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Chapter 6 – Introduction

Toxicity & Risk Assessment of Nanomaterials in the Environment

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Integral to a discussion of nanotechnology is consideration of the implications of using these novel materials. One critical implication, nanomaterial toxicity, was the focus of plenary, concurrent, and poster presentations. The importance of this topic was clearly laid out in the conference opening when Jeff Morris challenged the meeting attendees to seek to understand which properties and characteristics of nanomaterials relate to their toxicity and to identify and investigate doses relevant to real-world exposures.

Plenary speaker Anne Fairbrother observed that much data are needed, not only on numerous classes of nanomaterials but on key variables that influence toxicity. Examples of these variables are biological species tested, toxicity tests used, test sample preparations, entry portals, dose metrics, and modes of action. In a complementary plenary, JoAnn Shatkin focused attention on risk analysis and placing priority on establishing what we know about toxicity and dose-response in the context of realistic exposures.

These plenary presentations set the stage for the concurrent sessions, which provided insights on how the field is responding to this pressing issue. One theme that emerged throughout the talks was the importance of characterizing nanomaterials. The sheer variety of nanomaterials presents huge obstacles for comprehensive assessments of these novel substances.

The papers demonstrated that investigations have progressed beyond evaluating the effects on toxicity by fundamental nanomaterial characteristics such as size, shape, charge and purity. Investigations have broadened and matured to consider variables such as aggregate size, chlorination, coatings, dispersants, hydrophobicity, pH, reactivity, redox potential, surface area, and reactive oxygen species (ROS) generation. A wide range of nanomaterial types are being studied as well, including single- and multi-walled nanotubes, fullerenes, and nanoparticles composed of silver, nickel, titanium dioxide, iron, and aluminum oxides.

To conduct these studies, investigators have used a diverse array of test species from non-mammalian taxa such as microbes, aquatic species, and terrestrial invertebrates to mammalian systems such as mice, rats, and human cell lines. In addition to the diversity of test species being investigated, a multitude of endpoints are being used to assess toxicity, including oxygen uptake rates, gene expression, mobilization and internalization of particles, oxidation, neurotoxicity, fate and biological uptake, protein expression, and cell function.

Several general principles regarding nanomaterial toxicity emerged during the toxicity sessions:

- Nanotoxicity is often associated with ROS

- Characterization of the nanomaterials tested is critical to data interpretation
- Aggregation typically reduces toxicity
- Aggregation and agglomeration are dynamic processes, so dissociation can occur over time and, therefore, toxicity can also change over time

In summary, these studies demonstrate that nanomaterial toxicity is extremely complex. Despite the vibrant and maturing field of nanotoxicology, our understanding is very limited. Because our current understanding of the environmental implications of nanomaterials is so limited, there is a large gulf between it and the flourishing development of new nanomaterials and applications. A prudent approach to this disparity is to develop best practices and to be clear and transparent about what we know, as well as what we don't know but need to know.

Managing the Risks of Nanomaterials

Anne Fairbrother
Exponent, Bellevue, Washington, U.S.A.

My purpose in presenting this material is to provide a context within which to consider the risks of nanomaterials, either as they are applied directly to the environment for remediation of contaminated sites, or indirectly through manufacturing, use, and disposal. Although delegates to this conference are no doubt well informed about particular aspects of nanotechnology and its products, it often is useful to review basic principles. I will describe the classes of nanomaterials and general uses of the various types of manufactured nanoparticles. This will include a review of the properties of nanoparticles that differentiate them from macroscale products, particularly with regard to potential human health and environmental effects. Understanding how nanoparticles move through environmental media is fundamental to assessing risks, so I will review what is known and where research is needed to develop methods for quantifying nanoparticles and describing exposures. I will briefly discuss what currently is known about how nanoparticles affect people or plants and animals. Finally, I will review the current regulatory initiatives being considered for environment, health, and safety.

Classes and Types of Nanomaterials

Many nanoparticles occur as a result of natural processes. They are formed from sea-spray, volcanic eruptions, lightning strikes, and forest fires. Colloidal materials in streams contain nanosized particles, including humic and fulvic acids, proteins, and peptides. Hydrous iron and manganese oxides occur naturally in nanosized particulates, as do clays and minerals such as asbestos. Some nanoparticles enter the environment as combustion by-products, from fires, various types of industrial air pollution, and from running internal combustion engines (e.g., automobiles). These are referred to in regulatory language as <PM10 particulates, meaning they are particulate matter less than 10 microns in size.

Another class of nanomaterials is those that are deliberately produced, although when they were first manufactured it was not possible to do so precisely, so they were not uniform in size and shape. These include materials such as carbon black (a type of soot) in particle sizes of 10–400 nanometers for use in tires, rubber, plastics, pigments, and toner for laser printers and silica fumes (a byproduct of making silicon metal and alloys) that are used as additives for cement and in foods, plastics, and pharmaceuticals. With the invention of the scanning probe microscope, the size, shape, and surface characteristics of deliberately made nanomaterials could be more closely controlled and engineering of nanomaterials became possible. Nanomaterials also could be engineered as combinations of two or more substances, such as silicon coated titanium dioxide used for sunscreens.

Uses of Manufactured Nanomaterials

Once manufactured nanoparticles could be precisely engineered, their use in consumer products increased exponentially. At the time of this writing there are more than 800 such products (www.

nanotechproject.org/inventories/consumer/), and there likely will be thousands within the next few years. Examples include titanium dioxide and zinc oxide as photolytic and UV blockers in paints, sunscreens, cosmetics, bottle coatings, and cement. Metal nanoparticles also are used in mineral supplements (e.g., zinc, palladium, titanium, indium) and as combustion catalysts (such as cerium dioxide in diesel fuels and nickel dioxide in fuel cells), thin films on solar cells, gas sensors, refrigerants, magnets, and in medical imaging. Quantum dots are manufactured from metals or metal oxides surrounded by a silica shell and have unique electronic, optical, magnetic, and catalytic properties. They range in size from 1,000 to 10,000 atoms and are used, for example, in semiconductors, electronics and computing, photovoltaic cells, and light emitting diodes. Zerovalent iron is produced by reduction of solutions of metal salts and has been used to remove nitrates from soils or to detoxify organochlorine pesticides and polychlorinated biphenyls (PCBs) in contaminated soils or sediments. Nanosilver is the most well-known metallic nanomaterial because of its antimicrobial properties. It has been used, for example, as a deodorizer in socks, as a whitening agent in soaps, and in washing machines.

Nonmetallic nanomaterials include fullerenes. Fullerene is a generic term used to describe carbon nanoparticles that take the form of hollow spheres or tubes. They are similar in structure to graphite. While graphite is composed of flat sheets of carbon atoms arranged in hexagons, fullerenes contain different arrangements of the carbon atoms that form three-dimensional structures. The smallest and most common fullerene, C60, is a sphere of sixty carbon atoms. Nanotubes are similar in structure to C60 but are elongated to form a tubular structure, usually one to two nm in diameter and up to 1 mm long.

The simplest nanotubes are a single layer of carbon atoms arranged in a cylinder (single-walled carbon nanotubes). They also can consist of multiple concentric tubes (multi-walled carbon nanotubes) with diameters up to 20 nm and lengths greater than 1 mm. Carbon nanotubes are synthesized from graphite using an arch discharge or laser ablation process. They can be made more water soluble by the attachment of polyethylene glycol or phospholipids, which also reduces the tendency of all nanoparticles to agglomerate. Carbon nanotubes are very strong (nearly 500 times stronger than steel), very light (about one-sixth the weight of steel), and are about 10 times more conductive than copper. They have been used in plastics and other composites to increase strength, in flat panel displays, and in energy storage devices.

Why Are Nanomaterials Different?

Nanoscale substances behave differently than their macroscale counterparts because of their small size. An example can be seen in the color of gold. Macroscale gold is a shiny orange-yellow color. The same is true of a particle of gold 100 nm wide, but between 100 and about 30 nm, gold is purple, and at 30 nm in size, a gold particle is bright red. Smaller gold particles become brownish in color. Below about 100 nm, the behavior of elements follows the rules of quantum physics. Gravity is not important, and van der Waals forces (1) govern the attraction/repulsion behavior between particles. Surface charge becomes very important and agglomeration of particles occurs readily. Nanoscale particles easily penetrate cell walls and membranes without the requirement of phagocytosis for entering cells. Phase dispersion (such as octanol / water partitioning) is not predictable, because nanoparticles can get caught in the meniscus between the two phases. Nanoparticles are highly chemically reactive because of their high surface to volume ratio. They can be made efficiently and cheaply either through self-assembly of atoms

(an inherent property of nanomaterials) or through pulverization of macroscale materials.

Transport and Fate of Nanomaterials

Nanoparticles can be highly mobile in the environment, but their mobility tends to be reduced through agglomeration into large particles. Agglomeration increases deposition rates of nanoparticles as a result of their propensity to attach to mineral surfaces. Although there are good models to predict agglomeration and/or deposition of spherical particulates to form packed beds of spheres, such models are not yet available for agglomeration and behavior of nanoparticles that have nonspherical shapes and complex surfaces. This makes prediction of transport and fate in environmental media (e.g., water and sediment) difficult for nanosized particles. The field of colloid physics provides a basis for such studies, but traditionally has not studied solid state materials or the type of multi-layered, multi-chemical particles found in manufactured nanomaterials. However, for properly assessing risks of nanomaterials in the environment, it is critical to know how to measure and predict the persistence, agglomeration, binding to lipids and/or organic matter, hydrophobicity, and other properties of nanoparticles (Klaine et al. 2008).

It is likely that sediments are the final sink for nanoparticles in aquatic systems (Klaine et al. 2008). Adsorbed organic matter will stabilize charges on particles and cause fibrils to form, which may cause particles to agglomerate via bridging mechanisms. Sediments also will stabilize pH, and calcium ions and natural colloids (e.g., clays, fulvic and humic acids) will retain nanoparticles. Studies on nanoparticle mobility in porous media, such as groundwater aquifers or sand filters, have shown that mobility is a function of surface chemistry and particle size (Wiesner et al. 2006). High ionic strength and divalent ions will increase retention of nanoparticles in porous media. Groundwater aquifers and surface water with ionic strengths of >10–4 M and significant concentrations of calcium or magnesium should favor nanoparticle deposition (Wiesner et al. 2006). Even the most mobile nanoparticles should be removed in sand filters during municipal wastewater treatment using conventional technology. However, nanoparticles are frequently coated with polymers, polyelectrolytes, or surfactants that change surface charges. Nanoparticles intentionally released into the environment to remediate contaminated soils or sediments, such as zerovalent iron, have polymer coats to reduce agglomeration and increase water solubility. This decreases the efficacy of water treatment systems. Metal oxide quantum dots and other manufactured nanomaterials have similar coatings. Although not intentionally released into the environment, they may be released through wastewater or other disposal methods.

Salinity significantly increases agglomeration of nanoparticles (Niehof and Loeb 1972). This is important in studying in vivo exposures of people and animals to nanoparticles, as blood is a relatively saline solution. Marine organisms experience lower exposure to nanoparticles than do their freshwater counterparts because of the salinity-induced agglomeration, which results in lesser toxic responses (Kashiwada 2005). Additionally, currents and thermoclines in marine environments affect the transport and movement of nanoparticles in the water column. Therefore, predictions of transport and fate of nanoparticles in marine systems cannot be accurately predicted from studies in freshwater systems.

Redox transformations are very important in the degradation of organic compounds and also affect precipitation and dissolution reactions. These are mediated by microorganisms through

enzymatic activity and indirectly through formation of reactive oxygen species. It is not known if nanomaterials can be transformed by redox processes. It is plausible that fullerenes could be oxidized (e.g., hydroxylated) by soil fungal enzymes such as cytochrome P450, peroxidases, or lactases, as they have a high propensity to accept electrons (Wiesner et al. 2006). This is an area in need of research.

Measuring Nanomaterials in the Environment

A variety of methods are available for characterizing nanoparticles. These include high resolution imaging techniques such as scanning and transmission electron microscopy that can be used to measure the shape, size, and crystalline nature of the particles. Scanning probe techniques such as atomic force microscopy also measure the size and shape of nanoparticles. UV-vis spectroscopy, infrared spectroscopy, and x-ray photon spectroscopy are tools for studying the surface chemistry of nanoparticles. X-ray diffraction measures the surface area of particles. Solubility of nanoparticles can be measured by ultrafiltration, dialysis, and flow-field fractionation coupled to ICP-MS (Klaine et al. 2008). However, none of these methods is well-suited to quantifying the amount of nanoparticles in environmental media (soil, sediment, or water). Further, it can be difficult to separate nanomaterials from natural colloidal matter. Experimental methods such as near-field acoustic holography (Shekhawat and Dravid 2005) show promise, but currently are too expensive and time-consuming to be applicable to high throughput environmental screening. Measurement of nanoparticles in environmental media remains an area of high research need for environmental monitoring and risk assessment.

Effects of Nanomaterials

Studies of the effects of nanomaterials on biota and people began only a few years ago, but are increasing exponentially. This section of my presentation provides an overview of the types of effects that may be observed and considerations for standardizing and improving toxicity tests on different types of organisms.

Aquatic organisms are exposed to nanoparticles primarily through gut intake followed by translocation within the body (Roberts et al. 2007; Fernandes et al. 2007). Terrestrial animals are exposed through the lung (inhalation) and gut (diet), while plants are most likely to be exposed via root uptake. Nanoparticles can diffuse through the cell membrane or can be taken up by adhesion and endocytosis. They are not dependent upon the circulatory system but can move through the body via cell-to-cell contact. This is a very important consideration in understanding nanoparticle distribution and metabolism within organisms. Potential mechanisms of toxic action within an organism include: disruption of membranes or membrane potential, formation of reactive oxygen species, oxidation of proteins, interruption of energy transduction, release of toxic constituents, and genotoxicity (Klaine et al. 2008). Antibacterial activity occurs as a direct contact between a positively charged nanoparticle and the bacterial cell surface. This changes the surface phosphorylation and membrane permeability, causes oxidative stress and formation of highly reactive epoxides resulting in DNA damage, and affects the integrity of the bacterial cell membrane (Klaine et al. 2008).

Using cell cultures, fullerenes have been shown to have antibacterial properties, to be cytotoxic to human cell cultures, to cause DNA damage, and to inhibit protein folding. In laboratory

rodents, single-walled carbon nanotubes have been reported to cause lung granulomas and necrosis, pneumonitis, and activate immune cells (Wiesner et al. 2006). Metal nanoparticles have also been shown to cause pulmonary inflammation, alveolar degeneration, and DNA damage in rodents (Handy et al. 2008; Wiesner et al. 2006). Effects in aquatic organisms have been summarized by Handy et al. (2008), Klaine et al. (2008) and more recently by Ziccardi et al. (2008). These include oxidative stress and lipid peroxidation in liver, gill, and brain of fish, along with glutathione reduction in the gill reported as a result of fullerene exposure. Cytochrome P450 enzyme expression may be downregulated and gill pathology is evident in some fish exposed to fullerenes. Fullerenes and metal nanoparticles have also been reported to cause delayed hatch, reduced growth, increased molting, oxidative stress, and mortality in various aquatic invertebrates. However, evidence of effects appears to be very dependent upon test conditions, particularly methods used to solubilize the nanoparticles and get them into solution. Toxicity to terrestrial organisms has been studied in plants and soil microbes (summarized by Klaine et al. 2008). Exposure to metal nanoparticles has been reported to reduce root elongation and decrease seed germination, depending upon species, test conditions, and concentrations in soils. Conversely, Yang et al. (2007) showed increased seed germination and photosynthesis in spinach exposed to titanium dioxide nanoparticles. Soil microbes are not affected by exposure to fullerenes (Johansen et al. 2008).

Results of studies of effects of nanoparticles on both aquatic and terrestrial species depend greatly upon the test conditions. Because such tests have not yet been standardized, it is difficult to make comparisons among species exposed to the same nanomaterials or within species using different particle types. Questions for standardizing aquatic test systems include (but are not limited to): how do pH, hardness, ionic strength, or organic ligands affect toxicity? Is water the correct exposure route or is diet more appropriate? How should particles be solubilized (e.g., sonication, stirring, functionalization using tetrahydrofuran)? Should both water soluble forms and agglomerates be tested (e.g., water column versus sediment organisms)? What methods are available for measuring exposure concentrations? For terrestrial bioassays, should the particles be mixed into soil by spraying a solution onto the soil or by mixing in dry particles? Because particles adhere to the soil solid phase, how should exposure be measured and expressed?

For all studies, it is important to characterize the stock solution to quantify the average and range of particle sizes used in the study. It is also important to measure particle sizes throughout the experiment as changes may occur because of agglomeration and other factors. There also is a question about how exposure should be quantified. Should exposure be expressed in terms of surface area, or number of particles per unit volume (ng/L), or mass (ng/kg)? There is no apparent reason for not using standard aquatic and terrestrial test organisms in bioassays to assess nanoparticle toxicity, although it is not known which organisms represent the most (or least) sensitive species.

Regulatory Concerns for Nanomaterials

While the science of nanomaterials is not new, applications of nanoparticles to consumer products and their use in commerce is relatively recent. Recognizing the commercial potential of this emerging technology, governments are providing billions of dollars in research funding for nanomaterials science and commercialization. However, very little funding has been available to study the environmental fate, human health and safety, or ecological effects of

the manufacture and use of nanoparticles. In the U.S., only 5 percent of the 2009 $1.5 billion budget for the Nanotechnology Initiative is directed toward environmental issues and human health and safety. At the time of this writing, the U.S. has not proposed any new approaches to assessing the risk or regulating nanomaterials, as existing laws provide the framework for doing so. Products containing nanosilver used as a biocide (e.g., in washing machines or socks) are regulated under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) in the same manner as other biocidal products. Carbon nanotubes recently were determined to be new substances and, as such, require registration under the Toxic Substances Control Act (TSCA). Similarly, silica and alumina nanoparticles have been designated a "significant new use" and are regulated under section 5(a)(2) of TSCA. EPA's Nanoscale Materials Stewardship Program provides a framework for voluntary submission of exposure and effects information. California has asked manufacturers of carbon nanotubes to monitor worker health (though inhalation) and report exposures and associated effects. Canada recently instituted reporting requirements for nanomaterials used in quantities greater than 1 kg/yr. Australia has been working to develop an International Organization for Standardization (ISO) nanomaterial standard for occupational health and safety, but primarily funds innovation and development in manufacturing. In Britain, the Department of Environment, Food, and Rural Affairs (Defra) established a nanotechnology research coordination group and commissioned several white papers on the topics of health and safety (e.g., Crane and Handy 2007). At the time of this writing, Britain's Health and Safety Executive advised companies or universities supplying carbon nanotubes to include health and safety information on their materials (including "Caution: substance not yet fully tested") and an indication of the concentration of the substance in the material. Several nongovernmental organizations, industries, and governments have called for responsible production and use of nanomaterials through a better understanding of potential effects and standardized approaches to testing and regulation. Most notable are the German Chemical Industry Association (VCI) who published several white papers on the topic (VCI 2008), and Environmental Defense in partnership with DuPont, who published a nanorisk framework (Environmental Defense - Dupont 2008). The Scientific Committee on Emerging and Newly Identified Health Risks in the European Union has recognized that risk methodologies require modifications for use with nanomaterials, stating that additional guidance is needed on how to conduct standardized toxicity tests. The Organization for Economic Cooperation and Development (OECD) established a Working Party in 2006 to address these and other questions for evaluating risks of manufactured nanomaterials in the workplace and environment.

Conclusions

The science of nanomaterials and its commercial applications continues to grow exponentially, as do concerns about possible environmental, health, and worker safety risks from exposure to nanoparticles. Assessing potential risks is hampered by lack of information on transport and fate of nanoparticles in the environment and standard methods for assessing effects to aquatic or terrestrial organisms and human health. We need to know how to prepare and characterize test materials. We need information about the full life-cycle of products, and how the associated nanoparticles may move into and through the environment. We lack methods for measuring and tracking nanoparticles in water, soil, or sediment and have no standard quantification metric. Regulatory agencies continue to struggle with whether to define nanoparticles as new or existing substances or as substantially new uses of existing substances. Appropriate values that would

trigger requirements for additional testing or product controls are lacking. There is no doubt that the public has been calling for an increased level of precaution with regard to nanomaterials, as has happened with other emerging technologies (e.g., biotechnology). Although we can learn many basic principles from the science of colloidal physics and chemistry, manufactured nanomaterials have unique properties that affect both exposure and potential effects. Without appropriate funding to develop test and measurement methods and to address the risk-related questions described in this presentation, both regulators and the public are left without the necessary tools to make informed decisions about risk management of nanomaterials.

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Advancing Risk Analysis for Nanomaterials and Nanotechnologies

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Introduction

Decisions about the use and acceptable levels of substances in products and in the environment are increasingly determined using quantitative risk analysis. There are many questions and few answers about the toxicity of nanoparticles, stemming in part from an inability to adequately measure or predict the key characteristics of particles as they relate to toxicity.

Risk analysis as it relates to health and the environment encompasses a multi-disciplinary set of methods to assess the levels at which substances may cause harm and to judge whether the associated risks are acceptable. Risk analysis involves both science and judgment; the science of characterizing materials, their toxicity, and their exposure characteristics, is weighed against other materials and standards established as acceptable by society or within organizations. Terminology varies across disciplines and organizations, but the assessment phase of risk analysis generally consists of the following steps: hazard characterization, exposure assessment, dose response analysis, and risk assessment. Underlying each of these steps is the judgment of the available data, interpretation according to models, assessment of uncertainty, communication, and evaluation of the acceptability of risk levels in societal context.

The rapidly expanding development and use of materials in the nanoscale range has generated new challenges to the application of current risk analysis methods for environmental, health, and safety concerns. The unique properties that may exist for these materials potentially have significant implications for current approaches to the hazard identification, exposure assessment and dose-response components of the traditional risk assessment paradigm that informs risk management decisions, and may confound the accurate assessment of potential risks as well as require changes to the way such risks are communicated to stakeholders and managed by policymakers.

One of the challenges for risk assessments of nanoscale materials is that the small size of materials conveys a much greater ratio of surface area to mass, which affects behavior in biological and environmental systems, generally increasing activity over larger scale materials. Currently, there is a poor understanding of the key factors that contribute to these behaviors, which have been noted in literature and among convened experts to potentially include a breadth of interrelated factors, including: surface area, surface charge, surface chemistry, porosity, aggregation and agglomeration state, particle size and distribution, and level of contamination. A further complication with assessing risks at the nanoscale is that materials produced are best described as mixtures, that is, as produced they tend to have a breadth of sizes, impurities, and characteristics, and they are generally combined with other materials for applications. The field of risk analysis has been challenged by the issues raised in analyzing and assessing the risks from mixtures, and this complexity is compounded with poorly characterized mixtures at the nanoscale.

The biggest issue in using risk analysis for decision making about nanomaterials is the current lack of data to inform assessments. There is a lack of agreement on how to characterize engineered nanoscale materials for toxicological studies, combined with limitations in the availability of methods for measuring them. The available data are generally in systems with limited applicability to real world exposures, such as in vitro assays that have not been validated against in vivo data. Only a few studies have measured exposure levels, and environmental models for estimating exposures to nanomaterials also have limitations, since the unique properties of nanomaterials appear to also extend into the properties that dominate transport and fate in complex biological and environmental systems.

To address these challenges, the Emerging Materials and Nanomaterials Specialty group (EMNMS) of the Society for Risk Analysis organized a public/expert workshop, Nano Risk Analysis: Advancing the Science for Nanoscale Material Risk Management held on September 10-11, 2008, in Washington, DC. The workshop created a multidisciplinary discussion among experts in risk analysis, nanotechnology researchers, environmental science, other key stakeholders and members of the public interested in risk analysis, risk communication, and nanotechnology to identify approaches for risk analysis that assess the unique aspects of nanotechnology and nanomaterials. To facilitate the discussion, five topical white papers, each co-authored by a combination of nanotechnology and risk experts, were drafted and presented on topics of: hazard identification and uncertainty; toxicology; exposure assessment; risk characterization; and risk communication. These papers were vetted in plenary and facilitated deliberative discussion sessions and will become a series of publications. For each topic, authors considered the challenges posed by nanomaterials and nanotechnologies, and the opportunities to apply the tools and methods developed for risk analysis to these current concerns.

One conclusion of the workshop was that despite current limitations, risk analysis remains a valid approach to identifying, assessing and informing the management of engineered nanoscale materials. This was also a conclusion of the European Food Safety Authority. Below, the issues nanotechnology and nanomaterials raise for each step of the risk analysis process are identified, and ideas discussed and issues raised at the Nano Risk Workshop are summarized, in the context of other international efforts to address risks from nanomaterials and nanotechnologies.

Specific Considerations For Each Step Of Risk Assessment

Many of the uncertainties introduced by nanomaterials are not novel for risk assessment. Radiation, respirable fibers and other particles also challenge classical toxicology models, required defining new metrics and tolerable levels. Many of the complexities remain issues for legacy and emerging substances in the environment – determining threshold levels versus background (need to monitor background levels), age dependent dosimetry, body burdens from non-engineered sources, defining internal dose –as was done for radiation, fractionation of mixtures, and consideration of cumulative exposures. None of these sources of complexity are specific to nanoscale materials.

Every step of the risk analysis process includes uncertainty. There are additional challenges due to the physical dimensions of nanoparticles and the limited understanding and ability to predict their impact on biological and environmental risks. New tools are needed to estimate and measure real world exposures. Others have addressed the need to define and prioritize research

needs for dose-response, exposure assessment and material characterization of engineered nanomaterials in the short and long term. In particular, needs include fate and transport models, biomarkers, and incorporating new scenarios for exposure (e.g. worker exposure during recycling).

A few key points to keep in mind regarding the state of the science of nanomaterial EHS risks:

- Nanomaterials may behave like particles and have physical effects in addition to chemical effects, which may require new measurements and assessments to determine;
- Material properties such as surface area, particle size, particle number, and aggregation state may be important determinants of toxicity at the nanoscale;
- Existing mass- and concentration-based environmental health and safety (EHS) thresholds may not be adequate to assess health and environmental risks of nanoscale particles to workers and others;
- Small changes in particle size and surface properties may drastically affect toxicity;
- Nanomaterials' small size means they have the potential to "translocate" in the body (e.g., cross the blood/brain barrier); and
- There is a poor understanding of the environmental fate and effects of nanomaterials, in part because larger particle dynamics are not likely predictive for nanoscale particles.

Uncertainty about Material Characterization Challenges the Identification of Hazard

Hazard characterization forms the question to be addressed in the risk assessment. Hazard characterization questions for nanomaterials can be similar to chemical substances and mixtures; the essential differences include the currently limited understanding of the key particle attributes to measure, and the ability to measure them. Generally, chemicals are reported in mass quantities, as a concentration (e.g. mass of a chemical per unit of volume in air, food, water, or blood). For nanomaterials, the surface characteristics appear to be important parameters to characterize, and at the moment, the measurement priorities are poorly understood. For nanomaterials, mass may not be the best measure for characterizing risk. It may be important for risk characterization to measure several surface properties (surface area, charge, level of contamination), but this is not standardized, and measurement methods vary. Risk screening strategies proposed by ILSI RF, OECD and ISO have defined over a dozen potential measures to characterize nanoscale materials and are working toward standardizing methodologies. One proposal is to use a minimum set of characterization parameters in all studies, to ensure comparability of data until the key relationships between physical characteristics and toxicity are identified.

The SRA Nano Risk workshop addressed issues of nanomaterial characterization in the context of uncertainty analysis, because this affects the exposure assessment, dose/response analysis and risk characterization stages of risk assessment. Paoli and Shatkin suggested that while traditional techniques for quantitative uncertainty analysis may be helpful for interpreting and

estimating the uncertainty associated with nanomaterial characterization, it will be more fruitful to conduct quantitative evaluations of uncertainty when the relationships between nanomaterial characteristics and risk are better identified.

