solution of 30% dextran in MQ-water. When the fluid in the tubing was depleted, a second 6 mL wash of MQ-water was added, and the tubing was agitated to remove nanoparticles adhered to the sides and to break up large agglomerations. A new counter dialysis solution was prepared, and the process was repeated for a total of three rinses. The nanoparticles were removed from the tubing by adding 1 mL of MQ-water, agitating the nanoparticles, and pipetting the suspension into a Falcon tube. This process was repeated twice with MQ-water for a final volume of 3 mL. The suspension was diluted 1:10 to reduce self-quenching, and the fluorescent area was determined using a fluorescent spectrophotometer (ex: 304 nm; em: 610 nm). A standard curve, obtained by spiking MQ-water with seven different concentrations of polystyrene nanoparticles, was used to calculate nanoparticle concentrations from the fluorescent area values.

A protocol by Shanks and Edmondson (1989) was modified to determine the percent incorporation of nanoparticles into marine snow using a direct and an indirect sampling method. Four liters of a $1.7 \times 10^{12}$ b/ml suspension were prepared by adding pre-washed 22-nm polystyrene particles to 210-μm sieved seawater. A total of 16, 250 mL bottles were filled in ¼ volume increments, placing the stock solution on a magnetic stir plate after each pour to prevent agglomeration and settling. Eight of the bottles were put on a roller table (Figure 3) for 96 hours at 15 rpm, while the other 8 bottles were placed next to the roller table. After 24 hours, the Day 1 bottles were removed from the roller table, while unrolled bottles were inverted gently three times; all were allowed to settle for two hours. The process was repeated for each day’s bottles.

Figure 3

The aim of the direct method was to sample the concentration of nanoparticles incorporated in the marine snow. To start, a glass transfer pipette was used to gently remove marine snow from the bottom of each bottle and place it into a Falcon tube. An equal volume of water was removed from the bottom of the unrolled bottles and placed into separate Falcon tubes. Samples were centrifuged at 3220x g for 15 minutes, the supernatant removed, and the marine snow was gently washed with 5 mL of MQ-water to remove salts. The samples were centrifuged again at 3220x g, the MQ-water removed, and the marine snow was placed in 3 mL of 1N NaOH to digest the organic matrix of the marine snow. After a 1-week digestion, samples were analyzed for fluorescence on a fluorescent spectrophotometer. When sampling marine snow, a fraction of the overlying water was also taken. A conservative method for estimating percent incorporation would assume that all the nanoparticles pelleted in the marine snow during centrifugation, while a less conservative method would assume that
some of the nanoparticles settled in the marine snow during centrifugation. The conservative calculation of nanoparticles in marine snow subtracted the number of nanoparticles in the overlying water fraction from the number of nanoparticles in the marine snow; the less conservative calculation made no adjustments to the number of nanoparticles in the marine snow.

In the indirect method, the remaining seawater was used to calculate percent incorporation. The seawater was mixed on a magnetic stir plate for 5 minutes, and a 3 mL volume was removed from each bottle for analysis on the fluorescent spectrophotometer. The process was repeated for each day’s samples.

**Results**

Following rinsing with dialysis tubing, the nanoparticles in MQ-water settled to the bottom of the Falcon tube within minutes, and the white precipitate did not form when the washed nanoparticles were added to sieved seawater.

The direct method indicated that nanoparticle concentrations increased in the marine snow with time until a point of saturation was reached whereupon the percent incorporation of nanoparticles in marine snow decreased. The direct method had percent incorporations of 15%, 30%, 48%, and 44% for days 1 through 4, respectively. The indirect method had percent incorporations of 21%, 15%, 43%, and 48% for days 1 through 4, respectively. The indirect method closely followed the direct method except on Day 2 where the nanoparticle uptake in marine snow decreased resulting in lower percent incorporation. Data were mean ± range of two replicate bottles (Figure 4). Additionally, results showed the less conservative method for calculating percent incorporation was nearly identical to the conservative method. Data were mean ± range of two replicate bottles (Figure 5).

**Discussion and Conclusions**

The precipitate that formed when the nanoparticles were added to the sieved seawater was likely caused by the manufacturer-supplied surfactant. Experiments showed washing the nanoparticles in MQ-water using dialysis tubing prevented formation of the white precipitate. Either the direct or indirect sampling method could be employed to calculate the percent incorporation of nanoparticles in marine snow. The slight variations in percent incorporation were attributed to differences in the number of nanoparticles initially added to each bottle and daily variations in nanoparticle uptake. Furthermore, the curve of the less conservative method for estimating percent incorporation was very similar to that of the conservative method signifying little, if any, of the 22-nm spheres were incorporated in the marine snow following centrifugation at 3220x g. Thus, the less conservative method for calculating percent incorporation of nanoparticles in marine snow was as valid as the conservative method.
In the near future, marine snow containing polystyrene and metal nanoparticles will be fed to several species of bivalves and the gills and digestive glands will be isolated. These organs will be assessed for nanoparticle accumulation using the above techniques and imaged with scanning electron microscopy to determine the incorporation of nanoparticles by suspension-feeding bivalves. Results of this work will provide insight into how a potential anthropogenic contaminant is affecting living marine resources and human health.

Acknowledgements
Evan Ward, Bridget Holohan, NSF Selection Grant IOS 0718820, and NOAA Oceans and Human Health S08-67963

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Altered Gene Expression Profiles in Murine Brains Following Exposure to Inhaled Nickel Nanoparticles

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Keywords: brain; oxidative stress; inflammation; nickel; nanoparticles; inhalation; arrays

Abstract
Transcriptional response was assessed in three regions of the mouse brain following exposure to inhaled nickel nanoparticles (Ni NPs, CMD= 40nm). Utilizing a whole-body exposure system, C57 male mice inhaled either 100 µg/m³ of Ni NPs or filtered air for 5h/d, 5d/w, for up to 5m. The olfactory bulb, midbrain, and cerebellum were collected 1w, 3m, and 5m, post exposure (24h) for gene expression analyses. Pathway focused PCR profiling arrays for oxidative stress and inflammation were used to evaluate the change in expression for 168 genes. A change of 2-fold or higher was considered important. More changes in expression were observed in the olfactory bulb, followed by, almost equally, the cerebellum and midbrain. Array results were confirmed using individual real time RT-PCR on these selected genes: Fmo2, Ccl2, and Il-1β. Nickel content was found to be present in the highest concentration in the olfactory bulb, followed by the midbrain and cerebellum. These results suggest that at concentrations below the current OSHA permissible exposure limit (1 mg Ni/m³), Ni NPs can penetrate specific regions of the brain and induced oxidative stress and inflammatory responses; however, the olfactory bulb may be the more sensitive target to adverse effects of Ni nanoparticle inhalation.

Introduction
Nanotechnology is an exciting new field that uses numerous forms of nanomaterials to discover new applications. Included are nanoparticles (NPs, particle diameter <100 nm), which have gained tremendous interest due to their catalytic, optical, and electronic properties (1). With rapid advancements in this field, there are legitimate concerns about the potential health effects that NPs may cause. For this reason, among others, it is important that research be conducted that addresses specific questions concerning their potential hazards and toxicities.

The respiratory tract is the primary target for inhaled NPs; but, there is evidence that once deposition occurs, these particles can escape clearance mechanisms and target secondary organs like the brain (2). In addition, there is data illustrating that direct penetration into the central nervous system is also possible (3). Since it has been suggested that many of the observed effects that result from inhalation exposures to NPs are likely to involve oxidative stress (OS) and inflammatory mechanisms, the aim of this study was to examine the transcriptional response of OS and inflammatory related genes in three regions (olfactory bulb, midbrain, and cerebellum) of the mouse brain following exposure to inhaled Ni NPs. Ni NPs were chosen because they are used widely in industrial applications (4). Chemical composition analysis revealed our particles to be nickel hydroxide which has become attractive for use in energy markets, especially as an additive to electrodes to enhance the discharge specific capacity in batteries (5).

Methods
Animals: Male C57BL/6 mice (12 weeks old, body weight 20-25 g) were obtained from Taconic Farms (Germantown, NY) and housed in our AALAC accredited housing facility. Animals were kept on normal 12h light/ dark cycles and received food and water ad libitum,
except during exposure. After a two week acclimation period, mice were exposed in whole body inhalation chambers described previously (6) to either filtered air (FA) or nano-Ni(OH)$_2$ for 5h/d, 5d/w to either 78.1 ± 19.7 µg Ni/m$^3$ for 1w, 81.5 ± 5.9 µg Ni/m$^3$ for 3m (12 weeks) or 78.4 ± 2.6 µg Ni/m$^3$ for 5m (20 weeks). For nickel content analysis, animals were exposed for 3 days to 670 ± 20 µg Ni/m$^3$. Animals were euthanized with an overdose of sodium pentobarbital (150-200 mg/kg) via an intraperitoneal injection at 24 h post-exposure.

**Generation of Nano-Ni(OH)$_2$:** Particles were produced by opposing metallic nickel electrodes (99.995% purity, ESPI, Ashland, OR) in an ultra pure argon chamber using a Palas® GmbH arc furnace (Model GFG-1000, Karlsruhe, Germany). Ultra pure oxygen was added to filtered dilution air in order to keep the exposure atmosphere at 20% oxygen. The count mean diameter of generated nano-Ni(OH)$_2$ was 40 nm. The chemical composition was determined by X-ray photoelectron spectroscopy (XPS) and particle size by a scanning mobility particle sizer (SMPS) and transmission electron microscopy (TEM).

**Brain Tissue Nickel Content:** Olfactory bulb, midbrain, and cerebellum were removed from mice, regionally pooled (n=4), weighed, and wet ashed using optima grade nitric acid and hydrogen peroxide (Fisher Scientific, Pittsburgh, PA). Analytes were diluted to 0.2% with deionized water. Using graphite furnace atomic absorption spectroscopy (GF95, Thermo Scientific, Waltham, MA) nickel content was determined using a 5-point calibration curve constructed from certified nickel reference standards (Fisher Scientific, Pittsburgh, PA).

**Quantitative RT-PCR:** Using RNeasy mini kits (Qiagen, Valencia, CA), total RNA was extracted and treated for genomic DNA contamination. The quality and concentration was determined using a Nanodrop 1000 spectrometer (Thermo Fisher Scientific, Wilmington, DE). PCR profiling arrays cDNA was prepared (pooled samples, n=4/region) and used as outlined in the user manual (SABiosciences, Frederick, MD). Individual RT-PCR (n=4/region), cDNA was prepared with Applied Biosystems transcription kits. Relative mRNA levels were quantified using a 7300 Real-Time PCR instrument (Applied Biosystems, Foster City, CA), under the following conditions: 10 min at 95°C followed by 40 cycles at 95°C and 60°C (15 sec and 1 min, respectively). Using primer/probe sets from TaqMan® Gene Expression Assays, the forward and reverse primers for flavin containing monoxygenase 2 (Fmo2, reference sequence, NM_018881.3); chemokine (C-C motif) ligand 2 (Ccl2, NM_011333.3); and interleukin 1, beta (Il-1β, NM_008361.3) were used to determine relative mRNA levels under the aforementioned conditions. Relative mRNA levels were established for arrays and individual RT-PCR using the comparative C$T$ method. All expression levels were normalized to the housekeeping gene, hypoxanthine phosphoribosyltransferase [Hprt-1, (NM_013556.2)], and reported as a relative fold change over their respective FA controls.

**Statistical Analyses:** Student’s t-test was used to evaluate the difference in means (p≥ 0.05). Data are expressed as mean ± standard error of the mean (SEM).

**Results and Discussion**
Pathway focused arrays for oxidative stress & antioxidant defense and inflammatory cytokines & receptors were used in order to profile the expression of 168 genes in three regions of the mouse brain: olfactory bulb, midbrain and cerebellum. After 1w of exposure to inhaled nano-Ni(OH)$_2$, approximately 50 genes were either up or down-regulated in all regions. Following 3 and 5m of exposure, more changes in gene expression were observed in the olfactory bulb (85 and 72, respectively) followed by the cerebellum (45 and 57,
respectively) and the midbrain (32 and 60, respectively). The total number of altered genes in the cerebellum and midbrain after 3 and 5m of exposure were similar. Out of all of the examined genes, less than four were commonly up or down-regulated after all exposures. This differentially expression in response to Ni exposure indicates that in each region of the brain, distinct toxicities may have occurred.

Array results were confirmed using quantitative individual RT-PCR for selected genes: Fmo2, Ccl2, and II-1β. These genes were chosen based on whether change in expression was observed in all three regions after every exposure period; or a robust change in expression (at least twice the 2-fold regulation deemed as important) was observed in at least two regions of the brain following any of the exposure periods. Fmo2, which is an OS related gene, was up-regulated in all three regions of the brain after all exposures (Figure 1). Fmo2 is a microsomal enzyme, similar to cytochromes P450 in its role in the oxidation of xenobiotics (7). While metabolic protection is suggested by the up-regulation of this gene, there is also evidence for Fmo2 involvement in the oxidation of glutathione (GSH) to glutathione disulfide (GSSG) (8). Increased levels of GSSG as compared to GSH have been shown to be indicative of OS (9). Ccl2 and II-1β are both inflammatory related genes that were up-regulated in at least two brain regions after Ni NPs exposure; but the exposure period in which altered expression was observed varied between regions (Figure 2). Ccl2, also known as monocyte chemottractant protein-1 (Mcp-1), is a chemokine involved in the migration of leukocytes to sites of inflammation and injury (10); and II-1β is a major proinflammatory cytokine shown to be associated with neuroinflammation and in the progression of neurodegenerative diseases (e.g. Parkinson’s disease) (11). The induction of both genes are indicators of region specific inflammatory states, possible marked by the infiltration of immune related cells. For all assessed genes, the individual regulation patterns were in good agreement with array findings.

![Figure 1](image1.png)

**Figure 1.** Relative mRNA expression levels of Fmo2 in the olfactory bulb, midbrain, and cerebellum of mice exposed to nano-Ni(OH)₂ (100 µg/m3) for 1w, 3m or 5m. Data are reported at 24h post exposure as a normalized relative fold change over control (mean ± SD for individual RT-PCR values; n=4/group). Asterisks indicate a statistically significant difference between nickel exposed and control groups (*P<0.05). Abbreviations: Fmo2 containing monoxygenase 2 (Fmo2); ind. (individual).
Deposition of nickel was also assessed in which the greatest amount was detected in the olfactory bulb (364 ng/g of tissue), followed by the midbrain (176 ng/g of tissue) and cerebellum (31 ng/g of tissue; for each region, control levels were subtracted). A correlation was observed between the amount of nickel detected in each region and the pattern of expression observed with increasing exposure periods. However, this same correlation was not observed for the inflammatory array data possible due to its role as a downstream event to OS or compensatory mechanisms not captured by this PCR profiling system.

**Conclusion**

Collectively, these results suggest that the olfactory bulb, midbrain, and cerebellum are affected by inhaled nano-Ni(OH)$_2$ exposures via OS and inflammatory mediated pathways. While region sensitivity cannot be conclusively ranked, the olfactory bulb may be the more sensitive target given the total number of altered genes observed and the highest nickel content detected in this region. Additional research is warranted in order to correlate the observed mRNA expression levels to phenotypic effects.

**Literature Cited**


MultiCriteria Mapping of Stakeholder Preferences in Regulating Nanotechnology

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Keywords: nanotechnology, regulatory options, multicriteria mapping, stakeholders

Abstract

A number of recent publications on governance of nanomaterials have pointed to stakeholder deliberation as a key element for nanotechnology to reach its full potential and to secure democratic and transparent decision-making process. In order to promote and/or facilitate such discussions on whether or not and how to regulate nanotechnology, we used the open-source program termed MultiCriteria Mapping (MCM) to structure and facilitate 26 interviews with various stakeholders in the US including academics, NGOs, public interest groups, regulators and global, medium and small nanomaterial producing companies. MCM offers a systematic part quantitative, part qualitative approach to clarify why some regulatory options were deemed to be acceptable/unacceptable by various stakeholders and which criteria are used to evaluate the different regulatory options. In order to set the stage equally for all the interviews, a number of policy options on how to regulate/not regulate nanotechnology had been pre-defined ranging from a ban for nanotechnology and materials to relying on voluntary measures and no additional regulation over moratorium on R&D and/or commercialization. After considering the policy options, interviewees were asked to list their “criteria” for assessing the pros and cons of the different policy options after which they performed a quantitative evaluation of the relative performance of the options under each of the criteria. Finally, interviewees were asked to assign values concerning the relative importance of the different criteria and a final ranking of the various policy options was generated and discussed. Of the predefined options, adopting an incremental approach and implementing a new regulatory framework scored the highest whereas a complete ban and no additional regulation of nanotechnology scored the lowest. Criteria applied by various stakeholders differ substantially and included environmental, health, safety, social, ethics, and regulatory issues. Reading across the ranking of each of the individual stakeholders, opinions of how to move forward in regard to regulation of nanotechnology seems to be far less polarized than expected.

Introduction

A number of recent publications on governance of nanomaterials have pointed to stakeholder deliberation as a key element for nanotechnology to reach its full potential and to secure democratic and transparent decision-making process (Greenwood 2007). In order to promote and/or facilitate such discussions on whether or not and how to regulate nanotechnology, we used the open-source program termed MultiCriteria Mapping (MCM) to structure and facilitate 26 interviews with various stakeholders in the U.S including academics, NGOs, public interest groups, regulators and global, medium and small nanomaterial producing companies.
Methods

MCM is an open-source program developed by Andy Stirling, University of Sussex and it offers a systematic part quantitative, part qualitative approach to clarify why some regulatory options were deemed to be acceptable/unacceptable by various stakeholders and which criteria are used to evaluate the different regulatory options. MCM has been used in the past to evaluate policy options in regard to genetically modified crops and obesity among other (Mayer and Stirling 1999, Lobstein and Millstone 2006).

The interviews were completed in a 3-month period between May and August, 2007 leading up to the U.S. Environmental Protection Agency’s publication of their voluntary nanomaterials stewardship program. Stakeholders included various forms of NGOs and academics, local and government regulators and decision-makers, and small, medium, and large companies involved in R & D and commercializing of products containing nanomaterials. Stakeholders were identified and contacted through a two-step process. First, contacts were taken with stakeholders and specialists (NGOs, academics, regulators, industry, etc.) that had publicly expressed their views on whether or not and how to regulate nanotechnology. Second, all interviewees were asked to help identify additional stakeholders relevant to the investigation.

In order to set the stage equally for all the interviews, a number of policy options on how to regulate/not regulate nanotechnology had been pre-defined. These options have been identified through a literature study and represent a wide range of diverging views of different stakeholders. These options were:

1. Ban nanotechnology
2. Ban some nanotechnologies and material based on hazard assessment
3. Moratorium on R&D and commercialization
4. Moratorium of commercialization: Moratorium of commercialization of all nanomaterials until safety has been tested
5. Relying on voluntary measures
6. Forming and implementing a new regulatory framework
7. Launching an incremental process using existing legislative structures to the maximum, revisiting them, and, when appropriate only, amending them
8. No additional regulation needed.