Currently, there is a pervasive uncertainty associated with the behavior of nanomaterials, including which factors are associated with absorption and adverse effects, and the limits of those factors. For example, is there a difference in effects from exposure to particles with an average diameter of 25 nanometers versus particles with average diameter of 50 nanometers, and if so, what if the particle size distributions overlap; can these be distinguished? The current limitations for measurement and analysis contribute to the uncertainty, since we are both uncertain about what to measure, and how to measure it. This "model uncertainty" contributes as much to the current inability to conduct quantitative risk and uncertainty estimates as the limitations in the current database. Further, it is clear from existing studies that we are not able to generalize about these properties across different types of nanomaterials.

Nanomaterial characterization is not more uncertain than other substances, it is simply less understood. There are plenty of examples in recent history that have required new units of measurement for risk characterization. There are at least two possible factors that complicate measurement: nanomaterials tend to exist as mixtures of particle sizes, purity, and aggregation rates, and their properties may vary when incorporated into products; and some particles exhibit dynamic behavior, that is, they associate and dissociate with each other and with biological molecules such as proteins. Again, these concerns are not necessarily unique to nanoscale materials. Pathogenic organisms grow, and ecosystems are dynamic. Further, mixtures have been accommodated in risk analysis using toxicity equivalents and other measures of relative potency. The complexity of contributing factors suggests nanoscale materials and nanotechnologies need to be assessed under realistic exposure conditions.

There is a diversity of views on the issue of the importance of particle size. For example, there is no evidence to suggest that 100 nanometers (nm), the generally accepted upper limit of nanoscale particles, has any biological relevance. It is merely a scale. However, it is widely recognized that people are investigating applications of nanoscale materials for a reason - material properties change at the nanoscale. Some suggest that even aggregated nanoparticles, that can be several hundred nanometers or more in diameter, still possess nanoscale properties. What is not clear is when size matters, and how much, compared to surface and other physical properties. To date, it has not been possible to generalize the findings from one study or type of material to another.

Exposure Assessment for Nanomaterials

Characterizing exposures to nanomaterials requires new measurement metrics be developed. The current techniques available for sample analysis may not be sensitive or specific enough to detect the low exposure levels of nanoscale materials, where relatively larger effects may be seen from lower mass quantities. Historically, risk assessment has considered the mass or a concentration of substances. As we've discussed, the number of particles, the total surface area, and the reactivity of the surface area may become key exposure parameters. In occupational environments, techniques such as particle counting and surface area measures apply, but in toxicology experiments, and in broader scenarios, it is more difficult to measure these physical attributes of nanoscale particles, particularly if they are not spherical. There may be

a need to distinguish engineered from incidental nanoparticles. Many methods use established relationships, e.g. the Brauner, Emmet and Teller (BET) method for calculating surface area, or visual tools, such as TEM, that are less precise than may be warranted for this situation where small subtle changes may have significant effects on particle behavior.

During the SRA Nano Risk workshop, participants suggested that the current situation of exposure assessment for nanoparticles is not dire, and should try to rely on mass as the key measure, and focus on evaluating how the environment affects nanoparticles. It will be important to assess the relative bioavailability of nanoscale particles compared with larger particles for specific exposure pathways. Another suggestion was to make a "metric matrix" to compare exposures and inform dosimetry for nanomaterials. The needs for nanomaterial exposure assessment include a host of uncertainties that have been addressed for other agents, and include: internal dosing, body burdens, thresholds of toxicity and comparison to background levels. Several participants called for measures to simplify exposure assessment.

"Nanotoxicology"

One widely observed effect from exposure to nanoparticles is inflammation, an immune system response resulting from the generation of reactive oxygen species (ROS) when cells encounter the surface-active nanoparticles. Inflammation is associated with the development of many diseases such as asthma, cardiovascular disease, and immune system diseases. Inflammation has been observed in whole animal studies (in vivo) and in cellular assays (in vitro) with a diverse array of nanomaterials. It is presently unclear whether or in what ways the chemical composition, size, shape, or surface characteristics affect the toxicity of nanoscale materials. Existing studies are inconsistent in their findings. Differences observed between engineered nanoparticles and their counterparts (besides the many as yet characterized) may include:

• dose metrics, absorption, distribution and excretion, as a result of size and/or external modifications; and

• mechanisms of toxicity, as a result of increased access to cell matrices or generation of reactive oxygen species — because of new characteristics such as increased surface area.

Consequently, traditional testing and detection methodologies may be inappropriately applied to nanoparticles. Of the three routes of exposure relevant to humans, the majority of studies address the inhalation route of exposure, considering occupational exposures during production. There is relatively little evidence on the uptake of nanoparticles across the skin, with the exception of photoactive compounds in sunscreens (nanoscale titanium and zinc oxides), which have not been demonstrated to cross the skin barrier, and few studies assessing the toxicity of nanoparticles once ingested. The data suggest that effects relate both to particle, as well as chemical, attributes.

A number of researchers are trying to develop predictive approaches to toxicity studies that don't involve testing in whole animals. In vitro assays have to date not shown to be relatable to whole animal studies for nanoparticles. This may relate in part to the difficulties of getting nanoparticles dispersed in dosing solutions. Many nanoparticles are very sticky, and tend to agglomerate, or aggregate. When they do, it increases the difficulty of measuring the toxic effects, or the confidence level about the exposure levels associated with them. Some researchers use aggressive techniques to separate the particles prior to dosing, so it then becomes a

question of whether the findings can be related to real world exposures, where particles may be aggregated. A recent study showed that some nanoparticles interfere with the reactive agents in an in vitro assay, producing a false positive (Wörle-Knirsch et. al, 2006). The lack of an agreed-upon standard for material characterization, as well as for standardized types of test assays, has led to a diversity of approaches such that the existing studies are of limited comparability. This is problematic because the results tend to be equivocal - in one test system an effect is observed, while in the next, no effect is observed.

Because the data are equivocal, there is no conclusive evidence that particle size is the main driver of toxicity. This has led some organizations (e.g. FDA) to avoid defining nanoscale materials by size, and others to acknowledge the challenges for communication and management raised by establishing a bright line for nanoscale material (EFSA 2009). In the short term, agencies likely will adopt a case-by-case approach to nanomaterial reviews. One suggestion from the SRA Nano Risk workshop was to test the toxicity across ranges of particle sizes, with an eye toward developing likelihood functions rather than bright lines. That is, design experiments to define size ranges where nanoscale effects must be considered. This may involve tracking size, rather than using size to classify materials, since size is not currently a good a predictor of hazard. This approach would limit the effect of "lumping materials", and allow categorization to be defined by variables as they occur. Categories of hazard may relate to uses, not sizes, which would classify exposure types. Other participants suggested that nanomaterials may represent a phase, not a class, of materials. The key need is to determine when mass-based doses result in different effects.

Another significant issue for assessing toxicity is not unique to nanoscale substances, but may be more pronounced because subtle changes have greater relative effects at the nanoscale. That is, most nanoparticles tend to be present in a distribution of sizes and purity levels resulting from manufacturing or processing that affect toxicity and behavior. The small scale makes nanomaterials vulnerable to changes during handling, and the choice of media used for handling may affect the state of aggregation and surface properties of some nanomaterials. However, treating nanomaterials as mixtures may help determine the key parameters, relationships, and defining criteria for variables other than mass and size.

The equivocal reports of nanomaterial toxicity beg for a standardized set of criteria and measurements to be reported for toxicity studies, that can help to probe the key criteria, and understand differences in study outcomes for seemingly similar materials. These criteria need to be reported along with estimates of measurement error, or property tolerance levels, to inform the importance to overall material toxicity and the associated variability. Many workshop participants suggested approaches for ensuring standardization of parameter reporting, including: broad analysis of the forthcoming data sets; adoption of a single harmonized standard for nanomaterials reporting; research efforts to improve analytical measurements; grant funding agencies adopting requirements for all toxicological studies; international harmonization of data collection approaches; editorial requirements on manuscript submission, and adoption of standards by professional organizations such as Society for Risk Analysis, Society of Toxicology, The Society for Environmental Toxicology and Chemistry, and others.

Risk Characterization for Nanomaterials

Considering risks from nanomaterials and nanotechnologies in the context of risk management decisions may mean comparison to existing standards for more common chemical substances for which there has been more discussion and debate. There are few available risk assessments for nano materials. Those currently available are generally comparative—that is, compare risks for nanoscale versions of substances to those from larger particles. For example, the National Institute for Occupational Safety and Health (NIOSH) compared the risks from nanoscale titanium dioxide to larger particles Niosh, 2005). Warheit et al. (2007) compared the properties and effects of three types of nanoscale titanium dioxide in a breadth of in vitro and in vivo toxicity assays.

In the workplace, exposure to substances can be more easily managed – it is a fairly controlled environment, but in the broader environment, it may be the products that need to be managed, not the substances, since their applications and uses will vary so greatly. Most consumer exposure to nanomaterials is likely to occur when people use products that contain nanomaterials. This is an added challenge for risk management and risk assessment, to determine the potential risks associated with the use of nanomaterials in products, where materials have different levels of bioavailability and exposure profiles.

Typically, one considers exposure to the active substance only, not the effects of the entire matrix on exposure. Secondary pathways that release (nano)materials in the environment under poorly controlled conditions increase the number of potential receptors and pathways. For example, a packaging material that contains a layer of antimicrobial nanoparticles may have a protective coating that ensures no direct contact with the user, or the packaged item. However, this material can be recycled, introducing the nanoparticles into new matrices with indeterminate exposure pathways. Alternatively, if the package is disposed of as solid waste, nanoparticles could be released to ambient air by incineration, to water from landfill leachate, or to sewage sludge that is applied as fertilizer to crops, depending on the method of disposal.

Representatives of regulatory agencies in Europe, Canada and the U.S. at the SRA Nano Risk Workshop were in general agreement that existing risk models must be considered in terms of the unique attributes of nanomaterials, and adapted, but not replaced. It will be critical to understand exposure potential, and nuances of dose response assessments, which require an adaptive approach to management. A case-by-case approach is the most logical and likely path to understanding the dimensions of risk important for nanomaterials and nanotechnologies, but there is need for an overarching framework, or a “road map” for developing data and environmental health and safety research. There is general agreement that a life cycle framework for risk analysis is warranted and appropriate. The importance of clear and proactive communication about the potential risks was repeatedly highlighted.

Participants were in general agreement that the current risk characterization framework is appropriate for nanomaterials. However, “nanotechnology is not old wine in a new bottle.” Dose metrics and chemical characteristics are novel, including the dynamism of the materials, that is, the observation that characteristics can change with time and in response to the environment.

The combined aspects of dynamism and novelty suggest a dynamic decision framework – so information, characteristics, and decisions can be updated when more is understood. Further, the desire to assess risks in real time, that is to ensure materials and technologies that are in use today will be tested for indications of toxicity, means that early assessments may include some uncertainties that will be updated when greater understanding of cause and effect relationships are elucidated. These factors require an adaptive management approach to risk analysis.

Several frameworks have been proposed that are explicitly designed to address the adaptive needs of evolving scientific understanding, and evaluate exposure across the life cycle of nanoscale materials, in particular: Comprehensive Environmental Assessment (Davis, 2007); NanoLCRA (Shatkin, 2008) and the Nano Risk Framework (EDF DuPont, 2007). There is a need for public vetting of these and other frameworks. There is also a need for a coordinating entity to prepare to assess a breadth of anticipated data sets from members of the Organization for Economic Cooperation and Development, and from various data calls in the United Kingdom, United States, and Canada.

Communicating About Nanotechnology and Nanomaterials

As with other aspects of risk analysis, communicating about the risks of nanomaterials and nanotechnologies is not inherently different from communicating about other classes of substances. However, the breadth of uncertainty and lack of agreement about terminologyconfound clear communication. It is easier to communicate facts than uncertainty. Workshop participants expressed concern that while the technical and scientific issues are being addressed, with scientists and risk analysts identifying, characterizing and assessing the unique attributes of nanomaterials, public perceptions may not recognize this progress. For example, it is a myth that nothing is known about the risks of nanomaterials. Similarly, scientists do not agree there is a need to change the risk analysis paradigm. One repeated myth is that nanotechnology is present in foods everywhere; another is that regulation of nanomaterials is lacking.

The risk analysis community has addressed significant uncertainty for other sources of risk, and generally manages risk by making conservative assumptions that are revised when data gaps are filled. Proactive approaches for informing and educating people about the risks and benefits of nanomaterials and nanotechnologies is a critical component of any risk management strategy, but is not typically how things are done.

Suggestions for improving the transparency and public trust of the management of risks from nanotechnologies include new organizations or partnerships that are privately funded but publicly conducted, outside of traditional regulatory agencies. There is a need for independent review and communication of data and risks in context. This independent evaluation requires adaptations to current models. The complexity of issues raised, some of which are not unique to nanomaterials or nanotechnologies, require new thinking about how to be proactive and transparent, establishing new relationships, and new approaches for integrating research, problem framing, risk assessment, management, and communications. Extensive efforts in Germany and Japan to engage the public have led to increased public awareness, acceptance and perceived benefits of nanotechnologies relative to risk.

One difficulty is the need to consider how to discuss risks from products, versus substances.

Because nanotechnology is not a market, its an enabling technology, it is not possible to define risks, or benefits, out of context. That is, the risks and benefits of medical applications of nanotechnology are evaluated in a different context than consumer or cosmetic products, however this is often presented under a single umbrella.

The perception of risk and of benefits will vary and is likely to influence public, regulatory, and non-governmental activities regarding risk and benefit evaluations. If there are benefits, life-saving benefits, these should be considered in the decision frame. This sentiment was echoed by workshop participants who recognize the public will accept some risks more than others depending on the benefits conveyed. Thus, risks associated with life-saving technologies may be perceived differently than purely cosmetic applications. There is a great need to explain these needs to risk managers.

Finally, there is a need for an independent and trusted entity to organize and filter information. The proliferation of informal and decentralized communications via the internet is a double edged sword. On the one hand, the internet and sources such as blogs flatten the landscape for obtaining information, on the other, unreviewed, biased and incorrect information is easily proliferated, and it is difficult to identify the sources and trustworthiness of much of the current data. This means that conflicting information abounds and confuses the reader.

There is a need for an independent and trusted entity to make new findings about risk transparent and understandable. Visual and graphical tools improve communication about complex topics. One suggestion was for Society for Risk Analysis to bring the extensive resources, international representation, and independence to this issue.

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Methods and Tools for Environmental Risk Assessment, and Decision-Making for Nanomaterials

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Abstract

Nanomaterials and their associated technologies hold promising opportunities for the development of new materials and applications in a wide variety of disciplines, including medicine, environmental remediation, waste treatment, and energy conservation. However, current information regarding the environmental effects and health risks associated with nanotechnology is limited and sometimes contradictory. This paper summarizes the conclusions of a 2008 NATO workshop designed to evaluate the wide-scale implications of nanotechnology on human health and the environment. A unique feature of this workshop was its interdisciplinary nature and focus on the practical needs of policy decision makers. Workshop presentations and discussion panels were structured along four main themes: technology and innovation, human health risk, environmental risk, and policy implications. Four corresponding working groups were formed to develop detailed summaries of the state-of-the-science in their respective areas and to identify emerging gaps and research needs. Gaps between the rapid advances in nanotechnology and the slower pace of human health and environmental risk science were identified, along with strategies to reduce the associated uncertainties.

Introduction

Many potential questions are associated with the current state of development and use of nanomaterials. For example, with the availability of over 600 consumer products worldwide claiming to contain nanomaterials, what information exists that identifies their risk to human health and the environment? What engineering and other personal and environmental protection controls can be deployed to minimize the potential human and environmental health and safety impacts of nanomaterials throughout the manufacturing and product lifecycles? How can the potential environmental and health benefits of nanotechnology be realized? To discuss and develop expert answers to questions such as these, the NATO Advanced Research Workshop "Nanomaterials: Environmental Risks and Benefits and Emerging Consumer Products" brought together 70 scientists and engineers from 19 different nations and multiple fields, reflecting the global and interdisciplinary nature of nanotechnology and nanomaterials research.

State-of-the-science reviews of nanotechnology were presented during the plenary sessions by renowned experts in the field, and over 20 poster presentations provided insight regarding specific projects and issues of interest to the nanotechnology community. Discussion panels were held to debate the implications of this information and to begin clarifying gaps in current knowledge, and four working groups (WGs) were formed to detail these gaps and propose solutions to address them. The WGs discussed methods and applications specific to the following areas: (i) technology and benefits, (ii) human health risks, (iii) environmental risks,

and (iv) policy implications. Prior to the conference, WG chairs prepared and circulated topical white papers, providing a starting point for the detailed WG discussions during the meeting. This summary paper was initially drafted by the workshop organizers and WG chairs and rapporteurs during a one-day meeting immediately following the workshop. The conclusions described for each WG are based on a prioritized list agreed upon during the post-workshop session. These efforts highlight the significant challenges to professionals in assessing the risks associated with nanotechnology; such assessments will almost certainly require a highly integrative and adaptive process of decision-making for nanomaterial risk assessment. The full reports from each WG are published in Linkov and Steevens (2009), but the concepts discussed and conclusions made are summarized in the following pages.

Nanotechnology, its Applications, Consumer Products, and Benefits

Nanotechnologies already provide exciting new applications in materials science, communications, electronics, medicine, energy, and the environment, to name just a few areas. Nanotechnology represents a platform technology that utilizes the properties of matter that arise at the nanometer scale. Many nanomaterials are currently being produced (some have been for many years), such as carbon black, fumed silica, carbon nanotubes, fullerenes, silver nanoparticles, polymer nanocomposites, dendrimers, metal oxides, organic and inorganic semiconductors, and nanocatalysts. Nanomaterials are used, for example, in coatings, emulsions, dispersions and films in automobile components, paper, cosmetics, textiles, and electronic displays. The unique physicochemical characteristics of nanomaterials, particularly the high surface-to-volume ratio (influencing solubility, chemical reactivity, and catalytic activity) and quantum effects (influencing colour, magnetism, hardness, and electronic properties), make them important drivers of innovation with the potential to benefit the world's entire population. Nanotechnology can thus be viewed as a cross-sectional and enabling technology.

In addition to enabling a new manufacturing paradigm, another benefit of nanotechnology would be its potential to help sustain the world's resources. At the workshop, this benefit was discussed along with the view of Petersen and Egan (2002), who believe that nanotechnology is a technology which, for the first time in history, holds the promise of providing inexpensive energy, food, and clean water for everyone on the planet; it could thus also be used in innovative ways to encourage political stability and responsibility.

Human Health Risk and Implications

The purpose of the Human Health WG was not to re-review extensive literature, but to consider important findings in the context of a rapid reduction in the uncertainties of the risk assessment process. Participants discussed mechanisms by which nanomaterials might pose a risk to human health, including nanosized particles penetrating epithelial barriers at the portal of entry and inducing oxidative stress. Both of these processes are fundamentally tied to the physical and chemical nature of the material itself. An important point is that there is no such thing as a generic "nanomaterial," as factors such as size, shape, chemistry, and solubility all affect the biological interactions and consequences of exposure to a specific nanoparticle. This is highlighted by recent reports of impacts from carbon nanotubes (Poland et al., 2008) and nano silver (Benn and Westerhoff, 2008). The goal that should be kept in sight, similar to a recent commentary (Hansen et al., 2008), is to facilitate actions taken by regulatory bodies that are

charged with protecting human and environmental health through the reduction in uncertainties and prioritization of health-based research.

It is neither feasible nor sensible to conduct safety evaluations for all nanomaterials in current or future production; therefore, a risk assessment paradigm should be flexible and based on current knowledge of similar materials (Linkov et al., 2008b). Along these lines, people are regularly exposed to nanosized particles in ambient air (i.e., ultrafine particles) that are derived from combustion processes. Although there are physicochemical differences between engineered nanomaterials and ambient ultrafine particles, the large body of toxicological literature regarding the latter provides a framework for understanding nanomaterial risks. In addition, large-volume production of nanosized titanium dioxide and carbon black particles has been carried out for several years, and it is possible that aspects of the risk assessment paradigms for these materials could be applied more generally to nanomaterials. Useful predictive guidance can also be gained from the literature regarding interactions of nanosized particles with skin, focusing on penetration of the stratum corneum and drug delivery. Although this approach focuses mainly on the respiratory tract and skin, such simplification is reasonable because of the ways in which humans are likely to be exposed to nanomaterials, namely in occupational and environmental settings and via consumer products.

Ecological Risk

This WG recognized that traditional risk assessment procedures are inadequate for predicting the ecological risks associated with the release of nanomaterials. The WG discussed a number of past case studies where the traditional approach to risk assessment failed to reveal unforeseen risks. The WG emphasized their belief that the root of the problem lies in an inadequate application of solid phase chemical principles (e.g., particle size, shape, and functionality) in the risk assessment of nanomaterials. The group felt strongly that the "solubility" paradigm used to evaluate the risks associated with inorganic or organic contaminants must be replaced by a "dispersivity" paradigm for evaluating the risks associated with nanomaterials.

In the opinion of the working group, the pace of development of nanomaterials will exceed the capacity to conduct adequate risk assessments using current methods and approaches. "New generation" products will include materials with targeted nanotechnology-biology interactions, DNA-scaffolded devices, composite materials with biological functions or photovoltaic properties, materials for new environmental remediation technologies, self-assembling devices, and polymer-based nanomaterials. These nanomaterials could be available in a variety of size classes and with different surface functionalizations, probably requiring multiple risk assessments for each material.

Considerations for Implementation of Manufactured Nanomaterial Policy and Governance

The participants in this working group agreed to focus discussions on policy frameworks, rather than on the gaps of regulation which have been analyzed elsewhere. Further, the scope of discussion was narrowed to focus on guidance deemed helpful for developing policies, and on the information and tools (e.g., databases and web portals) that (i) support the development of policies by regulators, industry, and others, and (ii) disseminate information to the public and others.

The WG agreed that while many different policy frameworks for manufactured nanomaterials have been developed globally (Table 1), a significant lag period remains between the development of nanotechnologies and the development and implementation of new policies. While policy initiatives range from voluntary measures to mandatory legislative frameworks, the WG recognized that governments and industry actually develop very few policies. The WG agreed that developing regulatory tools is an important gap in the knowledge necessary for manufactured nanomaterial regulation. Further, the WG agreed with that the starting point for development of these tools is the set of policies and procedures already developed by regulatory agencies and industry for traditional industrial materials, e.g., surfactants and other chemical substances.

Conclusions

Workshop attendees shared basic agreements on policy and risk assessment needs across countries. Attendees identified the need for a common, standardized taxonomy and terminology for nanomaterials in which key aspects should include nanomaterial physical and chemical characteristics, with the view that such a system would facilitate the development of informational resources (e.g., publications, other documents, and databases) to provide easy access and sharing across international borders as regulators attempt to understand and assess the properties of these new materials. Attendees also agreed that assessments covering the entire lifecycle would best inform and guide risk assessment for engineered nanomaterials and related nanotechnologies, and that consumer and occupational health protection policies needed additional development as well. Given the proprietary nature of these rapidly evolving technologies, and current voluntary reporting requirements, a mechanism is needed for regularly providing and updating information to scientists and policy makers regarding the safety profiles and characteristics of these current and emerging nanomaterials. Attendees were very aware that a serious nanomaterials-related health issue in one nation or region of the world would greatly promote a negative public perception of nanomaterials risk in every other nation or area.

Simultaneous advances in different disciplines are necessary to advance nanomaterials risk assessment and risk management. Risk assessment is an interdisciplinary field, but progress in risk assessment has historically occurred due to advances in individual disciplines. For example, toxicology has been central to human health risk assessment, and advances in exposure assessment have been important for environmental risk assessment and risk management. Nanotechnology, however, ideally involves the planned and coordinated development of knowledge across fields such as biology, chemistry, materials science, and medicine.

Likewise, a risk assessment of nanomaterials and related technologies requires a lifecycle approach, meaning a comprehensive assessment of the impact of nanomaterials at different stages of production, use, and disposal/recycling. The current state of knowledge makes the identification of major risk drivers challenging. This includes understanding environmental pathways, fate and transport processes, and reasonably foreseeable exposures. An integrated, holistic approach is needed to consider an individual's total exposure from relevant environments expressed in different units across receptor groups. This would lead to risk characterizations that are systematic and more inclusive, accommodating non-traditional information sources, measures, and endpoints.

Table 1: Elements of nanomaterial regulation frameworks discussed in each document (criteria are numbered 1 to 4 under each category; for each document and criterion, ■ = document discussed the criterion, ▪ = document mentioned the criterion, and (blank) = document did not address the criterion; adapted from Linkov and Satterstrom, 2008).

	Science and Research Aspects				Legal and Regulatory Aspects				Social Engagement and Partnerships				Leadership and Governance			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
US EPA, 2007	■	■	■	■	■	▪	▪	▪	■	▪	■	▪	▪	■	▪	
US FDA, 2007	■		■			■	■		■	▪	▪		▪			
Davies, 2006	▪	▪	■	▪	■	■	■	▪	■	■	■			▪	■	▪
ED-DuPont, 2007	■	■	■	■	■	▪			▪	▪			■	▪	■	▪
Québec Commission, 2006	▪	■	■	■	■	■			■	▪	■	■	■	■	▪	■
UK Royal Society, 2004	■	■	■	■	▪	■	■	▪	■	■	▪	■	▪	■	▪	▪
UK DEFRA, 2006	▪	▪	▪	▪	■				▪	▪	▪					
Responsible NanoCode, 2006			▪		■				▪	▪	▪					
EC SCENIHR, 2007	■	■	■	■												
EC Action Plan, 2005	▪	▪	▪	▪		▪			■	■	■	■		▪	▪	
IRGC, 2005, 2006, 2007	▪	■	■	■	■	▪			■	■	■	■	■	■	■	■
US NNI, 2008	■	■	■	■					▪		▪		▪	■	▪	▪
REACH, 2006	■	■	■	■	▪	▪	■	■	■	■	■	■	▪	■	■	▪

Sub-criteria for the table are as follows:

- **Science and Research Aspects**
  1. Development of methods for detection / characterization / data collection
  2. Assessment of environmental fate & transport / impacts
  3. Assessment of toxicology / human health impacts
  4. Assessment of health and environmental exposure
- **Legal and Regulatory Aspects**
  1. Voluntary regulatory and best-practices measures
  2. Information-based regulatory tools (*e.g.*, labeling)
  3. Economic-based regulatory tools (*e.g.*, tax or fee for safety testing)
  4. Liability-based regulatory tools (*e.g.*, penalty for pollution)
- **Category 3: Social Engagement and Partnerships**
  1. Promotion of education and distribution of information / use of risk communication tools
  2. Use of stakeholder engagement tools
  3. Development of partnerships with academia, industry, public organizations, provinces, and international regulators
  4. Emphasis of ethical conduct
- **Category 4: Leadership and Governance**
  1. Transparency in nanotechnology-related decisions
  2. Consideration of benefits of nanotechnology
  3. Adaptive modification of existing or development of new legislation
  4. Consideration of precautionary principle

The attendees agreed that while existing chemical risk assessment and risk management frameworks may provide a starting point, the unique properties of nanomaterials adds a significant level of complexity to this process. The goals of this workshop included the identification of strategies and tools that could currently be implemented to reduce technical uncertainty and prioritize research to address the immediate needs of the regulatory and risk assessment communities. Such tools include advanced risk assessment, comprehensive environmental assessment, risk characterization methods, decision analysis techniques, and other approaches to help focus research and inform policymakers benefiting the world at large.

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The Toxicology of Nanomaterials: Where Do We Go from Here?

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Dr. Philbert opened the lunch plenary session by stating that he was going to challenge his audience with controversial statements that would point to areas for further consideration. The first statement, "There is no such thing as nanotechnology," was followed by a reprise of earlier and existing technologies. Early incarnations of nanotechnology were used in the defense arena for reinforcing at-risk joints, for example, in the enhanced soldier program. Another earlier application was electronics, for example, a flash drive that can store 24 gigabytes of information. Current applications of nanotechnology include sunscreen and zinc oxide coated windows. Language implying that nanotechnology is on the horizon is inaccurate.

There are more than 800 self-identified products containing nanomaterials. Here Dr. Philbert noted an interesting dichotomy. Some manufacturers use the term "nanomaterials" in labeling their products, even though the products do not contain them. Other manufacturers have abandoned the term, even when their products contain them. They opt, instead, for terms such as "ultrafine" or "microfine" in an attempt to distance themselves from nanomaterials and any potential public backlash. This raises the issue of perceived risk versus actual risk. Although concerns are expressed for the impacts of nanotechnology on toxicology and risk assessment, greater concern may be evident if nanotechnology is used to circumvent some protective biological processes, for example, to modify defective sperm to enable fertilization.

The statement "There is no such thing as nanotoxicology" could be called, in legal parlance, an excited utterance against interest. Nanotoxicology could be termed a loose constellation of poorly coordinated activities, with a lack of coherent, meaningful experimental standards. Dosing metrics are only clear for particles of regular geometry. However, zinc oxide particles have been shown to have widely disparate geometries, and each geometry may have a different effect. CAS RNs are not useful for materials with many different geometries. An evolutionary taxonomy is required for nanomaterials.

Much current work in the field may be considered preliminary, and more work is required on the metrics of exposure: duration, frequency, route, and magnitude. It is not known whether absorption, distribution, metabolism, and excretion are the same for all species. Complicating this is the difficulty of labeling nanomaterials for identification without changing their surface characteristics. How then does one track nanomaterials in vitro and in vivo?

The current high level doses used for mechanistic studies should be avoided, as these swamp any effects that might be seen at concentrations that could be encountered in the environment. Critical information can be missed, and undue emphasis could be given to effects that would not be seen at environmental concentrations. Acute studies have a place, but time will be critical for nanotoxicity studies, and even two-year rodent bioassays may be insufficient for some materials. Positive controls are essential and should be reported for all studies in addition to new findings.

An issue that requires attention is the establishment of de minimus standards for toxicological studies that can be used in risk assessment. It should be recognized that not all biological change is harmful, and between a normal state and pathology there is a gap that can be regarded as adaptation. A high quality database for all negative data could provide considerable cost savings.

Risk management is required for nanomaterials, and, while risk cannot be eliminated, the concept that risk can be managed needs to be communicated.

Conference Questions and Answers

Question:

There may be no such thing as nanotechnology, but what about nano-epidemiology? An exposure registry is required for people exposed to nanomaterials in the workplace to establish risks.

Answer:

The prefix "nano" gives these materials unwarranted importance, and the issue needs to be placed in context more are killed by cars than nanomaterials. Remember that nanoscale materials are ambient and are present in filtered drinking water.

Comment:

We cannot make the statement that nobody has been killed by nanomaterials; we do not know enough.

Reactive Oxygen Species Related Microbial Growth Inhibition By Silver Nanoparticles

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Abstract

Nanotechnology enhanced consumer products containing silver nanoparticles are emerging but the fate and effect of silver nanoparticles in the environment remain largely unknown. To evaluate the toxicity of silver nanoparticles to microorganisms involved in wastewater treatment, we measured and compared specific oxygen uptake rates of nitrifying bacteria before and after their exposure to silver nanoparticles. The active oxygen species in the presence or absence of silver was determined in parallel by using ROS-sensitive fluorescence dyes. Silver nanoparticles significantly inhibited the growth of nitrifying bacteria at silver concentrations less than 1 mg/L. The inhibition was well correlated with the intracellular ROS concentrations and more ROS was generated when it was exposed to silver nanoparticles, suggesting that the microbial growth inhibition by silver nanoparticles is related to ROS generation in the cell.

Introduction

Silver nanoparticles are used in many consumer products because of their strong antimicrobial activity (Benn and Westerhoff 2008; Mueller and Nowack 2008). We recently showed that at 1mg/L Ag, silver nanoparticles (average size, 14 ± 6 nm) significantly inhibited nitrifying bacterial growth (Choi et al. 2008). Although the mode of antimicrobial activity is still not clear, it is believed that silver species may induce to generate intracellular reactive oxygen species that can damage protein, DNA and membrane (Sondi and Salopek-Sondi 2004; Hussain et al. 2005; Lok et al. 2006). The reactive oxygen species including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$) are generated under oxygen-limited conditions or in the presence of environmental toxicants. For instance, semiconductive nanoparticles such as TiO_2 produce photocatalytic ROS at near UV. Silver ion was also reported to induce intracellular ROS (Inoue et al. 2002). The accumulation of high level intracellular ROS can damage cellular components and disrupt cell functions. To help elucidate the inhibition mechanism, we measured nitrification inhibition by various forms of silver including silver nanoparticles, silver ions, and silver chloride colloids to evaluate the relationship between silver concentrations and ROS production. Nitrification involving ammonia oxidation and nitrite oxidation by typically nitrifying bacteria is important in wastewater treatment and global nitrogen cycling. Nitrifying bacteria were chosen as model microbes because of sensitivity to environmental change like as pH, temperature, and several toxicants (Blum and Speece 1991). The quantitative description of the relationship between ROS and nanosilver toxicity will

therefore help better understand the antimicrobial mechanism of silver nanoparticles.

Methods

Autotrophic nitrifying bacteria were cultivated in a continuously stirred tank reactor (14L) operated at solids retention time (SRT) of 20d and hydraulic retention time (HRT) of 1d. Silver nanoparticles were made from reduction of silver ion (silver nitrate) with sodium borohydride in 0.06 % (wt) PVA (polyvinyl alcohol) solution used to control silver particle size (Choi et al. 2008). The average size of the synthesized silver nanoparticles was 15 nm.

The toxicity of silver nanoparticles to nitrification was investigated by measuring specific oxygen uptake rate (SOUR) after ammonium (10 mg-N/L) injection to aliquots of nitrifying cultures (60 mL) in the presence or absence of silver nanoparticles in a closed respirometric vessel. The degree of inhibition (%) was calculated based on the relatively decrease of SOUR in the presence silver (Equation 1).

$$Degree\ of\ Inhibition\ (\%) = 100 \times \frac{SOUR_{without\ nAg} - SOUR_{with\ nAg}}{SOUR_{without\ nAg}} \qquad (Equation\ 1)$$

To determine intracellular ROS concentrations, aliquots of nitrifying biomass suspensions were removed from the nitrifying bioreactor, centrifuged and resuspended in a loading buffer solution containing 10 μM H_2DCFDA (dichlorodihydrofluorescein diacetate, Invitrogen, OR, USA) for 30 minutes. After the centrifugation, the pellet cells were inoculated with prewarmed growth medium, amended with nanosilver (average size: 15 nm) or silver bulk species (for comparison) at predetermined concentrations, and plated into 96-well plates. The fluorescence of the cells from each well was measured with 485 nm excitation and 535 nm emission filters using a microreader (VICTOR^3, Perkin Elmer, Shelton, USA). Fluorescence data were taken automatically after 30 min incubation. Hydrogen peroxide (30%, Fisher Scientific) was used as a standard for ROS measurements and intracellular ROS concentrations were normalized in H_2O_2 unit.

To determine photocatalytic ROS concentrations, APF (3'-(p-aminophenyl) fluorescein, Invitrogen, OR, USA) was used in cell free condition to measure ROS generated by nanosilver itself and compared ROS generation before and after exposure to fluorescent lab light for 30 minutes. The APF was added at a final concentration of 5 μM and the photocatalytic ROS that is mainly related to $OH^{\cdot}$ production was determined in mole units of OCl^- in the solution.

Results

All forms of silver tested inhibited nitrification. At the same silver concentrations, silver nanoparticles presented the highest degree of inhibition (Figure 1). As the silver concentrations increased, the inhibition appeared to follow a saturation curve with R^2 range from 0.91 to 0.97. The concentrations of silver nanoparticle, silver chloride, and silver ion causing 50% inhibition were determined to be 0.14 mg/L, 0.25 mg/L, and 0.27 mg/L.

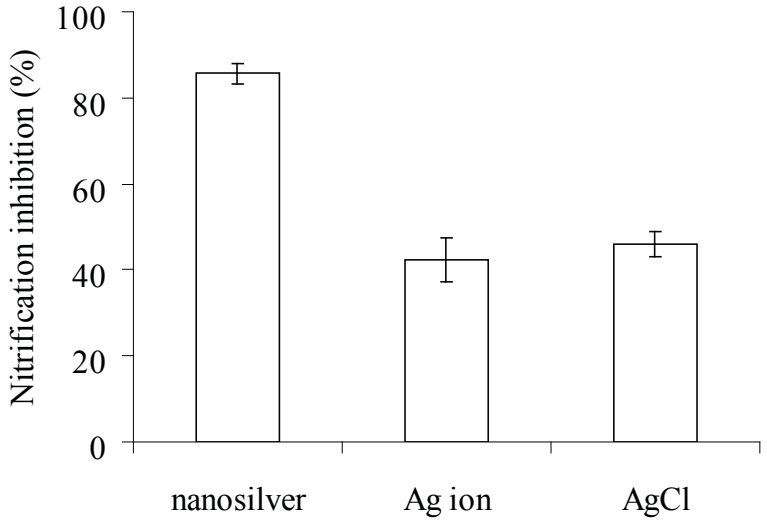

Figure 1. The toxicity of silver to nitrifying bacteria at 1mg/L Ag. Error bars indicate one standard deviation.

The ROS concentrations (normalized in H_2O_2 concentration) increased when the nitrifying biomass suspensions were exposed to silver nanoparticles (Figure 2). Inhibition by Ag nanoparticles as well as other forms of silver (AgCl colloids and Ag^+ ions) correlated well with the intracellular ROS concentrations (Figure 3) by using a saturation-type model. Poor correlation, however, was noticed between the observed inhibition and the photocatalytic ROS concentrations. Therefore, photocatalytic ROS concentrations were not a good predictor of inhibition by Ag nanoparticles.

Discussion

Nanosilver toxicity was well correlated with intracellular ROS concentrations. All forms of silver including Ag^+ ion, silver chloride and nanosilver induced intracellular ROS but the patterns of their correlations with inhibition were different. Ag nanoparticles appeared to be more toxic than Ag^+ ions at the same level of intracellular ROS or the same total Ag concentrations, suggesting that factors other than ROS are also important in determining nanosilver toxicity.

Nanoparticles are very mobile and active because of their small size. Recently, it has been shown that gold nanoparticles coated with negative charged and hydrophobic ligands could penetrate into the cell membrane without disruption (Verma et al. 2008). It was also suggested that nanoparticles could be more toxic via a Trojan-horse type mechanism (Limbach et al. 2007). Silver nanoparticles induced more ROS production and higher toxicity at the same Ag concentration than that of silver bulk species. It is therefore possible that these nano-size particles may have a different transport mechanism from that of silver ion to enter or interact with the cell.

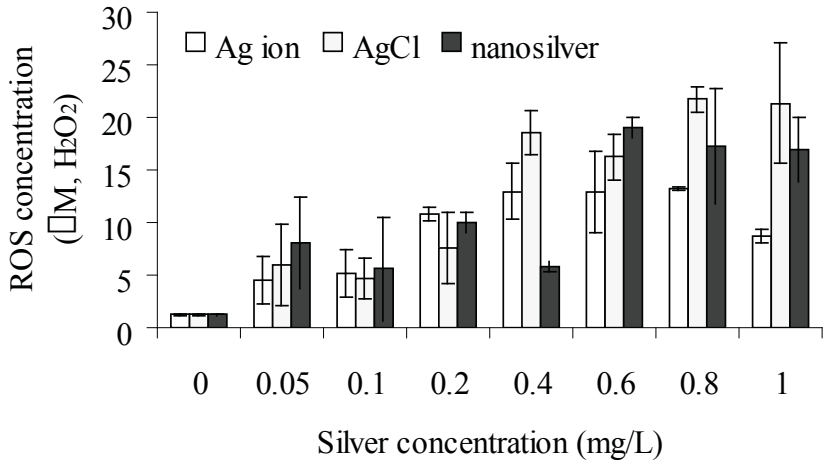

Figure 2. Changes of intracellular ROS concentrations in nitrifying bacteria exposed to different forms of silver. ROS concentration was measured in H_2O_2 units. Error bars indicate one standard deviation.

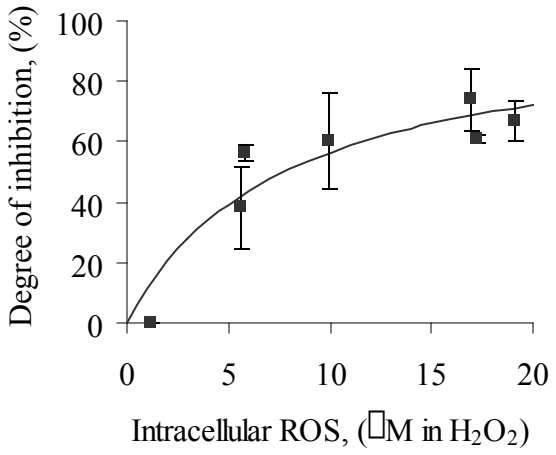

Figure 3. ROS-related nanosilver toxicity to nitrifying bacteria. The ROS concentrations were measured in H_2O_2 units. Error bars indicate one standard deviation. R square value of 0.86 was calculated using a saturated model.

Conclusions

Silver nanoparticle was more toxic to nitrifying bacteria than silver ion or silver chloride colloid.

At the same silver concentrations, silver nanoparticles tended to generate more ROS than the bulk silver species. The toxicity of silver nanoparticles was correlated with the intracellular ROS concentrations.

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Conference Questions and Answers

Question:
Do you have evidence that silver enters bacteria?

Answer:
We did not do that experiment, but previous publications of TM (transverse magnetic) studies indicate that particle size <10 nm enters the cell.

Question:
How did you make sure that the toxicity effect was due to the nanoparticles rather than silver ions left in solution?

Answer:
After preparing the nanoparticles, we used an ion-specific electrode to determine the concentration of remaining silver ions, and the concentration was negligible.

Question:
If there is an uptake difference in the rate nanosilver and silver ions cross the cell membrane, the toxicity of nanosilver could be underestimated if it moves more slowly into the interior of the cell. Did you perform any enzyme inhibition studies to look at this?

Answer:
Yes. Nanosilver inhibited AMO (ammonia monooxygenase) located on the cell membrane more than HAO (hydroxylamine oxidoreductase) located between the periplasma and cell membrane.

Identification of Biomarkers of Exposure to Metal-based Nanoparticles through Gene Expression Profiling Using Daphnia magna MicroArrays

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As new emerging contaminants are developed and gradually replace past environmental pollutants, we are faced with an unprecedented challenge. We are currently able to confront the issue of emerging contaminants before they become an environmental problem and develop strategies to mitigate the risk that may be associated with their release into the environment. One area where attention should be directed is the rapidly growing field of nanotechnology. The emergence of genomic techniques has presented many exciting new possibilities in ecotoxicology including the ability to classify chemicals based on their expression pattern or fingerprint. Previous research focused on three well-characterized metal pollutants: copper, cadmium, and zinc, and the invertebrate indicator species, Daphnia magna. Using a custom D. magna cDNA microarray containing approximately 5000 cDNA clones, which identified distinct expression fingerprints in response to sublethal copper, cadmium, and zinc exposures and validated several genes as biomarkers of exposure (Poynton et al., 2007). The goal of this current study was aimed at developing biomarkers of exposure that can be applied to study the bioavailability and environmental exposure of metal based nanoparticles. The questions we wanted to answer were: 1. Can we identify biological indicators of exposure to nanoparticles using a similar approach as Poynton et al. 2007? 2. Can we distinguish between the particle induced effects and chemical composition effects through comparative gene expression profiling? 3. What exposure time produces the most robust and specific gene expression pattern?

Conference Questions and Answers

Question:
What were the genes shown that were different, and what were they?

Answer:
We do not know; they were just coded with in-house descriptors, and we have not identified their particular functions. However, a Daphnia genome chip is now under development.

Question:
Can you speculate how genes being switched on could be used in a regulatory framework?

Answer:
Some literature suggests a “No Observable Transcription Expression Level” (NOTEL). The NOTEL could be used as a regulatory driver, or a “No Observable Protein Expression Level” (NOPEL). Once proteins are turned on there could be a cascade effect with adverse effects.

Save

Using Microarrays to Test the Effects of Acute Exposure to Multiwalled Carbon Nanotubes (MWCNTs) on Gene Expression in Fathead Minnows (*Pimephales promelas*)

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Abstract

There are increasing concerns regarding the release of nanomaterials, in general, and nanotubes, in particular, into the environment and their potential effects on fish and wildlife. Researchers are only recently evaluating the potential for exposure and adverse effects of nanoparticles on fish and wildlife through toxicological testing, and data are extremely limited. Under an EPA Phase 1 SBIR grant, we have been given the opportunity to use state-of-the-art oligonucleotide microarrays to examine the toxicity and gene expression patterns in fathead minnows (*Pimephales promelas*) exposed to two different sizes (< 8 nm and 50-80 nm outer diameter) of multiwalled carbon nanotubes (MWCNTs).

We exposed adult female fathead minnows for 48-hours to three concentrations of each nanotube (0.1 mg/L, 0.3 mg/L, and 1.0 mg/L), a (water) control, and a carrier control (1.2 mg/L NaDDBS for the 8 nm MWCNT, 2.2 mg/L for the 80 nm) under aerated static renewal conditions. After exposure, we harvested gill, gonad and liver, and then measured gene expression using a fully annotated 15,208 gene oligonucleotide microarray. We used GeneSpring (version 9.0.3) to analyze the data, looking for differentially regulated genes ($p \geq 0.05$ and fold change ≥ 2).

At these concentrations, we found no toxicity and observed no gross organ abnormalities. From the microarray analysis, we did observe a substantial transcriptional response to exposure in each of the tissues, with over 400 genes exhibiting altered expression in each of the tissues. However, there was very little commonality in the transcriptional response of these three tissues to a given MWCNT. The majority of the differentially expressed genes from each tissue are involved in the biological process category 0006xxx, regulating transport, transcription and protein functions. In addition, the response of a single tissue (gill) to both sizes of nanotubes showed as many differences as similarities.

Introduction

Nanoparticles have increased in manufacturing, industry, and commercial products over the past two decades. However, assessing the potential effects of nanoparticles on human health is not an easy task, as the properties of nanoparticles depend not only on the size of the particle, but also on the structure, microstructure, and surface properties (coating) (Moore 2006, Yin et al. 2005, Burleson et al. 2004). Invariably, industrial products and wastes, including some aerosols,

tend to end up in waterways despite safeguards; it is inevitable that nanoscale products and by-products will also enter aquatic environments as nanotechnology industries scale up production (Moore 2006, Borm et al. 2006). Thus, uptake of nanoparticles into the aquatic biota is a major concern.

However, the evaluation of the potential for exposure and adverse effects of nanoparticles on fish and wildlife has begun to be addressed only recently through toxicological testing, and data are extremely limited. Concern about environmental contaminants that adversely affect health, development and reproduction of exposed wildlife has led to the development of both specific in vitro and in vivo assays to test for these effects. Gene microarrays integrate in vivo exposures with mechanistic outcomes. Using this technology, we can test thousands of genes at one time with mRNAs isolated from tissues of exposed animals. Under an EPA Phase 1 SBIR grant (EP-D-08-026), we have been given the opportunity to use state-of-the-art oligonucleotide microarrays to examine gene expression patterns in female fathead minnows (FHM, *Pimephales promelas*) exposed to two different sizes of multiwalled carbon nanotubes (MWCNTs).

Materials and Methods

Nanotube suspension. We purchased two dry nanotube powder samples, <8 nm and 50-80 nm outer diameter (O.D.), from Cheaptubes (www.cheaptubes.com, Brattleboro, VT). We suspended each sample in water containing 10mg/ml sodium dodecylbenzene sulfonate (NaDDBS), at a nominal concentration of 10 mg/ml. We bath-sonicated the nanotubes suspensions for four hours, and then centrifuged at 600 rcf for 30 minutes. We retained the supernatant, and then calculated that between 35-50% of the nanotubes remained in suspension, approximately what was expected according to the method described by Attal et al. (2006).

Nanotube exposures/tissue collection. We performed all exposures as aerated 48 hour static bioassays in 2L beakers, with 4 replicate beakers per concentration, and 3 adult female fathead minnows per beaker. For each nanotube, we used five different exposure conditions: (water) control, carrier control (1.2 mg/L NaDDBS for the 8 nm MWCNT, 2.2 mg/L for the 80 nm), 0.1 mg/L, 0.3 mg/L, and 1.0 mg/L MWCNTs. After 48 hours, we euthanized two fish from each beaker by immersing them in 100 mg/L MS-222 (Tricaine) buffered with 10 mg/L NaHCO3 for five minutes. We opened the carcass by ventral incision, removed the liver (partial), ovary (left horn), and gill (left), and immediately placed the tissues in 1 ml RNALater (Ambion, Inc., Austin, TX), storing samples at -20°C.

Hybridization of microarrays. We isolated total RNA using the RNEasy Plus Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. We determined the quality of the RNA by running a 1.0 μL aliquot on a 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). As this was a reference design experiment, we labeled the exposed samples with cyanine (Cy) 5-CTP (Perkin Elmer, Wellesley, MA, USA) and the reference sample with Cy-3-CTP. We labeled, hybridized, and washed the arrays according to Agilent's Two-Color Microarray-Based Gene Expression Analysis (Quick Amp labeling) Protocol (version 5.7, March 2008). The FHM microarrays used in this experiment were developed by EcoArray and manufactured by Agilent Technologies, Inc. Each array contains probes for 15,208 annotated gene sequences; there are 8 arrays per glass slide. We scanned the slides with an Agilent DNA microarray scanner, which processes the raw images and converts the data into .txt files using Agilent's Feature Extraction

Software, Version 9.5.3.

Normalization of microarray data and statistical analysis. The resultant data was analyzed using Gene Spring version 9.0.3 (Agilent Technologies, Inc., Santa Clara, CA). For this project we considered the two sizes of nanotubes as different experiments, and we analyzed the tissues, gill, liver, and ovary, within each size category independent of each other. We accepted as differentially regulated all genes with a P-value of 0.05 or lower (statistically significant) and a fold change >2.0.

Results

We observed no mortality in adult female fathead minnows exposed for 48 hours to dispersed suspensions of two sizes of MWCNT at concentrations up to 1 mg/L. At necropsy, we did not note any gross pathology in any of the organs examined.

We used gene expression analysis to investigate the response of gill, liver, and ovary to each nanotube. We observed a substantial transcriptional response to exposure in each of the tissues, with over 400 genes exhibiting altered expression in each of the tissues (Figures 1 and 2).

Figure 1. Differential gene expression in female fathead minnows exposed to 1.0 mg/L <8 nm O.D. MWCNTs compared to controls.

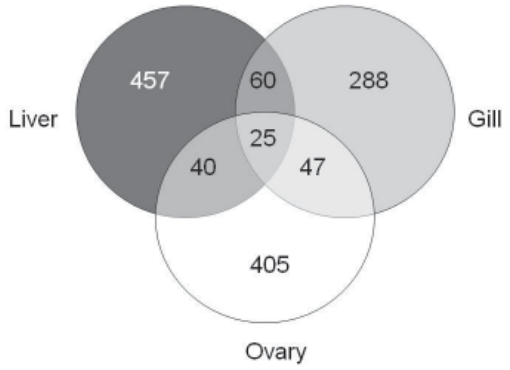
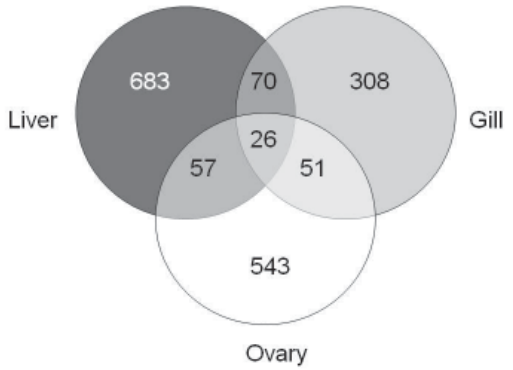


Figure 2. Differential gene expression in female fathead minnows exposed to 1.0 mg/L >50 nm O.D. MWCNTs compared to controls.



However, there was very little commonality in the transcriptional response of these three tissues to a given MWCNT. The majority of the differentially expressed genes from each tissue are involved in the biological process category 0006xxx, regulating transport, transcription and protein functions (Figure 3 and Table 1).

To determine whether MWCNT with different diameters produced different responses, we compared the transcriptional response in the gill to MWCNT of <8nm O.D. and 50-80 nm O.D. Our analysis found 60 genes whose expression was significantly altered that were common to both exposures. Analysis of this subset of genes reveals that some genes show very similar regulation, however there are a number which reveal dramatically different responses between the two treatments (e.g., , AF236669 which is active in transport, AL929504 and AC132256). Genes which show similar responses between the two treatments are involved in, among other things, transcription, cell adhesion and protein transport.

Discussion

The results of this study demonstrate that MWCNT which are well dispersed in water are not acutely lethal to fathead minnows at concentrations up to 1 mg/L. Toxicity studies for MWCNTs

Figure 3. GO (Biological Process) pathways in gill after exposure to 1.0 mg/L >50 nm MWCNTs

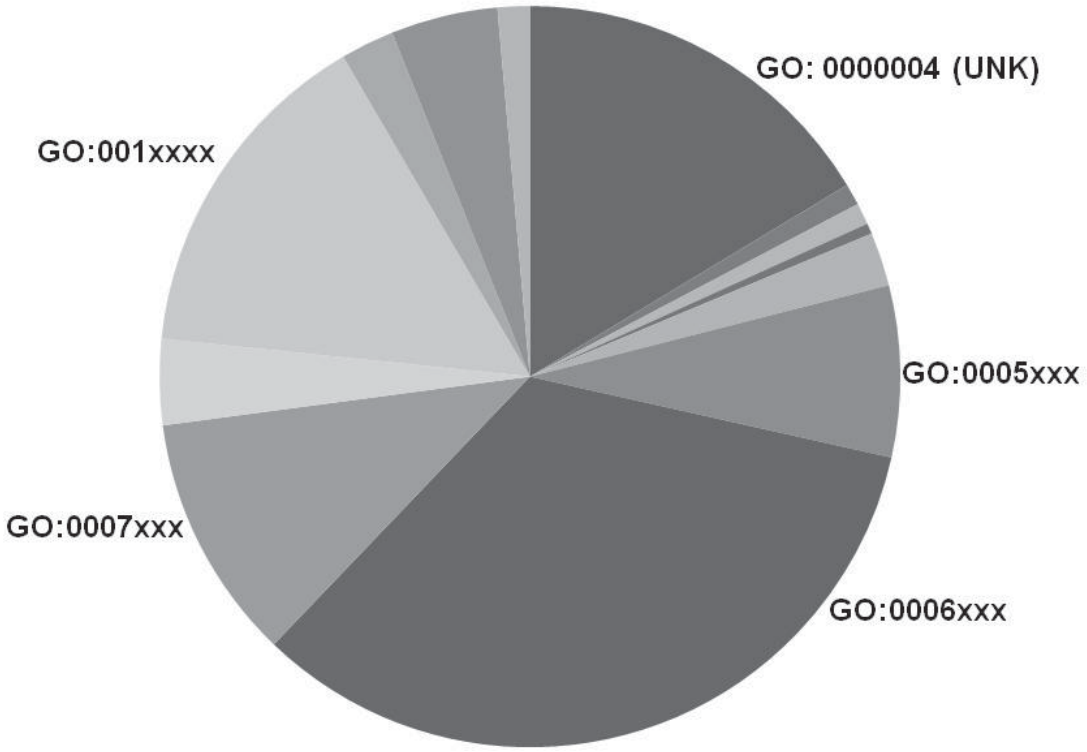
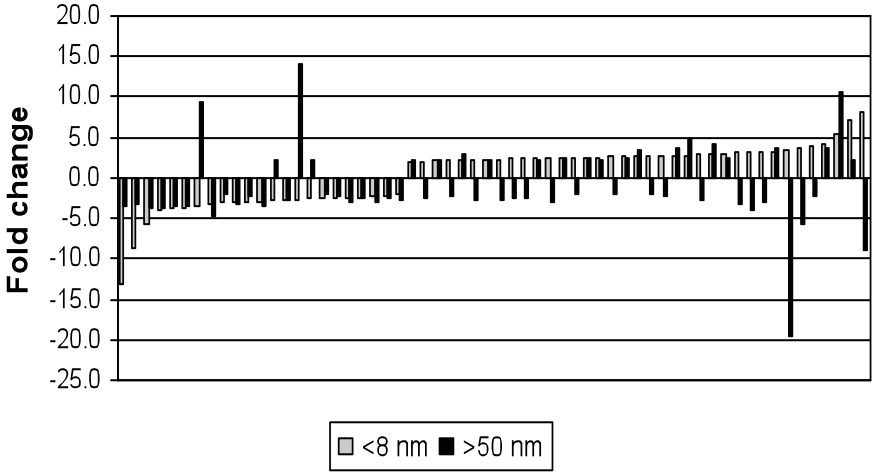


Table 1. Biological processes represented in the gill under GO category 0006xxx. While this specific list is from gill tissue exposed to >50 nm OD MWCNTs, genes differentially expressed in the liver and ovary are also predominantly from GO category 0006xxx. Gill = 34%, liver = 35%, ovary = 26%. The second highest category is GO: 0000004, biological process unknown.