The list is not meant to be a complete list, but rather a starting point for discussion. After considering the policy options, interviewees where asked to list their “criteria” for assessing the pros and cons of the different policy options, after which they performed a quantitative evaluation of the relative performance of the options under each of the criteria. For instance, how well does the option of banning nanotechnology protect human health on a scale from 0-100, where 100 is the best possible and 0 is the worst possible. In order to allow for uncertainty in the estimation, the interviewees were allowed to give a range rather than a single number.

Finally, interviewees were asked to assign values concerning the relative importance of the different criteria, and a final ranking of the various policy options was generated and discussed (see Figure 1 for an example).
Results

By combining each of the individual interviewees’ ranking of the various options, an overall ranking of the options was generated (see Figure 2). Of the predefined options, adopting an incremental approach and implementing a new regulatory framework scored the highest, whereas a complete ban and no additional regulation of nanotechnology scored the lowest. Although relying on voluntary measures ranked third, it was ranked as the most preferable option by only one stakeholder. The criteria applied by various stakeholders to evaluate the different policy options differed substantially. In total, stakeholders listed a total of 216 criteria that clustered into environmental, health, safety, social, ethical, and regulatory issues.
Discussion

Reading across the ranking of each of the individual stakeholders, opinions of how to move forward in regard to regulation of nanotechnology seems to be far less polarized than expected. In general there was agreement about the best and the worst policy options although the criteria on which various stakeholders evaluate these options differ substantially. Relying on voluntary measures is currently the primary option chosen by the Environmental Protection Agency in the US. It is interesting to note that this option in general scored low although it was mentioned by several stakeholder as a starting point and a key component of future regulatory polices regarding nanotechnology.

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Keywords: policy, regulation, risk management, economic analysis, insurance

Abstract
Nanoscale science has emerged as a key technology frontier, yet poses new challenges due to our limited understanding of potential risks. Refining the implementation of the existing and politically viable regulatory framework would reduce risks to firms, consumers, workers, and the environment, thereby ensuring continued market growth and innovation. Examining the economic efficiencies associated with a wide range of policy alternatives produces new policy options for reducing risks and improving efficiencies under the existing regulatory regime. Strengthening private risk instruments, incentivizing improved processes, and improving transparency all provide significant benefits to consumers, workers, the environment, and firms.

I. Current Regulatory Frameworks
The emergent nanotechnology industry is at a crossroads and faces three significant challenges. First, the industry faces economic challenges due to increasing risk uncertainty, inefficient commercialization, and environmental, health, and safety (EHS) externalities. Secondly, legal challenges stem from a legal framework that fails to adequately address unique nanomaterial properties, and a lack of scientific research to adequately define risk and to inform more effective regulation. Finally, the U.S. and the EU have built a strong consensus that current frameworks are sufficient, yet there is a potential for standards disharmonization from various governmental and NGO groups.

Much of the current literature focuses on the applicability of specific regulations to nanotechnology without comprehensively analyzing all existing regulatory tools. Such limited analysis leads to an improper focus on specific regulatory sectors, a failure to recognize private risk management as an important tool, and a call for incremental change to the existing framework. Each of these outcomes fails to leverage maximum efficiency and risk management opportunities.

II. Methodology
This assessment flows from a comprehensive analysis of the existing regulatory framework in the U.S. and the EU. A literature review from a wide range of academic disciplines produced a set of 31 U.S and 40 EU consumer, worker, environmental, intellectual property, and standards and measurements laws cited as potentially applicable to regulating nanotechnology. Additionally, all U.S. and EU industry self-regulation in the form of codes of conduct and risk management tools, as well as special interest group calls for increased regulation, are included in this analysis. A systematic comparison of corollary regulations produced a set of common features and significant variations between U.S. and EU law. Subsequently, this comprehensive set of existing policies was subjected to a 1st-order rudimentary economic analysis. The resulting work creates a clear picture of the current challenges and the need for an integrated public-private regulatory framework in order to manage risk efficiently in the face of insufficient scientific data to properly develop new regulation.
III. Current Regulation: Many Tools, Significant Opportunities

A wide range of existing chemical, worker, and environmental regulations can apply to nanotechnology, thereby ensuring a strong regulatory net if fully implemented as allowed by law (see Table 1). There are currently no nano-specific regulations (aside from a Berkeley, CA disclosure ordinance, and forthcoming registries from California and Canada). Although this list contains all laws cited as potentially applicable, only a few regulations are frequently cited as likely to be used (noted in italics; regulations not cited in literature yet still corollary or potentially applicable noted in parentheses).

A comprehensive look at these U.S. and EU regulations shows that despite the many tools available, some risks still exist. The U.S. and the EU have comparable regulatory frameworks, although the EU and Member States regulated “human contact and consumption” goods—cosmetics, food, and drugs—with more stringency. There is strong corollary law, with essentially no areas covered by only the U.S. or the EU.

A deeper analysis of the corollary laws indicates generally strong similarities in the implementation level, with some significant differences. Common to both systems are a governmental-level consensus that existing regulatory systems are adequate. Some nano-specific issues, such as a volume triggers, could be shifted at the implementation level. Of the noticeable differences, EU regulation is more likely to require industry rather than consumers to bear the costs of ensuring safety through various mechanisms as pre-manufacture notification or requiring a level of proved safety before products can be released to market, but there are important exceptions to this trend in U.S. regulation.

Our current understanding of the physical characteristics and behavior of nanomaterials and their impact on health and environmental safety is limited, and frustrates our ability to design effective regulation today.

For example, particle size (below 20 nm) is increasingly understood to have significant health and safety impacts, yet early research indicates surface area or structure are potentially more important in determining toxicity. Given the lack of scientific evidence to adequately define risks for at least the next five years, a broader look at regulatory goals could produce better risk management in the near future.

IV. Objectives of a Strong Regulatory Framework

Stepping back from specific existing regulations, we must ask: what is an optimal regulatory framework? An effective framework would achieve three goals: (1) protect consumers, workers, and the environment from harmful substances, (2) provide a stable and predictable business environment to support robust growth and innovation, and (3) provide all stakeholders with information to make informed decisions about use and exposures risks.

Such a framework would be built from the ground up, using dose/response curves to determine risk assessment and risk management structures, which would then inform regulatory standards, triggers, processes and other safety mandates and incentives.

The current system fails to adequately reduce risk; much of this risk is due to the infancy of this emerging technology and the subsequent lack of scientific information to adequately assess risk. Consequently, producers are not well incentivized to actively participate in the assessment and management of risk. To date, the most effective environmental regulations, such as the Toxics Release Inventory (TRI), have focused on enforcing minimum standards and incentivizing the disclosure of information. Such modern regulation focuses on regulatory processes rather than strict standards which produces greater efficiencies and better outcomes.
Ultimately, an effective nanotechnology regulatory structure would increase the efficiency of the market; reduce risk to industry, consumers, workers, and the environment; reduce the perceived risk to consumers and industry in order to reduce costs; and set high expectations for safe and responsible development. Critical to the successful implementation of these changes is finding low-cost or no-cost mechanisms which can reside easily within the existing regulatory structures, which improves the likelihood of implementation. To be politically feasible, the changes must respond to the existing consensus in the U.S. and the EU that current regulations are generally adequate, but that that shifts in implementation are tolerable.

Table 1. U.S. and EU Regulation Applicable to Nano (partial list).

<table>
<thead>
<tr>
<th>U.S. Regulation</th>
<th>EU (UK Implemented)</th>
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<tr>
<td><strong>Consumers</strong></td>
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<tr>
<td>TSCA Chemicals</td>
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<td>FIFRA (PRIA)</td>
<td>Chemicals (Hazard Information and Packaging for Supply) 2002</td>
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<td></td>
<td>Biocidal Products 2001 (a.a.)*</td>
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<td></td>
<td>Control of Pesticides 1986</td>
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<tr>
<td>FFDCA Foods, Drugs, Cosmetics</td>
<td>Food Safety Act 1990 (a.a.)</td>
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<td>FQPA</td>
<td>Cosmetics Products (Safety) 2004</td>
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<tr>
<td>DSHEA</td>
<td>Articles in Contact with Food 1987 (a.a.)</td>
</tr>
<tr>
<td></td>
<td>Novel Foods and Novel Food Ingredients 1997 (a.a.)</td>
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<tr>
<td></td>
<td>Plastic Materials and Articles in Contact with Food 1998 (a.a.)</td>
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<tr>
<td></td>
<td>EC 178/2002 on General Principles of Food Law</td>
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<tr>
<td></td>
<td>Colours in Food 1995 (a.a.)</td>
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<td></td>
<td>Miscellaneous Food Additives 1995 (a.a.)</td>
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<td>Biomedical</td>
<td>Medical Devices 2002 (a.a.)</td>
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<td></td>
<td>Medicines Act 1968</td>
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<td>Medicines for Human Use (Marketing Authorisations) 1994 (a.a.)</td>
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<td>FHSA</td>
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<td>MSDS Reporting</td>
<td>SDS</td>
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VI. Strengthening the Existing Regulatory Framework

A rudimentary 1st-order economic analysis points to a very clear set of policy shifts that can be easily implemented within the existing regulatory structure to improve efficiencies and reduce consumer, worker, and environmental risks today. Bolstering the weakening insurance market for nanotechnology is a critical first step in ensuring the continued growth of the emerging market while providing a safe and responsible development of novel materials. The
creation of a separate risk market can provide an added incentive for continued innovation. Both of these options provide significant benefits at essentially no costs.

Less easily implemented but still highly effective are the creation of subsidies for clean productions processes and materials registries that can reduce information asymmetry and incentivize higher standards while avoiding inefficiencies. Private-public partnering to provide rigorous third-party certification and the support of voluntary agreements will also ensure a vibrant and responsible market development. By ensuring that risks are managed and reduced, nanotechnology can continue on its very promising path in transforming basic manufacturing processes. Further work using real data on an industry and sector level is critical to verifying this assessment.

Ultimately, science will provide the necessary answers to develop responsive regulatory shifts. In the interim, we must use the tools available to ensure a robust and responsible industry will can fulfill the remarkable promise of nanotechnology.

Table 2. Weighing Various Policy Options within the Existing Regulatory Framework (partial listing)

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<td>Yes</td>
<td>No</td>
<td>Unlikely</td>
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<td>Yes</td>
<td>No</td>
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<td>Set standards</td>
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<td>Possibly</td>
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Detection and Bio-Distribution of Inhaled Carbon Nanotubes in Lungs by Raman Spectroscopy

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Keywords: carbon nanotubes, Raman spectroscopy, inhalation, pulmonary

Abstract

Although mechanisms by which the carbon nanotubes (CNTs) bio-distribute into tissues are poorly understood, they appear to be absorbed by cells and accumulate in different organs. Because of their small size and limited miscibility in water, inhalation is a probable entry route into living organisms. Once inhaled, CNTs may enter the blood and lymph systems and bio-distribute throughout the body. However, their small size and composition make detection in intact tissues difficult. We have successfully used Raman spectroscopy (RS) to detect inhaled CNTs in samples of lung tissue.

BALB/c mice were exposed to an aerosol of purified single-walled CNTs dispersed in distilled water. Adult mice were exposed by placing them in a 2 L exposure chamber into which 5 ml of a 20 ppm CNT solution was introduced as an aerosol for 15 min. Mice were sacrificed immediately or maintained for 8 or 24 hours after exposure. Following the 4 or 24 hour waiting period, animals were sacrificed, an incision was made on the frontal side of the body cavity and a ligature was tightened around the trachea and the inflated lungs removed. After removal, lungs were immediately frozen in liquid nitrogen. Slices from frozen lung tissue were analyzed by RS. Comparison of scattering at specific wave numbers by samples from exposed and unexposed animals showed a prominent band between 1500 and 1600 cm\(^{-1}\). This signal is diagnostic for the presence of CNTs. These preliminary studies indicated that the CNTs penetrate deep into lung tissue in a very short time (within 60 min) and agglomerate in all sections of the lungs, including the alveolar regions.

Introduction

As the production and use of engineered nanoparticles increases, the risk of unintended environmental and human exposure also increases. To understand the consequences of these exposures, studies to characterize the bioavailability, bioaccumulation, mechanisms of action, and clearance of nanoparticles from living organisms are needed. While recent reports have shown how carbon nanotubes (CNTs) distribute when injected into mice (1) the experiments described herein were done to characterize absorption and distribution of CNTs after exposure by inhalation exposure. Because of their composition (2) and minute size, CNTs can be difficult to detect in biological matrices. Raman spectrometry has been shown to be capable of detecting CNTs in biological samples (3). This study was designed to investigate the pulmonary intake, uptake, distribution and clearance of CNTs by mice exposed to water aerosols containing CNT suspensions.

Methods

Single wall carbon nanotubes were suspended in water solution by sonification. Individual, alert, active animals were placed in a Plexi-glass™ box (volume ~2 L) and exposed to a water aerosol containing measured amounts of CNTs. Mice were exposed to nebulizer-generated aerosol containing 20 ppm CNTs for 15 min. ~5 mL of aerosol was introduced into the exposure chamber during this time. Although this technique does not allow the amount of
inhaled CNTs to be determined, the maximum amount of CNTs that could have been inhaled was 100 µg (~4000 µg/kg). Obviously animals did not inhale all aerosol resulting in a lower but unmeasured amount entering into animal. Unexposed (control) animals were treated identically except that aerosol contained no CNTs. After exposure, animals were transferred to a clean cage and allowed to move freely and undisturbed for 4 or 24 hrs before tissue collection. For tissue collection animals were anesthetized and euthanized followed by blood sample collection and the immediate removal of inflated lungs. Tissue was immediately frozen in liquid N₂ and stored at -80°C. Lung samples were sectioned into slices (~150 nm thick) from frozen tissue at ~ -20°C using a cryomicrotome. Slices were placed on clean dry slides and dried at room temperature. Slices were taken from four lungs from four unexposed animal (n= 12 slices), from an exposed animal at 8 hours post exposure (n=3 slices) and 24 hours post exposure (n=3 slices). Tissue was analyzed using Raman spectroscopy: (632.81nm laser excitation, 250µm spot size, 6 scans, 25 seconds per scan). Previous experiments have shown that under these conditions, SWCNTs exhibit a strong, characteristic peak at ~1580 cm⁻¹. Although this peak is present in unexposed animals, it occurs at a much lower intensity. Spectra were processed using LabSpec® (Version 5.23.24) during collection. Comparisons among spectra from different animals were made by averaging all spectra from lung tissue slices of all slices taken from each animal group. To allow comparisons among spectra, the peak intensities were normalized using a ratio to average intensity at both an adjacent peak and an adjacent trough. The adjacent peak was between wave numbers 1485 and 1515 cm⁻¹ (Fig 2), the adjacent trough was between wave numbers 1485 and 1515 cm⁻¹ (Fig 3).

Results

Single wall CNTs used in this experiment express a characteristic peak at ~1580 cm⁻¹. Spectra of unexposed control animals are shown in figure 1. A peak is observed at ~1580 cm⁻¹ but expresses relatively lowered intensity levels then the intensities of spectra from exposed animals. Data collected from exposed animals is shown in figures 2 and 3. Figure 2 represents data that was normalized to recorded intensities at an adjacent peak (wave numbers 1350 to 1450 cm⁻¹). Figure 3 represents data normalized data normalized to recorded intensities at an adjacent trough (wave numbers 1485 to 1515 cm⁻¹).

![Raman spectra from sections of lungs from unexposed animals (n=12) the red and blue bars illustrate regions to which data was normalized (See Figs 2 & 3).](image)
Figure 2. Raman spectra of lung sections from animals exposed to water aerosol containing 20 ppm SWCNTs. Lungs of exposed animals were collected 8 or 24 hours post exposure. Data from all sections collected from each animal (8 h n=3; 24 h n=3; Unexposed n=12) were averaged and normalized to average intensities at an adjacent peak (wave numbers 1350 to 1450 cm\(^{-1}\) indicated by red bar on Fig 1).

Figure 3. Raman spectra of lung sections from animals exposed to water aerosol containing 20 ppm SWCNTs. Lungs of exposed animals were collected 8 or 24 hours post exposure. Data from all sections collected from each animal (8 h n=3; 24 h n=3; Unexposed n=12) were averaged and normalized to average intensities at an adjacent peak (wave numbers 1485 to 1515 cm\(^{-1}\) indicated by blue bar on Fig 1).

**Conclusions**

Collected data indicates an overall increase of intensity at wave number ~1615 cm\(^{-1}\) in lungs of exposed animals compared to unexposed animals. This suggests that the animals inhaled and bioaccumulated CNTs from the aerosol and that these nanotubes were detectable using Raman spectroscopy. This finding strongly suggests that accumulation of CNTs within lungs following inhalation is a potential source of exposure. The reduction in intensity between 8 and 24 hrs after exposure suggests that CNTs may be cleared from lungs rather quickly. However, the fate of CNTs after inhalation requires further study.
Literature Cited


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Keywords: nano zerovalent iron, emulsified nano zerovalent iron, micro zerovalent iron, hexavalent chromium, trichloroethylene

Abstract

Groundwater pollution by volatile organic contaminants (VOC) and heavy metals is a serious environmental problem and the application of zero valent iron (ZVI) is one of the more promising solutions. The purpose of this research is to assess the efficiency of Nano zerovalent iron (nZVI) as compared to Emulsified nano zerovalent Iron (EZVI) and micro zerovalent iron (MZVI) for the remediation of trichloroethylene (TCE) and Cr(VI) contaminated groundwater. Test results revealed that nZVI achieved satisfactory reduction of TCE compared to EZVI within the experimental time frame. It also appears that for some of the nZVI treatments, the reduction of TCE was biologically mediated; i.e., minimal or no TCE reduction was observed for the sterilized duplicates or in the control samples with no nZVI. The treatability results for low Cr(VI) concentrations (< 1 mg/L) indicated that all the reductants performed satisfactorily. However, only partial reduction was observed for some of the corresponding sterilized samples, which may indicate that part of the reduction could be biologically mediated. For high Cr(VI) concentrations (8.5 mg/L), only nZVI performed satisfactorily and it seems that the reduction was not biologically mediated. EZVI achieved a borderline performance, whereas MZVI achieved poor performance.

Introduction

Hexavalent chromium [Cr(VI)] and trichloroethylene (TCE) compounds are common pollutants that could be found at many contaminated sites. Biotic and abiotic processes have been used to remediate Cr(VI) and TCE contaminated water. The biotic or abiotic reduction of the highly mobile Cr(VI) to the less soluble Cr(III) is a proven and an effective method for the remediation of Cr(VI) contaminated water. Conversely, the reductive dehalogenation of TCE to ethene and chloride is an effective remediation method. Zero valent iron (ZVI) under different microbial conditions was successfully used to treat TCE, PCE, and Cr(VI) contaminated groundwater (Gandhi et al., 2002; Cho et al., 2006). On the other hand, abiotic reduction was also reported to remediate TCE, PCE, and Cr(VI) contaminated water (Cao et al., 2006; Janda et al., 2004). However, the formation of an oxide passivation layer that coats the iron particle at neutral to alkaline pH may affect the long term activity of ZVI. The passivation effect can be significantly reduced by using self catalyzed bimetallic iron particles or by using high surface area ZVI, such as nano-scale ZVI (nZVI). EZVI utilizes a combination of stimulated biological activity and direct reduction of contaminants by ZVI for the treatment of various chlorinated compounds and chromate (Milum et al., 2004; Quinn et al., 2005). Encapsulating the ZVI in a hydrophobic membrane protects the nZVI from other groundwater constituents that would otherwise exhaust much of the reducing capacity of the
iron. This reduces the mass of EZVI required for treatment relative to unprotected ZVI. Until the oil membrane is consumed by biological activity, EZVI will combine directly with the target contaminants. In addition to the abiotic degradation associated with the ZVI, the injection of EZVI will result in enhanced biodegradation of dissolved chlorinated ethenes because the vegetable oil and surfactant act as electron donors to promote anaerobic biodegradation processes.

The purpose of this research was to assess the efficiency of nZVI, EZVI and MZVI for the remediation of Cr(VI) and TCE contaminated groundwater obtained from a study area located in Gloucester County, New Jersey. The samples were collected from three monitoring wells at the site. The objective of the study was to meet the groundwater quality standards of 70 ppb for Cr(VI) and 1 ppb for TCE. Batch experiments were conducted to evaluate the effectiveness of the three reductants under sterilized and non sterilized conditions for the reduction of the target contaminants. Sacrificial samples were used and analyzed at each set time interval.

Materials and Methods

nZVI (NanoFe™), supplied by Pars Environmental, Inc., consists of sub-micron (< 10^-6 m) particles of zero valent iron with a noble metal catalyst. MZVI was investigated in a few batch tests as a possible replacement to nZVI for the reduction of Cr(VI) in contaminated groundwater due to potential cost savings. The selected MZVI, HC15, was obtained from Hepure Technologies, Inc. and has an average particle size of 12 to 13 microns. EZVI (SRS-DNAPL™ Emulsified Zero Valent Iron) was supplied by Terra Systems. The basic SRS-DNAPL™ EZVI package contains: 17% Zero Valent Iron (nano-scale is 100-200 nanometers; micro-scale is 3 microns); 34.5% soybean oil; 1.4% food grade emulsifier package; and 47.1% water.

The concentration of TCE in the aqueous phase was measured using Gas Chromatography - Mass Spectrometry (GC/MS) equipped with TEKMAR-2016 purge and trap autosampler. pH, Eh, and DO were monitored with standard probes and meters. All redox potential measurements are reported relative to a platinum electrode combination probe. In order to convert the readings relative to H2 redox potential, 199 mV has to be added to each measurement. Dissolved Cr(VI) and total Cr were analyzed by the colorimetric method (EPA Method 7196A) and ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometry), respectively. Batch test experiments were conducted to investigate the reductive remediation of the contaminated groundwater in soil-groundwater composite samples using nZVI, EZVI and MZVI. The contaminated soil and groundwater samples were mixed at a liquid to solid ratio of five. The composite samples were placed into 250-mL amber glass bottles with Teflon lined caps with the set amendment dosages. The bottles were capped with no head space and were placed on a planetary shaker at 20 °C. Sacrificial samples were used and analyzed at each set time interval. The treatment dosages used for nZVI were 1, 2 and 4 g/L; EZVI were 2.25, 4.5 and 9 g/L and MZVI were 2 and 4 g/L.

Results and Discussion

The initial characterization of the groundwater in wells MW1, MW2 and MW3 is presented in Table 1. The summary of the treatment results is presented in Table 2. The treatability results for MW1 indicated that only nZVI performed satisfactorily. The best Cr(VI) treatment results were achieved using nZVI at 2 g/L or 4 g/L. It seems that the reduction was not biologically mediated because the reduction was immediate (1 day, Figure 1). The Cr(VI) concentration after treatment were 22 µg/L, 10 µg/L, and 10 µg/L for testing conditions of 2 g/L - sterilized, 4 g/L – non-sterilized, and 4 g/L – sterilized, respectively. EZVI achieved
Table 1. Initial characterization of contaminated ground water

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MW1</th>
<th>MW2</th>
<th>MW3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.19</td>
<td>6.63</td>
<td>6.65</td>
</tr>
<tr>
<td>Eh RMV</td>
<td>140.9</td>
<td>-1.05</td>
<td>22.2</td>
</tr>
<tr>
<td>DO mg/L</td>
<td>6.2</td>
<td>5.17</td>
<td>6.45</td>
</tr>
</tbody>
</table>

borderline performance with Cr(VI) concentration of 100µg/L attained using 9 g/L under non-sterilized conditions, after a curing period of 21 days. The reduction of Cr(VI) was gradual and it was not immediate as was the case for nZVI (Figures 1 and 2).

![Figure 1](image)

The performance of all other EZVI tests was marginal with the next best performance achieving Cr(VI) concentration of 4.29 mg/L when 4.5 g/L was used, after a curing period of 1 week. MZVI performed rather poorly as compared to the other two reductants. Minimal or no Cr(VI) removal was observed even at dosages of 4 g/L and a curing period of 7 days. The treatability results for MW2 indicated that both nZVI and EZVI performed satisfactorily. The best treatment result was obtained using 9 g/L of EZVI for the non-sterilized conditions. Cr(VI) was reduced to approximately 10 µg/L, after a curing period of 7 days. There was some decrease in Cr(VI) concentration for the lower dosage of 4.5 g/L. Cr(VI) concentration decreased to approximately 0.449 mg/L, after a curing period of 7 days. Satisfactory removal rates were observed using MZVI for MW2 using a dosage of 2 g/L under both sterilization conditions where Cr(VI) concentration was measured at approximately 20 µg/L after a curing period of 2 days. It appeared that Cr(VI) removal was immediate and not biologically mediated as there was no difference in the test results between the sterilized and non-
sterilized samples. For MW3, it appears that only nZVI achieved complete reduction of TCE whereas EZVI did not achieve complete removal within the experimental time frame of 28 days. It also appears that for nZVI, the reduction was biologically mediated; i.e., minimal or no TCE reduction was observed for the sterilized duplicates and the control samples with no nZVI. Even the lower nZVI dosage of 1 g/L was able to completely reduce TCE. EZVI achieved partial reduction of TCE for the non-sterilized samples where TCE concentration was reduced to 1.03 µg/L using a dosage of 2.25 g/L; however, a higher dosage of 4.5 g/L did not achieve better results within the experimental time frame of 28 days (3.46 µg/L). Partial reduction was observed for the corresponding sterilized EZVI treated samples. The comparative results of the highest dosage used for the treatment of Cr(VI) and TCE in MW1, MW2 and MW3 under non-sterilized conditions are presented in Figures 1-3, respectively.

Figure 2
Table 2. Summary of the experimental results for MW1, MW2, MW3

<table>
<thead>
<tr>
<th>Percent Reduction of Cr(VI) for MW1</th>
<th>Percent Reduction of Cr(VI) for MW2</th>
<th>Percent Reduction of TCE for MW3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Concentration mg/L</td>
<td>Initial Concentration mg/L</td>
<td>Initial Concentration µg/L</td>
</tr>
<tr>
<td>Sterilized</td>
<td>Sterilized</td>
<td>Sterilized</td>
</tr>
<tr>
<td>Non Sterilized</td>
<td>Non Sterilized</td>
<td>Non Sterilized</td>
</tr>
<tr>
<td>2 g/L</td>
<td>0.5 g/L</td>
<td>1 g/L</td>
</tr>
<tr>
<td>NZVI</td>
<td>NZVI</td>
<td>NZVI</td>
</tr>
<tr>
<td>Sterilized 99.97</td>
<td>Sterilized 54.00</td>
<td>Sterilized 9.89</td>
</tr>
<tr>
<td>Non Sterilized 41.77</td>
<td>Non Sterilized 86.00</td>
<td>Non Sterilized 11.54</td>
</tr>
<tr>
<td>4 g/L</td>
<td>1 g/L</td>
<td>2 g/L</td>
</tr>
<tr>
<td>Sterilized 99.87</td>
<td>Sterilized 96.45</td>
<td>Sterilized 34.07</td>
</tr>
<tr>
<td>Non Sterilized 99.87</td>
<td>Non Sterilized 95.82</td>
<td>Non Sterilized 100.00</td>
</tr>
<tr>
<td>EZVI</td>
<td>EZVI</td>
<td>EZVI</td>
</tr>
<tr>
<td>4.5 g/L</td>
<td>4.5 g/L</td>
<td>2.25 g/L</td>
</tr>
<tr>
<td>Non Sterilized 45.70</td>
<td>Non Sterilized 74.00</td>
<td>Sterilized 52.13</td>
</tr>
<tr>
<td>9.0 g/L</td>
<td>9.0 g/L</td>
<td>4.5 g/L</td>
</tr>
<tr>
<td>Sterilized 17.85</td>
<td>Sterilized 15.82</td>
<td>Sterilized 84.80</td>
</tr>
<tr>
<td>Non Sterilized 22.78</td>
<td>Non Sterilized 99.09</td>
<td>Non Sterilized 93.13</td>
</tr>
<tr>
<td>MZVI</td>
<td>MZVI</td>
<td>MZVI</td>
</tr>
<tr>
<td>2 g/L</td>
<td>1 g/L</td>
<td>1 g/L</td>
</tr>
<tr>
<td>Sterilized 18.61</td>
<td>Sterilized 17.09</td>
<td>Sterilized</td>
</tr>
<tr>
<td>Non Sterilized 17.34</td>
<td>Non Sterilized 20.91</td>
<td>Non Sterilized</td>
</tr>
<tr>
<td>4 g/L</td>
<td>2 g/L</td>
<td>2 g/L</td>
</tr>
<tr>
<td>Sterilized 43.04</td>
<td>Sterilized 98.36</td>
<td>Sterilized</td>
</tr>
<tr>
<td>Non Sterilized 13.42</td>
<td>Non Sterilized 98.18</td>
<td>Non Sterilized</td>
</tr>
</tbody>
</table>

Not tested
Conclusions

The treatability study test results revealed that nZVI achieved satisfactory reduction of TCE whereas the other two reductants did not achieve complete removal within the experimental time frame. It also appears that for the nZVI, the reduction of TCE was biologically mediated; i.e., minimal or no TCE reduction was observed for the sterilized duplicates and the control samples with no nZVI. The treatability results for low Cr(VI) concentrations (MW2) indicated that all the reductants performed satisfactorily. However, only partial reduction was observed for some of the corresponding sterilized samples, which may indicate that part of the reduction could be biologically mediated. For high Cr(VI) concentrations (MW1), only nZVI performed satisfactorily in the treatment of Cr(VI) and it seems that the reduction was not biologically mediated. EZVI achieved a borderline performance, whereas MZVI achieved poor performance.

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Effects of Electrolytes and Suwannee River Humic Acid on the Aggregation Behavior of Silicon Nanoparticles

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Keywords: nanoparticles, silicon, aggregation, humic acid, calcium, DLVO

Abstract
Studies about the impact of the release of silicon nanomaterials to the environment and on human health by the microelectronics and biomedical devices industries are urgently needed. The aggregation kinetics of silicon NPs was investigated using dynamic light scattering (DLS) and electron microscope (EM) in the presence of Ca²⁺ and Suwannee River Humic Acid (SRHA). The reaction-controlled regime and diffusion-controlled regime can be observed in the absence as well as presence of SRHA, which is qualitatively consistent with the traditional Derjaguin-Landau-Verwey-Overbeek (DLVO) model. The aggregation rates in the presence of SRHA were higher than that in the absence of SRHA at the same Ca²⁺ concentrations and the critical coagulation concentrations (CCC) decreased from 0.4 M to 0.1 M. The enhanced aggregation was attributed to bridging caused by Ca²⁺ and SRHA.

Introduction
Silicon nanoparticles (NPs) have great potential applications in biomedical devices and microelectronics. E.g., silicon NPs facilitate the conjugation of DNA or protein probes in cell biology and medicine. Luminescent silicon NPs are used in imaging of tumor and other organs. In addition, there have been many studies with silicon NPs for electronic devices, secondary batteries, supercapacitors and solar cells for energy devices. Despite the relatively low toxicity of silicon, silicon oxide NPs can be toxic in high doses. Therefore, the environmental behavior of silicon NPs needs to be investigated before its wide application. In this paper, we report on the aggregation of silicon NPs in natural aqueous environment using dynamic light scattering (DLS), transmission electron microscopy (TEM) in the presence of calcium ions and Suwannee River humic acid (SRHA). The classical DLVO model and the traditional filtration model were applied to simulate the aggregation of silicon NPs in aqueous environment.

Methods
Silicon NP dispersions were ultrasonicated in an ultrasonic bath for 60 min to break up aggregates formed during storage. The dispersion was allowed to settle down for 96 hours and was separated for subsequent experiments. The dispersion concentration kept stable after settling down for 24 hours in the sedimentation curve (Figure 1).
Electrolyte (CaCl₂) stock solutions were prepared using analytical reagents (Fisher Scientific) and filtered through 0.2 μm filters (Whatman, Inc., Clifton, NJ) before use. All experiments and measurements were conducted at pH 4.2 ± 0.1. The SRHA (standard II, International Humic Substances Society) solutions were made by dissolving 22.9 mg SRHA standard II into 50 mL DI water and stirred overnight. The solutions were then filtered through 0.2 μm filters and pH was adjusted from the initial value of 3.4 to 10.2 by addition of NaOH. The SRHA solution was stocked in the dark at 4 °C. The total organic carbon content was measured at 232.76 mg/L (Phoenix 8000 TOC analyzer, Teledyne Tekmar, Ohio, USA).

Hydrodynamic size and surface potential were measured using Malvern Zetasizer. The attachment efficiency \( \alpha \) (or inverse stability ratio, 1/W) is defined as the aggregation rate constant of interest normalized by the rate constant derived under diffusion-limited (fast) aggregation conditions.

\[
\alpha = 1/W = \frac{k_{11}}{(k_{11})_{fast}} = \frac{1}{N_0} \frac{(dr_\alpha(t))_{t \to 0}}{dt} = \frac{1}{(N_0)_{fast}} \frac{(dr_\alpha(t))_{t \to 0, fast}}{dt}
\]

**Results and Discussion**

As shown in the SEM image (Figure 2-a), the particle size of the silicon NPs was around 100 nm. This result is consistent with the size measurement from TEM images (Figure 2-b). Based on the electron diffraction analysis, these Si NPs are crystalline particles. SEM-EDS microanalysis suggests that Si NPs are pure silicon.
At 0.08M CaCl$_2$, silicon NPs started to aggregate induced by the screening of the electrostatic forces. Aggregation rate and attachment efficiency increased with increasing CaCl$_2$ concentration from 0.08M to 0.4M (Figure 3). With further increase of CaCl$_2$ concentrations from 0.4M to 1M, the attachment efficiency did not change greatly indicating a diffusion-controlled regime. Therefore, the critical coagulation concentration (CCC) of Ca$^{2+}$ was determined as 0.4M. The aggregation curve of silicon NPs at various CaCl$_2$ concentrations qualitatively corresponded with the DLVO theory.

In the presence of SRHA, silicon NPs started to aggregate at 0.01M CaCl$_2$. The attachment efficiency increased with increasing CaCl$_2$ concentration to 0.1M and kept constant when CaCl$_2$ concentration increased further to 1M. However, the attachment efficiency in the presence of SRHA was higher than that in the absence of SRHA at the same IS in both regimes. In addition, CCC of Ca$^{2+}$ in the presence of SRHA (0.1M) was smaller than that (0.4M) in the absence of SRHA. This indicates that the presence of SRHA greatly enhanced the aggregation of silicon NPs in Ca$^{2+}$ solutions. It appears that the TEM image provided evidence for the assumption that gel cluster complexation can contribute to the observed enhancement in aggregation (Figure 4).
Conclusions
The aggregation of silicon NPs is consistent with colloidal DLVO theory qualitatively. However, the presence of NOMs, such as SRHA, plays an important role in the transportation of silicon NPs. SRHA enhanced the aggregation kinetics of silicon NPs due to the gel cluster in Ca\textsuperscript{2+} solutions. The study finds that Ca\textsuperscript{2+} concentrations and SRHA determine the transport of silicon NPs. In natural aqueous environment, the presence of various ions and NOMs can make the prediction of transportation of NPs even more complicated.

Literature Cited
**Effects of Electrolytes and Natural Organic Matter on the Aggregation Kinetics of Boron Nanoparticles**

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E-mail address: mwazne@stevens.edu

**Keywords:** aggregation, nanoparticles, boron, natural organic matter, DLVO, attachment efficiency

**Abstract:**

The effects of electrolytes and natural organic matter on the aggregation of boron nanoparticles were investigated in aggregation experiments. Aggregation studies help to assess the fate, transport, and exposure pathways of various nanoparticles in aquatic environments. The aggregation kinetics of boron nanoparticles were investigated in the presence of monovalent (NaCl) and divalent electrolytes (CaCl₂ and MgCl₂), and two kinds of natural organic matter (NOM) — Suwannee River humic acid (SRHA) and sodium alginate through time-resolved dynamic light scattering (DLS). The presence of SRHA stabilized the boron nanoparticles and resulted in a decrease in the values of the attachment efficiency, in the reaction-limited regime, and an increase in the values of the critical coagulation concentration (CCC). Electrostatic repulsion was suggested as a cause for the induced stabilization. In the presence of alginate, similar behavior was observed for suspensions with high and low MgCl₂ concentrations and for suspensions with low CaCl₂ concentration. However, in the presence of alginate and high Ca²⁺ concentration, the attachment efficiency values kept increasing and attained values greater than unity. There was no clear distinction between the reaction-controlled and diffusion-controlled regimes in the presence of alginate. The induced destabilization might be attributed to the affiliation of adsorbed alginate with high concentration of Ca²⁺ ions. For the sodium chloride solutions, the aggregation kinetics and surface charge did not vary greatly in the presence of alginate. Results from this study suggest that various NOM and electrolytes play critical roles in boron nanoparticles aggregation and exposure pathways in natural aquatic environment.

**Introduction**

Boron nanoparticle is being considered as a solid fuel for rockets and as a gun propellant due to its desirable combustion heat and fast energy release rate. In a hybrid rocket motor, using 23% of nano-boron particles in hydroxyl terminated polybutadiene (HTPB)-based solid fuels, an increase of mass burning rate of 44% for nano-sized boron particles and 111% for nano-sized B₄C particles was observed.

Upon release to the environment, the engineered nanomaterials are expected to come in contact with NOM, which is ubiquitous in natural waters. In addition, various kinds of NOM play critical and different roles in the transport of nanoparticles. The stabilization or destabilization of many nanoparticles due to the presence of NOM under various circumstances was attributed to different mechanisms, such as electrostatic forces, steric repulsion, or bridging. The predominate mechanism in the transport of nanomaterials depends on many factors, such as the surface nature of nanoparticles, electrolyte types and
concentrations, NOM types and concentrations, and the thickness or the length of the NOM chains.