GO: Biological process
GO:0006071; glycerol metabolism
GO:0006118; electron transport
GO:0006122; mitochondrial electron transport, ubiquinol to cytochrome c
GO:0006139; nucleobase, nucleoside, nucleotide and nucleic acid metabolism
GO:0006259; DNA metabolism
GO:0006268; DNA unwinding during replication
GO:0006281; DNA repair
GO:0006289; nucleotide-excision repair
GO:0006306; DNA methylation
GO:0006334; nucleosome assembly
GO:0006350; transcription
GO:0006355; regulation of transcription, DNA-dependent
GO:0006366; transcription from RNA polymerase II promoter
GO:0006398; histone mRNA 3'-end processing
GO:0006405; RNA export from nucleus
GO:0006412; protein biosynthesis
GO:0006429; leucyl-tRNA aminoacylation
GO:0006457; protein folding
GO:0006464; protein modification
GO:0006468; protein amino acid phosphorylation
GO:0006508; proteolysis
GO:0006605; protein targeting
GO:0006629; lipid metabolism
GO:0006810; transport
GO:0006811; ion transport
GO:0006836; neurotransmitter transport
GO:0006865; amino acid transport
GO:0006915; apoptosis
GO:0006916; anti-apoptosis
GO:0006928; cell motility
GO:0006936; muscle contraction
GO:0006937; regulation of muscle contraction
GO:0006955; immune response

Figure 4. Gill: 60 genes common to both exposures



in aqueous environments are scarce, yet we found our lack of toxicity consistent with previous work on other nanoparticles (e.g., Zhu et al., 2008; Blaise et al., 2008). It is important to note that without use of a dispersing agent, suspensions of MWCNT rapidly aggregated and settled out of the water column, resulting in little or no exposure of pelagic organisms. The dispersal agent itself can cause toxicity. This highlights the importance of testing the form of the nanomaterials that will actually be released into the environment in order to accurately assess risk.

Exposure to MWCNT produced significant transcriptional effects on gill, liver, and ovary of adult females. The responses in these organs were quite different, suggesting that the tissues are responding differently, though it is unclear whether MWCNT were absorbed and reached internal organs or if responses of liver and ovary are secondary to physiological stress due to effects on gill. We are currently performing histopathological analysis of several tissues to help answer these questions.

Comparing the response of the gill to different size MWCNT suggests that nanotubes of different diameters can cause different responses. The biggest differences between the two types of tubes were in genes involved in transport of calcium ions or cations; genes integral to transmembrane movement of substances. The differences may be due to size, but the properties of nanoparticles depend not only on the size of the particle, but also on the structure, microstructure, and surface properties (coatings) (Moore 2006, Yin et al. 2005, Burleson et al. 2004).

Conclusions

The results of this study suggest that while acute exposure to MWCNTs is not toxic to fathead minnows at concentrations up to 1.0 mg/L, such exposure does result in differential gene expression in gill, liver and ovary tissue. Many of the genes that do change are involved in regulating transport, transcription and protein functions. However, this is a preliminary examination of the data, and we will be undertaking a much more detailed analysis. We intend to

analyze the effects of the NaDDBS solvent, the 0.3 mg/L concentration, on the differential gene expression. In addition, we are analyzing stained tissue sections for histopathologies, and will correlate those findings with the expression data across tissues.

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Conference Questions and Answers

Question:
You showed morphological changes within a section of gill. Did you show any effect on oxygen transport?

Answer:
We think it does have an effect, but we have not gotten that far yet. We can relate the changes in the gill to other studies (rat inhalation) that have indicated toxic effects in the lung.

Save

Integrative Strategies to Understand Nanomaterial-Biological Interactions

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Abstract

The rapid rate of discovery and development in nanotechnology will undoubtedly increase the potential for both human and environmental exposures to novel nanomaterials. While numerous applications promise benefit to human health or the environment, the potential health and environmental risks associated with the unique properties of nanoscale materials are unknown and may lead to unintended health and safety consequences. The current gap in nanoparticle toxicological data dictates the need to develop rapid, relevant and efficient testing strategies to assess these emerging materials of concern prior to large-scale exposures. Here we present a novel approach that utilizes a dynamic whole animal (*in vivo*) assay to reveal whether a nanomaterial produces adverse responses at multiple levels of biological organization (i.e. molecular, cellular, systems, organismal). Early developmental life stages are often uniquely sensitive to environmental insult, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. Most toxic responses result from disruption of proper molecular signaling, thus, early developmental life stages are perhaps the ideal life stage to determine if chemicals or nanomaterials are toxic. Therefore, the embryonic zebrafish model was chosen to investigate nanomaterial biological activity and toxic potential. Investigations using this model system can reveal subtle interactions at multiple levels of biological organization, thus we have developed an EZ (embryonic zebrafish) metric for nanomaterial toxicity (EZ-metric) that takes into account the types and frequency of sublethal effects in addition to overt mortality. The EZ-metric was used to compare morbidity and mortality elicited from exposure to over 100 novel engineered nanomaterials using the Nanomaterial-Biological Interactions (NBI) knowledgebase at Oregon State University.

Introduction

Scientists and engineers, whether in industry, government or academia, have a common need to understand how nanomaterials interact with biological systems. The importance of this information is most obvious for applications in biomedicine such as targeted drug delivery, novel therapies using nanomaterials as agents, prosthetics, regenerative medicine, diagnostics and imaging. Information gained at the interface of nanomaterials with biological systems

can be used to guide materials design (e.g., biomimicry), optimize synthesis processes (e.g., nanomanufacturing architecture), and integrate 'soft' and 'hard' platforms (e.g., bionics). However, consideration is now being given to the environmental and health implications of nanotechnology so information on how and why nanomaterials may interact with, and potentially alter, biological processes is of critical importance. Ultimately, this foundational knowledge can be used to direct the safe development of future nanomaterials and nanotechnologies and provide input into the regulatory process, two strategies that could improve public perception of nanotechnology.

Investigations to understand nanomaterial-biological interactions are fraught with complexities. First and foremost would be the lack of relevant baseline data on nanomaterial characteristics, biological effects, and determinants of the unique properties that are so desired. There are few standard methodologies and materials that are specific for nanotechnology and nanomaterials. Recent studies conducted by the Nanotechnology Characterization Laboratory (NCL) indicate that adaptations of standard protocols in toxicology may be necessary for the correct interpretation of test results [1]. Additional complexity is derived from the sheer diversity of nanomaterials that are being/or will be tested in a broad array of animal systems and cell based assays. Obtaining comprehensive knowledge of nanomaterial-biological interactions and responses will likely require consideration and inclusion of the entire body of data produced from global efforts in this research area[2]. Thus, the Oregon Nanoscience and Microtechnologies Institute (ONAMI) is working jointly with Oregon State University to develop a knowledgebase of Nanomaterial-Biological Interactions (NBI) to address such critical infrastructure needs for nanotechnology.

The goal of ONAMI's Safer Nanomaterials and Nanomanufacturing Initiative (SNNI) is to develop new nanomaterials and nanomanufacturing approaches that offer a high level of performance, yet pose minimal harm to human health or the environment. The SNNI research paradigm is a testing-redesign loop that utilizes multiple whole-animal systems (e.g., zebrafish, water flea, fruit fly) to rapidly evaluate the biological responses to nanomaterial exposure[2]. One such rapid testing platform is the embryonic zebrafish assay. In this assay, developing zebrafish embryos serve as an integrated sensing and amplification system that is sensitive to perturbation. This experimental platform offers the power of whole-animal investigations (e.g., intact organism, functional homeostatic feedback mechanisms and intercellular signaling) with the convenience of cell culture (e.g., cost- and time-efficient, 96-well plate exposure chambers, minimal infrastructure, small quantities of nanomaterial solutions required). Here we present data on a variety of nanomaterials that were testing using this novel approach. Oftentimes, we are able to test a series of materials that differ in only one aspect, for instance, size or surface groups. Such an approach is preferred when the aim is to develop design rules for benign nanomaterials.

Methods

We used the embryonic zebrafish assay to perform screening-level toxicity evaluations of carbon fullerenes, carbon nanotubes, nanoparticulate metal oxides, nanoscale polystyrene spheres, CdSe Quantum Dots®, PbS nanoparticles, fluorescein-labeled cowpea mosaic viral nanoparticles, tobacco mosaic viral nanoparticles, multi-functional dendrimers, gold nanoparticles, silver nanoparticles, nanocrystalline cellulose and silicon nitride nanoparticles. Basically, zebrafish

Danio rerio embryos were collected from group spawns [3] and staged [4]. The chorion surrounding the embryo was removed enzymatically [5] at 6 hours post fertilization (hpf) and embryos were placed in 96-well exposure plates, one animal per well, at 8 hpf. Embryos were exposed to 100µl of nanomaterial solution over a broad concentration range, typically 5-fold serial dilutions ranging from 16 parts per billion (ppb) to 250 parts per million (ppm).

Waterborne-exposed embryos were evaluated at 24 hpf for viability, developmental progression and spontaneous movement (earliest behavior in zebrafish). At 120 hpf, behavioral endpoints (motility, tactile response) were thoroughly evaluated *in vivo* and larval morphology (body axis, eye, snout, jaw, otic vesicle, notochord, heart, brain, somite, fin, yolk sac, trunk, circulation, pigment, swim bladder) was evaluated and scored in a binary fashion (present or absent). Control and nanomaterial-exposed groups are statistically compared using a standard proportionality test, the Fisher Exact test. Based on the lethal and sublethal effects data from our embryonic zebrafish assays, we have developed an EZ (embryonic zebrafish) metric for nanomaterial toxicity (EZ-metric) that takes into account the types and frequency of sublethal effects (morbidity) in addition to overt mortality elicited from exposure.

Results

The toxicity of gold nanoparticles was influenced by synthesis methods, purity, core size, and surface functionalization (charge). The vast majority of dendrimers (12 out of 17) did not elicit a response independent of the generation (~size). Those that elicited a significant response were amine terminated but not of a specific generation. No response was elicited from exposure to a series of 10 viral nanoparticles except for the tobacco mosaic viral capsid functionalized with polyethylene glycol. Of eleven nanoparticulate metal oxides tested, approximately half were benign to embryonic zebrafish and toxicity appeared to be related to particle shape and reactivity.

Discussion

The embryonic zebrafish model is extremely useful for rapidly assessing the potential of nanomaterials to interact with and alter biological processes. Using this model, we determined differential toxicity profiles for diverse groups of nanomaterials in an effort to define relationships between nanomaterial physicochemical properties and the biological responses they elicit. The EZ-metric was established to provide a relative comparison of nanomaterial-elicited effects on integrated living systems. Our calculated EZ-metrics were consistent with other statistical measures. However, for the majority of nanomaterials tested, we did not observe significant adverse biological outcomes. There were some unique observations from those nanomaterials that did elicit significant responses. Extremely small changes in size, such as an increase in size from 0.8 to 1.5 nm, can significantly affect the biological response to gold nanoparticle exposure. Dendrimers which have potential applications for drug delivery were relatively benign to the developing embryonic zebrafish. Viral nanoparticles that were functionalized with polyethylene glycol (PEG) elicited significant deleterious effects while the other viral nanoparticles did not. This may be due to the increased residence time of nanomaterials functionalized with PEG. The toxicity of nanoparticulate metal oxides appears to be related to differences in shape of the materials but was not correlated with zeta potential.

Conclusions

The embryonic zebrafish model system can be employed to rapidly gain information and provide feedback to engineers designing novel nanomaterials. Incorporating toxicological evaluations early in research and development schemes will allow us to close the testing-redesign loop and favor the development of nanomaterials with minimal toxicity. Metrics that combine data on morbidity and mortality are valuable for understanding overall whole organism response and relating it to cellular and molecular level responses. Our results revealed that characteristics such as purity, size, surface functionalization, synthesis method, particle shape, and reactivity are important parameters governing nanomaterial toxic potential.

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Conference Questions and Answers

Question:
Are you basically using Multi-criterion Decision Analysis to evaluate adverse effects on the zebra fish screening-level assay?

Answer:
Yes, the EZ Metrics system is basically the same as Multi-criterion Decision Analysis.

Question:
How does the zebra fish assay relate to other toxicity assays that are closer to human exposure?

Answer:
Other researchers are using the zebra fish for biomedical research and believe that it correlates well; there is about 80 percent gene homology. The zebra fish assay gives a target area for further investigations. Having said that, it is difficult to see what an isolated hepatocyte preparation tells the investigator about human physiology.

Question:
Which effects seem to be the most sensitive in this assay?

Answer:
Pericardial edema (swelling of the heart) seems to be very sensitive and is commonly seen; however, this effect may be mediated by several mechanisms. We have also seen effects on the notochord that cause it to push out to the exterior.

Save

Nanotoxicology: Developing a Responsible Technology

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Abstract

Nanotechnology is not only an emerging field of study, it is now an industry. Because of this, we now see an abundance of nanomaterials in numerous consumer goods. Still further, well established industries, such as food packaging, forestry and paper, plastics and paints, and electronics are beginning to use nanotechnology's scientific and engineering-based advances to better their products, profit, and marketability. The research presented here describes basic concepts of nanotechnology and its potential health and safety risks, the current status of nanomaterial-containing consumer products in the marketplace, and nanomaterial synthesis and physico-chemical properties important to toxicological and ecotoxicological evaluations. This material aims to prepare chemists, toxicologists, risk assessors, and policy-makers to meet the rapidly growing need to understand and evaluate the risks that engineered nanomaterials may pose to human health and the environment. A toxicological and risk assessment of nanomaterials requires an understanding of the unique differences between these "new" materials and their previously studied chemicals or larger-particle predecessors. For example, studies on the biocompatibility of various metal oxide nanoparticles (such as titanium dioxide, aluminum oxide, and iron oxide) in various crystalline forms exposed to whole animal and cultured cells are compared and contrasted to the more commonly used micro-sized particles. Results show that, depending on chemical composition, crystalline structure, and type (and degree of) surface modifaction, nano-scale metal oxide particles may induce elevated levels of alkaline or acid phosphatase and increase levels of lactate dehydrogenase (an indicator for "leaky" membranes). However, larger metal oxide particles remain relatively inert in cultured cells, the lungs of rats, and algae test systems. Current methods for, and challenges to, toxicological and ecotoxicological testing of nanomaterials will be covered. Most importantly, this work identifies strategies in the material design process that minimize potential human health and safety risks when working with nano-scale materials.

Introduction

The use of nanomaterials is expected to have great potential to advances devices and procedures in the medical field, improve consumer goods and industrial products, and tackle rising energy requirements. This opportunity is based on the unique physical and quantum properties that vary continuously with changes in the size of some materials produced between 1 and 100 nanometers. As with any new technology, the potential risks associated with nano-based products is needed in order to properly assess the safety of the novel materials being developed. The potential human and environmental health risks are determined by the hazards posed and the potential exposures to the nanomaterials that are developed for use in products. Both hazard and exposure potential will vary widely for different nanomaterials and for different products that

incorporate nanomaterials. This work focuses on relating nanomaterial physical and chemical properties to its potential hazards in human health and the environment. The properties of nanomaterials are predominantly associated with their nanometer size scale, structure-dependent electronic configurations, and extremely large surface area-to-volume ratios relative to larger-sized chemicals and materials. Size falls in the region between individual atoms or molecules and their corresponding bulk materials (Colvin 2004). Particle size and surface area are important properties from a toxicological perspective because as the size of a particle decreases, its surface area increases. This allows for a greater portion of the total atomic make-up to be located on the material's surface rather than within its interior. These atoms on the surface of the material may be chemically and biologically active, potentially contributing to the development of adverse health effects. Other physical and chemical properties such as shape, surface coating, aggregation potential, and solubility may also affect the reactivity and mobility of nanomaterials, with the possibility of negating or amplifying any associated size-related effects.

Nanotoxicology. The evaluation of the safety of nanomaterials will likely require a multidisciplinary approach between toxicologists and experts in materials science, chemistry, physics, biotechnology, engineering, and/or other appropriate disciplines. The physical and chemical properties of nanomaterials can modify cellular uptake, protein binding, translocation from portal of entry to the target site, and the potential for causing tissue injury (Oberdorster et al. 2005a). The unique chemical and physical properties of nanomaterials may present special challenges to the toxicologist or ecotoxicologist when designing studies to accurately and reproducibly identify adverse biological interactions or effects. Scientific experimentation in this area is complicated by several factors including: the need to (1) characterize nanomaterials during several stages of toxicological testing (e.g., before, during after nanomaterial administration); (2) express and/or administer the dose of nanomaterials (e.g., mass, surface area, or particle number; (3) confirm that the nanomaterial aggregation state at time of administration; and (4) identify analytical difficulties in detecting and quantifying nanomaterials in biology and the environment.

Risk Assessment Process for Nanomaterials. Risk assessment is the systematic scientific characterization of potential adverse health effects resulting from human or environmental exposures to hazardous agents or situations (NRC 1983, 1994). As with larger-sized chemical substances, risk assessment will be the basis of assessing and regulating nanomaterials to protect human health and the environment. A risk assessment consists of four components, 1) hazard identification – qualitative evaluation of the adverse effects of a substance, 2) exposure assessment – evaluation of the types (routes and media) and magnitude or levels of exposure, 3) dose–response evaluation – relationship between dose and incidence (or severity) of an adverse effect, and 4) risk characterization – quantitative estimation of the probable incidence of adverse health effects under various conditions of exposure, including a description of the uncertainties involved (Purchase 2000). Thus, the distinction between the *hazard* (an inherent toxic property of a chemical that may or may not be manifested, depending on exposure potential) and *risk* (the consequences of being exposed to a hazardous chemical at a particular exposure level) is critical (Purchase 2000).

Characterizing Nanomaterials for Toxicological Evaluation. One obstacle in developing safety information on nanomaterials involves technical issues associated with conducting

reliable and reproducible toxicity assessment (Bucher et al. 2004) and among these issues is the characterization of materials to be tested (Powers et al. 2007). Particle characterization in solution or suspension is just as important as characterization the dry, as-received phase. In addition, a characterization profile of the nanomaterial within the in-life test system should be included. Characterization of nanomaterials can be divided into three categories based upon the physical state of the nanomaterial (i.e. dry, wet, or in life) and are referred to as primary, secondary, and tertiary. Primary characterization is performed on particles or materials as-received in the dry native state. Secondary characterization is performed on particles or materials in the wet phase as a solution or suspension. Tertiary characterization is performed on particles or materials either *in vivo* or *ex vivo*.

Methods

The Nanomaterials. In this paper, we describe the characterization methods used to determine the physical properties of five metal oxide nanomaterial systems: titanium dioxide, zinc oxide, aluminum oxide, silicon dioxide, and iron oxide. All five materials were produced in either liquid phase or aerosol phase. All five materials were designed to be crystalline, spherical, and ~30 nm

Table 1. Techniques for evaluating physical properties of nanomaterials.

Property	Definition	Technique
Particle Size	The range of sizes of particles within a sample; this measurements gives an indication of the aggregation/agglomeration state	o Specific surface area (SSA BET) o Dynamic light scattering (DLS) o Electron microscopy (TEM & SEM)
Chemical Composition	Information on the material's intrinsic chemical toxicity can be attained; includes both composition of the particle's core and its surface	o Spectroscopy: X-ray photoelectric (XPS), Raman, Inductively coupled plasma atomic emission (ICP-AES), Fourier-transform infrared (FTIR), Differential thermal analysis (DTA)
Morphology	Information on aspect ratio for non-spherical particles; crystal structure for crystalline materials; allotropic forms for materials of similar chemical composition	o X-ray diffraction (XRD) o Electron diffraction (ED) o X-ray Photoemission Spectroscopy (XPS)
Surface	Information about the interface of the solid particle and liquid solvent can be attained	o Zeta potential & isoelectric point (IEP) o Electron spin resonance (ESR) o Chemiluminescence
This list is intended for establishing a relative metric of the **physical** properties of **nano**materials.		

in diameter at the time of production. Each system was suspended in Milli-Q ultrapure water, phosphate buffered saline solution, and cell culture media. Concentrations ranged 0.001 to 1000 mg/L (milligrams of material per liter of solvent).

Characterization Techniques. Transmission electron microscopy (TEM) is one of the most common and informative methods used to size nanoparticles in the dry state, in wet phase, and in cultured cells. TEM pictures were taken on three different sample states using the JEOL JEM-2010 microscope for dry state and cellular association analyses. For wet phase analyses, the FEI Tecnai G2 F20 FE-TEM microscope for wet phase analyses; each TEM sample was prepared by flash freezing 2 µl of 5 and 50 mg/mL nano-TiO_2 suspension via FEI Vitrobot at liquid nitrogen temperature (196°C) onto a 300-mesh copper/carbon grid (Ted Pella, Inc., Redding, CA). Alternative to the TEM method is dynamic light scattering (DLS). Although traditional methodologies are limited to the particle's size (data is less reliable as the size of the particle decreases), recent advances in the technique have improved light scattering measurements (Powers et al. 2006). Here, we used the Malvern ZetaSizer Nano ZS to determine size, size distribution, and surface charge of each nanomaterial system. For the specific surface area measurements (SSA) measurements, we used the BET (Braunauer, Emmett, and Teller) method to attain an area measurement that can then be converted (through stoichiometry) to a primary particle size. There is an inverse squared relationship between surface area and radius of a nanoparticle, so as the radius of the nanoparticle decreases, the surface area increases exponentially (Brunauer et al., 1938). We utilized the Micromeritics ASAP 2020 instruments for these studies. The materials were also characterized for morphology using X-ray diffraction (XRD) (Otwinowski and Minor, 1997). XRD patterns were collected using a Siemens Platform-Model General Area Detector Diffraction System with a Cu Ka source.

Surface Activity. Determining the surface activity of nanomaterial is non-trivial. The assay used depends on the electron configuration of the material being tested. Here, we briefly describe the use of luminal for TiO2 particles, but other dyes and techniques may be used to determine reactivity of other metal oxide systems. The chemiluminescence of luminol was used to qualitatively probe the production of RS over 20 min. This method, while not quantitative, does provide an indicator of RS production and is completed in the dark (Arnhold et al., 1991; Hadjimitova et al., 2002). Luminol (< 99%, Sigma) was prepared using Milli-Q water at 0.140 M NaCl, 10.0 mM PBS, and adjusted to pH 7.30 (Allen and Loose, 1976; Hallett and Campbell, 1983). Chemiluminescence intensities were measured with a SpectraMax M2 (Molecular Devices, Sunnyvale, CA).

Results

Results show that, depending on chemical composition, crystalline structure, and type (and degree of) surface modifaction, nano-scale metal oxide particles change in aggregation state, may induce elevated levels of some enzymes, and increase levels of cytotoxicity (as measured by cell density, viability, and oxidative stress).

Discussion

There are several challenges associated with the evaluation of the potential human and environmental health risks from the development and use of novel nanomaterials include

Table 2. Summary of results.

Metal Oxide Nanomaterial	*Surface Area (m^2/g)*	*Density (g/cm^3)*	*Calculated Size in Dry State (nm)*	*Particle Size in Dry State from TEM (nm)*	*Size in Wet Phase (Milli-Q water) (nm)*			*Surface Charge in Wet Phase (mV)*
					1 ppm	10 ppm	100 ppm	
titanium dioxide (TiO2)	51.19	3.88	30.2	27	125.0	280.8	298.5	+1.62
zinc oxide (ZnO)	32.1	5.6	33.3	39	150.6	176.1	167.5	-55.0
aluminum oxide (Al2O3)	54.350	3.8	29	48	42.1	110.3	870.1	-21.8
silicon dioxide (SiO2)	31.4	2.66	71.8	30	52.0	50.1	60.3	-
iron oxide (Fe2O3)	48.6	5.2	23.5	30	188.3	237.2	750.7	-

All five materials were designed to be crystalline, spherical, and ~30 nm in diameter at the time of production.

appropriate toxicological studies for the hazard evaluation of nanomaterials, characteristics of nanomaterial-containing products, increased funding for EHS research and opportunities for collaborations.

Conclusions

At this point, it appears that the research, development, and production of nanomaterials are greatly outpacing the speed by which toxicological and exposure information is being acquired on nanomaterials. An understanding of the mammalian and ecotoxicological profiles of nanomaterials will be necessary to prioritize those nanomaterials that are safe for use and to establish appropriate safety procedures for handling those nanomaterials that may pose potential health hazards if there is sufficient exposure in the workplace, to consumers, or in the environment. When conducting physical and chemical characterization of nanomaterial properties, each material property should be measured using more the most appropriate technique, and when possible, results should be confirmed with an additional analytical technique. No single technique can accurately describe the properties of a nanomaterial. Methodological limitations, non-trivial sample preparation, and incorporation of the appropriate controls are all issues investigators should consider when analyzing nanomaterial samples.

It is important that nanomaterials are developed responsibly, with optimization of benefits and minimization of risks, with international cooperation to identify and resolve gaps in knowledge. To maintain a high level of public health, occupational health, and environmental protection, it will likely be necessary to conduct nanomaterial-specific risk assessments to evaluate any potential human and environmental health effects and to ensure the development of safe nanomaterial-containing consumer products.

Conference Questions and Answers

Question:
The characterization matrix for nanoparticles studies is very extensive, especially for their surface chemistry, and is time consuming. Have you thought of using a hypothesis-driven approach?

Answer:
Yes, the hypothesis-driven approach needs to be maintained and incorporated by new and experienced researchers to avoid the unnecessary expenditure of time and materials. However, the characterization of nanomaterials may differ with time-at time zero, 24 hours, 30 days, and post-exposure-but this does not answer the question.

Question:
Is the difference in the persistence of the inflammatory response due to the severity of the response and its time to resolve, or to the persistence in the lungs of the nanomaterials?

Answer:
The nanomaterials will be coated with lung surfactants; however, when they are acid washed, the nanomaterials appear to be unchanged. They maintain their catalytic ability and do not appear to be degraded. The coating nanomaterials receive in the lung does not apparently degrade the particles.

Use of Multi-Criteria Decision Analysis for Classification of Nanomaterials

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Abstract

There is rapidly growing interest by regulatory agencies and stakeholders in the potential risks associated with nanomaterials throughout the different stages of products' life cycle (e.g., development, production, use and disposal). Risk assessment methods and tools developed and applied to chemical and biological agents may not be readily adaptable for nanomaterials because of the current uncertainty in identifying the relevant physico-chemical and biological properties that adequately describe the materials. Such uncertainty is further driven by the substantial variations in the properties of the original material because of the variable manufacturing processes employed in nanomaterial production. We propose a decision support system for classifying nanomaterials into different risk categories. The classification system is based on a set of performance metrics that measure both the toxicity and physico-chemical characteristics of the original materials, as well as the expected environmental impacts through the product life cycle. The stochastic multicriteria acceptability analysis (SMAA-TRI), a formal decision analysis method, was used as the foundation for this task. This method allowed us to cluster various nanomaterials in different risk categories based on our current knowledge of nanomaterial's physico-chemical characteristics, variation in produced material, and expert estimates. SMAA-TRI used Monte Carlo simulations to explore all feasible values for weights, criteria measurements, and other model parameters to assess the robustness of nanomaterial grouping for risk management purposes.