Methods

Average particle size of boron nanoparticles ranged from 10 to 20 nanometers. Boron dispersion was prepared in D.I. water followed by ultrasonication for 30 minutes to break down any aggregates. All experiments and measurements were conducted at pH 5.7±0.1 and 25 °C. The particle size distribution and \( \zeta \)-potential were obtained by Nano Zetasizer (Malvern) using dynamic light scattering (DLS) and Helmholtz- Smoluchowski relationship. The attachment efficiency \( \alpha \) is defined as the aggregation rate constant of interest normalized by the rate constant derived under diffusion-limited (fast) aggregation conditions.

\[
\alpha = \frac{1}{W} \frac{k_{11}}{(k_{11})_{fast}} = \frac{1}{N_0} \frac{(dr_s(t))_{t \to 0}}{(N_0)_{fast} (dt)_{t \to 0, fast}}
\]

Results

A DLVO-type aggregation behavior was observed for boron nanoparticles in the presence of SRHA as evidenced by the presence of both reaction- and diffusion-controlled regimes (Figure 1). The attachment efficiency in the presence of SRHA was smaller than that in the absence of SRHA for the reaction-controlled regime. However, in the diffusion-controlled regime, the attachment efficiency was similar to that in the absence of SRHA. In addition, the CCC for electrolytes increased.

The addition of alginate did not have a significant effect on the attachment efficiency profile for the NaCl solution (Figure 2). The aggregation of boron nanoparticles in the presence of alginate and MgCl\(_2\) is similar to that in the presence of SRHA. There was no clear distinction between the reaction-controlled and diffusion-controlled regimes in the presence of alginate and CaCl\(_2\).
Figure 1
Figure 2
Figure 3 shows boron nanoparticles in the presence of alginate (4.4mg/L TOC) in 0.8mM CaCl₂ solutions where boron nanoparticles were well dispersed and stabilized. Figure 4 is TEM image for boron nanoparticles in the presence of alginate (4.4mg/L TOC) in 6 mM CaCl₂ solutions. It appears that the boron nanoparticles aggregation was induced by the alginate network binding junctions shown as the shadowy region in the TEM image.

Surface potential of boron nanoparticles was studied in the absence and presence of 4.4ppmC alginate in CaCl₂ and MgCl₂ solutions. The surface potential was more negative in the presence of alginate than in the absence of alginate. This indicates that the adsorbed functional groups of alginate on the surface of boron nanoparticles, such as carboxylate functional group, led to the decreased surface potential. However in Na⁺ electrolytes, the addition of alginate did not change surface potential. Surface potential decreased in the presence of SRHA, similar to that in the presence of alginate.

Discussion
The effects of larger collision radii of the alginate-coated boron nanoparticles and formation of the alginate gel cluster accelerate the rate of aggregation in the presence of alginate and high Ca²⁺ concentrations. Conversely, the affiliation of alginate with Mg²⁺ did not result with the “egg-box” gel structure and therefore the boron nanoparticles were stabilized.

Conclusions
The results of this study revealed that the aggregation behavior of boron nanoparticles differed greatly in the presence of various NOM and electrolytes. Two important and opposite effects are observed—stabilization and enhanced aggregation. In natural aquatic systems, the coexistence of various electrolytes and NOM can make the aggregation of nanoparticles rather complicated. The determination of the type of electrolytes and NOM is equally important as the type of nanomaterials for the prediction of the fate and transport of nanoparticles.
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Considerations for Interpreting Nanomaterial Toxicity Studies for Use in Environmental Risk Assessment

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Keywords: nanoparticles, risk assessment, ecotoxicity

Abstract
As the use of commercial nanomaterials rapidly increases, it is important to improve our understanding of their potential effects on biota and our ability to conduct meaningful environmental risk assessments on these materials. The toxicity of nanoparticles (NPs) can be influenced by chemical composition, particle size and shape (including agglomerates), surface area, surface charge, stability, solubility, and the presence of contaminants such as metals and solvents used in the synthesis of NPs. Because of their highly reactive surface chemistry, traditional mass measurements of exposure (concentration or dose) may not be adequate for characterizing stressor/response relationships. Therefore, to better elucidate potential toxicity and to provide data that are useful in the context of risk assessment, studies are needed to determine whether the toxicity of a particular material is related to the nano-characteristics or the chemistry of the material. When interpreting studies for use in environmental risk assessment, consideration should be given to the confounding effects of the test methods used to prepare the material (e.g., solvent vehicles used to maintain aquatic dispersion), the presence of trace contaminants, and the potential influence of particle aggregation, among other factors. Other confounding issues that must be addressed include the lack of standard reference materials, standard test materials, and standard methods for preparation and testing of suspensions, including chemical methods that are used to increase the stability of particle suspensions. Because of the high reactivity of some types of NPs, physical and chemical characterization of NPs is needed at both the start and end of any experiments conducted to gain an appropriate understanding of exposure. More research is clearly needed to understand the potential for impacts of NPs on ecological receptors and systems, but emerging research should be conducted in a manner that allows for a meaningful interpretation of results. Until a paradigm for research on NPs has been developed, the results of available studies should be used with caution for characterizing environmental risk. This paper highlights some of the factors that should be considered when interpreting the effects of NPs, and provides example cases where toxicity test results indicate confounding factors that may limit their utility for risk assessment.

Introduction
Nanomaterials include naturally occurring nano-sized particles (e.g., those from volcano ash and forest fires), nanoparticles (NPs) from combustion byproducts (e.g., diesel exhaust), and manufactured nanomaterials (U.S. EPA, 2007). Organisms can be exposed to NPs from deliberate environmental application, as in the use of nanotechnology for remediation of contaminated water and soil, as well as from unintentional releases from fuel additives, or industrial and domestic waste streams. The available ecoxicological studies on NPs have focused on the toxicity of metal oxide particles, carbon nanotubes, and fullerenes, primarily in aquatic, plant, or microbial toxicity tests. These studies have shown that NPs can be taken up by biota, and that dose-response relationships and patterns of relative toxicity among types of particles are beginning to emerge (see Moore et al., 2006; Klaine et al., 2008). However,
NP toxicity can be influenced by chemical composition, particle size and shape (including agglomerates), surface area, surface charge, stability, solubility, and the presence of contaminants such as metals and solvents used in the synthesis of NPs. Such influences should be considered when interpreting the effects of NPs on biota. Here, we provide examples of toxicity tests for which confounding factors may limit the utility of the tests for assessing risk.

Discussion
Factors that affect the interpretation of nanoparticle toxicity tests

The behavior and toxicity of NPs can be influenced by particle size and shape. For instance, particle size can influence the location of NP deposition within the body, such as the nose, throat, lungs, bloodstream, or organs. Some NPs may behave more like gas molecules than particles, and because of their small size, may pass easily into the bloodstream (Oberdörster et al., 2005, as cited in Bell, 2007). Often, but not always, smaller particle size is associated with greater toxicity. In a 12-week inhalation study, rats exposed to 20-nm titanium dioxide (TiO$_2$) particles exhibited persistently higher inflammation of the lung than those exposed to 250-nm TiO$_2$ particles (Oberdörster et al., 1994). Similarly, the effects of TiO$_2$ in water on green algae were greater for 25-nm particles than for 100-nm particles (Hund-Rinke and Simon, 2006). Unfortunately, the controls included in such studies do not always allow for determination of whether the increased toxicity is directly related to size, or if it is related to higher dissolution rates of toxic chemicals from smaller particles. Particle morphology may also be important; one study showed that long and straight carbon nanotubes (CNTs) introduced into the abdominal cavity of mice caused mesothelioma-like lesions, but this effect was not observed from exposure to CNTs that were tangled (Poland et al., 2008).

NPs often adhere together, forming agglomerates, which can significantly change their chemical behavior and toxicity. For instance, agglomeration can rapidly reduce NP concentrations, resulting in overestimates of exposure for various materials, including airborne CNTs (Maynard et al., 2004), TiO$_2$, zinc oxide, aluminum oxide, fumed silicon, silicon dioxide, carbon black, and copper (II) oxide (Ma-Hock et al., 2007). Agglomeration also reduced penetration of CNT into the chorion of zebrafish embryos (Cheng et al., 2007).

Surface structure, particle coating, and functionalization are important factors when interpreting NP studies. For example, decreased toxicity of C$_{60}$ and a fullerol in water to zebrafish embryos was observed with an increased number of attached functional groups (Zhu et al., 2007). Daphnid behavior was affected by the presence of fullerene functional groups; C$_{60}$.Hx.C$_{70}$.Hx (a fullerene derivative), but not C$_{60}$, affected the ability of the daphnids to recover to pre-exposure behavior levels (Lovern et al., 2007). In addition, settling and agglomeration in water were shown to be faster for raw CNTs than for those that were functionalized (Kennedy et al., 2008).

Surface charge affects NP behavior (e.g., agglomeration, dispersal, partitioning, sorption, interactions with other substances) and influences the bioavailability, mobility, and penetration of NPs into organism tissues (Royal Commission on Environmental Pollution, 2008). In addition, NPs that are soluble in water can move readily through aqueous environments, although solubility and transport may be complicated by the formation of agglomerates (Brant et al. 2005; Hyung et al., 2007).

Chemicals used in the production of commercial NPs and solvent carriers used to prepare experimental solutions can also confound causality of effects (Oberdörster, 2004; Smith et al., 2007; Henry et al., 2007; Lovern and Klaper, 2006). For example, the hatching delay of zebrafish embryos from exposure to CNTs in water may have been the result of nickel and cobalt catalysts used in CNT synthesis, as opposed to the direct result of exposure to CNTs.
(Cheng et al., 2007). Tetrahydrofuran, a solvent that was used to dissolve the C$_{60}$ in water, likely increased the acute toxicity of C$_{60}$ in water to daphnids and fathead minnows (Zhu et al., 2006).

**Challenges in characterizing exposure**

Because of their highly reactive surface chemistry, traditional mass measurements of exposure (concentration or dose) may not be adequate for characterizing stressor/response relationships for NPs. Some effects are better correlated with particle surface area than with mass (NIOSH, 2005). To better elucidate NP toxicity, and to provide data that are useful in the context of risk assessment, studies are needed to determine whether the toxicity of a particular material is related to its nano-characteristics or the chemical composition of the material. In addition, it is important to conduct physical and chemical characterization of NPs throughout the duration of the experiments, because NP characteristics can change over time (Royal Commission on Environmental Pollution 2008). It is also important to note that the true size of NPs used in experiments may differ significantly from the advertised size of the commercially supplied materials (Adam et al., 2006); therefore, careful particle measurements are important in characterizing NPs and conducting laboratory testing.

**Conclusions**

Results of available studies on NPs should be used with caution in environmental risk assessments because of the many factors that may affect toxicity (e.g., chemical composition, agglomeration potential, etc.). Standard methods and test materials are needed for future studies to ensure their utility in risk assessments. Materials science analysis and quantitative structure activity relationships (QSARs) may be useful, but require further consideration before application to NP risk assessment.

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An Examination of Existing Data for the Industrial Manufacture and Use of Nanocomponents and Their Role in the Life Cycle Impact of Nanoproducts

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Keywords: nanomanufacturing, nanoparticles, life cycle assessment

Abstract: This work examines the manufacture and use of nanocomponents and how they can affect the life cycle impact of resulting nanoproducts. Available data on the production of nanoproducts and nanocomponents are used to identify the major groups of nanocomponents studied: inorganic nanoparticles, carbon-based nanomaterials, and specialty/composite materials. A comparison of existing results for life cycle assessments of nanocomponents and nanoproducts is used to possibly identify trends in nanomanufacturing based on material grouping with regard to non-renewable energy use and greenhouse gas emissions. Continuing work is needed in this area to incorporate other factors such as toxicity and resource consumption in addition to energy use and global warming potential to fully understand the role of nanomanufacturing in the life cycle of nanoproducts.

The development of methods and processes to mass produce nanocomponents, materials with characteristic lengths less than 100 nm, has led to the emergence of a large number of consumer goods (nanoproducts) containing these materials (1-3). In addition to toxicological concerns, there is a growing desire to better understand how the manufacture and incorporation of nanocomponents in consumer products will contribute to other potential environmental concerns such as global warming and land use. An assessment tool that can be used to address this is Life Cycle Assessment (LCA) (4-9). An LCA can establish the comparative impact of products or processes in terms of specified impact categories using a well-defined and documented methodology (10-13). Typical impact categories include global warming/climate change, stratospheric ozone depletion, human toxicity, ecotoxicity, photooxidant formation, acidification, eutrophication, land use, and resource depletion (12). The potential advantage of LCA-based evaluations for nanoproducts is that they can address both the health and environmental consequences associated with the inclusion of nanocomponents. The ultimate goal is to ensure that the potential benefits of nanocomponents are realized in a manner that is safe for both consumers and the environment without resulting in unintended consequences.

For any nanocomponent, there are four main aspects of its life-cycle that must be considered with its use: material selection, manufacturing, application and disposal/recycle. The present work focuses on manufacturing. The manufacture of nanocomponents involves a variety of synthesis techniques. These techniques can be classified based on the type of approach (top-down or bottom-up) and nature of the chemistry involved (wet or dry). The various process options can each have an impact on the overall environmental and toxicological impact. Although LCA is a well-established methodology for evaluating the environmental impact of products, materials, and processes, potential shortcomings have been raised regarding its application to nanotechnology (4, 14, 15, 16). These include functional unit selection, inventory data collection and/or estimation, allocation, and toxicity assessment. Also evident is the need for either a refinement to LCA methodology or the development of a complementary approach to account for socio-economic impacts/benefits.
when accessing nanoproducts \((14, 15, 16)\). Presently, a large number of data gaps exist when considering the application of LCA to nanoproducts \((4, 14, 16, 17)\). Specifically, only minimal data exist detailing the material inputs and environmental releases related to the manufacture, release, transport, and ultimate fate of nanocomponents.

Based on the assessment of global nanomanufacturing, the largest groups of nanocomponents in use are nanoparticles (NPs) and carbon nanotubes (CNTs). These materials are used both in dispersive form and in composite materials. Therefore, preliminary LCAs dealing with nanotechnology have focused primarily on the use of these materials. The current work examines specific cases representing the major groups that have appeared in published LCA studies: inorganic nanoparticles, carbon-based nanomaterials, and specialty/composite materials. A comparison of relevant data for all cases is then used to evaluate the general production of nanoproducts. Although some assessments have attempted to be more robust than others with regard to impact analysis, only data pertaining to the subsequent discussion are presented. Specifically, the focus is on global warming potential based on energy and fossil fuel requirements because it was the only impact category consistently covered in all studies.

Osterwalder and coworkers performed a cradle-to-gate assessment of titanium dioxide \((\text{TiO}_2)\) nanoparticle production \((8)\). The goal of the study was to compare energy requirements and greenhouse gas production for the classical milling process with a novel flame synthesis technique. The functional unit for energy discussions was 1 metric ton of \(\text{TiO}_2\) while the unit for greenhouse gas emission was 1 kg of \(\text{TiO}_2\). Classical titanium ore processing is carried out using either the liquid phase sulfate process or gas phase chloride process. For both processes, the product, purified \(\text{TiO}_2\), is milled to its final size. Flame spray synthesis involves the combustion of an organo-metallic precursor with subsequent condensation of the metal residue in nanoparticle form. The sulfate process requires 32-40 \(\text{GJ.ton TiO}_2^{\text{-1}}\) while the chloride process uses only 19 \(\text{GJ.ton TiO}_2^{\text{-1}}\). Although this was a cradle-to-gate study, it is not clear if the energy needed to obtain the raw ore was included. The total \(\text{CO}_2\) emissions for the sulfate and chloride processes are 5 and 4 kg \(\text{CO}_2.\text{kg TiO}_2^{\text{-1}}\), respectively. Unfortunately, the total energy requirements for flame synthesis were not presented. Instead, only a discussion of \(\text{CO}_2\) emissions was provided for three different organo-metallic precursors. The total \(\text{CO}_2\) emissions attributed to this process range from 15-30 kg \(\text{CO}_2.\text{kg TiO}_2^{\text{-1}}\). Khanna and coworkers \((4)\) have performed a cradle-to-gate assessment of carbon nanofiber (CNF) production. The goal of the assessment was to determine the non-renewable energy requirements and environmental impacts associated with the production of 1 kg of CNFs. A vapor phase process involving the catalytic pyrolysis of hydrocarbons in the presence of a transition metal catalyst and sulfur was identified as the suitable method for continuous production of CNFs. Various hydrocarbon feedstocks were considered. The amount of energy required to produce 1 kg of CNFs ranges from 654 to 1,807 MJ.kg\(^{-1}\) depending on the feedstock. The global warming potential ranged from 70-93 kg \(\text{CO}_2\) equivalent.kg CNF\(^{-1}\). Perhaps the most noted use of nanotechnology currently is semiconductor manufacturing in the electronics industry. A cradle-to-gate life cycle assessment of energy requirements and global warming potential of nano-scale semiconductor manufacturing has been presented by Krishnan and coworkers \((5)\). The goal of the study was to identify potential process improvements. The functional unit selected was 1 silicon wafer with a 300-mm diameter that can be used to produce 442 processor chips. The assessment was gated at the point where wafer processing is complete, prior to cutting and packaging. The total energy required for the process, excluding the manufacture of fabrication equipment, is 11,600 MJ.wafer\(^{-1}\). The emission of global warming gases is 13 kg \(\text{CO}_2\) equivalent.wafer\(^{-1}\).
The data above can provide some insight regarding the potential burdens that must be addressed if the large-scale use of these types of nanocomponents is to continue. First, it is necessary to express the data from the three studies in a common mass-based unit. Accordingly, energy consumption is presented in MJ.kg⁻¹ and global warming potential is expressed as kg CO₂ equivalent.kg⁻¹. Energy consumption during the product life cycle can be important because it relates to the consumption of fossil fuels and the generation of greenhouse gases. Therefore, it is desirable to design manufacturing processes that minimize energy use. The data for energy consumption of the materials discussed above is shown in Figure 1.

Figure 1. A comparison of the total energy requirements for various nanomanufacturing processes (18).

The production of semiconductors (excluding equipment fabrication) is by far the most energy intensive process of the three nanocomponents examined. In fact, the energy used to manufacture NPs by milling appears to be insignificant when compared with the production of other materials. This is very encouraging given that NPs are the most frequently used nanocomponent. However, the validity of this conclusion is dependent upon two points. First, a clear explanation of how the extraction and beneficiation of ores was included is needed. Second, data is needed to prove that the energy required to achieve near “nano” sizes by grinding is not substantially larger than the energy associated with grinding true NPs. One thing that is certain is that the large energy demand for semiconductors can be attributed to the use of specialty processes such as vapor deposition and thermal oxidation during formation of circuit layers (5). This suggests that all of the intricate gas phase processes proposed for nanocomponent production could be subject to high energy demand, extending the findings of Osterwalder and coworkers regarding NP production to all nanocomponents (8). Although the traditional milling process for TiO₂ did not require significant energy, this process is not suitable for nanocomponents involving surface functionalization or specialty blending (8). Instead, more intricate processes for the manufacture of these materials are necessary. The production of nanocomponents using these techniques (i.e. flame and plasma synthesis, vapor deposition, etc.) needs further study to address the issue of energy consumption and how to best optimize it. The global warming potential associated with the life cycle of materials is becoming increasingly important as concern for global warming escalates. A comparison of the global warming potential for the various materials is shown in Figure 2.
Figure 2. A comparison of the global warming potential for various nanomanufacturing processes (18).