Abstract

There is rapidly growing interest by regulatory agencies and stakeholders in the potential toxicity and other risks associated with nanomaterials throughout the different stages of the product's life cycle (e.g., development, production, use and disposal). Risk assessment methods and tools developed and applied to chemical and biological materials may not be readily adaptable for nanomaterials because of the current uncertainty in identifying the relevant physico-chemical and biological properties that adequately describe the materials. Such uncertainty is further driven by the substantial variations in the properties of the original material because of the variable manufacturing processes employed in nanomaterial production. To guide scientists and engineers in nanomaterial research and application as well as promote the safe use/handling

of these materials, we propose a decision support system for classifying nanomaterials into different risk categories. The classification system is based on a set of performance metrics that measure both the toxicity and physico-chemical characteristics of the original materials, as well as the expected environmental impacts through the product life cycle. The stochastic multicriteria acceptability analysis (SMAA-TRI), a formal decision analysis method, was used as the foundation for this task. This method allowed us to cluster various nanomaterials in different ecological risk categories based on our current knowledge of nanomaterial's physico-chemical characteristics, variation in produced material, and best professional judgments. SMAA-TRI uses Monte Carlo simulations to explore all feasible values for weights, criteria measurements, and other model parameters to assess the robustness of nanomaterial grouping for risk management purposes.

Introduction

Nanotechnology is a rapidly growing field of research that is already demonstrating a great impact on consumer products. The field of nanotechnology can be defined as the production and use of materials at the nano-scale, normally characterized as smaller than 100 nm in one dimension (Oberdörster et al., 2007). Nanomaterials are formed through both natural (e.g., combustion by-products) and synthetic processes. For the purposes of this paper, we focus our discussion solely on engineered nanomaterials, which are currently used in more than 600 different consumer products (Woodrow Wilson Institute, Online database, 2008). In spite of their potential commercial benefits, some nanomaterials have been identified as toxic in in vivo and in vitro tests. Clearly, our knowledge of the potential toxicity of these materials is far from comprehensive (Oberdörster et al., 2005; Thomas and Sayre, 2005). The potential environmental fate and toxicity (as well as potential for exposure and risk) of nanomaterials may be strongly impacted by the material's physico-chemical characteristics. For example, potentially toxic nanoparticles that tightly bind to soil surfaces may exhibit limited movement through the environment. In this case, such materials may be deemed relatively safe for certain specific uses. Such information is important as a lack of understanding of nanomaterial toxicity and risks may delay full-scale industrial application of nano-enabled technologies.

Nanomaterial research and regulations could be guided by a systematic characterization of factors leading to toxicity and risks in the absence of definitive data. In this paper we propose a risk-based classification system for nanomaterials that takes into account several parameters commonly associated with ecotoxicity and environmental risk of nanomaterials. These parameters vary from nanomaterial physico-chemical characteristics to expected environmental concentrations to fate and transport mechanisms. In this work, we focus primarily on ecological risks although the same methodology could be applied to human health risk assessment. This work does not attempt to draw exact conclusions about the environmental risks associated with different nanomaterials, but rather to provide reasonable recommendations about which nanomaterials may need more precise measurements and testing to be safely deployed in consumer products.

MCDA Approaches to Classification

Clustering nanomaterials into ordered risk categories can be treated as a sorting problem in the context of multi-criteria decision analysis (MCDA). MCDA refers to a group of methods used

to impart structure to the decision-making process. Generally, the MCDA process consists of four steps: (1) structuring the problem by identifying stakeholders and criteria (nanomaterial properties in this case) relevant to the decision at hand, (2) eliciting the parameters of the model (weights, thresholds, etc.), and assigning measurements for each alternative (e.g., nanomaterial risk group), (3) executing the model through computer software, and (4) interpreting results of the model and possibly re-iterating the process from step 1 or 2 by re-evaluating the model. The goal of this MCDA process was not to select a single best alternative, but to rank or group alternatives through a structured process. A detailed analysis of the theoretical foundations for different MCDA methods and their comparative strengths and weaknesses is presented in Belton and Stewart (2002). A review of MCDA applications to environmental management can be found in Linkov et al., 2006.

The SMAA-TRI sorting method (Tervonen et al., 2009) is well suited for the proposed classification system given the uncertainty of available information regarding the physico-chemical characteristics of nanomaterials (see Figueira et al., 2005a, for a review of other MCDA sorting methods). Many of the characteristics attributed to nanomaterials are limited to a solely qualitative assessment. We used SMAA-TRI, an outranking model based on ELECTRE TRI (see e.g., Figueira et al., 2005b) for the assignment procedure. If an alternative outranked another, then the alternative was considered at least as good or better than another alternative. We preferred SMAA-TRI as it extends the capabilities of ELECTRE TRI by allowing the use of imprecise parameter values. ELECTRE TRI assigns the alternatives (different nanomaterials in this study) to ordered categories (risk classes). Three types of thresholds are used to construct the outranking relationships by defining preferences with respect to a single criterion. The indifference threshold defines the difference in a criterion that is deemed insignificant. The preference threshold is the smallest difference that would change the expert preference. Between these two lay a zone of "hesitation" or indifference. The veto threshold is the smallest difference that completely nullifies (raises a "veto" against) the outranking relation. The assignment procedure involves comparing the properties associated with a specific nanomaterial (g1, g2, …, gm) against a profile that includes ranges of criteria metric values corresponding to several risk classes. Comparisons are performed with respect to each criterion, taking into account the specified thresholds. The final classification decision is based on the profile criteria weights and specified cutoff level (lambda). For example, Class 4 represents the highest risk while Class 1 is the lowest risk (Figure 1).

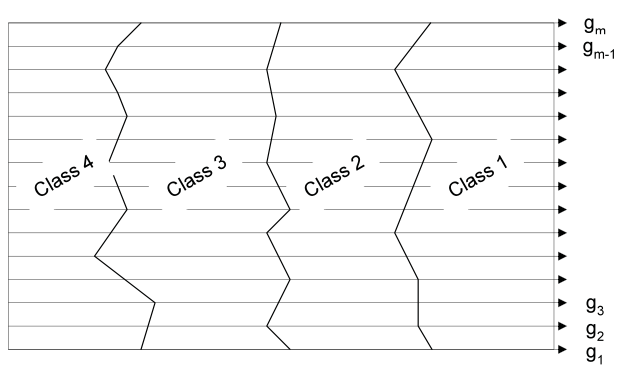

Figure 1. Example measurements of profiles for each criterion gj (adapted from Merad et al., 2004). Profiles are marked with horizontal lines.

The assigned criteria weights represent the subjective importance of the criteria. For this reason, ELECTRE TRI was particularly attractive for these classifications because the weights represent "votes" for each criteria which are not affected by criteria scales. The lambda cutting level represents the minimum weighted sum of criteria that have to be in concordance with the outranking relation for it to hold: the lambda cutting level is used to transform the "fuzzy" outranking relation into an exact one (whether an alternative outranks a profile or not). For example, a lambda cutting level of 0.6 means that 60% of the weighted criteria have to be "at least as good" for the outranking relation to hold.

Alternatives were compared by accounting for the three thresholds. An alternative and profile with scores of 0.4 and 0.6 (for the same criterion) respectively, and an indifference threshold of at least 0.2, demonstrates that this criterion fully supports the conclusion that the alternative outranks the profile. Sometimes the support is not binary, but is further affected by linear interpolation in the hesitation zone of both veto and preference thresholds (see e.g. Tervonen, 2007). In this case the support can have real values between 0 (no support) and 1 (full support).

All the parameters of ELECTRE TRI can be imprecise and represented by arbitrary joint distributions in SMAA-TRI. This feature allows us to make conclusions about risks related to different nanomaterials even though the information about their characteristics is limited. Monte Carlo simulations were used in SMAA-TRI to compute acceptability indices for alternative categorizations (i.e., for assigning nanomaterials in different risk classes). SMAA-TRI allows performing automatic sensitivity analysis.

Output of SMAA-TRI comes as a set of category acceptability indices which describes the share of feasible parameter values that assign alternatives to each category. The category acceptability indices are measures indicating the stability of the parameters, i.e, if the parameters are too uncertain to make informed decisions. A high index (>95%) signals a reasonably safe assignment of the alternative into the corresponding category. With lower indices, the risk attitude of the decision maker defines the final assignment. For example, if an alternative has a 80% acceptability for the lowest risk category, and a 20% acceptability for the second lowest risk category, a risk-averse decision maker could assign the alternative to the higher risk category.

SMAA-TRI conducts the numerical simulation by comparing the effect of changing parameter values and criteria evaluations on the modeling outcomes. Parameter imprecision can be quantified by Monte Carlo simulations using different probability distributions (uniform, normal, log-normal, etc). Gaussian or uniform distributions are typically used (for more information about SMAA methods, see Tervonen and Figueira, 2007).

If some model parameters need to have their sensitivity assessed, they can be considered as imprecise and defined as probability distributions.

Criteria

Recent articles, as well as the frameworks reviewed in this study, generally propose several different characteristics in the risk assessment of nanomaterials. These characteristics are generally based on extrinsic particle characteristics (size, agglomeration, surface reactivity,

number of critical function groups, dissociation abilities), (Biswas and Wu, 2005; Borm and Müller-Schulte, 2006; Borm et al., 2006; Gwinn and Vallyathan, 2006; Kreyling et al., 2006; Medina et al., 2007; Nel et al., 2006; Oberdorster et al., 2005; Thomas and Sayre, 2005). These various parameters are critical because they define the fate and relevant intact exposure pathways as well as internal dose required to assess risk (Powers, 2007; Tsuji et al., 2006). Summary descriptions of five basic extrinsic nanomaterial properties, agglomeration and aggregation, reactivity, critical functional groups, particle size, and contaminant dissociation are presented below:

• Agglomeration, weakly bound particles, and aggregregation, strongly bound or fused particles, (ISO, 2008) are important criteria of risk because it provides a description of the physical state of nanoparticles (NP) in the aquatic system (Kennedy et al., 2008); Wang et al., 2008). In aqueous solutions, NP agglomeration generally occurs by two mechanisms: colloid settling and flocculation. Flocculation occurs when Brownian-driven collisions bind unassociated particles together through Van der Walls forces by dehydrating the interacting surfaces. Consequently, the particle separates out of solution containing the mass of the previously unassociated particles. Settling, on the other hand, occurs due to the pull of gravity, as described by Stokes law relationships. Particles may settle but remain non-flocculated, settling at interparticle distances with the lowest free energies. In the absence of surfactive agents, particle flocculation is fairly predictable by particle charge. Charged functional groups give way to the development of a surface electrostatic potential which extends out a few nanometers at the solid-liquid interface forming a diffuse double layer or DDL (Bowden et al., 1977; Uehara and Gillman, 1981). Classical DLVO theory predicts that repulsive forces between particles (arising from overlapping DDLs) increase with increasing ion concentrations (or increasing ionic strength, I) because of rising osmotic pressures at the solid-solution interface force the DDL to swell (Evangelou, 1998, and references therein). Yet, classical Debeye-Huckel theory predicts a competing case where increasing ion concentration decreases DDL thickness, throwing a system into flocculation. Thus, at a fundamental level, the process of agglomeration represents the balance of these two competing charge interactions.

• Reactivity/Charge. Charge may be expressed on NP either by design (such as through functionalization) or by spontaneous degradative reactions. NPs may be functionalized with various types of groups, such as COOH, NH2, and SH2 through standard organic synthesis methods. Such functionalizations may be useful for manufacturing processes. For example, single-walled carbon nanotubes (SWNTs) are typically carboxylated at their ends as part of the isolation/purification processs (Anita Lewin, RTI International, personal communication). The type of charge occurring on functionalized NPs is called variable charge, which means that the magnitude of the surface electrostatic potential varies with solution pH (Uehara and Gillman, 1981). Variably charged groups characteristically exhibit a surface pKa. Thus, variably charged surface groups may be speciated (e.g., protonated vs. deprotonated) by the classical Henderson-Hasselbauch equation. Furthermore, the magnitude of the surface electrical potential may be suppressed by increasing I, as described previously. Thus, the reactivity of variably charged functional groups varies with the difference in solution pH from the surface pKa and the magnitude of I.

• Critical functional groups: Related to the reactivity/charge, critical functional groups make up

an important criterion given the fact that nanomaterial functionality and bioavailabilty is directly related to chemical species. Basing risk criteria on elemental speciation is superior to elemental composition alone because it identifies the unique set of reactions available to each species. For example, suspended zero-valent Fe nanoparticles have been shown to catalyze reductive degradations of aqueous organic contaminants (Joo et al., 2004). The same degradative ability has been shown for structural Fe2+ (higher oxidation state than zero-valent Fe but different speciation in terms of its complexation environment) domains at clay-edge and -interlayer nano-sites in soil (Hofstetter et al., 1999; 2003). The Cd2+ cation in quantum dots exhibits no toxicity to organisms as long as it remains complexed with Se (Derfus et al., 2004). Speciation also determines solubility or potential dissociation of nanomaterials.

• Contaminant dissociation: This criterion describes risk associated with residual impurities contained within the NP. For example, Fe oxide NP may contain S impurities depending on whether FeCl3 or Fe2(SO4)3 was used in manufacturing. Carbon nanotubes may contain Ni, Y, or Rb metal cation impurities (Bortoleto et al., 2007; Chen et al., 2004), which may either be entrained within or adsorbed onto the surface of the tubes. However, little is actually known about the extent in which metallic and organic contaminants remain with the manufactured product. Thus, the assignment of this risk criterion could change depending on better information.

• Size: Particle size is a criterion related to the agglomeration and reactivity criteria. Obviously, smaller particles agglomerate at slower rates. However, agglomeration is also related to the particle size distribution or polydispersivity. For example, greater monodispersivity of particles sizes appears to promote more stable dispersions (Chappell et al., 2008). Also, nanoparticle reactivity is also impacted by the size of NP surface relative to the bulk of the solid. While the surface is the reactive portion of solids, the bulk component may suppress the surface reactivity through internal reorganizations, etc. NPs are essentially surfaces with limited bulk. Thus, the smaller particle size, the lower bulk to potentially limit surface reactivity. Surfaces with low accompanying bulk have been shown to possess enhanced reactivities, such as high-affinity adsorption of metals or unique structures of assembly during agglomeration (Auffan et al., 2008; Erbs et al., 2008). Particle size is particularly important in terms of distinguishing the unique size-dependent chemistry of nanoparticles from classical colloid chemistry.

Processes that may influence the potential hazards of engineered nanomaterials include bioavailability potential, bioaccumulation and translocation potential, and potential for toxicity. These processes have been described in empirical studies and are dependent on the characteristics of the particles as described above. It is difficult to predict the behavior of these materials, however, in the future computational approaches are expected to provide additional tools to estimate these processes from the physical and chemical parameters.

• Bioavailability potential: Bioavailability describes the amount of material absorbed across cell membranes from the various exposure routes (e.g., dermal, inhalation, and oral exposures) into system circulation in an organism (Medinsky and Valentine, 2001). This process is controlled by the characteristics described above. For example, charge of the particles may influence the agglomeration of the particles and hence limit the ability of the particle to cross the gastrointestinal membranes after oral ingestion. There are however, several pathways which nanoparticles may cross cell membranes ranging from pinocytosis, endocytosis, and

diffusion as summarized by Unfried et al. (2007). The mechanism by which these particles are absorbed are highly dependent on the particle composition, surface modification, size, shape, and agglomeration.

• Bioaccumulation Potential: Bioaccumulation is the net accumulation of particles absorbed from all sources (soil, water, air, and food) and exposure routes listed above into an organism. Accumulation must consider the temporal aspects of exposure and include kinetic factors such as exposure concentration, duration of exposure, clearance, biotransformation, and degradation. Most studies to date have focused on the potential for uptake and translocation in specific tissues (Ryman-Rasmussen et al., 2006; Gopee et al., 2007; Kashiwada, 2006) and have not addressed the toxicokinetics of nanoparticles.

• Toxic Potential: Toxicity of engineered nanomaterials and particles in mammalian and other animal systems has been assessed primarily through cytotoxicity screening assays; although some in vivo studies have been completed. It is proposed that toxicological effects of nanomaterials occurs through oxidative stress, inflammation from physical irritation, dissolution of free metal from metal nanoparticles, and from impurities in nanomaterials (e.g., catalysts) (Oberdorster et al. 2007). The characteristics of nanoparticles that influence toxicity include the size, surface area, morphology, and dissolution. To date, screening studies using in vitro approaches have observed toxicity from metal nanoparticles at lower concentrations (Bradich-Stolle et al., 2007) than toxicity from carbon-based nanoparticles (Murr et al., 2005; Grabinski et al., 2007).

Proposed Classification Framework

The purpose of the proposed classification system is to preliminarily group nanomaterials in risk classes for screening level risk assessments. Such groupings should aid in prioritizing materials for further study. In this paper, we considered five risk categories: extreme, high, medium, low, and very low risk. In order to assign particular nanomaterials to these categories, we need to define criteria scales, thresholds, and measurements.

The quantitative criterion, particle size, was evaluated as the mean size of the material in units of nanometers as obtained from literature review and expert estimates. Bioavailability, bioaccumulation, and toxic potential were measured through subjective probabilities that the nanomaterial has significant potential in the criterion. These, as well as rest of the criteria (agglomeration, reactivity/charge, critical function groups) were measures based on expert judgments. The qualitative criteria, agglomeration, reactivity/charge, and critical function groups, were measured in terms of ordinal classes: 1 was the most favorable (least risk) value class, while 5 the least favorable (highest risk).

For the qualitative criteria, we encoded the classes with integers. The indifference thresholds were set to 0 and the preference thresholds to 1. This choice of thresholds represented an ordinal scale: a smaller number was preferred to a larger one, but the intervals did not carry any information (e.g. 1 is as much preferred to 2 as 2 is to 3). If there were multiple possible classes for an alternative, the measurement was modeled with a discrete uniform distribution, meaning that the density function for the distribution was such that the integers corresponding to these classes were equiprobable. Veto thresholds were not used in this phase of the framework, but will

be added later when more information about the criteria becomes available. Size is a criterion that should have some veto associated with it, so that very small materials cannot be assigned to the safer (lower risk) categories.

Even though nanomaterial size is believed to be a factor influencing toxicity, there is little specific information available characterizing toxic effects relative to the 1 to 100 nm size range (Powers et al., 2007). More research is needed to define the thresholds in a more exact manner. If a "smaller"-sized nanoparticle represents higher risk, it follows that a larger size is "more preferable" because of its inherently lower risk. Due to these knowledge gaps, imprecise thresholds were used for nanomaterial size with indifference threshold of 10 ± 5% and preference threshold of 25 ± 5%.

Bioavailability, bioaccumulation, and toxic potential were all measured using a cardinal but subjective scale as described above. Because of the subjectivity of this scale, we applied imprecise thresholds. Indifference thresholds were set to vary uniformly from 0 to 10, and preference thresholds from 10 to 20.

The SMAA-TRI model separated the risk categories using profiles formed from measurements of the same criteria as the alternatives. In our framework, the profile measurements were all exact (Table 2).

Our model applied imprecise preference information in the form of weight bounds. For more information on how these were implemented, see Tervonen and Lahdelma (2007). We judged the toxic potential to be the most important criterion, and thus it was assigned weight bounds of 0.3–0.5. Bioavailability and bioaccumulation potentials were deemed the least important criteria, and as a result, we were undecided on their relative importance. Both of these criteria were given weight bounds ranging from 0.02–0.08. The rest of the criteria were assigned weight bounds of 0.05–0.15.

We used imprecise values for the lambda cutting level within the range of 0.65–0.85. Lambda defines the minimum sum of weights for the criteria that must be in concordance with the outranking relation to hold. The classification was performed according to the pessimistic assignment rule, which in risk assessment applications represents a more conservative approach.

Criteria measurements. The first four criteria are measured as ordinal classes. Measurements of reactivity/charge have associated uncertainty in that the materials can belong to either of the indicated classes. The following three criteria have linear imprecision of 10 in both directions from the indicated mean value. Size has uncertainty of 10% of the shown mean value.

Example

We demonstrated application of the framework by classifying five nanomaterials: nC60 (a fullerene), MWCNT (Multi-Walled Carbon Nanotube), CdSe (quantum dot), Ag NP (Silver-Nanoparticles), and Al NP (Aluminum Nanoparticles). Typical size ranges for these materials were estimated based on in situ measurements from the available literature. Other properties were assessed using authors expert judgments, taking into account the characteristics for each criterion described in Section 3. Metrics for the five materials used in our case study (Table 2) as well other model parameters were input into the SMAA-TRI software. Even though

Profile	Agglomeration	Reactivity /charge	Critical Function groups	Contaminant Dissociation	Bioavailability potential	Bioaccumulation potential	Toxic potential	Size
Extreme-high	4	4	4	4	100	100	100	5
High-medium	3	3	3	3	80	80	80	50
Medium-low	2	2	2	2	70	70	70	100
Low-very low	1	1	1	1	60	60	60	200

Table 1: Profile measurements. Each row corresponds to a profile differentiating the categories presented in the first column.

	Agglomeration	Reactivity /charge	Critical Function groups	Contaminant Dissociation	Bioavailability potential (±10)	Bioaccumulation potential (±10)	Toxic potential (±10)	Size (±10%)
C60	4	2, 3	3	2	25	50	10	100
MWCNT	4	2, 3	4	3	25	50	25	50
CdSe	4	4, 5	1	4	50	75	75	20
Ag NP	3	4, 5	1	4	50	75	75	50
Al NP	5	1, 2	1	1	25	75	10	50

Table 2: Criteria measurements. The first four criteria are measured as ordinal classes. Measurements of reactivity/charge have associated uncertainty in that the materials can belong to either of the indicated classes. The following three criteria have linear imprecision of 10 in both directions from the indicated mean value. Size has uncertainty of 10% of the shown mean value.

criteria metrics used in the paper were assessed using expert judgment and its objectivity can be questioned, the outranking algorithms used in SMAA-TRI together with the the choice of absolute thresholds implemented in this study allows to obtain robust results (Tervonen (2007).

Category acceptability indices obtained from the simulation are presented in Figure 2. These indices show that the data was too imprecise to make definite decisions about the risks related to the different nanomaterials. However, there was sufficient data to make preliminary classifications. For example, CdSe exhibited a very high index in the high risk-class. On the other hand, Al NP may be considered relatively safe, its category acceptability indices for low and very low risk were 34 and 34, respectively. Summing these indices gave the material an estimated 68 percent probability of being classified as "low to very low risk". C60 showed a reasonable acceptability index (49%) for the low risk category. In terms of making risk-aware decisions for C60 and Al NPs, we feel that further studies into expanding the potential applications of Al NP and C60 (as opposed to CdSe) are justified.

It is important to point out that in spite of the high uncertainty of the above results, this work represents a reasonable starting point for a more thorough follow-up analysis. And indeed, more data is required to improve our estimates. Risk estimates based on acceptability indices below a certain threshold (e.g. 80 %) should be viewed with caution. For example, should C60 be deemed viable for further research and application, additional measurements will be required to further refine the risk estimates. In spite of its limitations, the quantified risk values determined from our simulations are helpful in characterizing the risk and uncertainty for limited and variable data.

	Extreme risk	High risk	Medium risk	Low risk	Very low risk
C60	0	0	51	49	0
MWCNT	0	26	73	1	0
CdSe	0	98	1	1	0
Ag NP	0	29	71	1	0
Al NP	0	0	33	34	34

Figure 2. Category acceptability indices of the example. A high index means, that the material is assigned to corresponding category with a higher confidence as measured by larger share of possible parameter values corresponding to this category.

Concluding remarks

Nanotechnology is a fast growing research field with an increasing impact on our everyday

lives. Although nanomaterials are used in common consumer products, the lack of information about human health and environmental risks may hamper the full-scale implementation of this technology. We presented in this paper a systematic multi-criteria approach that allows for assigning nanomaterials into ordered risk classes. Materials assigned to the highest risk class potentially represent areas of important future toxicological studies while materials exhibiting low risk may be recommended for targets of research aiming at commercial use. The proposed framework takes into account measurements and expert estimates for multiple criteria that are known to impact the toxicity of the material.

The use of SMAA-TRI approach allows for the explicit incorporation of uncertainty parameters in the model. An appealing characteristic of the outranking model applied in SMAA-TRI is that it allows veto effect to be modeled, meaning that a nanomaterial's poor performance in one criterion cannot be compensated by good performance in other criteria (as is the case for compensatory MCDA models, e.g. utility theory). This convention prevents decisions about the risk of a particular nanomaterial being unduly based on one particular criterion (such as size vs. surface reactivity relationships) as the material may have other physico-chemical characteristics related to size that exhibit a greater impact on its toxicity.

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Conference Questions and Answers

No Questions.

MSDSs Fail to Communicate the Hazards of Nanotechnology to Workers

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Background

In the United States, the Occupational Safety and Health Administration's Hazard Communication Standard (29 CFR 1910.1200) requires that employers inform their workers of the chemical hazards to which they are exposed and how they should protect themselves. The 2006 European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) initiative on chemical hazard communication is more comprehensive and ambitious than the OSHA requirements.

Nanomaterials are widely believed to have begun a new revolution in manufacturing that will broadly provide improved products and capabilities in areas as diverse as sports equipment and biomedical sensors. Engineered nanoparticles, however, have been shown in animal studies "to reach the alveolar region; avoid macrophage engulfment; cause oxidative stress, inflammation, and fibrosis; and translocate into the blood." (1) The National Institute for Occupational Safety and Health (NIOSH) has raised concerns about what prevention and control actions should be taken while toxicological research is ongoing. (2)

A more fundamental question is what should workers currently manufacturing these products be told about the risks they face? With $88 billion worth of products containing nanomaterials reportedly sold in 2007, there are clearly many workers potentially exposed. (3) Their numbers have been projected by the U.S. government to grow to 2 million worldwide over the next 15 years. (4) Given the limited toxicological information that is available for most nanomaterials, the task of effectively communicating the risks of handling these materials is daunting. Material Safety Data Sheets (MSDSs) are required for nanomaterials that meet the definitions of hazardous chemicals under OSHA's Hazard Communication standard. MSDSs from suppliers are the preferred source of risk information for nanotechnology firms, according to a survey of firms in Massachusetts. (5) The Wilson Center for Scholars website maintains the most comprehensive, publicly-available online inventory of commercial nanoproducts. (6) As of May 31, 2008, there were 609 materials in the Wilson Center nanoproduct database, which is growing by 3-4 products each week.

Unfortunately, industry hasn't done a good job of communicating the hazards of standard industrial chemicals despite the two and a half decades since the promulgation of OSHA's Hazard Communication standard in 1983 to get it right. This author participated in an OSHA-funded 1997 study of the peer-reviewed hazard communication literature. The results (which are still on OSHA's website) indicated broad shortcomings with the research methods, which generally relied on self-reported preferences rather than observations of actual behaviors and on students as test subject, rather than workers. (7) One representative study employed an expert panel to review the accuracy of the technical information in randomly-chosen MSDSs and found that only 11 percent of the MSDSs were accurate in all of the following four key areas: health effects, first aid, personal protective equipment, and exposure limits. Particularly pertinent to

nanomaterials, the health effects data on the MSDSs frequently were incomplete and the chronic data – the biggest unknown for nanoparticles - were often incorrect or less complete than the acute data. (8) Of significant concern, three separate studies found that literate workers only comprehended roughly 60 percent of the health and safety information on sample MSDSs. (9, 10, 11)

A recent review of more current literature regarding the accuracy, comprehensibility and use of MSDSs unfortunately did not show improvements over the 1997 review. Accuracy and completeness were found to be relatively poor: the majority of studies showed that the MSDSs did not contain information on all the chemicals present and workers showed low comprehensibility because of overly complex language. (12)

A key role of MSDSs is to communicate the government's regulatory requirements for specific chemicals. The U.S. governmental efforts to research and regulate chemicals have not kept up with industry's impressive ability to develop and produce new ones. Nanotechnology appears to be a tsunami wave heading towards this badly leaking ship. In the U.S. there are currently around 600 OSHA Permissible Exposure Limits (PELs) for individual chemicals, most of which haven't been updated in 40 years, despite new research findings. The Bush administration created only one health standard for a chemical (hexavalent chromium) in eight years and only after receiving a court order to do so. (13) There is no definitive count of the number of chemicals in regular use today, but an often cited estimate is around 100,000. The Chemical Abstract Service had registered 37,966,182 organic and inorganic substances developed by industry as of September 18, 2008. (14) Scanning Tunneling Electron Microscopy allows the manipulation of individual atoms. Given the 118 elements available for combination, an estimate of between 10^{200} to 10^{900} distinct nanoscale particles has been posited - an unimaginable number (particularly for regulators). (15)

Methods

The National Institute for Occupational Safety and Health (NIOSH) appears to maintain the most complete collection of MSDSs for nanomaterials and provided the Lippy Group a copy of the 49 MSDSs collected as of September 2007. All of these documents were then individually assessed to answer the following questions:

1. Is the actual component or components that contain nanoparticles clearly identified?
2. Is there cautionary language provided about nanomaterials?
3. What, if any, Permissible Exposure Limits or Threshold Limit Values are provided?
4. What ventilation is recommended?
5. What personal protective equipment is recommended?
6. Are explosive hazards noted where appropriate?

Results

- 33 percent of the MSDSs did not identify the nano-sized component in the material.
- 56 percent did not have any cautionary language pertaining to the nanosized component.
- 67 percent listed an OSHA Permissible Exposure Limit (PEL) or American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value, but all were tied

to the normal form of the nanomaterial (e.g. carbon black or graphite rather than carbon nanotubes).

- 89 percent recommended using respiratory protection, but tied it to the normal OSHA PELs or TLVs, which are often based on the OSHA nuisance dust standards.
- 79 percent recommended using local exhaust ventilation; of those that did, 25 percent recommended a face velocity greater than 100 feet per minute even though NIOSH has indicated that standard fume hoods operated at that rate tend to create too much turbulence to fully contain nanoparticles, which when dry are extraordinarily buoyant.
- None indicated that nanoparticles pose a much greater flammability risk even though the minimum ignition energy decreases exponentially with particle diameter. As the British Health and Safety Executive noted, "An increasing range of materials that are capable of producing explosive dust clouds are being produced as nanopowders." (16)

Discussion

Manufacturers of nanomaterials have an opportunity to learn from the hazard communication failures of the past and create informative tools that workers and employers find helpful, despite the acknowledged gaps in our current understanding of the toxicology of nanoparticles. This will require honestly describing what we know and don't know. At a minimum, manufacturers must identify which components in their formulations contain engineered nanoparticles. Listing OSHA PELs for macro-sized materials without any conditional statements may meet regulatory requirements, but borders on the unethical. For instance, the OSHA PEL for synthetic graphite is 15 milligrams per cubic meter, but Oberdorster reported "profound cytotoxity" for single walled carbon nanotubes in animal instillation studies for exposures at 0.38 micrograms per square centimeter and noted that even the low mass-based concentrations of nano-sized materials measured in workplace air (generally less than 50 micrograms per cubic centimeter) represent "very high particle number concentrations." (17) NIOSH has flatly stated that "… the occupational exposure limit for graphite should not be used to allow extensive exposure to carbon nanotubes that appear far more toxic than graphite," but this practice appears to be common among manufacturers of carbon nanotubes. (18)

Manufacturers of nanomaterials can have an informed and safer workforce, able to respond rationally to this remarkable new trend in manufacturing, but they must do a better job with MSDSs and government should help.

Recommendations

1. OSHA should require that all nanoscale materials be identified on MSDSs, to answer a question posed at a national conference by one of their nanotechnology experts. (19)
2. Given the absence of occupational exposure limits for nanomaterials, OSHA should require conditional language be included in all MSDSs containing nanomaterials to explain the inadvisability of using PELs derived for normal forms of the materials. One example proffered by a hazard communication expert: "Established exposure values do not address the small size of particles found in this product and may not provide adequate protection against occupational exposures." (20)

3. The Nanotechnology Environment and Health Implications Working Group of the National Nanotechnology Initiative should consider the hazard communications needs of workers currently creating nanoproducts, rather than wait until the toxicology data are conclusive. Technology Safety Data Sheets, informational tools developed for the U.S. Department of Energy to inform workers of the risks posed by new remediation technologies, can serve as an example of an alternative, creative approach. A majority of surveyed populations of technology developers, state environmental regulators and heavy equipment operators found these tools "quite valuable." (21) Given the abysmal results demonstrated thus far with the standard MSDS approach, it may be time to consider creating Nanotechnology Safety Data Sheets.

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Conference Questions and Answers

Question:
Material Safety Data Sheets (MSDSs) are based on Chemical Abstracts Service (CAS) Registration Numbers (RNs). How will nanomaterials be organized-as a Technology Safety Data Sheet (TSDS) for hazardous communications (HAZCOM)?

Answer:
A change to a TSDS is probably not necessary. However, an easily accessible document is needed that uses standard phrases. This approach has been adopted by the Europeans and the United States' National Institute of Occupational Safety and Health (NIOSH). Standard phrases incorporated in a safety communication document will help, but the CASRN will probably need to be added.

Question:
How is the term "nanotechnology" used in a product description?

Answer:
Some manufacturers use the term "nanotechnology," but there are no nanomaterials in the product. I do not know of any regulation affecting the use of the term.

Question:
How is risk communicated in the absence of information?

Answer:
We do not want to lose the potential of the field by being overly cautious, but standard industrial hygiene practice-for example, do not dump nanomaterials; use HEPA filters to keep particles down-can be used.

Q and A: Perspectives on Nanotoxicology

Panelists:

Dr. Christi Sayes, Dr. Igor Linkov, Dr. Bruce Lippy, Dr. Stacey Harper

Question:
We (Emory University Rollins School of Public Health) have been working with NIOSH nanotechnology industry field team studies looking at occupational exposure to carbon nanotubes, but have no characterization of different job titles that would help characterize exposure.

Answer (Lippy):
This is a key problem. In the past, we characterized the work force by who's doing what. Now, nobody seems to be doing this, but it should be done. NIOSH should be taking a lead.

Question:
This is a question for Igor Linkov. Your case studies look at many different types of nanomaterials, metals and metal oxides, various forms of carbon. If you just focused on one, aluminum oxide for example, what factors or features would you look at to determine toxicity as compared to looking at a range of materials?

Answer (Linkov):
We have already done this. To determine a range of toxicity, you can do the same "weight-of-evidence" procedure to develop a matrix, use professional judgment to weight the lines of evidence, and analyze the data.

Question:
U.S. EPA and the Organization for Economic Cooperation and Development (OECD) are working together to understand how to test nanomaterials for ecotoxicity, so that we can tell industry how to test materials before they are marketed. In Stacey Harper's presentation there appeared to be a mixture of exposure and response, but little characterization of the materials in the exposure medium. Could a nanomaterial be misrepresented if it appeared to be potent in a screen, but was, in fact, agglomerated in an exposure medium and, as a result, was less toxic?

Answer (Harper):
For many nanomaterials, we have moved on to the next level of investigation. We characterize the materials as received and characterize them again in the exposure medium, so we are addressing this point.

(Sayes):
It needs to be understood that nanoparticles will aggregate, agglomerate, but over time 95 percent of the nanoparticles I work with will de-aggregate.

(Harper):
However, are the characterizations we can do at the present time sufficient? Do we have sufficient data to be predictive? I do not think so.

(Sayes):
The methods we and the DuPont laboratory are using are adequate. However, there is a need to be open about data, and research should be collaborative.

Question:
Where is the European Union on assessing the toxicity of nanomaterials? Are they taking the same approach as the United States?

Answer:
The EU is taking a basically similar approach. There is a framework for judgment of toxicity, but a verbal framework may not translate into a quantitative risk assessment. Further discussions between the U.S. and the EU Commission are to be held in Brussels later his fall. An OECD member-country has made a strong call for regulation and enforcement of manufacturers in the area of product labeling. Products containing nanomaterials should be labeled with what nanomaterials and coatings they actually contain. Some studies suggest that the Europeans are taking a management approach and are spending more effort on looking at the implications of nanomaterials rather than on the applications.

Question:
Given where we are now in our understanding of the toxicity of nanomaterials, how do we communicate hazards to workers? How are hazards communicated and managed in research and engineering laboratories?

Answer:
Many laboratories engaged in the production of nanomaterials take no precautions, and engineering laboratory staff have been observed picking up nanomaterials with their bare hands. We must get the message out about uncertainty, and communicate "our best guess," until we have precise information.

(Lippy):
The National Institute for Occupational Safety and Health (NIOSH) is working on best practices for these materials. In the meantime, good work practices are still important, and local exhaust ventilation should be used in the work area. Uncertainty should be emphasized, and we must be honest about what we do not know.

Question:
Given that risk is a product of hazard and exposure, are we looking at the wrong end of this? We have little information on the raw materials, what they eventually turn into in the environment, and what happens in the event of a release.

Answer:
None.

Question:
What happens to these materials at the end of their life cycle, when they are cycling in the environment?

Answer:
This is being studied, but as yet there is little information in this area.

Question:
There is a term "over engineering." Are we in danger of "over-toxicity" for nanomaterials?

Answer:
I do not think so. What we learn from nanotoxicity could be applied to nanomedicine.

Comment:
There is a fundamental difference between the nanotoxicity of consumer products and nanomedicine that is regulated by the Food and Drug Administration (FDA). The FDA demands that materials are safe and effective, and that you know what happens to nanomaterials in the body after use. There is a requirement that nanomaterials are tracked through the body. However, consumer products containing nanomaterials may degrade and end up in a landfill, subject to the exotic chemistry of that location, and may end up in water streams.

(Linkov):
You cannot stop industrial progress, but you can go ahead with structured hypothesis testing. We will learn from mistakes. We need to continue studies and collect information.

Comment:
It is important to assess risk over the life cycle of nanomaterials. As pharmaceuticals are now turning up in drinking water, the question of where nanomaterials will end up is not irrelevant. Worker exposure is probably the most important current exposure at the moment.

Question:
Nanotechnology is a great source of federal funding. Not all nanotechnology is recent; it has been termed colloid science and catalytic science in the past. Colloidal gold was used before the Romans in the production of colored glass, and nanomaterials were released when the first log was set on fire. How do you assess the risk from nanomaterials, given that we are bombarded by them all the time? How do you adjust the risk assessment to take them into account?

Comment:
At last year's Society of Toxicology (SOT) meeting, a member from the National Aeronautics and Space Administration (NASA) stated that all human cancers are due to nanoparticles. That was an outlandish statement. Perhaps if you can isolate antibodies to specific nanomaterials and

look at their interactions, you might be able to isolate cause and effect.

Comment:
There is a need to work with industry. They could be given quick information on toxicity in return for their maintenance of a national database on nanomaterial toxicity.

Comment:
That is a beautiful idea, but there is a problem with proprietary information. Regulators want full information, but industry is usually unwilling to provide it. Maybe confidentiality agreements could be signed. There is a need for a database that will enable us to be predictive.

Comment:
A database could be constructed using metadata analysis of the characterization and effects of nanomaterials, without being explicit about the precise nature of the materials. Proprietary information need not be revealed, and QSARs (quantitative structure-activity relationships) need not be tied tightly to a particular product.

Question:
Are there any messages for average consumers that can be distilled down to help them estimate their own risk?

Answer:
We can divide nanomaterials into broad categories-benign and more risky. It may then be possible to engineer materials with properties that make them less toxic. We can try to integrate risk within set properties and ranges.

Question:
Then this is a development process to aid industry-this material may cause too much risk to continue to develop?

Answer:
Igor Linkov's models may be used to help industry to decide what to use or proceed with by providing "GO"/"NO GO" indications. Eventually consumers may be able to use this information.

Linkov:
The drug companies exploit QSAR to engineer and design drugs. It should be relatively inexpensive for a manufacturer to perform some up-front toxicity tests.

Question:
Is this what the nanorisk framework is about, "GO"/"NO GO" decisions?

Answer:
Yes.

Question:
How do nanomaterials affect consumers? We need to look first at potential health effects for workers and then move towards consumers.

Answer:
Look at work in Europe by Peter Wick. Look at sensitive sub-populations, such as pregnant women. Nanomaterials move across the placenta. That's where some real progress can be made.

Question:
What are we looking at for an exposure metric-concentration, chirality, surface charge?

Answer:
We should look at all of them. We need someone to perform a search of the current literature.

Save

Bioavailability and Toxicity of Nickel in Metallic Nanoparticles

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Abstract

Catalytic routes are used for arc synthesis of commercial carbon nanotubes and nickel is a common metallic catalyst. Nickel and nickel oxide nanoparticles are also used commercially in nanomagnetic devices, batteries, fuel cells, catalytic convertors, and solar cells. Nickel is classified as a known human carcinogen and is a common industrial and environmental pollutant. Recent studies have shown that metallic nickel nanoparticles induce greater acute lung toxicity and inflammation than micron-sized nickel particles following intratracheal instillation in rodents that has been attributed to elevated surface area and reactivity of nanoparticles. Lung epithelial cells are a primary target for nickel-induced toxicity following inhalation of poorly soluble nickel particles. The proposed mechanism responsible for nickel toxicity is phagocytosis or endocytosis of particulate nickel by target cells. It is postulated that intracellular release of Ni (II) ions from the acidic environment of endosomes could interact with cytoplasmic or nuclear protein targets. In acellular assays, Ni (II) ions were mobilized from metallic nickel nanoparticles and mobilization was enhanced at acidic pH. Human lung epithelial cells (H460) internalized metallic nickel nanoparticles within 24 hours in vitro. Intracellular mobilization of Ni (II) ions was greater from metallic nanoparticles than from micron-sized particles. Metallic nickel nanoparticles also induced dose-dependent toxicity in parallel with intracellular Ni (II) ion mobilization as assessed by morphology, Syto-10/ethidium homodimer viability assay, and Pico Green fluorescence to quantitate cellular DNA. These experiments support a role for mobilization of Ni (II) ions from nickel nanoparticles in the enhanced toxicity of these metallic nanomaterials.

Introduction

Carbon nanotubes and metallic nanoparticles have great potential as novel chemical sensors and for new remediation technologies at Superfund and other toxic waste sites. Adverse human health effects due to occupational and environmental exposure to nanomaterials are a major concern and a potential threat to their successful commercialization and environmental and biomedical applications (Duffin et al., 2007). Realization of their commercial potential

will require a better understanding of the interactions of nanomaterials with biological systems and the development of new strategies to minimize human health risk. Manufactured nanomaterials are highly variable with respect to chemical and physical properties, state of aggregation, and purity. Toxicological screening is urgently needed to identify potentially hazardous nanomaterials and to re-engineer or post process these materials to minimize adverse environmental and health impacts (Borm et al., 2006).

Transition metal catalysts including Fe, Ni, Y, Co, or Mo are used in the manufacture of commercial carbon nanotubes (Donaldson et al., 2006). Transition metals in the form of metallic nanoparticles or metal oxides are also useful as high-efficiency catalysts for chemical remediation of contaminated ground water (Kanel et al., 2005), nanomagnetic devices, and the next generation of energy technologies including biomass gasification (Li et al., 2008), fuel cells, catalytic convertors, and solar cells (Irwin et al., 2008). Transition metals in various chemical and physical forms are known to be toxic and in the case of nickel and cobalt, known human carcinogens (Lison, 1996; Salnikow and Zhitkovich, 2008). Recent studies have shown that metallic nickel nanoparticles induce greater acute lung toxicity and inflammation than micron-sized nickel particles following intratracheal instillation in rodents that has been attributed to elevated surface area and reactivity of nanoparticles (Zhang et al., 2003; Monteiller et al., 2007).

Human lung epithelial cells (H460) were used as target cells to investigate bioavailability and toxicity of nickel in metallic nanoparticles. These experiments had three objectives: 1) to assess whether nickel (II) ions could be mobilized extracellularly from metallic nickel particles, 2) to determine whether human lung epithelial cells internalize metallic nickel particles and whether nickel (II) ions are mobilized intracellularly, and 3) to assess the acute toxicity of metallic nickel particles relative to soluble $NiCl_2$ in this model system.

Methods

Characterization of Nickel Particles

Nickel (II) chloride hexahydrate and metallic nickel particles were purchased from Sigma-Aldrich and sterilized at 400°C for 15 minutes under nitrogen gas. Surface areas were measured by the Brunauer-Emmett-Teller (BET) method and were determined to be 0.8 m^2/g for nickel microparticles and 4.4 m^2/g for nickel nanoparticles. Zeta potential was measured as 27 mV for the microparticles and -29 mV for the nanoparticles suspended in phosphate buffered saline, pH 7.2. Samples were sonicated for one hour in a Branson 2510 sonicating water bath before exposure to cells.

Mobilization of Nickel (II) Ions

Nickel (II) mobilization into cell culture medium was determined directly using ICP-OES analysis as described in Liu et al., 2007 over a range of doses equivalent to 1-10 ug/cm^2 used in the cell toxicity assays. Intracellular mobilization of soluble nickel (II) ions was visualized by Newport Green fluorescence as described previously (Ke et al., 2007). Newport Green dichlorofluorescein diacetate (Molecular Probes) is a cell permeant fluorescent probe that was diluted in dimethylsufloxide and Pluronic F127 detergent at a 1:1 ratio prior to loading at a final concentration of 5 um for 30 minutes at 37C. The cells and all cell culture labware were washed with Hank's balanced salt solution/1mM EDTA, pH 7.2 to chelate any extracellular metal ions

prior to loading. Fluorescence was visualized using a Nikon Eclipse E800 microscope and digital images were recorded.

Cell Culture and Toxicity Assays

The human lung epithelial cell line NCI-H460 was purchased from ATCC and cultured in monolayer in RPMI 1640 (GIBCO) containing 2% fetal bovine serum (Atlanta Biologicals) and 1% penicillin/streptomycin at 37°C in 6% CO_2, 94% air.

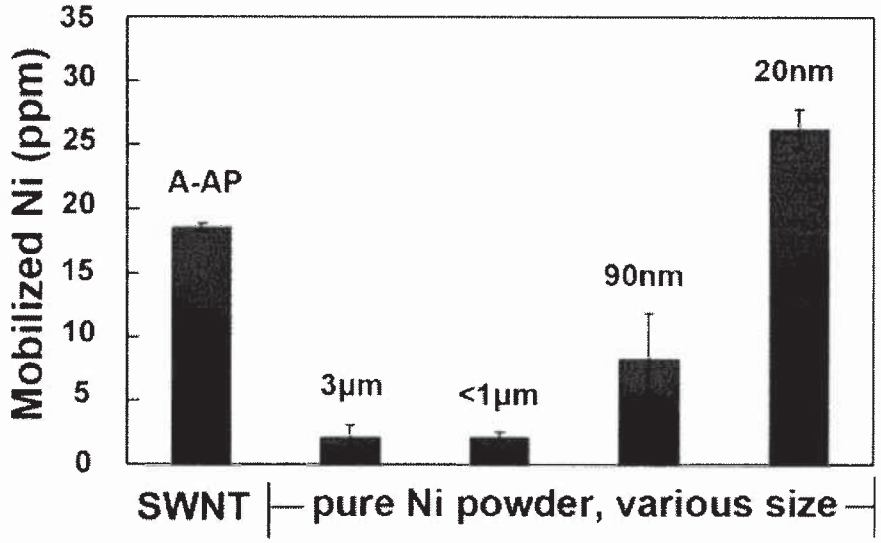
Cell Toxicity

Cell toxicity was evaluated by transmission electron microscopy, phase contrast microscopy, Syto-10/ethidium homodimer viability assay (Molecular Probes), and PicoGreen (Invitrogen) fluorescence induced by binding to cellular DNA.

Results

Previous studies (Liu et al., 2007) demonstrated mobilization of nickel at pH 5.5 in acetate buffer from commercial nickel particles at a dose of 200 ug nickel/ml (Figure1). As particle size decreases, significantly more nickel is mobilized from nickel nanoparticles compared to nickel microparticles. Based on this acellular assay, it is hypothesized that metallic nickel nanoparticles would lead to intracellular nickel mobilization and produce greater toxicity than nickel microparticles. Lung epithelial cells are a primary target for nickel-induced toxicity and carcinogenicity in humans following inhalation of poorly-soluble nickel particles (Oller et al., 1997). A human lung epithelial cell line (H460) was used to test this hypothesis. Cellular uptake of <100 nm and 3 um nickel particles was demonstrated by transmission electron microscopy after 24 hours of exposure (Figure 2). Agglomerates of metallic nickel nanoparticles were seen in cytoplasmic vacuoles. It is hypothesized that enhanced release of nickel (II) ions from nanoparticles would occur in the acidic environment of endosomes or phagolysosomes (Costa

Figure 1. Nickel mobilization from carbon nanotubes and nickel particles (Liu et al., 2007)



et al., 2005). After 48-72 hours of exposure to metallic nickel nanoparticles, there was a dose-dependent increase in Newport Green fluorescence that was not observed following exposure to 1 um metallic nickel particles (Figure 3).

Nickel (II) ions were also mobilized from nickel nanoparticles after 72 hours in cell culture medium; however, at the highest dose equivalent to 10 ug/cm^2 used in the cell toxicity assay, only 0.45-0.68 ppm of nickel was mobilized. This extracellular level of nickel is too low to induce toxicity in human lung epithelial cells. Direct exposure to 50 uM -100 uM $NiCl_2$ is required to induce necrosis in H460 cells after 24 hours (Figure 4). Exposure to H460 cells to metallic nickel nanoparticles induced dose-dependent toxicity after 48-72 hours as assessed by Syto-10/ethidium homodimer viability assay (Figure 3), phase contrast microscopy (Figure 3), and PicoGreen fluorescence (Figure 4). No toxicity was induced by exposure to H460 cells to metallic nickel microparticles over this range of doses.

Discussion and Conclusions

These studies show enhanced mobilization of nickel (II) ions from metallic nanoparticles in comparison with metallic micoparticles. Intracellular nickel (II) mobilization occurred after 24 hours of exposure of human lung epithelial cells to metallic nickel nanoparticles, followed by cell toxicity and necrotic cell death after 48-72 hours.

The proposed mechanism responsible for nickel toxicity is phagocytosis or endocytosis of poorly-soluble nickel compounds by target cells (Oller, 2002). Nickel may be delivered to cells following uptake of metallic particles, either as single particles or as respirable agglomerates. Intracellular mobilization of nickel (II) ions is postulated to occur in the acidic environment of endocytic or phagocytic vacuoles leading to release of nickel (II) ions into the cytoplasm and

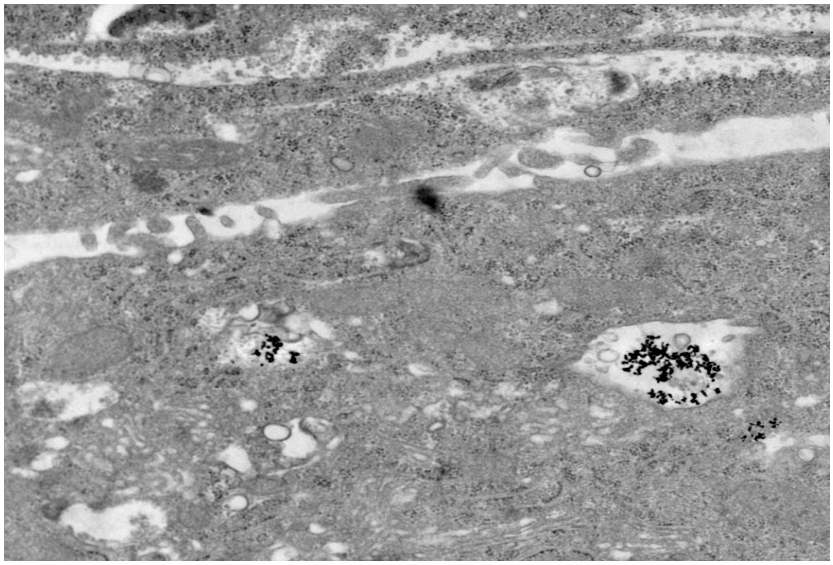


Figure 2. Internalization of metallic nickel nanoparticles by human lung epithelial cells in monolayer culture.

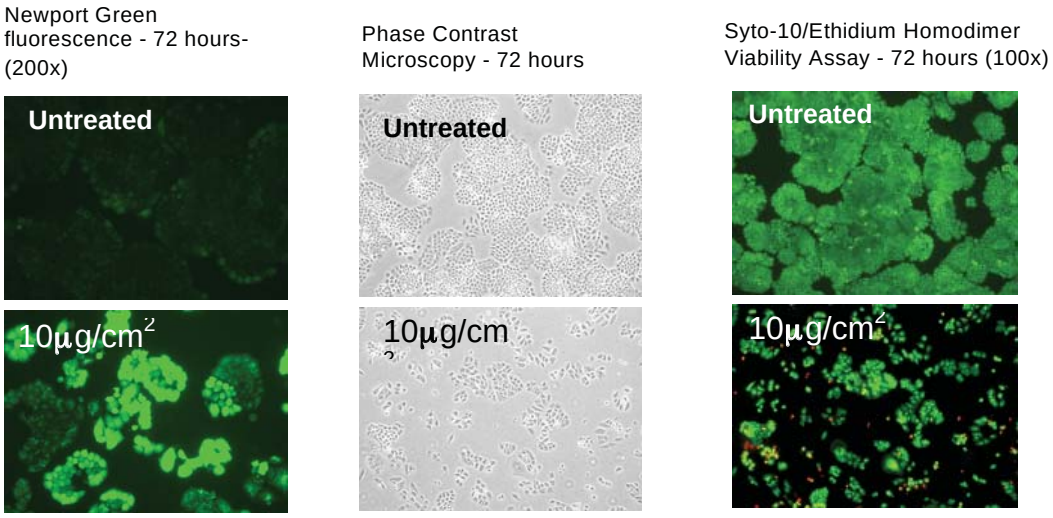


Figure 3. Exposure of human lung epithelial cells to metallic nickel nanoparticles leads to intracellular release of nickel (II) and toxicity after 48-72 hours**.**

nucleus (Costa et al., 2005). This nickel-ion hypothesis has been proposed as the mechanistic basis for nickel toxicity and carcinogenicity (Kasprzak et al., 2003). Nickel (II) ions have been shown to have both genetic and epigenetic effects that contribute to development of lung cancer (Denkhaus and Salnikow, 2002; Salnikow and Zhitkovich, 2008).

Bioavailability of nickel has been demonstrated in metallic nickel nanoparticles using acellular assays (Liu et al., 2007) and has been confirmed in a human lung epithelial cell assay in these

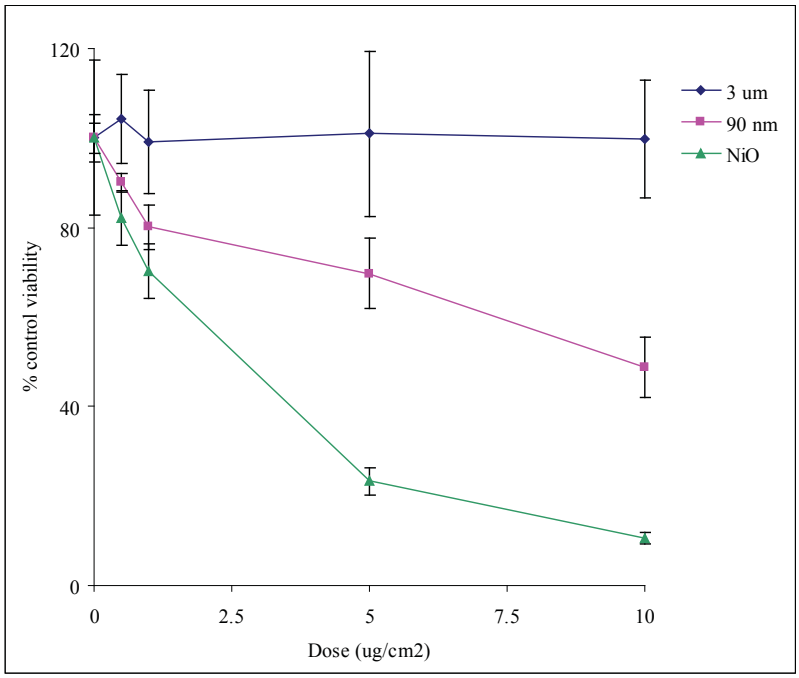


Figure 4. Toxicity of metallic nickel nanoparticles and microparticles after exposure of human lung epithelial cells for 72 hours.

studies. These results raise concern regarding potential toxicity and carcinogenicity of metallic nickel catalyst residues mobilized from carbon nanotubes or nickel nanoparticles following inhalation during manufacture and use of these nanomaterials.