Again, the manufacture of semiconductors has the largest impact when compared to the other materials. However, CNF production demonstrates comparable global warming potential. This should be expected though, considering the CNF process involves hydrocarbon combustion. This also explains why the equivalent CO$_2$ emissions increase when comparing production of TiO$_2$ using organic precursors and milling processes. The other reason for the larger global warming potentials for CNF and semiconductor manufacturing is the much larger energy requirements when compared to NP production. Additional thought must also be given to address the socio-economic impacts/benefits which should be integrated with the LCA framework to provide a more comprehensive assessment tool for decision making when considering the use of nanocomponents and nanoproducts. A full understanding of the role of nanomanufacturing in the life cycle of nanomaterials will only be possible through continuing work that focuses on not only energy use and global warming potential, but also on unresolved questions concerning the impact of nanomaterials on resource depletion and land use, work place exposure, and the widespread release of nanomaterials into the environment.

Disclaimer
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Quantifying Hormetic (Biphasic) Dose-Responses in the Assessment of Nanoparticle Toxicology

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Keywords: hormesis, nanoparticles, toxicology, high-throughput, biphasic

Abstract
The term hormesis describes a dose-response relationship that is characterized by stimulation below the toxic threshold. Previous reports have shown that this relationship is ubiquitous in the dose-response assessment of a variety of pharmaceuticals, metals, organic chemicals, radiation, and physical stressor agents. Recent reports have also indicated that certain nanoparticles (NPs) may exhibit a hormetic dose-response. We describe here the application of three previously described hormesis evaluative methodologies to quantify the magnitude of the hormetic dose-response for biphasic responses. This methodology may be useful in screening assays that attempt to parse the observed toxicological dose-response data into categories based on the magnitude of hormesis in the evaluation of NPs.

Introduction
Hormesis is a dose-response relationship that is characterized by differing results at high and low doses. For example, when considering an endpoint such as cell proliferation, the response at low concentrations may be a modest stimulation above the controls, followed by inhibition at higher concentrations (Calabrese and Baldwin, 2002). When represented on a graph, this dose-response resembles an inverted U-shaped dose response, or a β-curve (Figure 1).

Hormesis has been observed in a number of studies involving the biological effects of nanoparticles. For example, Jan et al. (2008) describe a hormetic response of murine neuroblastoma (NG108-15) neurite growth after exposure to a 25 nM concentration of thioglycolic acid (TGA)-capped cadmium telluride (CdTe) quantum dots produced in the presence of gelatin. In addition, Drobne et al. (2009) discuss statistically significant stimulation of food assimilation, feeding rate and catalase activity in an animal model (arthropod) following exposure to titanium dioxide (TiO₂) particles.

Here we describe three methodologies that have been previously used to evaluate such data for the presence of hormesis.
Methods
Part I
Primary Assessment (Calabrese et al., 2006)

- Count the number of times replicates in each experiment were above and below (or equal to) 100% and compare the counted values to expected values assuming a threshold model (each repetition would have either one of two different responses: an H, responses above 100%; or an L, response less than or equal to 100%).

- Then use a “fair coin” model for the responses below the bench mark dose 10 (BMD_{10}) where each single replication would have a 50% probability of being above or below (or equal to) 100% and determine statistical independence across responses.
Test the agreement between the observed data and this hypothesized model

Secondary Assessment (Calabrese et al., 2006)

- Evaluate which concentration-response model best describes the observed data, construct above-control (>100% response) and below-control (<100% response) ratio.
- Evaluations would be made relative to responses above 100, 105, 110, 115 and 120% and then to below-the-appropriate-control group response using the formula:
  \[
  \text{Control/(Above Response Level)} = \text{Below Response Comparison}
  \]
  (e.g., 100%/120% = 83.33%).
- This method accounts for the possibility of an unrestricted stimulatory response while at the same time fixes the maximum inhibitory response at zero.
- Calculate the mean and standard deviation of the responses at doses below the BMD

Part II (Calabrese et al., 2008)

- Estimate the average magnitude of response for each dose-response using a linear mixed model to predict the average response in the low-concentration zone for each NP.
- Present the average response for each of the NPs using the best linear unbiased prediction (BLUP) or empirical Bayes approach (presented with prediction intervals).
- This assessment provides a more accurate prediction of the true NP response compared to a simple mean due to the fact that the regression towards the mean affects only NPs whose predictor differs from the mean, and not the mean itself.
- Fit separate models for each NP by the number of concentrations below the toxic threshold (e.g., BMD or ZEP).
- Assuming at each concentration, there were two replicates, the model would have 2 variance components: (1) variance of the distribution of the mean response for the agent and (2) replication variance, an estimate averaged over agents and concentrations.

Part III (Nascarella et al., 2009)

- Evaluate the quantitative features of hormetic dose-responses
- This methodology selects only dose-responses that have a low-dose response that is stimulated above the controls, followed by a toxic response at higher concentrations.
- The quantitative features of hormetic concentration-response curves using this methodology are (Figure 2):
  (A) Maximum stimulation (mean stimulation compared to a control of 100%)
  (B) Width of the concentration showing stimulation above 100%
  (C) Width from the maximum stimulation to the ZEP
Results and Discussion

This presentation describes a multi-part evaluation of hormesis that has been previously applied to a single, albeit large, high-throughput study of chemicals (*i.e.*, NCI YACDS; Holbeck and Simon, 2007). This is a novel approach to evaluate dose-responses in a high-throughput study on features such as reproducibility, magnitude of stimulation, and the consistency of responses below the toxic threshold; while at the same time quantifying the specific parameters of the hormetic dose-responses (amplitude, width, ZEP/BMD, *etc.*). However, as Oberdörster et al. (2005) has reviewed, the prerequisites for determining the dose-response relationship for NPs include a sufficient number of data points in the dose-response continuum. Oberdörster *et al.* have also commented on how the paucity of data in the low-dose range can result in severe misinterpretation of the response model. Calabrese and Blain (2005) have similarly stressed the importance of study design in the assessment of hormesis. Namely, that the hormetic response is typically modest, with the maximum stimulatory response being only 30-60% greater than the controls, with an optimal range of hormetic responses beginning at \( \frac{1}{3} - \frac{1}{4} \) of the estimated toxic threshold (Calabrese and Blain, 2005; Nascarella *et al.*, 2009). This observation is consistent with both *In vitro* and *In vivo*
studies. For example, the stimulation of neurite growth in murine cells exposed to a 25 nM concentration of TGA-capped CdTe NPs was 20% greater than the controls (Jan et al., 2008), and the stimulation of feeding rate was 57-70% greater than the controls in arthropods (Porcellio scaber) exposed to 10-1000 μg/g of TiO₂ NPs in food (Drobne et al., 2009).

Hormesis is a stimulatory response at low concentrations that is generally the result of an initial disruption in homeostasis, and appears to represent a modest overcompensation response (Calabrese and Baldwin, 2001). While there is no single mechanism to explain all examples of hormesis, a common molecular approach is that of a 2-receptor subtype model. In this model, one receptor would have high and the other low affinity for the agonist however, because there are more low affinity receptors, these receptors have more capacity. This would lead to a biphasic dose-response with the high affinity receptor becoming activated at the lower concentrations and the low affinity/high capacity receptor becoming dominant at the higher concentrations (Szabadi, 1977).

**Conclusion**

While this paper describes several methodologies that have been used in dose-response assessments of chemical agents, the aim of this article is to provide a short summary of the evaluative methodology to encourage their use in NP toxicology evaluations. Such evaluations are most fruitful when study design consideration for the evaluation of hormesis are applied *a priori*. Design considerations such as adequate dose spacing below the toxic threshold (e.g., NOAEL, BMD, or ZEP) are critical to detect a dose-response pattern consistent with the hormesis model. Calabrese and Baldwin (2003) have shown that the detection of a threshold is essential as it provides an opportunity to denote the dose-response transition from a hormetic response (just below the threshold), to the traditional toxic response as the threshold is exceeded. Given that the majority of dose-response studies involving NPs are conducted in high-throughput *in vitro* screens, this creates an excellent opportunity to evaluate hormesis as it is less cost prohibitive than it may be in whole animal studies. While most NP evaluations are focused on determining toxicity values, the relationship between hormesis and toxicity (as measured by the IC₅₀) remains to be fully characterized. Initial reports in this area indicate that these parameters are inversely related (Nascarella *et al.*, 2009b).

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Nanotechnology for Site Remediation

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**Keywords:** nanotechnology, remediation, nanoscale zero-valent iron, environmental applications of nanotechnology

**Abstract**

The Technology Innovation and Field Services Division (TIFSD) of the U.S. Environmental Protection Agency’s (EPA’s) Office of Solid Waste and Emergency Response recently published a fact sheet on the use of nanotechnology for site remediation. (see [http://clu-in.org/542F08009](http://clu-in.org/542F08009).) Nanotechnology holds promise in remediating hazardous waste sites cost-effectively and in addressing challenging site conditions, such as where dense nonaqueous-phase liquids (DNAPLs) are present in contaminated aquifers. Nanoscale iron is already in use in full-scale projects with an encouraging measure of success. EPA has collected information on 26 sites where nanoscale zero-valent iron has been tested for site remediation. The presentation would summarize the Agency’s findings to date, including both successes and limitations encountered in the field applications and also questions about fate, transport, and toxicity of nanomaterials.

The presentation would also include a summary of what EPA’s various program offices are doing with regard to applications and implications of nanotechnology.

According to the National Nanotechnology Initiative, nanotechnology is defined as the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications (NNI, 2008). Nanomaterials may form naturally and include erosion and dust particles. Anthropogenic sources of nanoparticles include engineered (e.g., carbon nanotubes, quantum dots, metal oxides) and incidental materials (e.g., particles from industrial processes, combustion).

Nanomaterials may exhibit unique properties not found in the same materials in macroscopic form. Because of a vastly increased surface area per unit mass, nanomaterials may be highly reactive. They may also exhibit unique magnetic, electronic, mechanical, or photonic properties. Because of their unique properties, engineered nanomaterials are being incorporated into a wide variety of consumer products. The Woodrow Wilson International Center for Scholars’ Project on Emerging Nanotechnologies has a database with over 800 nanotechnology-based consumer products currently on the market (See: [http://www.nanotechproject.org/consumerproducts](http://www.nanotechproject.org/consumerproducts)).

The U.S. Environmental Protection Agency (EPA) is interested in both the potential environmental applications of nanotechnology and the potential implications, including potential toxicity, exposure, fate and transport, and impacts for regulatory responsibilities. EPA’s Office of Research and Development has funded research in both applications and implications of nanotechnology. (See: [http://www.epa.gov/ncer/nano/](http://www.epa.gov/ncer/nano/))

**Environmental Applications**

Researchers are taking advantage of special properties of nanomaterials to develop new technologies for addressing environmental problems. One example is the use of nanotechnology to develop membranes for water treatment, desalination, and water reclamation. These membranes incorporate a wide variety of nanomaterials, including nanoparticles made of alumina, zero-valent iron, and gold (Theron, 2008). Arbon nanotubes
can be aligned to form membranes with nanoscale pores to filter organic contaminants from groundwater (Mauter, 2008; Meridian Institute, 2006).

Companies are also developing nanomaterials for use in cleaning up spills or other accidental releases of hazardous materials. NanoScale Corporation is marketing its product, FAST-ACT®, as a chemical containment and neutralization system that first responders can use to clean up toxic chemical releases of industrial chemicals or chemical warfare agents (NanoScale, 2008).

A group of researchers at the Massachusetts Institute for Technology (MIT) have developed a “paper towel” for oil spills that is comprised of a membrane or mat of potassium manganese nanowires. According to the researchers, the nanowire membrane selectively absorbs oil with high efficiency. The oil can be recovered by heating the mat, which can then be reused. The membrane, which appears to be impervious to water, may have additional uses in water filtration (Thomson, 2008).

**Nanoscale Zero-Valent Iron and Site Remediation**

Environmental applications of nanotechnology include new methods to clean up hazardous waste sites. Early treatment remedies for groundwater contamination were primarily pump-and-treat operations. Because of the relatively high cost and often lengthy operating periods for these remedies, the use of *in situ* treatment technologies is increasing.

Nanoscale zero-valent iron (nZVI) holds promise in remediating sites cost effectively and in addressing challenging site conditions, such as where dense nonaqueous-phase liquids (DNAPLs) are present in contaminated aquifers. Research indicates that injecting nZVI particles into areas within aquifers that are sources of chlorinated hydrocarbon contamination may result in faster, more effective groundwater cleanups than traditional pump-and-treat cleanup methods.

Nanoparticles can be highly reactive due to their large surface area to volume ratio and the presence of a large number of reactive sites. This allows for increased contact with contaminants, thereby potentially resulting in rapid reduction of contaminant concentrations. Because of their minute size, nanoparticles may pervade very small spaces in the subsurface and remain suspended in groundwater, which could allow the particles to travel farther than macro-sized particles and achieve wider distribution.

Nanoparticles such as nZVI, bi-metallic nanoscale particles (BNPs), and emulsified zero-valent iron (EZVI) may chemically reduce the following contaminants effectively: perchloroethylene (PCE), TCE, cis-1, 2-dichloroethylene (c-DCE), vinyl chloride (VC), and 1,1,1-trichloroethane (TCA), along with polychlorinated biphenyls (PCBs), halogenated aromatics, nitroaromatics, and metals such as arsenic or chromium. Two of the important degradation reactions for chlorinated solvents are reductive dechlorination and beta elimination. Beta elimination, which occurs most frequently when the contaminant comes into direct contact with the iron, follows the pathway of 

\[
TCE + Fe^0 \rightarrow HC Products + Cl^- + Fe^{2+}/Fe^{3+} \quad (U.S. EPA, 2008)
\]

Reductive dechlorination, which occurs under the reducing conditions fostered by nZVI in groundwater, follows the pathway of 

\[
PCE \rightarrow TCE \rightarrow DCE \rightarrow VC \rightarrow ethene \quad (Elliot, 2006).
\]

**Advances**

Nanoscale iron particles can be modified to include coatings such as polyelectrolyte or triblock polymers (Saleh, 2007), or can be encased in emulsified vegetable oil droplets (Hydutsky, 2007; He, 2007). Some nanoparticles are made with catalysts that enhance the intrinsic reactivity of the surface sites (Tratnyek, 2006).
BNPs can be injected by gravity or by pressure feed (Gill, 2006). Research is ongoing into methods of injection that will allow nanoparticles to better maintain their reactivity and increase their access to recalcitrant contaminants by achieving wider distribution in the subsurface. Creating nZVI on site reduces the amount of oxidation the iron undergoes, thereby reducing loss in reactivity. Researchers in green chemistry have successfully created nZVI in soil columns using a wide range of plant phenols, which, according to the researchers, allows greater access to the contaminant and creates less hazardous waste in the manufacturing process (Varma, 2008).

Limitations

Studies have shown that nanoparticles may not achieve widespread distribution in the subsurface due to agglomeration prior to complete dispersion within the soil or groundwater matrix, limiting the radius of influence. Passivation is another factor that may limit the effectiveness of iron nanoparticles. If nZVI is being used, improper handling can result in the iron becoming oxidized and passivated prior to reacting with the contaminants. Researchers have developed methods, some of which are in use commercially, to improve the mobility of iron nanoparticles within aquifers and to optimize contact between the nanoparticle and contaminant. Ongoing studies are evaluating surface coatings and other modifications that would reduce agglomeration of nanoparticles and maximize subsurface mobility (Phenrat, 2008).

A challenge with evaluating the effectiveness of nanoparticle injection is monitoring the distribution of injected particles in the subsurface. It is therefore important to identify the appropriate parameters to measure performance. Also, additional research is needed regarding the safety of the technology and the fate, transport, and transformation of nanoscale zero-valent iron particles during and after field application (U.S. EPA 2008).

Field Studies

EPA obtained information on 26 sites where nanoparticles have been tested or used for site remediation. These include 7 full-scale and 19 pilot-scale projects. In the majority of these studies, the primary contaminant of concern was trichloroethene. Most of these projects used gravity-feed or low pressure injection and focused on source zone remediation. More information is available at http://clu-in.org/542f08009.

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Use of NZVI for the Reductive Dehalogenation of Some Chlorinated Pesticides

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Keywords: nano-scale zero-valent iron, environmental remediation, reductive dechlorination

Abstract
Nano-scale zero-valent iron (NZVI) can degrade or transform a wide range of pollutants both organic (e.g., chlorinated pesticides) and inorganic (e.g., nitrate, chromate, perchlorate, metal ions) by chemical reduction. Due to its non-toxic nature, NZVI is an environmentally friendly, yet strong reductant. Its high surface-to-volume ratio promotes mass transfer to and from the solid surface and increases the reaction capacity for contaminant removal and degradation. NZVI-based techniques have been receiving attention recently as a cost-effective environmental remediation tool due to its combined advantages of easy mixing and potential mobility in groundwater and other treatment processes. The present study assesses the feasibility of degradation of some organochloro pesticides by starch stabilized laboratory synthesized NZVI particles. The main objective of this study was to ascertain the feasibility of using NZVI for reductive dehalogenation of some pesticides that do not interact appreciably with micron sized ZVI. Two isomers of the pesticide endosulfan, viz., Endosulfan-1, Endosulfan-2, Endosulfan sulfate, Endosulfan Ether and Lindane were tested for their reactivity with NZVI. This study indicates that the use of NZVI has capability for partial or complete dehalogenation of the organochloro pesticides.

Introduction
Over the last decade many researchers have shown that a wide variety of halogenated organic carbon (HOC) compounds in aqueous solution could be dehalogenated through interaction with metallic or zero valent iron (ZVI) surface. Compounds tested include chloroethanes, chloroethenes, chlorophenols, chlorinated pesticides and herbicides, polychlorinated biphenyls, etc. For a particular HOC, the rate of dehalogenation in a batch system depends on the surface area of ZVI provided and the surface characteristics of the ZVI. ZVI surfaces with little or no iron oxide coating have been demonstrated to support faster dehalogenation of HOCs as compared to ZVI surfaces with substantial amount of oxide coatings. In addition, several classes of HOCs, e.g., chlorobenzenes and several pesticides and herbicides do no undergo ZVI mediated dehalogenation. Further, in a compound class, compounds with lesser number of particles, with specific surface areas of up to an order of magnitude more that micron sized ZVI particles, have been shown to allow faster rate of dehalogenation of HOCs as compared to ZVI surfaces with substantial amount of oxide coatings. In addition, several classes of HOCs, e.g., chlorobenzenes and several pesticides and herbicides do no undergo ZVI mediated dehalogenation. Further, in a compound class, compounds with lesser number of particles, with specific surface areas of up to an order of magnitude more that micron sized ZVI particles, have been shown to allow faster rate of dehalogenation of HOCs. Furthermore, the surface of NZVI particles has been reported to be more reactive, thus allowing HOCs which were un-reactive towards micron sized ZVI to be dehalogenated by NZVI. This constitutes the so called, “Nano” effect of the NZVI particles. The use of NZVI particles thus represents a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems. Halogen atoms are generally dehalogenated more slowly than compounds with more numbers of halogen atoms, e.g., pentachlorophenol is dehalogenated faster than chlorophenols. Most of the variability in observed degradation rates for a particular HOC with different batches of ZVI is attributable to the differences in iron
surface area and variations in ZVI surface characteristics. Iron particles with high specific surface area are likely to support faster dehalogenation of HOCs.

Materials and Methods
Pure compounds (>99% purity), viz., endosulfan 1 (CAS No.: 959-98-8), endosulfan 2 (CAS No.: 33213-65-9), viz., Lindane (CAS No.: 58-89-9) were purchased from Sigma–Aldrich Ltd., USA. Various solvents, viz, n-hexane and methanol, of HPLC grade (>99% purity) were purchased from Merck, Germany. Ferric chloride (96% purity) was purchased from Qualigens, India, while sodium borohydride, NaBH₄ (97% purity) and soluble starch (>99.5% purity) were procured from Loba chemicals, India. Experiments were carried out in 16 mL borosilicate glass vials confirming to ASTM type-I grade, purchased from Wheaton Science, USA. The glass vials were equipped with screw caps with 18 mm teflon faced re-sealable septa. Clear 2 mL gas chromatograph (GC) auto sampler vials with 11 mm aluminum seals and PTFE rubber lined septa (Wheaton Science, USA) were used GC analysis. These vials were used only once before disposal. NZVI Prepared by wet chemical process and its sizes majored by TEM and surface area by BET surface area analyzer (Coulter SA 3100, USA).

Result and Discussion
Synthesized NZVI particles varied in size from less than 10 nm to 40 nm and the average particle size was around 17 nm. Specific surface area are of such particles was estimated to be approximately 38 m²g⁻¹. In contrast, specific surface area of electrolytic iron particles of 100 mesh (~150 mm) size was 2 m²g⁻¹ approximately. Endosulfan 1 was readily dehalogenated by a 0.1 g dm⁻³ NZVI suspension, with the observed normalized pseudo first order reaction rate (k_N) and half-life (t_{1/2}) being 0.72 h⁻¹g⁻¹ NZVI L and 9.5 hours respectively (Figure-1). When Endosulfan-1 was contacted with 0.25 g dm⁻³ NZVI particles, the observed normalized pseudo first order reaction rate (k_N) was 0.352 h⁻¹g⁻¹ NZVI L, which is considerably less than what was observed with 0.10 g dm⁻³ NZVI suspension. This decline was attributed to the agglomeration of NZVI particles in the 0.25 g dm⁻³ NZVI suspension, resulting in the reduction in specific surface area of NZVI (Figure 2).

Endosulfan-2 was degraded readily on interaction with 0.1 g dm⁻³ NZVI solution, with the respective normalized pseudo first order reaction rates (k_N) being 0.62 h⁻¹g⁻¹ NZVI L and the corresponding half lives (t_{1/2}) for degradation being 11.1 hours (Figure 3). Both Endosulfan Sulfate and Endosulfan Ether were readily degraded by NZVI, the observed normalized
pseudo first order reaction rate \( (K_N) \) and half-life \( (t_{1/2}) \) for Endosulfan sulfate and Endosulfan ether dehalogenation by NZVI was 1.32 \( \text{h}^{-1} \text{g}^{-1} \) NZVI L, 5.22 hours and 0.93 \( \text{h}^{-1} \text{g}^{-1} \) NZVI L and 7.5 hours respectively (Figures 4 & 5). Lindane degraded slowly when contacted with 0.1 \( \text{g dm}^{-3} \) NZVI suspension, with the observed normalized pseudo first order reaction rate \( (K_N) \) and half-life \( (t_{1/2}) \) for Lindane degradation by NZVI was 0.014 \( \text{h}^{-1} \text{g}^{-1} \) NZVI L and 50 hours respectively (Figure-6).

**Conclusion**

Results of this study indicate that the use of NZVI for partial or complete dehalogenation of otherwise recalcitrant pesticides and herbicides is a viable option in conditions pertaining to natural subsurface environment. NZVI particles can be suspended in slurry and directly pumped to the contaminated site for environmental remediation. Environmental technologies involving NZVI could thus provide cost-effective solutions to some of the most challenging environmental cleanup problems.
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Nanotechnological Solutions for Monitoring and Treatment of Drinking Water and Groundwater

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Keywords: water quality, biosensors, nanofiltration, photocatalysis

Abstract
Sensitive analytical methods for multi-residue monitoring of water pollutants are required to implement and verify compliance with environmental legislation. Advanced water quality monitoring techniques incorporating integrated biosensor, optical, microfluidic and information technologies can be expected to lead to significant changes in the way we control and manage aquatic environments. Traditional filtration methods used to purify drinking water and remove contaminants from wastewater are of limited effectiveness because of the relative inefficiency of the active materials employed. Because of their greater specific surface area, nanoparticles and nanoporous membranes are more efficient than conventional materials for water purification. Potential applications include provision of clean drinking water, remediation of contaminated aquifers and treatment of wastewater. The advantages and disadvantages of nanofiltration and photocatalysis for the removal of organic and inorganic pollutants from water are discussed.

Introduction
There is a need for analytical methods and monitoring tools for detecting and analyzing common water pollutants such as endocrine disruptors, phenols, pesticides, pharmaceuticals, dyes and sulfonates in the presence of many other chemicals. Innovative solutions based on miniaturized, inexpensive biosensor systems are currently under development. Recent advances in biosensor, bioassay and immunoassay techniques for water monitoring and environmental sampling for detection of organic and inorganic pollutants have been reviewed by Farré et al. (2005) and Rodriguez-Mozaz et al. (2006) Immunosensors employing antibodies or antigens for detecting organic pollutants, endocrine disruptors, and pesticides have high selectivity and sensitivity, are suitable for remote operation, provide rapid data and are able to detect many compounds of interest at levels lower than the current legal limits.

Photocatalysis is emerging as a valid alternative to conventional water treatment technologies and titanium dioxide is the most commonly used material. Photocatalytic water treatment has been shown to be an effective method for the degradation of pollutants and the destruction of micro-organisms (Dunlop et al., McMurray et al., 2005). The power required for the UV light source represents the most significant operating cost and hence solar photocatalysis has been the focus of a significant research and development effort. Nanofiltration is another promising method for water treatment. The membrane pore size lies in the range between ultrafiltration and reverse osmosis and is similar to that of pesticide molecules and other chemicals typically present in water. It is useful for removal of natural organic matter, micropollutants and heavy metals, disinfection, desalination, and ion separation (Rickerby and Morrison). Potential applications include discharge and reuse of wastewater, high quality drinking water, groundwater treatment, removal of organic and inorganic pollutants in surface water, and recycling of process water.
Biosensors for Water Quality Monitoring

Tschmelak et al. (2004) have developed an automated immunoassay to detect organic compounds such as endocrine disruptors, various hormones, pesticides and antibiotics. The system uses labelled antibodies and a detection method based on total internal reflection fluorescence (TIRF). Limits of detection were determined from a multi-analyte calibration with atrazine, bisphenol A, and estrone. A limit of detection of less than 0.020 μg L⁻¹ was obtained for all three compounds in a simultaneous analysis, which is comparable with the sensitivity attainable with the traditional analytical techniques GC–MS and HPLC–DAD.

An improved system was introduced by Proll et al. (2005) employing an integrated optical chip able to detect 32 compounds simultaneously. It used microfluidics technology for sample injection and an optical waveguide to conduct light from a solid state laser to the sensing windows on the chip surface. Its performance was evaluated in comparison to that of the conventional analytical techniques SPE–LC–DAD UV, SPE–LC–MS and GC–MS for atrazine, bisphenol A and estrone in surface water, groundwater, drinking water and wastewater. The detection limits for all three analytes were in the nanogram per litre range.

The robustness of biosensors can be improved by using molecular imprinting to create artificial receptors in synthetic polymers using appropriate templates. Removal of the template by solute extraction leaves binding sites in the polymer containing complementary functional groups that can selectively bind the target molecule in a similar manner to natural receptors (Haupt, 2003). The specificity and stability of molecular imprinted polymers make them alternatives to biological receptors in many immunoassay and biosensor applications. Piletsky et al. (2001) have reported the use of molecularly imprinted polymer immunoassays for analysing atrazine and epinephrine. These devices showed remarkable stability, with affinities that decreased only 10-20% over a period of two months.

Nanomaterials for Water Treatment

Anatase titanium dioxide is the most widely used material for photocatalysis because it is highly photoreactive, inexpensive, of low toxicity, chemically and biologically inert, and photostable. Illumination with UV light causes formation of electron-hole pairs and hydroxyl radicals to be generated at the surface, which destroy organic pollutants by converting them to carbon dioxide and water. The photocatalytic efficiency is typically only around 0.01% but can be increased by doping with gold nanoparticles, which increase the photocatalytic activity at concentrations as low as ~1 at% (Orlov et al., 2006).

McMurray et al. (2006) carried out laboratory scale studies of electrochemically assisted photocatalysis of persistent organic pollutants such as atrazine. Dip coating, spray coating, or electrophoretic coating was used to immobilize titanium dioxide nanoparticles on the substrate. Photocatalysis allows disinfection of drinking water without chemicals; inactivation of bacterial spores takes place within a few hours and the method is effective also against chlorine-resistant organisms. Significantly increased disinfection efficiencies are obtained (Dunlop et al., 2005) when photocatalysis is used rather than UV irradiation alone. A solar photocatalytic reactor was designed by Sichel et al. (2007) as a solution to the problem of providing safe drinking water in developing countries and emergency situations. An immobilized catalyst was used to avoid the presence of nanoparticles in the water after treatment.

Nanofiltration membranes work by transferring one component more readily than another due to differential transport, causing separation into a retentate, enriched in less mobile components, and a permeate, enriched in faster components (Van der Bruggen and Vandecasteele, 2003). They have applications in both drinking water purification and wastewater treatment and generally consist of a multilayer structure. Separation takes place in
the top layer, which is mechanically supported by a series of asymmetric layers. The pore size is of the order of 1 nm and the pressure required is lower than for reverse osmosis due to the comparatively larger free volumes in the membranes.

While nanofiltration is an effective method for production of high-quality water, it suffers from inherent problems related to the treatment of the concentrate, membrane fouling, incomplete separation, fabrication of membrane structures, process design, and high levels of heavy metals and toxins in the retentate and/or permeate. Membrane fouling limits efficiency but a possible solution is to modify the structure with a hydrophilic surface to reduce fouling and allow stable operation. Alternative membrane designs with a colloidal interlayer and an oxide top layer on a ceramic support are being investigated. Techniques are currently being studied that employ membrane cascades and recycling of the permeate or concentrate to obtain a better separation between ions and organic solutes.

Systems using photocatalytic methods for water purification are already commercially available and nanosized titanium dioxide is being produced by several companies on a commercial scale. Further research is needed in order to control the treatment to eliminate any toxic by-products. Investigation is also needed of the potential long term health and environmental risks of using nanoparticles for water purification. Nanofiltration requires further modifications to improve the efficiency of the process. The health and environmental risks for nanofiltration are related not to nanostructured materials but to disposal of the toxic products in the concentrate or retentate and the contaminated filters after use.

**Conclusions**

Environmental nanotechnologies can contribute to achieving sustainability while protecting the environment. Applications of nanotechnologies for water monitoring and treatment have been demonstrated both at the laboratory scale and in field trials. Biosensor systems are enabling the real time monitoring of water quality. Effective methods for drinking water purification and groundwater or wastewater treatment, employing photocatalytic nanoparticles or nanofiltration membranes, are currently under development. Potential risks are represented by the products of water treatment, which might be more toxic than the original pollutants, and the entry of these compounds into the environment.

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Uptake and Distribution of Quantum Dots in *Artemia Franciscana* and *Pimephales Promelas* and a Comparative Study of Dietary vs. Aqueous Uptake in *Pimephales Promelas*

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Keywords: nanomaterials, quantum dots (QDs), bioaccumulation, *Artemia franciscana* and *Pimephales promelas*.

Abstract

Understanding the underlying processes that lead to toxicity, persistence and bioaccumulation of nanoparticles, nanotubes, and nanocrystals are yet to be fully elucidated. Quantum Dots (QDs) are a class of nanocrystals that consist of a metalloid crystalline core (Cd/Se or Cd/Te crystal) surrounded by an organic “shell” or “cap”. There remains a vital question about the hazards and risks posed by these engineered particles, especially under conditions where oxidation and photolysis are likely to occur. These processes could result in the exposure of the toxic metallic core. The present study seeks to characterize the uptake, distribution, and potential bioaccumulation of QDs from an aquatic environment, using the standard test organisms-* Artemia franciscana* and *Pimephales promelas* along a simple two trophic level food chain. The average pixel intensities of the fluorescence images (measured using a Nikon epifluorescence microscope and MetaMorph© 6.10 Meta imaging series environment) confirms the uptake of QDs in both test species. Further, this research compares the dietary and the aqueous uptake of QDs in *P. promelas*. Results indicate a higher body burden of QDs from the dietary route of exposure. As indicated by the mean pixel intensities, the dietary uptake resulted in the accumulation of QDs along the gut tract of *P. promelas*. The results of the present study allow a better understanding of the environmental fate of these nanocrystals in aqueous food chains.

Introduction

Nanotechnology is the understanding and controlling of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications (1). A major breakthrough in nanotechnology is engineered nanomaterials (ENMs). ENMs have extraordinary physicochemical properties that bring along a concern about the adverse effects of such nanostructures on biological systems. These properties largely determine interactive mechanisms and the form in which these materials may be released (2) and in turn their potential toxicity to biological systems. The underlying processes that lead to toxicity, persistence, and bioaccumulation of manufactured nanoparticles are not well understood. Single organism and food web studies are needed to quantify the potential for bioavailability and bioaccumulation of ENMs. However, it is clear that organisms living in environments containing nanomaterials incorporate them within their bodies, mainly via gut tract (3-5). Further investigation and research also need to be done with nanomaterials that gain entry into the biological systems via different routes of uptake and tend to bioaccumulate within the various trophic levels of the food chain.

The focus of the present study was on the possibility of uptake and accumulation of one such engineered nanocrystal, QD. QDs are semiconductor fluorophores, 15-20 nm in overall diameter with a core diameter of approximately 4-5 nm. Currently QDs are applied in
biomedical imaging (6, 7), tools for site-specific gene and drug delivery (8), and electronics industries (9).

The commercially available QDs used in the present study consist of a Cd/Se crystalline core with a ZnS coating covered by an outer polymer layer. Product information provided by the manufacturer states that 1 ppb of QD contains approximately 260 ppb cadmium, and a 4:1 ratio to selenium (10) provides 65 ppb selenium per ppb QD. Due to the high concentration of cadmium within the core and the largely unknown environmental fate of these intact semiconductor nanocrystals, the potential exists for the toxic metal exposure to organisms on release of these nanocrystals into the environment, as indicated by some *in vitro* studies. For example, studies have found that three types of mercapto-undecanoic acid (MUA) substituted Cd/Se, QDs decreased viability in three types of cells *in vitro* (monkey kidney, HeLa cells, and human hepatocytes), and caused cell death after 4-6 h of incubation (11). Derfus et al. (12) indicated that Cd/Se QDs were toxic to rat liver hepatocytes if exposed to air or UV light, as a result of oxygen combining with Se and release of free Cd$^{2+}$ from the crystal lattice. Some other studies provide evidence of bioconcentration of QDs against the significant concentration gradients by the test organism, *Ceriodaphnia dubia* (13). Another recent study by Bouldin et al. (14) reported the aqueous toxicity and food chain transfer of QDs in freshwater algae (*Pseudokirchneriella subcapitatum*) and *C. dubia*.

**Materials & Methods**

All the exposure studies were done with QD® 545 ITK™ Carboxyl Quantum Dots (Fisher Scientific, USA; Fisher part no Q21391MP). These QDs are covered with a polymer coating that is treated so that carboxyl groups are available on their surface. The peak emission of these particles is between 541 nM and 549 nM. The QDs used for all nominal calculations in the study were based on 8.0 µM concentration (supplied by the vendor). All test organisms were cultured and maintained in the Ecotoxicology Research Facility at Arkansas State University (ASU ERF), Jonesboro, AR, USA, according to US EPA (15) protocol.

Aqueous exposure study: Three exposure groups of 1.0 ppb, 1.6 ppb and 2.0 ppb QD suspension and a control/unexposed group were used in the study set-up. For the aqueous exposure study with *P. promelas*, the organisms were directly exposed to a suspension of QD in de-chlorinated water for 24 h. Each exposure and control groups were in replicates of three with ten organisms in each replicate. *A. franciscana* were hatched from the cysts under controlled laboratory conditions and then exposed to QD suspension prepared in *Artemia* media for 8 h in a 24-microwell plate. Each exposure and the control group for *A. franciscana* were in replicates of six, with ten to fifteen organisms in each replicate.

Food chain exposure: For the dietary uptake study, the quantification of QD uptake was done after a 16-h feeding regime, where the *P. promelas* (age <24-h) were fed *A. franciscana* that had been previously exposed to QD for 8 h. To measure the QD uptake in the test organisms, a subsample of three to four live organisms were randomly chosen, rinsed with appropriate media to wash off excess QD attached to the exoskeleton and then examined under brightfield and fluorescence microscope to determine pixel intensity. All images were taken using a Nikon epifluorescence microscope (Nikon Eclipse E800, excitation λ 465-495: emission λ 515-555 nm) equipped with a mercury-vapor illumination and a Cascade Photometric digital camera. Images were digitized using Nikon ACT-1© software and stored for further analysis. MetaMorph™ 6.10 Meta Imaging Series Environment (Universal Imaging Corporation™) was used to quantify the average pixel intensity in the fluorescence images.
Results

Aqueous exposure: The average pixel intensity measured in QD-exposed *A. franciscana* after 8-h exposure revealed statistically significant differences in fluorescence among treatment and control groups.

Graph 1: Measured pixel intensity (mean ± SD) in *A. franciscana* exposed to various concentrations of Qdot® 545 ITK™ Carboxyl Quantum Dots for 8 h. (n=3) (p<0.005)

* = significantly different from unexposed (control)
** = significantly different from unexposed (control), 1.0 ppb & 1.6 ppb QD concentration.

Unexposed *A. franciscana* exhibited autofluorescence, average pixel intensity (±SD) ranged from 6.33±1.66. For the exposure groups, the average pixel intensities were 69.65±14.93 for 1.0 ppb QD, 101.60±26.41 for 1.6 ppb QD and 126.23±50.76 for 2.0 ppb QD exposure respectively. For the aqueous exposure with *P. promelas*, the pixel intensity of only the gut region was measured (for purposes of comparison with the dietary exposure results). Unexposed *P. promelas* exhibited autofluorescence along the gut tract (near the swim bladder); average pixel intensity was 7.70±0.50. The average pixel intensity for exposure groups was 13.0±1.72 for 1.0 ppb QD, 14.39±2.45 for 1.6 ppb QD and 11.41±1.83 for 2.0 ppb QD exposures respectively (Graph 2).

Dietary (food chain) transfer: No *P. promelas* mortality was noted following a 16-h feeding regime with QD-exposed *A. franciscana*. Unexposed *P. promelas* exhibited autofluorescence along gut tract (near the swim bladder), the average pixel intensity (±SD) of this region ranged from 7.52±0.70. For the exposure groups, the average pixel intensities of the gut tract of *P. promelas* (fed with 8-h QD exposed *A. franciscana*) were statistically different from the control group and ranged from 15.50±3.26 for 1.0 ppb QD, 33.56±5.61 for 1.6 ppb QD and 15.48±1.95 for 2.0 ppb QD exposures respectively.

Graph 3: Measure pixel intensity (± SD) of *P. promelas* after 16-h feeding regime with *A. franciscana* exposed to different concentrations of Qdot® 545 ITK™ Carboxyl Quantum Dots. (n=3) (p<0.009).