Acknowledgements

This research was supported by a Superfund Basic Research Program Grant from the National Institutes of Environmental Health Sciences (P42 ES013550) and a Nanoscale Interdisciplinary Research Team Grant from the National Science Foundation (NSF DMI-05066. Although this research was funded by NIEHS and NSF, it does not necessarily reflect the views of these agencies.

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Conference Questions and Answers

Question:
How can dose best be represented? You compared nano and microsize particles. Did you apply the same mass as concentration for both of these?

Answer:
Yes. All volumes, concentrations, and surface areas were the same.

Question:
Was this true if dose is expressed as particle concentration?

Answer:
A larger number of nanoparticles were used, and the number of microparticles was smaller, but the amount of nickel was the same for both.

Question:
How did you choose the dose and the dose response curves?

Answer:
Dose response curves were performed to optimize the dose. We also found mobilization in the cell at 200 micromoles (μmol) soluble nickel, so we used this information as well.

Save

Partial Oxidation (Aging) and Surface Modification Decrease the Toxicity of Nano-Sized Zero-Valent Ironz

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Abstract

Zero-valent iron (nZVI) is a redox-active nanomaterial used for the in-situ remediation of contaminated groundwater. To assess the effect of "aging" and surface modification on its potential neurotoxicity, rodent microglia and neurons were exposed in vitro to fresh nZVI, "aged" (>11 mo) nZVI, magnetite, and polyaspartate surface-modified (SM) nZVI. Increases in oxidative stress occurred in BV2 microglia in the following rank order: nZVI > "aged" nZVI > magnetite = SM nZVI. ATP levels were reduced in N27 neurons in the following rank order nZVI > SM-nZVI >"aged" nZVI = magnetite. nZVI and SM-ZVI nanoparticles produced ultrastructural changes in exposed neurons. Physicochemical properties of each material, measured under exposure conditions indicated that all had electronegative zeta potentials. nZVI sedimented and agglomerated more rapidly than SM-nZVI or other materials. Correlating these properties with toxicity indicates that oxidation of nZVI decreases its redox activity, agglomeration, sedimentation rate and toxicity to mammalian cells.

Introduction

Nano size, zero valent iron (nZVI) rapidly degrades contaminants relative to iron filings because of its high surface area, high redox activity and unique catalytic activity. nZVI also generates reactive oxygen species (ROS) though Fenton chemistry. In aqueous environments, nZVI oxidizes over time (i.e., "ages") to magnetite (Fe3O4), and other oxides such as hematite and goethite. For in situ applications, concentrated (~10 g/L) slurries of nZVI are injected directly into the ground at or near the source of contamination where they rapidly form immobile agglomerates due to attractive magnetic forces. Since the mobility of bare nZVI is limited to a few centimeters, "second generation" nZVI particles are being developed that can be surface-modified (SM) with polymers or surfactants to increase their migration and therefore proximity to the pollutant materials. This increased mobility, as well as nZVI's direct application to groundwater also increases the likelihood that that nZVI will disperse more widely in the environment and at low concentrations, could enter the ecosystem and food chain and impact biological systems.

Several studies indicate that ingested or inhaled nanoparticles can cross biological barriers (i.e., alveolar, intestinal, testes, dermal) and migrate in small numbers to various organs and tissues where they can potentially damage organ systems sensitive to oxidative stress (OS) such as brain. To examine the possible neurotoxicity of nZVI and its related products, OS_sensitive rodent brain cells (BV2 microglia and N27 neurons) were exposed in vitro, to fresh nZVI, "aged"

nZVI, magnetite, and polyaspartate (poly AA)SM-nZVI . Measures of OS and cytotoxicity were collected and related to their particle size distribution, zeta potential, and dispersion stability of material under exposure conditions.

Methods

Fresh nZVI and SM-nZVI (MRNIP) were purchased from Toda Kogyo (Onada, Japan). The preparation of the nZVI formulations, cell culture assays, morphological preparations and methods for PC measurements have been previously reported.

Results

OS and BV2 Microglia. Intracellular H2O2 generated from the oxidative burst, depolarization of the mitochondrial membrane, and increases in caspase 3/7 activity occurred only in response to fresh nZVI and/or "aged" nZVI. Reductions of ATP occurred in the following rank order: fresh nZVI > "aged" ZVI > magnetite. SM-nZVI did not produce OS or apoptosis. Ultrastructurally, large nZVI agglomerates, housed in membrane-bound vacuoles, occurred in close proximity to populations of disrupted mitochondria in BV2. Light microscopy indicated that their nuclei were swollen and centrally located, a morphology suggestive of apoptosis.

Neurotoxicity in N27 neurons. ATP levels of were reduced after a 6 h exposure in the following rank order: Fresh nZVI > SM-nZVI > "aged" nZVI = magnetite. Ultrastructurally, the nuclei of nZVI exposed N27 neurons displayed a perinuclear and pericellular distribution of floccular material. The cytoplasm of N27 neurons, treated with SM-nZVI appeared normal ultrastructurally, although small agglomerates (~200-300 nm) and single nanosize particles of electron dense nZVI appeared throughout and within the cell's nuclei and mitochondria. Frequent examples of electron dense membrane invaginations, suggestive of clathrin-lined endocytotic vesicles were also noted in SM-nZVI treated neurons.

Particle Characterization. Fresh nZVI, and MRNIP contained 35±1 and 24±2% Fe0 content , respectively and indicated that both were redox active. In contrast, the Fe0 content of "aged" nZVI was negligible or absent for magnetite. The zeta potential measured in exposure vehicle, was consistently electronegative. In physiological buffer,, the zeta potential ranged from -13.8 mV to -18.6 mV and in BV2 and N27 media they ranged -7.1 mV to -10.1 mV and -8.0 mV to -13.3 mV, respectively. The particle size distributions for the different nZVI materials, measured under exposure conditions indicated a bimodal distribution in all exposure vehicles and contained both small (hydrodynamic radius, (RH)<1 μm) and large (RH>2 μm) agglomerates. The sedimentation of particles measured in each exposure vehicle. indicated that fresh nZVI agglomerated and sedimented faster relative to the other materials. The slow agglomeration and sedimentation rate of polyAA-SM-nZVI was due to the electrosteric stabilization of its poly AA-coating.

Discussion

The present data show that nZVI materials (i.e., fresh, "aged" and SM-nZVI) are differentially toxic to mammalian nerve cells. In every instance, fresh nZVI was more toxic relative to its "aged", oxidized or surface modified counterpart. Fresh nZVI produced higher levels of ROS in microglia and was more cytotoxic to N27 neurons. Interactions between nanoparticles and

the biological response of cells are influenced by physicochemical properties, including size, aggregation state, surface charge, and the presence of surface coatings. Our data indicated that differences in bulk chemistry, redox activity surface chemistry and dispersion contribute to the differential toxicity of the nZVI formulations. The poly AA surface coating affected both particle agglomeration and surface charge as well as cellular internalization. The agglomeration and subsequent sedimentation plausibly affected the exposure of the particles to cells in culture by modifying the amount and rate at which the materials physically contacted the cells.

Conclusions

These results have important implications on using nZVI materials for ground water remediation. The unmodified nZVI particles are relatively immobile, oxidize in water and "age" over months into the less toxic magnetite and/or maghemite. This indicates that bare nZVI particles have a relatively low risk to ecosystems. Surface coating increases nZVI's dispersion to the subsurface and also appears to decrease its toxicity. However, the coating facilitates the particle's physical entry into into cultured neurons, a finding that may have long term neurotoxic consequences.

Conference Questions and Answers

No. Questions.

Save

Ecotoxicological Evaluation of Carbonaceous and Metal Nanoparticles Through Bioassays Relevant to Environmental Fate

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Abstract

This proceeding summarizes nanomaterial (NM) fate and toxicity investigations conducted at the U.S. Army Engineer Research and Development Center (ERDC) on several Army relevant NMs (fullerenes, nanotubes, aluminum oxides).

Introduction

As the novelty of nanoscience evolves into product development, ecotoxicologists are challenged to proactively gain fate and toxicology information for a diverse suite of nanomaterials (NMs). Unique NM properties may require flexibility in existing risk assessment (RA) approaches (EPA 1998, Davis 2007). Environmental RA includes key components (e.g., hazard identification, exposure and effects, risk characterization) that necessitate identification of NM specific research tools and information (Dale et al. 2008). Hazard identification uses a conceptual model to identify relevant pathways, media, and receptors; we provide a generic conceptual model for NM (Figure 1) to guide research towards relevant NM sources, media, and exposure pathways (Metcalf et al., 2008). It is hypothesized that NM surface and suspension chemistry are controlling factors determining exposure pathways in biphasic systems. The learning curve is steep for establishing appropriate quantification, dosimetry and presumably unique fate, transport and biological uptake information needed for RA. In the present paper, we summarize our study of the partitioning and toxicity of raw fullerenes (C60), raw and functionalized multi-walled nanotubes (MWNTs) and aluminum oxides (Al_2O_3) in aquatic and terrestrial systems, to begin to gain the type of information needed for RA.

Methods

Test materials. Dry fullerene (C60) powder (99.5% purity) was obtained from SES Corp (CAT No. 600-9950, Lot BT-6977). Raw MWNT, MWNT-OH and MWNT-COOH (Cheaptubes, Inc., Brattleboro, VT, USA) and reference carbon (carbon black, activated carbon) are described in Kennedy et al. (2008). Nano Al_2O_3 (11 nm) was obtained from Nanostructures and Amorphous Materials (Houston, TX, USA).

Analytical and fate. Dispersions of C60 were created by adding 200 mg/L dry powder to various waters (Milli-Q, moderately hard reconstituted water or MHRW, 3 ‰ and 20 ‰) and magnetically stirring for four weeks (Oberdorster et al. 2006). Three replicates were sampled at various timepoints, allowed to settle for 24-h and analyzed for concentration by extracting C60 particles in toluene after H_2O_2 oxidization (Deguchi et al 2001; Oberdorster et al 2006) and analyzing by photospectrometry (λ = 336 nm) against a standard curve of known concentration.

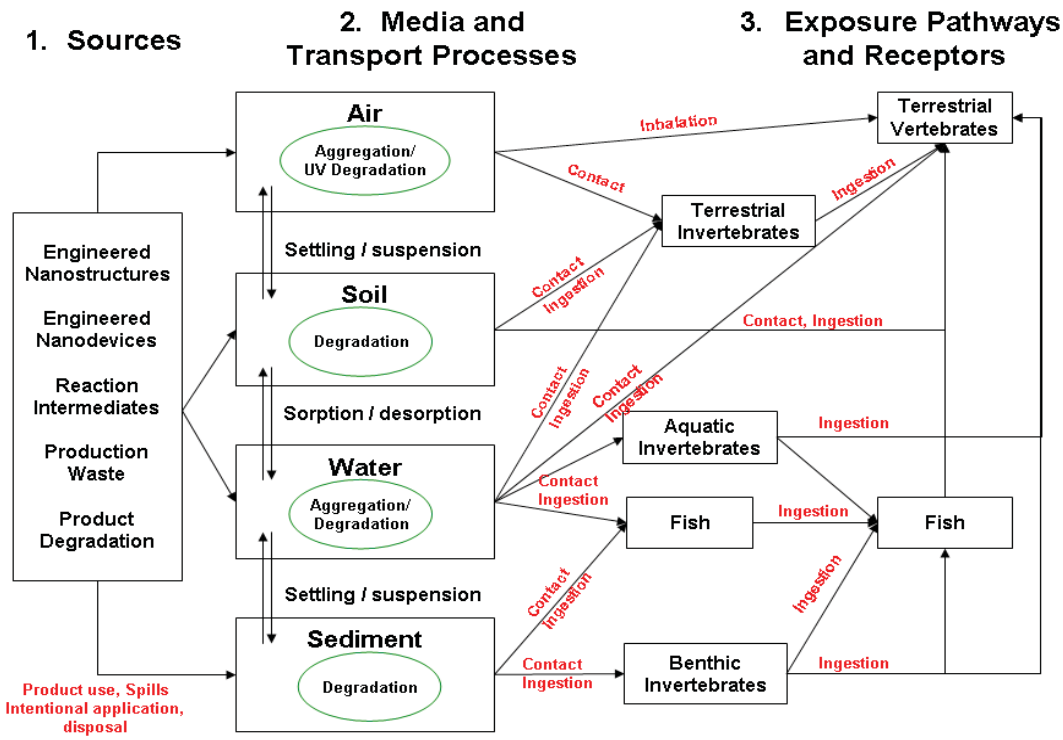

Figure 1. Conceptual model for potential nanoparticle and nanomaterial sources, fate, media and transport processes, and exposure pathways and receptors.

NMs were analyzed at ERDC using a Nova 3200e (Quantachorme Instruments, Boynton Beach, FL, USA), energy-dispersive X-ray (EDX) spectrometer (Quantax system, Bruker AXS, Ewing, NJ, USA) a 90Plus/BI-MAS (Brookhaven Instruments, Holtsville, NY, USA) and ZetaPALs (Brookhaven Instruments, Holtsville, NY, USA) for exposed surface area, elemental composition, aqueous particle diameter and particle charge, respectively. Particle concentrations (Al_2O_3) in tissue, sediment, and soil were quantified by inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS).

Bioassay. The cladoceran, *Ceriodaphnia dubia* (Aquatic Biosystems, Fort Collins, CO, USA) was the selected model for water toxicity studies on stable C60 and MWNT sols, tested at 25 ± 1° C for 48-h (U.S. EPA 2002). Methods for spiking sediments with MWNTs for *Leptocheirus plumulosus* and *Hyalella azteca* sediment tests (U.S. EPA 1994, 2000) are described in Kennedy et al. (2008). Nano and bulk Al_2O_3 were comparatively tested in sediment (*H. azteca*, *Tubifex tubifex*, *Lumbriculus variegatus*, *Corbicula fluminea*) and soil (*Eisenia fetida*). *Hyalella* response to nano or bulk Al_2O_3 was characterized in a 10-d exposure (survival, growth, bioaccumulation) in sediment homogenates (1-h rotary mixing at 1200 rpm) and a 14-d exposure to a thin surficial layer (0.625 or 2.5 g) on sediment or sand. A 10-d *T. tubifex* sediment toxicity (survival) bioassay and 28-d *L. variegates* and *C. fluminea* bioaccumulation bioassay were performed according to US EPA/US ACE (1998). Al_2O_3 was homogenized in

sediment for the *T. tubifex*, *L. variegatus*, and *C. fluminea* assays as above. Terrestrial exposures of *E. fetida* included a 28-d bioaccumulation, 48-h soil avoidance bioassay, and dermal uptake screening study. Bioaccumulation studies followed ASTM (1999) guidelines, with modifications. Soil (Grenada Loring (GL) Silty Loam, Learned, MS, USA) was spiked with increasing concentrations of bulk (tested range 0-13 g/kg) and nano (tested range 0-10 g/kg) Al_2O_3, tumbled for 24 h and hydrated prior to earthworm addition. Soil avoidance bioassays were conducted according to Environment Canada (2004). The acute (72-h) dermal uptake study followed a modified version of OECD guideline 207 (1984) using 1 ml of bulk and nano Al_2O_3 solutions (tested range 0-10,000 mg/L) to spike Whatman #1, 9 cm filter paper in 20 mL scintillation vials. Statistical significance was determined using one-way analysis of variance and Dunnett's test (SigmaStat 3.0, SSPS, Chicago, IL, USA). Lethal median concentration values (LC50) were determined by trimmed Spearman Karber (Toxstat, Gulley, 1996).

Results

Analytical and fate. For C60, we determined suspension characteristics such as high ionic strength (tested range: 0-20 ‰), low pH (tested range: 4-10) and low natural organic matter (NOM) concentrations (tested range: 0 – 100 mg/L) resulted in less stable C60 aggregates and reduced repulsive forces, measured as zeta potential (ζ). Dispersions of C60 were more stable in Milli-Q water relative to MHRW. Primary particles were not observed in any dispersion and concentration and aggregate size were charge related (Figure 2). The MWNT and reference carbon agglomerated and settled rapidly in fresh, estuarine and marine waters, and DLS indicated smaller effective diameter after sonication (Figure 3). However, NOM served as an effective surfactant, resulting in stable dispersions (ζ = -20 to -23 mV). The charge of Al_2O_3 particles in the dechlorinated tap (DTW) water used in sediment bioassays at neutral pH was negligible. Aqueous particle size characterization (DLS) of the nano Al_2O_3 in DTW yielded a bimodal particle distribution (120-170, 300-500 nm).

Bioassay. Water column exposure of stable C60 concentration (5 mg/L) did not result in toxicity to *C. dubia*. Neither Al_2O_3 particle size resulted in toxicity to *T. tubifex*. The growth of *H. azteca* was reduced only at the highest treatment of nano Al_2O_3 (100 g/kg). However, measured sediment Al concentrations from the bulk- and nano-spiked sediments differed substantially (55.1 ± 0.6 and 66.2 ± 0.6 g/kg, respectively). In thin layer exposures, nano Al_2O_3 was more toxic to *H. azteca* than bulk, with greater nano Al_2O_3 toxicity in sand relative to sediment. In 28-d bioaccumulation studies, biota-sediment accumulation factors were similar between nano and bulk Al_2O_3 for *Corbicula fluminea* but two-fold greater in *Lumbriculus variegatus* exposed to bulk Al_2O_3. Acute and chronic terrestrial toxicity and bioaccumulation studies with *E. fetida* determined nano Al_2O_3 reduced reproduction relative to controls and bulk Al_2O_3. Body burdens were higher in nano Al_2O_3 exposures relative to control and bulk exposures in chronic soil studies. Filter paper exposures resulted in greater uptake in both nano and bulk Al_2O_3 relative to control, although uptake was not dose dependent. Behavioral results suggested *E. fetida* preferred control to both nano and bulk amended soils (5, 10 g/kg).

Discussion

Particle dispersions with charges (ζ) greater than +20 or lower than -20 mV may be most appropriate for short- to long-term (> 48-h) water exposures while particle dispersions inside

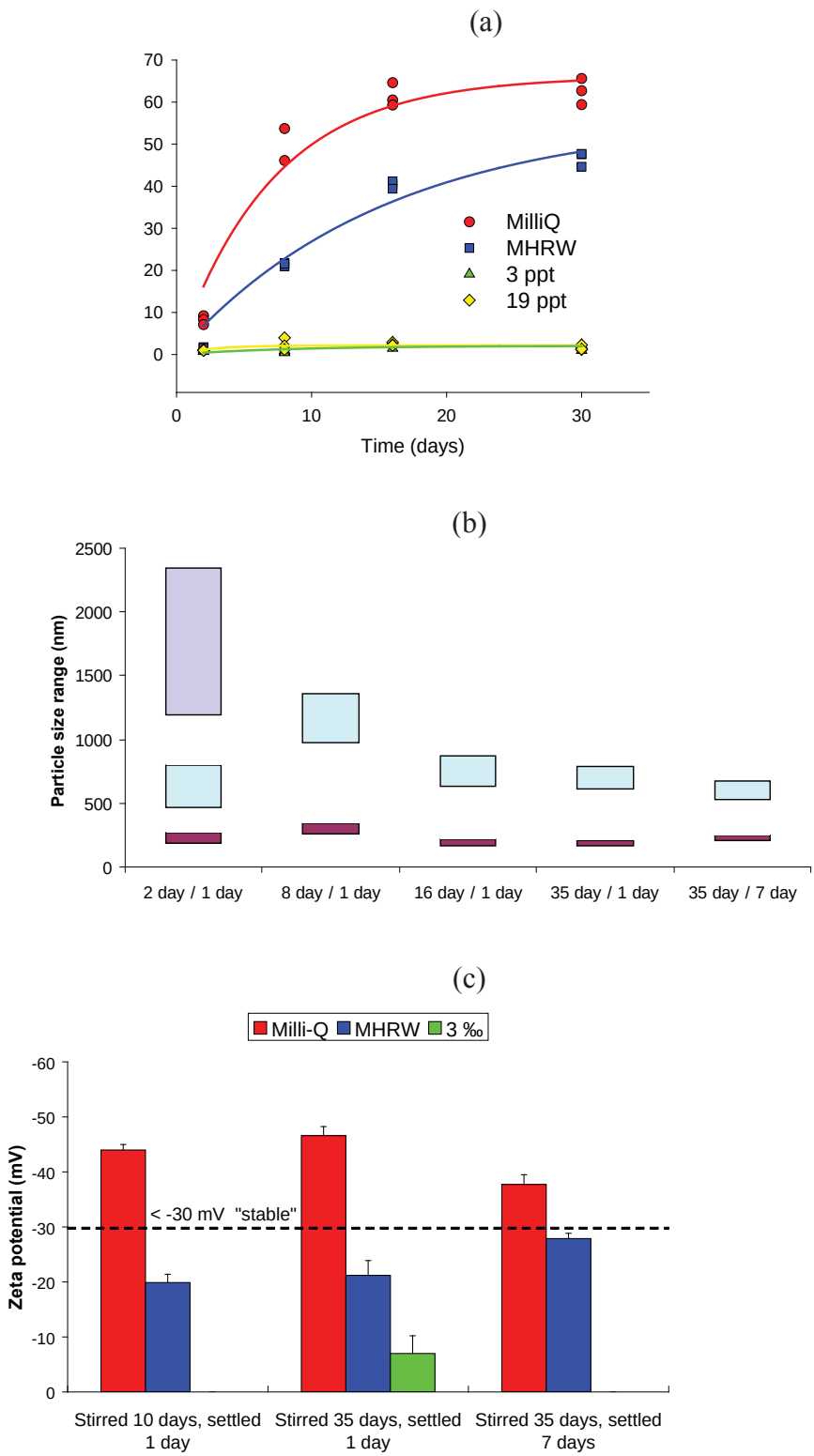

Figure 2. Aqueous fullerene (C60) dispersion characterization in milli-Q, moderately hard reconstituted water (MHRW), estuarine (3 ‰) and marine (19 ‰) waters. Panel (a) illustrates suspended concentration (C60), panel (b) shows the population size distribution in MHRW as determined by dynamic light scattering and panel (c) shows particle charge as zeta potential.

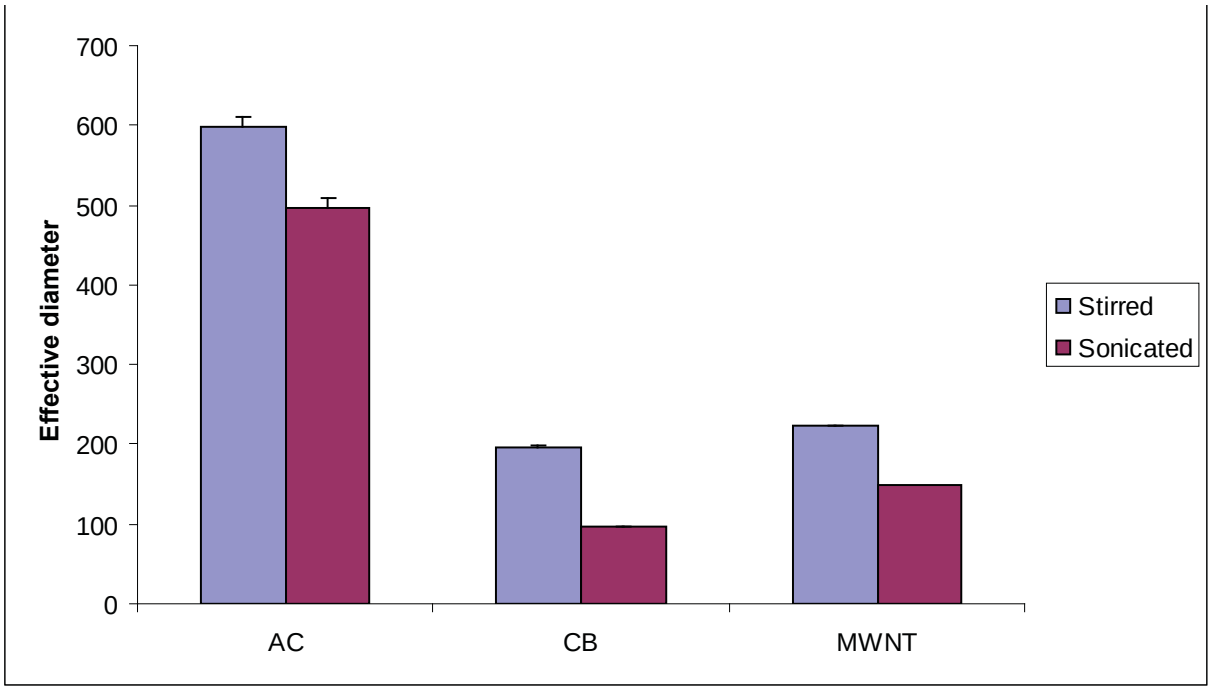

Figure 3. Effective diameters (nm) of carbon particles after magnetic stirring or probe sonication as indicated by dynamic light scattering. AC = activated carbon, CB = carbon black, MWNT = multi-walled nanotube.

this range may be less stable and more likely to partition to sediment. While fullerenes were not toxic at stable concentration (5 mg/L), it is likely higher stable C60 concentrations can be obtained by stirring concentrations less than 200 mg/L (Duncan et al. 2008). Additionally, exposure to C60 at higher, albeit unstable, concentrations (settling within 48-h) or longer exposures induce significant *C. dubia* mortality. Raw MWNT in NOM were toxic to *C. dubia* at 40-50 mg/L and 18 mg/L after 48-h and 96-h but MWNT-OH/COOH were not toxic up 80 mg/L (Kennedy et al 2008).

Since MWNTs in absence of NOM and Al_2O_3 dispersions were near the iso-electric point, sediment or soil bioassays were conducted. While MWNTs were toxic at very high concentrations (> 60 g/kg), they were less toxic than reference carbon (LC50: 19 - 27 g/kg) (Kennedy et al., 2008). This may occur for different reasons (e.g., micro-sized length dimension, stirring does not fully de-agglomerated MWNTs). Current studies in our laboratory indicate that sonication of MWNT in carriers allows for better dispersed, homogenous sediment. For Al_2O_3 sediment exposures, 10-d *H. azteca* growth was more sensitive to nano (100 g/kg) than bulk Al_2O_3 but substantial (~11 g/kg) differences in concentrations of bulk- and nano-spiked sediment precluded direct particle size toxicity comparison. A size-related toxicity effect was potentially indicated as nano Al_2O_3 reduced survival more than bulk in both sand and sediment in the thin layer exposure; greater toxicity in sand relative to sediment may indicate nano Al_2O_3 bioavailability to *H. azteca* may change with substrate type. Species-specific differences in relative bioaccumulation of bulk and nano Al_2O_3 were observed, potentially related to differing life characteristics and feeding (*H. azteca*, epibenthic detritivore; *L. variegates, infaunal*

sediment ingester, *C. fluminea*, facultative filter feeder). While the terrestrial fate of Al_2O_3 is yet to be fully characterized, Doshi et al (2007) reported that dissolution, formation of $Al(OH)_3$, and surface charge characteristics of nano Al coated with Al_2O_3 in a water/sand mixture may indicate increased transport in soil mixtures relative to pH. Nano Al_2O_3 chronic soil exposures resulted in decreased reproduction. Dermal (filter paper) exposures were difficult to interpret, as the high bulk exposures resulted in greater tissue concentrations than nano Al_2O_3. Aluminum recovery in high treatment bulk exposures may suggest ingestion of material or adherence of particles to the dermis. Earthworms avoided soils containing the highest concentrations for both nano and bulk Al_2O_3 (5, 10 g/kg). In combination with the chronic study, these data may indicate that impacts from nano Al_2O_3 on earthworms and aquatic benthos may occur at very high concentrations, unlikely to be found in the environment, in soil (2.5 g/kg) and sediment (66.2 g/kg Al). More studies are needed to determine the potential for longer term exposure, different NM preparation methods and dermal uptake to satiate risk assessment needs.