* = significantly different from unexposed (control)
** = significantly different from unexposed (control), 1.0 ppb, 2.0 ppb QD concentration.
Discussion

Aqueous toxicity study and similar food chain transfer of QDs have been reported in freshwater algae and C. dubia, where the transfer of QD from dosed algae to C. dubia was verified with fluorescence microscopy at 11.0 ppb and 55.0 ppb QD in aqueous suspension (13). In the present study, the evidence of dietary assimilation of nanocrystals was provided by the transfer of QDs from A. franciscana food source to the primary consumer, P. promelas, which in turn was quantified with increased pixel intensity. Evidence of bioconcentration was observed by the aqueous uptake of QDs by both A. franciscana and P. promelas. Similar bioconcentration studies have been done with C. dubia to demonstrate aqueous uptake of QDs (13). Models continue to be investigated to determine aqueous routes of uptake for ionic metals. Studies have reported assimilation efficiency of cadmium, a rate of aqueous uptake and food assimilation, in Daphnia magna resulting primarily from the aqueous phase and selenium assimilation resulting mostly from food uptake (16).

Conclusion

The results of the present research clearly indicate the uptake of QDs in A. franciscana and P. promelas via the two routes of uptake (Fig.1 & 2). Intact QDs possibly provided a measure of protection to these aquatic test organisms from the toxic core metals (Cd & Se) as there was absence of mortality. This observation was similar to those reported by Bouldin et al. in C. dubia (14). The present study also indicated that the QD accumulation in P. promelas (along the gut) from dietary exposure was higher than that of aqueous exposure. Also, in P. promelas, both the dietary and the aqueous uptake of QDs around the gut were the highest at 1.6 ppb QD concentration and decreased at the higher concentration (2.0 ppb QD), as indicated by the measure of their average pixel intensities. Further research can be done in this regard to study the changing dose-uptake relationship as well as the rate of depuration of the nanocrystals in these aquatic test species.

Figure 1

Brightfield image of the A. franciscana exposed to 1.6 ppb QD for 8 h.

Fluorescence image of A. franciscana exposed to 1.6 ppb QDs for 8 h.
Figure 2

Fluorescence image with highlighted gut tract of *P. promelas* fed with 1.6 ppb QDs exposed *A. franciscana* (16 h)

Fluorescence image with highlighted gut tract of *P. promelas* fed with 2.0 ppb QDs exposed *A. franciscana* (16 h)

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Carboxymethyl Cellulose Stabilized Magnetite Nanoparticles Are Nontoxic to Bacteria

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Keywords: nanoparticles, toxicity, magnetite, carboxymethyl cellulose, bacteria

Abstract
Stabilized iron oxide nanoparticles have potential for in-situ environmental remediation. However, little is known regarding the impact of these nanoparticles on soil microorganisms. This study aimed to investigate the effects of Fe₃O₄ nanoparticles on four pure cultures of bacteria, i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Enterococcus faecalis*. The effect of commercial and lab-prepared Fe₃O₄ nanoparticles was examined through growth experiments and observation of cell-nanoparticle interactions. The results show that Fe₃O₄ nanoparticles at concentrations of 0.3, 0.6, and 1.0 g Fe/L were neither toxic nor inhibitory to the four organisms tested. In some cases, growth stimulation was observed. After the addition of culture to nanoparticle suspensions, there was a loss of particle stability manifested by the formation of large aggregates. Scanning electron microscopy was performed on the aggregates formed in *E. coli* culture to examine the cell-nanoparticle interactions. Nanoparticles were found to cover cell surfaces and bridge cells together in clusters, but no damage to cell integrity was observed. *E. coli* cells blanketed by nanoparticles retained the shape of healthy cells. No cell damage or rupture was observed. Aggregates formed in the presence of bacteria consisted of nanoparticles and bacterial cells held in the nanoparticle matrix. We conclude that stabilized nanoparticles may interact with bacterial surfaces without causing damage sufficient to inhibit cell growth.

Introduction
Stabilized iron oxide nanoparticles have potential for in-situ environmental remediation. Specifically, magnetite (Fe₃O₄) nanoparticles when stabilized during synthesis can remove contaminants due to their small particle size resulting in high reactivity and mobility (4-7). Surface modification of nanoparticles by macromolecules enhances colloidal stability and dispersability in the soil. In the absence of a stabilizer, magnetite nanoparticles rapidly form large aggregates. Carboxymethyl cellulose (CMC) is a more effective stabilizing agent for Fe₃O₄ nanoparticles compared to other polymers such as starch and dextran due to its large size and negatively charged functional groups. Little is known concerning the effect Fe₃O₄ nanoparticles may have on microorganisms after they are applied to soil. Iron oxide nanoparticles (Fe₂O₃) display toxicity to human mesothelioma cells that cannot be explained by dissolved iron (3). Zero valent iron nanoparticles are toxic to bacteria due to their high redox reactivity. After entering the environment these particles are oxidized and form a shell of magnetite and other iron oxy-hydroxides on their surfaces (8, 10). Magnetite surfaces are more stable resulting in a longer life and contact with organisms (10). Auffan et al. demonstrated that Fe₃O₄ cytotoxic effects appear in doses higher than 700 mg/L for resting *E. coli* cells (1). This study aimed to investigate the effects of synthesized magnetite nanoparticles on bacteria through examining bacteria-nanoparticle interactions as well as monitoring toxicity in growing cultures.
Materials and Methods

Fe₃O₄ preparation: To prepare magnetite, ferric and ferrous salts were added in 2:1 molar ratio to achieve a final iron concentration of 1.5 g Fe/L using a procedure modified after several protocols (6, 11, 12). Ferric chloride hexahydrate (0.018 M) and ferrous sulfate heptahydrate (0.009 M) were prepared separately in distilled water purged with N₂ and mixed until dissolved. One percent carboxymethyl cellulose sodium salt (MW 90 kD) was bubbled with N₂ gas for 30 minutes. With continuous stirring ferrous and ferric iron solutions were added consecutively to the CMC solution and again purged with N₂ for 30 minutes. In one aliquot, 10 M NaOH was rapidly added to the solution turning it black instantly and reaching a final pH of 11. The solution mixture was bubbled with N₂ for an additional 30 minutes, sealed with a rubber stopper, and allowed to stand overnight. After 24 hours the solution pH was brought down to a biologically relevant pH of 7 with 5 M HCl. The final iron concentration was 1.5 g Fe/L with a final concentration of 0.5% CMC (w/v).

Commercial Fe₃O₄ preparation: Commercial Fe₃O₄ nanoparticles were purchased from NanoAmor (Houston, TX). Fe₃O₄ powder was sonicated using a Misonix sonicator in 200 mL of 0.5% autoclaved CMC for one hour (60 W) pulsing off every ten minutes for one minute.

Characterization of Fe₃O₄ nanoparticles: Transmission electron microscopy (TEM) was performed on a Zeiss EM 10C 10CR Transmission Electron Microscope. Ten µl of nanoparticle suspension (pH 7) was placed on a copper grid and allowed to dry down for 30 minutes. The average particle size was calculated based on three representative images for each treatment.

Cell growth study: P. aeruginosa, B. subtilis, E. faecalis and E. coli K12 were ATCC strains obtained from Auburn University Biological Sciences Microbiology culture collection. MOPS medium (9) was used as a minimal media with 0.01% yeast extract added to Enterococcus culture. Sterile disposable borosilicate glass vials (20 mL) were used to incubate cultures for 6 hours. Each vial contained an initial cell concentration of 10⁷ CFU/ml and 0.33% CMC. Vials were placed on a flat platform partially immersed into a 37°C shaking water bath. Since UV light may enhance toxic properties of iron nanoparticles, UV light was added to simulate solar UV radiation (290-400 nm). The 60 watt Mega-Ray UV lamp was placed 30.5 cm away from the vial platform. Borosilicate glass allowed UV light (>320 nm) to penetrate the vials and reach the nanoparticles.

Nanoparticle-bacterial cell interactions: Aggregates present in E. coli cultures containing nanoparticles were removed by centrifugation and dehydrated with ethanol. Dimethylsilizane was used to dry samples. Controls consisted of healthy E. coli cells as well as nanoparticles alone. Dried samples were placed onto grids, gold sputter coated and examined with a Zeiss EVO 50VP Scanning Electron Microscope (SEM).

Results and Discussion

CMC-stabilized Fe₃O₄ nanoparticles had an average diameter of 28.0 ± 5 nm. CMC-stabilized nanoparticles were observed in a single layer without aggregate formation (Figure 1A). Bare Fe₃O₄ nanoparticles synthesized in the absence of a stabilizing agent displayed an average particle size of 56.1 ± 12 nm. Commercial magnetite resuspended with CMC (Figure 1C) also showed a smaller average particle size of 49.0 ± 2 nm compared to commercial particles suspended in water (Figure 1D) having an average particle size of 52.4 ± 2 nm. CMC proved to be an effective stabilizer to keep particle size small during synthesis and aggregates dispersed in the commercial product. Compared to stabilized synthesized
magnetite nanoparticles, non-stabilized synthesized particles were much larger and more irregularly shaped.

Increasing concentrations of synthesized magnetite with and without 0.5% CMC resulted in stimulation of growth (Figures 2A and 2C) in spite of the fact that particles without stabilizer were much larger and settled much faster. As seen with the synthesized magnetite, the commercial CMC stabilized suspension also resulted in increased cell growth except for *E. faecalis* (Figure 2B). There were nanoparticle aggregates present in cell cultures that were not observed in the sterile controls. SEM was performed to examine constituents of an aggregate in *E. coli* cultures and no damage to cells was observed (Figure 3). Figure 3A shows the nanoparticle-bacteria interactions in a typical aggregate with stabilized synthesized magnetite nanoparticles. Figure 3B shows the interactions present in an aggregate of synthesized magnetite without the stabilizer. Commercial magnetite formed large aggregates in the presence (Figure 3C) or absence (Figure 3D) of stabilizer. Cells were covered in nanoparticles regardless of the particle type used. Non-stabilized aggregates were much looser than stabilized nanoparticles due to their larger size and inability to pack as closely. However, without stabilizer the aggregates were much larger and formed much more rapidly.
More aggregates were formed in bacterial cultures containing nanoparticles than sterile nanoparticle controls suggesting bacteria interact with and possibly promote nanoparticle aggregation. Aggregates formed in the presence of CMC could be easily re-dispersed. We hypothesize the CMC prevents nanoparticle aggregation as well as interactions between cells and nanoparticles. CMC stabilized synthesized Fe₃O₄ showed the least interaction with *E. coli* cells. Although ferrous is known to be involved in the Fenton reaction and produce hydroxyl radicals that cause oxidative stress to cells (2), both synthesized and commercial magnetite stimulated bacteria growth or had no effect in our study. As the total iron concentration increased cell growth increased as well. Lack of toxicity toward bacteria in our study is inconsistent with what was reported by Auffan et al. (1) perhaps due to our use of stabilizer and the use of growing cells in media instead of ultrapure water. Our SEM findings were consistent with Auffan et al.’s findings that magnetite nanoparticles interact with *E. coli* surfaces. However, we did not observe any damage to cell surfaces reported in their study (1).

**Conclusions**

Magnetite nanoparticles did not show any toxicity or inhibitory effects toward the four organisms tested in growth culture. There was interaction between cells and nanoparticles but magnetite nanoparticles did not damage *E. coli* or inhibit the growth of any of the organisms tested.

**Literature Cited**


Effects of Colloid Size and Concentration on Deposition Mechanisms in Saturated Natural Sand

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Keywords: straining, blocking, pore size distribution, saturated porous media, silica nanoparticles

Abstract
Straining is a geometrical retention mechanism of nanoparticles in porous media. In this study, column experiments were performed with synthetic fluorescent silica colloids with different sizes and concentration in natural saturated sand under conditions unfavourable to electrostatic attachment to quantify straining. Experiments with different colloid concentrations have highlighted a blocking mechanism: colloids are strained in sites until these sites are filled and then they are diverted from these regions. A colloid straining volume was determined from Scanning Electron Microscopy (SEM) images of porous media and used in a macroscopic model of transport and deposition.

Introduction
Natural or synthetic colloids are potential contaminants for groundwater. Colloids can be transported by groundwater flow and can also be retained by grains of soils. Their ability to be transported or deposited in saturated porous media depends on properties of both collector grain and colloid (size, surface potential, composition, shape) and chemical and physical properties of solution (pH, ionic strength, flow rate...).

Recently, Bradford and Torkzaban (2008) have reviewed retention processes that may occur at the interface, collector and pore scales. At the collector scale, colloid filtration theory (CFT) predicts a deposition rate linked to collector efficiency (Tufenkji and Elimelech, 2004; Yao et al., 1971). The CFT is based on the sphere-in-cell model (Happel, 1958) and does not include pore space geometry (Johnson et al., 2007). Straining occurs when colloids are transported to a constriction between adjacent grains that is too narrow to allow passage and is not included in the CFT (Bradford et al., 2002).

The objectives of this work is to investigate the influence of colloid concentration and size on straining in a natural soil and to determine the volume of the porous media available for straining in order to use this parameter in a macroscopic model.

Materials and Methods
Hostun sand ($d_{50} = 220 \mu m$) was used as porous medium in the column experiments. Pore size distribution (PSD) was evaluated with two different methods (see figure 1): Mercury Porosity Intrusion and scanning electron microscope (SEM) images analysis.
Figure 1: Pore size distribution (PSD) measured with mercury intrusion porosimetry (MIP) and scanning electron microscope (SEM) images analysis. Left Y axis corresponds to the relative pore area for SEM images method and right Y axis corresponds to pore volume for MIP method.

A sand column was coated, sawed in slices and observed with a SEM (MEB XL30, Philips) in back scattering electron (BSE) mode (best resolution 1pixel = 690 µm). PSD was then calculated. Foppen et al. (2005) used the PSD function to determine straining retention capacity of their sand. For a non-uniform porous medium with a PSD the volume of retention sites per volume of porous media is:

$$\sigma = \int_0^{d_p} \text{PSD}(x) dx \quad \text{Eq. 1}$$

Eq. 1 means that all pores with a diameter smaller than $d_p$ could be filled with colloids.

110, 260, 450 and 660 nm spherical silica fluorescent nanoparticles were synthesized from a Stöber synthesis (Ow et al., 2005). Concentration of colloids was evaluated by weighing three samples of colloidal solution before and after evaporating in an oven (90°C). Colloids density ($\rho_p$) was measured by differential sedimentation in a CPS disc centrifuge (CPS Instruments Europe) and was equal to 18 kg m$^{-3}$. DLVO profiles were calculated (Redman et al., 2004) for the silica colloids and quartz sand in deionised (DI) water. The profiles exhibited an energy barrier, meaning that these experimental conditions were unfavourable to attachment.

Classical column experiments were conducted (diameter: 2.6 cm and length: 15 cm) with DI water. Bulk density of the sand packed bed was $\rho_d = 1350$ kg m$^{-3}$. The porosity was $n = 0.48$. For each size of colloids, some experiments were duplicated. All experiments were conducted at a constant Darcy velocity of 1.88 cm h$^{-1}$, representative of natural flow conditions in a sandy aquifer. Silica colloids were injected during 0.5 pore volume at a constant input concentration $C_0$. To investigate input concentration influence on colloids retention, this parameter was ranged between 0.03 and 10 mg cm$^{-3}$. At the outlet of the column, colloids were detected by fluorescence (avantes, Avaspec 2048). The relative mass of colloids recovered in effluent $MB_w$ is calculated with eq. 2 from the breakthrough curve.

$$MB_w = \frac{\int_0^\infty C(L,t) dt}{C_0 \tau} \quad \text{Eq. 2}$$

$C_0$ (mg cm$^{-3}$) is the input concentration and, $L$ (cm) is the length of transport in the porous medium $\tau$ (h) is the input duration, $C$ (L,t) (mg cm$^{-3}$) is the colloid concentration measured at the outlet of the column. Following completion of the colloid transport experiment, spatial distribution of colloids in column was determined. Columns were cut in 1 cm slices. Each slice was placed into a 30 cm$^3$ vial. Vials were filled with DI water and shaken during 4 hours (Turbula WAB) to liberate strained colloids. The concentration of colloids in the excess
aqueous solution was measured using a spectrophotometer (Edinburgh Instrument).

The Convection-Dispersion Equation with a term of exchange with the solid matrix was used to model transport and retention (Eqs 3-5). It takes into account a depth dependent deposition rate with a blocking effect and is described by a second order kinetics law.

\[
\frac{n}{\partial t} + \rho_s \frac{\partial s}{\partial t} = (D \nabla c) - \nabla (cv) \tag{3}
\]

\[
\rho_s \frac{\partial s}{\partial z} = nk \left( 1 - \frac{s}{S_{\text{max}}} \right) \left( \frac{d_{\text{z0}} + z}{z} \right)^{-0.43} c^2 \tag{4}
\]

\[
S_{\text{max}} = \frac{\rho_s}{\rho_d} \tag{5}
\]

Where \( s \) (-) is the concentration of colloids on the solid phase, \( z \) is the space coordinate parallel to flow direction, \( D \) is the hydrodynamic dispersion coefficient which was measured with bromide ion experiment, \( k \) is the retention rate and \( S_{\text{max}} \) is the maximal mass of strained particles per unit mass of porous media (kg kg\(^{-1}\)).

**Results and Discussion**

Figure 2 presents the breakthrough curves of the different silica nanoparticles for several input concentrations. Figure 2 (a) refers to the smallest colloids, for all experiments that cover 2 orders of magnitude of input concentrations, more than 97% of the injected colloid mass was recovered from the column effluent meaning that no retention occurred in these conditions. Colloid retention is detectable in experiments with colloids of 260 nm and greater (Figure 2 (b) (c) and (d)). We assumed retention for 260 nm, 450 nm and 660 nm colloids is due to straining because -1- the conditions are unfavourable to attachment; -2- in the same conditions, 110 nm colloids are not retained at all although they have identical surface properties (data not shown).

![Figure 2](image)

**Figure 2.** Normalized breakthrough curves measured in experiments on the transport of (a) 110 nm colloids, (b) 260 nm colloids, (c) 460 nm colloids and (d) 660 nm colloids. For clarity, every 15th data point is plotted.
For each colloid size and concentration, resident and arrival times of breakthrough curves are similar. Only the value of $M_{BW}$ varied. Figure 3 presents experimental and modelled $M_{BW}$ as a function of $C_0$. For the duplicated experiments, the mean value is presented and error bars are standard deviation. For the 110 nm colloids, there is no retention but for the 260, 450 and 660 nm colloids, the retention is concentration dependent. For the 3 sizes considered, as input concentration increases, $M_{BW}$ first decreases until a critical $C_0$ value ($C_{0\text{crit}}$) and then increases. The second part of the curve is explained by the so called blocking effect (Ryan and Elimelech, 1996) and is characterized by $S_{max}$. In the first part of the curve, for $C_0 < C_{0\text{crit}}$, by increasing the flow rate of colloids, the number of colloids travelling through more tortuous pathways or taking low velocities streamlines increases. Indeed, $M_{BW}$ and thus retention rate depends on the input concentration before blocking starts. It means that the rate of filling the straining locations is concentration dependent (e.g. higher colloid concentrations fill straining sites more rapidly than low concentrations) and it justifies the second order kinetic law (Eq. 4). This was not previously shown but it was forecasted in Bradford and Torkzaban (2008).

![Figure 3: Experimental and calculated model described by equations 2-5 $M_{BW}$ as a function of input concentration $C_0$ and colloids diameters ($d_p$). Error bars are standard deviation for duplicated experiments.](image)

Figures 4 (a), (b) and (c) show the spatial distributions of 260, 450 and 660 nm sized colloids, respectively. For the smallest input concentrations, spatial distributions are rather hyperexponential. Under unfavourable attachment conditions, this shape, as reported by many authors (Bradford et al., 2003; Shen et al., 2008; Tufenkji et al., 2003) is an implication of straining (Bradford et al., 2006). The depth dependant retention function, eq 4, (Bradford and Bettahar, 2006) is the dominant mechanism at low concentrations, i.e. before blocking starts and describes the straining shaped profiles. When $C_0$ increases, blocking starts and profiles become more homogeneous. Blocking effect occurs when all sites of retention are filled. Thus, retention sites are homogeneously distributed along the column.
Figure 4: Spatial distribution profiles for (a) 260 nm, (b) 450 nm and (c) 660 nm silica colloids for various input concentrations. Normalized strained concentration (mass of colloid per mass of sand $S$, divided by the mass of silica colloids injected into the column $M_0$) is plotted as the function of distance from the column inlet.

The model takes into account the concentration dependant deposition which was experimentally highlighted. We used the same $S_{\text{max}}$ and $k$ values for each size of colloids (see table 1).

Table 1: Experimental conditions and results for the column experiments and parameters used for model simulations.

<table>
<thead>
<tr>
<th>$d_p$ (nm)</th>
<th>$C_0$ (kg m$^{-3}$)</th>
<th>$M_B$</th>
<th>$S_{\text{max}}$ (kg kg$^{-1}$)</th>
<th>$k$ (m$^3$ kg$^{-1}$ s$^{-1}$)</th>
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<tbody>
<tr>
<td>110</td>
<td>9.87</td>
<td>-</td>
<td>1.08</td>
<td>0</td>
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<tr>
<td>110</td>
<td>4.94</td>
<td>-</td>
<td>1.16</td>
<td></td>
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<tr>
<td>110</td>
<td>3.81</td>
<td>-</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>0.99</td>
<td>-</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>0.10</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>0.67</td>
<td>1</td>
<td>1.1e-5</td>
<td>9e-3</td>
</tr>
<tr>
<td>260</td>
<td>0.15</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>0.07</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>0.03</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>2.33</td>
<td>0.60</td>
<td>1.5e-4</td>
<td>2.15e-3</td>
</tr>
<tr>
<td>450</td>
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<td>0.58</td>
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<td>0.78</td>
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<tr>
<td>660</td>
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<td>660</td>
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<td>660</td>
<td>0.15</td>
<td>0.54</td>
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We used SEM images to measure an upper limit of $S_{\text{max}}$ with eq 5 ($S_{\text{max}} = 1.9 \times 10^{-4}$ kg kg$^{-1}$ for $d_p = 680$ nm). The simulations model rather well the experimental BTC and deposition profiles (see figure 5).

Figure 5: Examples of simulations with model described by equations 3-5, a) profiles of 450 nm colloids and b) breakthrough curves for 260 nm colloids

**Conclusion**

A concentration and depth dependent geometrical deposition was highlighted in natural sand with silica nanoparticles with various sizes and concentrations. A microscopic analysis
of the porous structure allowed to characterise a pore volume responsible for straining. A depth and time dependent deposition model with a second order kinetics reproduces rather well experimental results. $S_{\text{max}}$ parameter has been estimated on SEM images and corresponds to the maximal mass of particles retained in straining sites.

**Literature Cited**


Nanotechnology Regulation Under the Toxic Substances Control Act and the Precautionary Principle

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Keywords: Toxic Substances Control Act, TSCA, precautionary principle, risk, premanufacture

Abstract

The commercialization of nanotechnology has outpaced our understanding of the risks inherent in nanomaterials. The Project on Emerging Nanotechnologies has identified over 800 consumer products containing nanomaterials (2), yet there are significant questions about how to characterize the risks posed by the novel properties of these products (3). The question of how to balance the great commercial benefits of nanotechnology against the potential risks of nanomaterials has been debated for at least the last twenty years (4). The fundamental question is how to approach risk management so long as we have only imperfect information.

The precautionary principle suggests one answer to this question, and the United States is slowly moving towards the precautionary principle for nanotechnology regulation. In February 2009 Congress began hearings on restructuring the primary law relied on for regulating nanomaterials, and a significant impetus is a desire to implement the precautionary principle (5).

The Precautionary Principle

The precautionary principle is a policy and political concept providing guidance on how to regulate emerging technologies like nanotechnology, where there is a need to balance risks and benefits in the absence of complete risk information. Although there are many formulations of the principle, a prominent statement in the Canadian Environmental Protection Act, 1999, says: “Where there are threats of serious and irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.” The precautionary principle has been incorporated into the European Union’s Registration, Evaluation, Authorisation, and Restriction of Chemicals (6) (REACH) law. REACH adds the principle that the entity that intends to commercialize a chemical is responsible for conducting sufficient testing to permit a reasoned application of the precautionary principle.

In 2000 the European Commission wrote a guidance document on the application of the precautionary principle (7). The Commission wrote that the precautionary principle holds that lack of certainty is not a reason to refrain from mitigating risks. The Commission also noted that the precautionary principle operates in conjunction with the science of risk assessment and risk management instead of supplanting science. Consequently it only provides a policy solution when scientific information is incomplete. Risk management tools should still be applied, including a consideration of the proportionality of the response, cost benefit analysis, and analysis of new scientific developments. The Commission noted that all credible scientific opinions should be recognized, even if held by only a minority of the scientific community.

The precautionary principle can prevent the use of newer, safer chemicals if it requires extensive premarket testing for new chemicals while grandfathering older less safe chemicals.
US Laws
No US environmental law specifically regulates the environmental, health and safety effects of nanomaterials by name, but many permit agencies to assert regulatory jurisdiction over nanomaterials. Most of the laws are limited to specific environmental media or specific types of end products. For example, nanomaterials in air can be regulated under the Clean Air Act (8), in water they can be regulated under the Clean Water Act (9), and nanomaterials that are environmental contaminants can be cleaned up under the Comprehensive Environmental Response, Compensation, and Liability Act (10) (Superfund). Another set of laws governs the environmental, health and safety aspects of specific products. As examples, consumer products containing nanomaterials are regulated by the Consumer Product Safety Commission under several statutes, including the Consumer Product Safety Act (11). Pesticides that contain nanomaterials are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act(12), and foods, food additives, cosmetics, drugs and medical devices containing nanomaterials can be regulated under the Federal Food, Drug, and Cosmetic Act (13).

In contrast to the other statutes that give authority to regulate nanomaterials, the Toxic Substances Control Act (“TSCA”) (14) is not limited to specific environmental media or specific types of products (15). It became law in 1976, and was the last of the major environmental laws to be passed. It was designed to fill data gaps on a broad range of chemicals that were not already regulated, and so it gives the US Environmental Protection Agency (“EPA”) sweeping authority to collect existing data on chemicals. Because TSCA has such a broad scope, it is the best suited of all the existing environmental statutes to govern nanotechnology’s numerous applications.

Assessment of TSCA under the Precautionary Principle
TSCA does not incorporate the precautionary principle because it assumes that chemicals and nanomaterials are safe unless the EPA can demonstrate otherwise. It does not give the EPA a mechanism for collecting information when many nanomaterials are made, and imposes often insurmountable burdens on the EPA before it can require risk mitigation (16).

The entry point into the regulatory system for most chemicals is a Premanufacture Notice (“PMN”) which must be filed with the EPA before a chemical is first made or imported for commercial purposes. The vast majority of chemicals in commerce in the US were grandfathered and not subject to PMN requirements. In 1979 the EPA compiled a list of 62,000 chemicals that were already in commerce (17). That list is called the TSCA Inventory. The chemicals on the initial Inventory were not subject to any mandatory toxicity or safety testing (18). Under TSCA, no one can make or import a chemical substance that is not on the Inventory unless that person files a PMN with the EPA, with certain exemptions and exceptions (19). Since the initial Inventory was compiled approximately 21,000 new chemicals have been added to the Inventory through the PMN process (20). When the EPA reviews a PMN it has the opportunity to either negotiate or unilaterally impose restrictions on the subject chemical. A prospective manufacturer or importer must submit existing data it has with a PMN but there is no requirement to conduct new studies (21). Most PMNs do not contain any test or health and safety data. Sixty-seven percent have no test data of any kind, and eighty-five percent have no health and safety data (22). The EPA has taken the position that a nanomaterial that has the same “molecular identity” as a substance on the Inventory does not need to go through the PMN process (23). Therefore, the EPA will not have an opportunity to regulate many nanomaterials through the PMN process.
The EPA can require new studies on nanomaterials made from chemicals on the Inventory only if it follows a time consuming rulemaking procedure and can demonstrate that the substance “may present an unreasonable risk of injury to health or the environment.” (24). Alternatively the EPA can require new studies if a substance will be produced in “substantial quantities” and is expected to “be in the environment in substantial quantities”, or “there is or may be significant human exposure” (25) but it is unlikely these could be shown for nanomaterials which are typically made in small quantities. The EPA has issued only twelve test rules for specific chemical families (26) and four rules that cover multiple types of chemicals (27).

Moving Towards Implementation of the Precautionary Principle

It is apparent that the EPA is actively searching for additional means to both gather information and impose restrictions consistent with a precautionary approach to nanomaterial regulation. Faced with barriers to mandating studies on the properties of nanomaterials under TSCA, the EPA is using a voluntary program to collect data. EPA kicked off its Nanoscale Materials Stewardship Program (“NMSP”) in January of 2008 (28). Two of its objectives are to identify risk management practices for nanotechnology, and to gather test data to develop regulatory policies. The NMSP is in two phases – the basic program called for submission of existing data on material characterization, hazard, use, potential exposures, and risk management practices. Twenty-nine entities submitted existing data for the basic program, and seven more committed to submit information (29). The second phase is an in-depth program which is ongoing. Participants will sponsor new studies that may address physical characteristics, health and environmental effects, fate and transport, monitoring or estimating exposures or releases and an evaluation of engineering controls and personal protective equipment. To date only five companies have committed to conduct new studies (30). The EPA issued an interim report in January 2009 (31) and found the basic program a success, although there were few participants and the EPA acknowledged that very few submittals had toxicity or fate studies (32).

In October 2008 EPA clarified its position that unless a carbon nanotube is already on the Inventory as that specific nanotube, manufacturers and importers must submit a PMN before manufacturing or importing it (33). Therefore new nanotubes will have an entry point into the regulatory process. In fact, one company has announced that it has entered into two consent orders with the EPA following submission of PMNs on nanotubes (34). Typical consent orders impose restrictions on use and handling of substances until specified studies are performed.

In November 2008 the EPA issued a Significant New Use Rule on two nanoparticles (35). This rule requires further notification to the EPA before use of the nanomaterials for any purpose other than as an additive, or without specified personal protective equipment or in powder form. EPA stated the rule was issued because of data on “analogous respirable, poorly soluble, particulates”. This indicates that EPA will likely issue more rules requiring notification for nanoparticles and continue to issue rules that encourage, if not require, risk mitigation.

If the EPA’s attempts to implement aspects of the precautionary principle are unsuccessful, Congress may take action. As Congressman Bobby L. Rush said, “the statute is supposed to provide EPA with adequate regulatory tools to protect the public from unreasonable risk of injury to health or the environment. Unfortunately, the statute has seemingly been a failure ...” (36). Whether Congress will move in this direction, given the current economic challenges and the precautionary principle’s past history of keeping innovative products from quickly reaching the market, is yet to be seen.
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(1) With thanks to Miriam V. Gold, Esq. for her comments.
(2) The Project on Emerging Nanotechnologies is a project of the Woodrow Wilson International Center for Scholars. An analysis of data collected through August 2008 is available at http://www.nanotechproject.org/inventories/consumer/.
(8) 42 U.S.C. § 7401.
(9) 33 U.S.C. § 1251.
(10) 42 U.S.C. § 9601.
(15) The sole exceptions are that TSCA does not permit regulation of pesticides regulated under FIFRA, certain radioactive materials regulated under the Atomic Energy Act of 1954, 42 U.S.C. § 2011, food, food additives, drugs, cosmetics, or medical devices regulated under the FFDCA. Tobacco, firearms, shells and cartridges are also excluded from TSCA’s scope. TSCA § 3, 15 U.S.C. § 2602.
(18) EPA initiated a voluntary program for high production volume chemicals which resulted in analyses of 2,200 chemicals produced in volumes of one million pounds or more per year. United States Environmental Protection Agency (April, 2009). "High Production Volume (HPV) Challenge." http://www.epa.gov/oppt/chemrtk/index.htm.
(32) NMSP Interim Report, 9.
Investigation of the Adsorption Characteristics of Antimony, Cadmium, and Lead by Nano- and Microparticle Titania

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Abstract

The increasing ubiquity of nanotechnology, coupled with the fact that the toxicity of a nanomaterial is partially dependent on its adsorbed components, emphasizes the general importance and environmental significance of nanomaterial adsorption studies. The purpose of this research project was to investigate the adsorption characteristics of the environmentally significant toxic trace elements antimony, lead, and cadmium, using titania microparticles (µ-TiO2), and titania nanoparticles (n-TiO2) as potential metal sorbents. The Sb, Cd, and Pb adsorption affinity of nanoparticle titania was compared to that of microparticle titania (µ-TiO2) and activated carbon. In a typical adsorption experiment, a known amount of nanomaterial was shaken with a solution containing a predetermined concentration of antimony, lead, and cadmium. Following an equilibration period, the remaining solution analyzed using either inductively coupled plasma-mass spectrometry (ICP-MS) or atomic emission spectroscopy (ICP-AES). In the case of nanoparticle titania (n-TiO2) and microparticle titania (µ-TiO2), the effect of light and adsorption on antimony species [Sb(V) and Sb(III)] was also investigated, by using ion chromatography (IC) coupled to ICP-MS (IC-ICP-MS). Results indicate that Sb, Cd, and Pb were best adsorbed by nanoparticle titania, followed by activated carbon and microparticle titania. Adsorption data were fitted with Langmuir and Freundlich isotherms, and both provided good fits for the data. In general, trace element adsorption by activated carbon and nanoparticle titania was best fit by the isotherms. Results of the antimony speciation studies indicate that Sb(III) was oxidized to Sb(V) and adsorbed by microparticle titania; when nanoparticle titania was used as the adsorbent, however, Sb(III) oxidation cannot be confirmed because complete antimony adsorption always occurred at the concentrations studied. The presence of ambient light had only a small effect on adsorption and oxidation; antimony adsorption by microparticle titania was more complete in the absence of light. It is evident that nanoparticle titania could play a vital role in mobilizing trace metals such as lead, cadmium, and antimony.

Introduction

The application of nanotechnology is expected to increase significantly within the next decade. The increasing uses of nanotechnology, coupled with the fact that the toxicity of a nanomaterial is partially dependent on its adsorbed components, emphasizes the general importance, and environmental significance, of nanomaterial adsorption studies (Wiesner et al. 2006, Colvin, 2003, Dreher, 2004).

Nanoparticle titania is a nanomaterial that has been increasingly utilized. It has been used in paints as both a whitening agent and anantifungal. In the presence of ultraviolet light, it is capable of hydrolyzing water into hydrogen and oxygen. Dye-sensitized photovoltaic cells utilize titanium dioxide for its semiconducting properties. Because of the increased utilization of nanoparticle titania, the interaction of the toxic trace elements Pb, Cd, and Sb with...
nanoparticle titania was investigated. Furthermore, because speciation has been shown to be affected by adsorption to nanoparticle titania, the effects of nanoparticle titania adsorption on Sb adsorption were also explored (Mukhopadhyay and Lahiri, 2007, Giammar et al., 2007).

The purpose of this investigation was to investigate the adsorption characteristics of the environmentally significant toxic trace elements antimony and their inorganic species [Sb (III) and Sb (V)], lead, and cadmium, using titania microparticles (μ-TiO2), and titania nanoparticles (n-TiO2) as potential metal sorbents.

**Experimental Methods**

Solutions of either 50 ng/mL Sb(III) or Sb(V) were allowed to equilibrate during a 48 hour equilibration period in the presence of 2 mg/mL nanoparticle or 2 mg/mL microparticle titania at room temperature. Ion chromatography (IC) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) was used to separate antimony species in the solution phase (Steely et al., 2006, Cerrotti and Amarasiriwardena, 2009).

In order to study the adsorption affinity between nanoparticle (325 m²/g surface area) and microparticle titania and the trace elements Cd, Pb, and Sb, known concentrations of the given trace element absorbents were allowed to equilibrate a 7-day equilibration period at room temperature. Following the equilibration period, separated solutions were then analyzed using ICP-MS or inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

Using data obtained from the analysis, results were fit to Freundlich and Langmuir isotherms.

**Results and Discussion**

Results (see Figure 1a-d) indicate that, of the adsorbents studied, nanoparticle titania is the better adsorbent of cadmium, lead, and antimony, when compared to microparticle titania, and obviously it may be due to the high surface area of nano titania. For both micro and nano titania, at pH 6.3 both the Langmuir and Freundlich equations provided good fits of the data. In general, however, the fits for the microparticle titania were not as satisfactory as those for nanoparticle titania.

The calculated Langmuir parameters suggest that the cadmium monolayer adsorption capacity (μg/g) is higher (q_m n-TiO2 = 1.0 x 10⁶; q_m μ-TiO2 = 8.4 x 10⁵) than that of antimony (q_m n-TiO2 = 2.9 x 10⁴; q_m μ-TiO2 = 5.5 x 10³) or lead (q_m n-TiO2 = 1.0 x 10⁵; q_m μ-TiO2 = 1.4 x 10⁴), for both micro- and nanoparticle titania. However, this observation has some limitations, as the Langmuir model does not account for the possibility of multilayer adsorption.

Results of the antimony speciation studies indicate that microparticle titania (μ-TiO2) adsorbed the Sb(III), which was subsequently oxidized to Sb(V) (Figure 2a). In the case of nanoparticle titania (n-TiO2), it is unknown if Sb(III) was oxidized to Sb(V) in solution, as all antimony was completely adsorbed by the nanoparticles at the concentrations studied (Figure 2b). The presence of ambient light had only a small effect on adsorption and oxidation; antimony adsorption by microparticle titania was more complete in the absence of light (Figure 2c-d).
Conclusions

It is evident that nanoparticle titania could play a vital role in mobilizing trace metals such as lead, cadmium and antimony. As expected, micro titania has the capability to convert Sb(III) to the less toxic Sb(III). It is unknown if Sb(III) was converted to Sb(V) by n-TiO₂, as both antimony species were completely adsorbed. Nanoparticle titania was found to be a superior adsorbent of Cd, Pb, and Sb, but it is unknown if titania nanoparticles containing adsorbed toxic metals would be, in practice, any more toxic than pristine titania nanoparticles.

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Literature Cited