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Conference Questions and Answers

No. Questions.

Save

Impact of Nanomaterial Structure and Composition on the Ecotoxicology of Nanomaterials on Aquatic Species

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Abstract

There is a question as to how to predict the impact of exposure to nanomaterials on organisms, and specifically how changes in the structure of a particle will impact the physiological response of an organism to exposures. Our lab is using two models, Daphnia spp. and trout (Oncorhynchus mykiss) to examine the impacts of nanoparticle exposures on immune function, behavior, mortality and genomics of aquatic species. We investigated the impact of fullerene-based nanomaterials with different side chains and solubilities, as well as titanium dioxide, and gold nanomaterials on toxicity, physiology and gene expression response in Daphnia pulex. We have determined that core particle structure has a significant impact on toxicity and an affects the interaction of nanomaterials and Daphnia. Side chains and solubility have an impact on toxicity and physiological response that may provide a mechanism to mitigate the impacts of nanomaterials on aquatic species. However the impact of side chains may be tissue or assay dependent and in some cases the chemicals attached to the nanomaterials may have an independent impact. From this data we can summarize what characteristics of a particle may be the greater cause of toxicity so we can begin to make predictions about other types of particles to better inform risk assessment.

Conference Questions and Answers

Question:
Does Daphnia become a vector for C60s? If it is behaviorally inhibited from avoiding predation, it may be preyed upon more.

Answer:
Previous studies with gold nanoparticles show little tissue uptake by Daphnia. We may assume little tissue uptake of C60, but if Daphnia have C60 in their gut when eaten they could still be a vector.

Comment:
Other studies have shown cardiac effects in zebra fish.

Response:
We have not seen heart defects in Daphnia, as the heart is not visible, but hopping behavior may be related to C60.

Comment:
Tetrahydrofuran (THF) modified work with C60 and negated work on genetox (genetic toxicity).

Response:
THF does not negate the work, but it complicates the issue and needs further discussion. We still see genetox effects, and THF is important as it is still used to get C60 into products.

Evaluation of In Vitro Toxicity of Fullerene nC60 Derivatives Formed in Conditions that Simulate Disinfection Processes

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Abstract

The progress in the development of facile methods of producing water-soluble fullerene aggregates (nC60) brings about a higher likelihood of these materials' entering natural water reservoirs and drinking water supplies. Existing literature data on the potential toxicity of fullerenes and their derivatives show that the toxicity, if observed, is a strong function of the surface chemistry of fullerenes species. Disinfection processes such as ozonation and chlorination could, under certain conditions, modify surface properties of solubilized C60 nanoparticles and alter their toxicity. The study to be presented was aimed at the assessment of the toxicity of derivatized nC60 nanoparticles formed in conditions that simulate disinfection processes in a water treatment plant.

Conference Questions and Answers

Question:
C60 has oxidative stress ability. Why do you say you see no cytotoxicity to E.coli?

Answer:
The cytotoxicity of C60 depends on the method of preparation and is usually seen with solvent preparation.

Save

Carbon nanoparticle exposure alters protein expression and cell function in mouse renal principal cells

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Introduction

Carbon nanoparticles (CNP) are currently used in many industries, and their future application is likely to increase. Three common types include single wall carbon nanotubes (SWCNT), multiwall carbon nanotubes (MWCNT), and fullerenes (C_{60}). SWCNT consist of covalently bound carbon atoms arranged in a long, thin tube-like structure with a diameter of approximately 1.4 nm [1]. MWCNT have a similar structure, but they are longer than SWCNT and consist of several complex layers of nanotubes inside each other with a diameter of 10-20 nm [1]. C_{60} consist of 60 carbon atoms covalently linked together to form a spherical molecule.

Recent research has revealed diverse effects of CNPs on biological systems. One study indicated that SWCNT and MWCNT inhibit growth by apoptosis and loss of cell adhesion [2], while other studies suggest that carbon nanotubes seem to increase the growth of mesenchymal cells, cause fibrogenesis, and cause granuloma formation [3]. We have previously shown that MWCNT alter expression of genes for cellular transport, metabolism, cell cycle regulation, and stress response [4]. MWCNT are of special interest because of their structural similarity to asbestos [5]. Early experiments with fullerenes have shown them to be cytotoxic, and they have been shown to bind to ion channels [6]. Various types of nanoparticles are endocytosed and can alter the cytoskeletal organization [7].

The cell model used in the current study is the mouse principal cell type of the kidney cortical collecting duct, clone 4 ($mpkCCD_{cl4}$) cell line. $mpkCCD_{cl4}$ cells grow to form a confluent monolayer that simulates the barrier epithelial function and hormone responsiveness found in vivo in renal collecting ducts. These cells are of particular interest because they are responsible for much of the hormonally-regulated ion transport in the kidney. If the CNP exposure alters these cells, salt homeostasis could be modulated, resulting in changes in blood pressure.

We hypothesized that CNP exposure alters $mpkCCD_{cl4}$ cells resulting in abnormal cellular function. Experiments were conducted to determine functional, structural and proteomic changes induced by application of CNP to the renal barrier epithelial cells. Electrophysiological studies

were used to determine the effect of CNPs on transepithelial electrical resistance (TEER). Imaging studies were conducted to observe changes in specific cytoskeletal components and nuclear proliferation. Quantitative proteomic studies were conducted to correlate the observed structural and functional studies with CNP-induced changes in the expressed cellular proteome.

Methods

For the electrophysiological studies, SWCNT, MWCNT, and C_{60} were first sterilized by mixing in ethanol. After evaporation of ethanol under an ultraviolet germicidal lamp, the CNP were then diluted in fetal bovine serum to 5 mg/mL. Initially, CNP were diluted for each experiment in tissue culture media by vortexing. Later, to increase the efficiency of the CNP suspension, CNP/media mixing used sonication for electrophysiological, imaging, and LFQMS studies. Specifically, CNP were sonicated in FBS, sterilized via autoclave and diluted to 2% FBS-CNP in media and then diluted for each experiment. Cells were exposed to non-sonicated CNP at doses of 200 $\mu g/cm^2$ for 24 h in initial 2-DE studies and, in later experiments, exposed to sonicated CNP for 48 h at 20 $\mu g/cm^2$ or at 4 $\mu g/cm^2$ three times over 7 d.

Electrophysiological techniques were used to monitor TEER. Cells were grown to confluency over a period of 14 days on Transwell filters with CNP treatment as indicated in the figures. The filters were excised, mounted in a Ussing chamber, as described in detail previously [8]. The spontaneous transepithelial potential difference across the monolayer was measured and clamped to zero. The resulting short circuit current is a measure of net ion flux. Every 200 seconds, the zero holding potential was changed to a different holding potential and the resulting deflection in the short-circuit current (SCC) was measured and used to calculate the TEER by Ohm's law.

For imaging, replicate cellular monolayers were washed, blocked, exposed to mouse monoclonal PCNA antibodies overnight at 4°C, washed, and exposed to goat anti-mouse Alexofluor 488 secondary antibody. Another set of cells were exposed to Rhodamine-phalloidin and DAPI. All cells were visualized using a Nikon Eclipse TE2000-U Microscope fitted with a Nikon Digital Camera.

Proteins from duplicate samples exposed to non-sonicated SWNT and MWNT at 200 $\mu g/cm^2$ for 24 h, were analyzed by two-dimensional electrophoresis (2-DE) and altered proteins identified via mass spectrometry as described [4], and those from cells used in the TEER experiments (sonicated CNP at 20 $\mu g/cm^2$ for 48 h) by label-free quantitative mass spectrometry (LFQMS) [9]. Briefly, cells were lysed and solubilized in appropriate lysis buffers, and in the case of LFQMS, the resulting cell lysates then reduced, alkylated, and tryptically digested. Peptide concentration was determined by Bradford Protein Assay. Peptides were subjected to LC/MS analysis in random order by eluting with a linear gradient from 5 to 45% ACN developed over 2 h at a flow rate of 50 µL/min, and the effluent electro-sprayed into the LTQ mass spectrometer. The acquired data were filtered and analyzed, and database searches against the IPI (International Protein Index) mouse database used both the X!Tandem and SEQUEST algorithms. Protein quantification was carried out using a proprietary protein quantification algorithm licensed from Eli Lilly.

Results

A 48 hour exposure to 20 μg/cm^2 of either sonication suspended SWCNT or MWCNT significantly decreased the transepithelial resistance of the cellular monlayer (Figure 1). At this concentration, C_{60} had no significant effect.

Imaging studies revealed that CNP prepared by sonication and applied at a concentration of 20 μg/ml for 48 hours tended to agglomerate before settling on the monolayer. PCNA imaging showed that MWCNT and SWCNT agglomeration induced nuclear proliferation in cells surrounding the agglomerations (Figure 2, top). In a separate experiment, an increase in actin filaments was seen in cells surrounding agglomerations. Chronic, low-level exposure to MWCNT and SWCNT (5 μg/cm^2 thrice weekly) induced an increased number and size of large

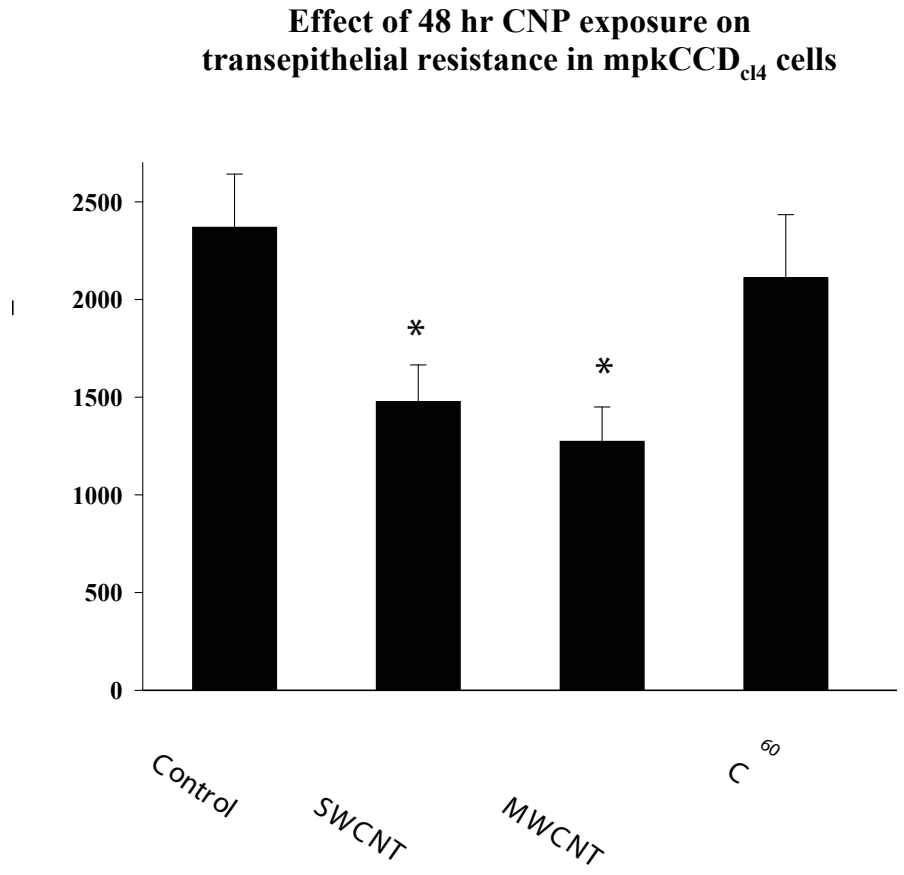
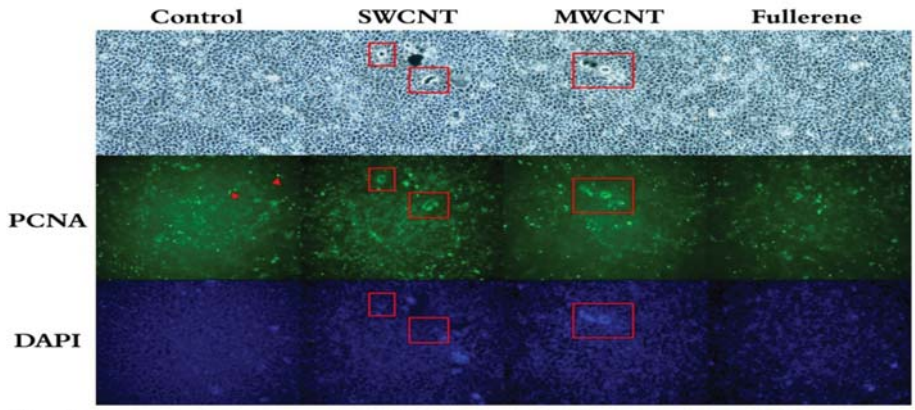


Figure 1. Effect of 48 hour CNP exposure on transepithelial resistance in mpkCCD$_{cl4}$ cells. Confluent monolayers of mpkCCD$_{cl4}$ cells were incubated for 48 hours with 20 μg/cm^2 CNP as indicated. Cells were removed from the Transwell chambers and mounted in Ussing chambers to monitor transepithelial electrical resistance. Bars indicate S.E.M. *Significantly different from matched control cultures ($p < 0.02$).



Qualitative analysis of changes in cell division via CNP exposure. mpkCCDI4 cells were grown to confluency and CNPS applied at 0.1mg/mL for 48hrs. The cells were fixed and stained for DNA (DAPI) and DNA replication (PCNA). Cells undergoing nuclear replication appear as intermediate green. Bright green spots appeared as an artifact of the staining procedure (arrowheads). Cells surrounding nanoparticle aglomerations showed an increase in DNA replication (red boxes).

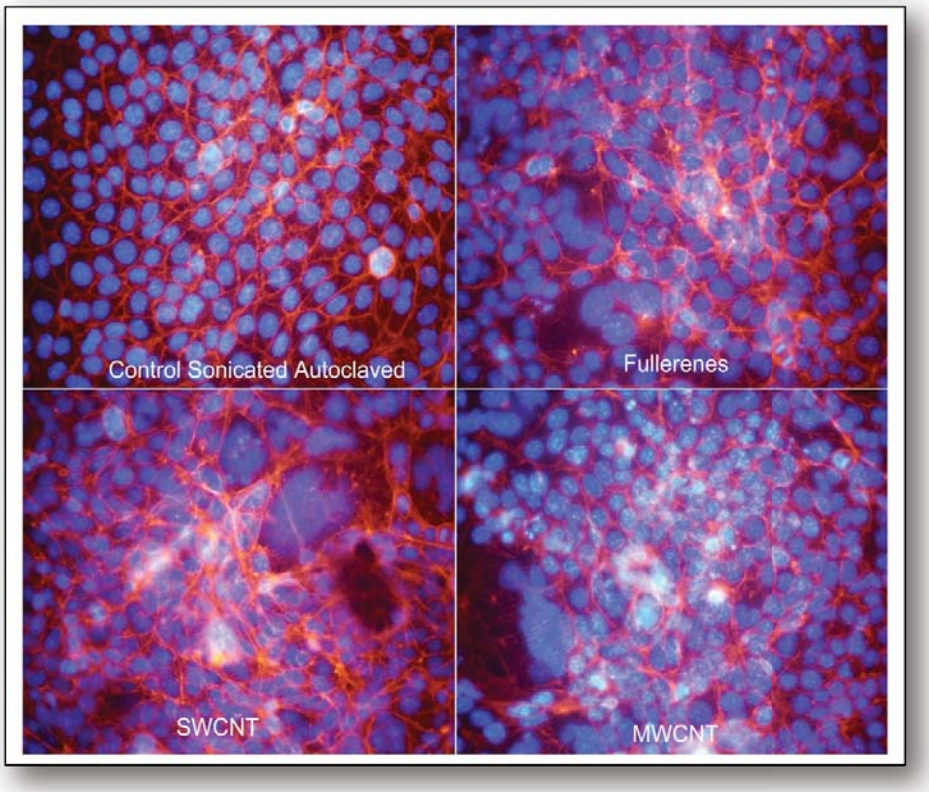


Figure 2. Top panel: $mpkCCD_{cl4}$ cells were grown to confluence in 6-well plates and then treated with sonication-suspended CNP at 20 µg/cm^2 for 48 hours. The cells were fixed and stained with proliferating cell nuclear antigen (PCNA) to indicate the nuclei of proliferating cells and with DAPI to stain all cell nuclei. The red boxes depict areas of CNP agglomeration and are the same in all fields. Bottom panel: $mpkCCD_{cl4}$ cells were seeded in 6-well plates and exposed for seven days to low levels (4 µg/cm^2) of the indicated nanopartical. The medium (containing fresh CNP) was renewed 3 times. The cells were fixed and actin was visualized using rhodamine-phalloidin (red) while the nuclei were stained with DAPI (blue). Cells treated with CNP showed an increased number of large, multinucleated cells.

multi-nucleated cells. Staining with rhodamine-phalloidin also revealed a general increase in expression of polymerized actin.

Differential protein expression at high dose SWCNT and MWCNT exposures determined by 2-DE is shown in Table 1. The expression of 11 proteins deemed either directly or indirectly associated with cell proliferation and function were altered by exposure. LFQMS analysis of CNP exposure for 48 h resulted in differential expression (via ANOVA) of 43 proteins variably effected by the different CNPs. Those whose expression differed from control are listed in Table 2.

Discussion

The electrophysiological studies and imaging studies indicate changes in cell function in cells treated with CNP. To more closely model *in vivo* exposures, functional changes were measured in $mpkCCD_{cl4}$ cells treated with CNP suspended via sonication. Previous studies have indicated that coated nanoparticles remain in solution, while unaltered nanoparticles agglomerate and settle out of solution. In the present study, visual observation confirmed that sonication increased the solubility and decreased agglomerate particle size although there is still a degree of agglomeration and precipitation onto the cellular monolayer.

TEER is a measure of monolayer integrity and is also a very sensitive measure of cellular viability. As cellular viability decreases, TEER falls precipitously. In the experiments shown in Figure 1, the decrease in TEER is substantial after treatment with either single- or multi-wall carbon nanotubes. However, these changes do not represent a decrease in cell viability. Control monolayers had an average TEER of $2370 \pm 815\ \Omega \cdot cm^2$. A decrease to 1477 ± 530 (SWCNT treated) or 1274 ± 465 (MWCNT treated) still exceed $1000\ \Omega \cdot cm^2$ which is considered a high resistance, intact epithelium. The changes in resistance indicate more subtle changes within the cells. Examples of cellular alterations which could be manifested as changes like these would be minor modifications of the cytoskeleton which is a major component in determining the impermeability of the junctional complexes or changes in the composition of the cellular membrane which would be sufficient to alter permeability.

Studies have suggested that CNP may have carcinogenic properties [10]. MWCNT have been specifically implicated due to their structural similarity to chrysotile asbestos that is widely accepted to cause carcinogenic responses in humans. After acute exposure to MWCNT and SWCNT, we observed changes in cells surrounding agglomerations of SWCNT and MWCNT. Cells seemed to exhibit proliferating nuclei as indicated by PCNA staining. In normal cells, once confluence is reached, cells no longer actively divide. These results indicate that SWCNT and MWCNT agglomerations cause cells to replicate abnormally, suggesting possible carcinogenic properties. Chronic treatment with CNP (especially MWCNT) suspended via sonication also revealed an increased number of large, multinucleated cells. Polyploidy, as observed here, is an indicator of genotoxicity and suggests that CNP (at these exposure levels) may be mutagenic.

$mpkCCD_{cl4}$ cells treated acutely with SWCNT and MWCNT as well as cells treated chronically with any of the three CNP types seemed to exhibit an increased expression of actin filaments. Previously, such changes in actin expression have contributed to a decrease in cell viability [11]. Our observed trends in cell function and abnormal nuclear proliferation seen via PCNA staining,

Table 1. Proteins altered by ethanol-sterilized, non-sonicated SWNT and MWNT at 200 µg/cm2 for 24 h, separated and analyzed by 2-DE and identified via mass spectrometry.

Increased expression of the proteins below, a response that may be directly or indirectly associated with cell proliferation and function	
Name	**Function**
stathmin-like 2	regulator of microtubule dynamics; renal expression is ↓ in with uranium toxicity
pseudouridine synthase 1	involved in protein synthesis, serves to stabilize required RNA conformations during translation
flotillin-2, isoform 1	scaffolding protein within caveolar membranes, functionally participating in formation of caveolae or caveolae-like vesicles; tethers growth factor receptors linked to signal transduction pathways, may also be involved in cell adhesion
Ran-binding protein 1	bi-directional transport of proteins and ribonucleoproteins through the nuclear pore complex, spindle formation, reassembly of the nuclear envelope; expressed at sites of mesenchymal/epithelial induction
enolase, 1 alpha	energy metabolism for proliferation; non-neuronal enolase is a diagnostic marker for many tumors

Decreased expression of the proteins below, a response that may be directly or indirectly associated with cell proliferation	
Name	**Function**
gap junction alpha-8	may contribute to minor changes observed in TEER
cyclin G2	acts as cell cycle inhibitors in certain cell types and may contribute in inducing cell cycle arrest
myotubularin-related protein 9	protein-tyrosine phosphatase that acts on the 2nd messenger IP3, localized on endosomes, and regulates intracellular vesicle trafficking and autophagy; dysregulation can effect trafficking (see stathmin-like 2)
olfactory receptor 586	part of the cell surface receptor mediated signal transduction process involving G-protein coupled receptors, including cyclic AMP and IP3 mediated processes
zona pellucida glycoprotein 4	cell adhesion molecule; intracellular matrix; may be involved in ↓ TEER
protein kinase, cAMP dependent regulatory, type I, alpha	in PRKAR1A mutant cells (↓ functional kinase) there is an increase in DNA transcription and/or activation of other pathways leading to abnormal growth and proliferation

support this. However, the proteomic results do not support differential expression of total actin. Taken together, these results suggest that the changes we have observed in the filamentous (phallodin-stained) actin are the result of changes in the filamentous/globular actin ratio in the cells.

Conclusion

The present study has shown that CNP induced significant alterations in renal collecting duct cell function, histology, and protein expression. CNP suspended via sonication cause histological changes including increased nuclear proliferation, elevated filamentous actin expression, and

Table 2. Proteins altered by autoclave sterilized, sonication-suspended C_{60}, SWCNT, or MCWNT at 20 μg/cm^2 for 48 h versus Control, analyzed by label-free quantitative mass spectrometry.

Name	C_{60}	SWCNT	MWCNT
acyloxyacyl hydrolase	↓	-	↓
alpha-kinase 3	-	↓	↓
catenin, β like 1			
chaperonin containing Tcp1, subunit 3 (γ)	↑	-	-
creatine kinase, mitochondrial 1, ubiquitous	↓	-	-
cytoskeleton associated protein 5	-	↑	-
F-box and leucine-rich repeat protein 13	-	↑	-
ferritin light chain 1	-	↓	-
ferritin light chain 2	-	↓	-
GrpE-like 1, mitochondrial	↓	-	-
GTPase activating RANGAP domain-like 3	-	↑	-
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), β subunit	-	↑	↑
isocitrate dehydrogenase 3 (NAD+) β	-	↑	↑
olfactory receptor 584	↑	-	-
peptidylprolyl isomerase B	-	↓	-
phosphate cytidylyltransferase 1, choline, α isoform	↑	-	-
prostaglandin E synthase 3	-	↓	-
proteasome subunit, β type 7	-	↑	↑
protein phosphatase 2, catalytic subunit, α isoform	-	↑	-
protein phosphatase 2, catalytic subunit, β isoform	-	↑	-
protein tyrosine phosphatase, receptor type, B	↓	-	↓
protein tyrosine phosphatase, receptor type, R	-	-	↓
Ras-GTPase-activating protein SH3-domain binding protein 1	-	-	↑
ribosomal protein L30	-	↓	-
serine (or cysteine) peptidase inhibitor, clade B (Serpin B10)	-	↑	↑
similar to DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	-	↓	-
sorting nexin 1	↑	-	-
SWI/SNF related, actin dependent regulator of chromatin c1	-	↑	↑
THO complex subunit 4, isoform 1	↑	-	-
transmembrane protein 202	↑	-	-
ubiquitin-conjugating enzyme E2 D2	↓	↓	↓
ubiquitin-like modifier activating enzyme 6	↓	↓	↓
voltage-dependent anion channel 2	↓	↓	↓
zinc finger, C3HC type 1	-	↑	-

multinucleation. The observed changes are subtle and likely represent cellular alterations that would have physiological effects over a prolonged time-course.

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Conference Questions and Answers

Question:
Does that system normally come into contact with natural particles?

Answer:
No. We will be running silica controls. We did not see evidence of inflammation. We are looking at lactate dehydrogenase (LDH) in the media and ILA (the human homologue of murine 4-1BB), but these data are not available yet. This system is not exposed to particles. The kidney does not filter large particles, and the filtrate is not exposed to particles, but is exposed to small molecules and metabolites.

Question:
Could the effects on endothelium-derived hyperpolarization (EDH)-induced ion channels be caused by an effect on the channels themselves or an indirect effect due to signaling?

Answer:
Perhaps with the fullerenes that might be the case. Changes in transport may be due to signaling mechanism change if the cell is injured or irritated.

Question:
Is the cell proliferation response due to toxicity or a signaling response?

Answer:
This remains to be seen.

Save

Can Nanoscale Particles Affect Plant Growth – Alfalfa Case Study

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Larry Kapustka, Golder Associates Ltd., Canada

Stephen Goudey, Golder Associates Ltd., Canada; HydroQual Laboratories Ltd., Canada

Abstract

Nanotechnology is a phenomenon that has the potential to change existing technologies and the ability to create new technologies that were previously unattainable. However, along with this potential is the added responsibility to understand and manage the hazards and risk associated with nanotechnology exposure. Regardless of the intended end use of nanotechnology, the environment will become the ultimate recipient of the products at the end of their life cycle. The impact of these nanoscale particles on the environment is not very well understood. What is the fate of these particles once they are in the environment? Do they biotransform, biomagnify or bioaccumulate? Or do they simply react with organic matter and become benign? Are these particles toxic to different ecological receptors? Currently very little data exists on the fate and effects of these particles once they are in the environment. Ecological receptors may be exposed to nanoscale particles in freshwater, saltwater, soil or sediment. Aquatic studies are beginning to demonstrate that nanoscale particle toxicity is highly organism and nanoscale particle dependent. However, within the terrestrial compartment there is still very little information on the toxicity of nanoscale particles on receptor organisms. We examined the effect several nanoscale particles had on plants using alfalfa inoculated with rhizobium. Measurement endpoints used were root growth, shoot elongation and nodule formation. The results of this study will be discussed. We will also discuss the development of a life cycle based risk assessment framework in the context of managing environmental risks associated with nanotechnology.

Conference Questions and Answers

Question:
What were your controls for plant growth?

Answer:
We used boric acid as a control.

Comment:
Based on the data presented today, you cannot argue there was an effect or no effect of nanomaterials on plant growth, as there were no controls for particle size, for the metals themselves, or for impurities left after material synthesis. Both positive and negative controls are needed.

Question:
You presented a slide showing theoreticians, researchers, engineers, producers and marketers, politicians, and the possible loss of public confidence due to an unplanned release. Do we have political or public opposition?

Answer:
We do not think we are at the point of political or public opposition, but we need to learn lessons from the nuclear power industry about managing concern.

Potential Toxicity of Nanomaterials and their Removal

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Abstract

The potential effects of chronic exposure of the aquatic organism—zebra fish embryo toFerric Oxide nanoparticles (nFe_2O_3) was investigated at the concentrations gradient (0 to 100 mg/L). The preliminary results showed that the survival rate of Zebra fish embryos were reduced when dosed 50 mg/L or higher nFe2O3. The hatch time was significantly delayed by the exposure of high concentrations (50 mg/L or higher) of nFe_2O_3. Zebra fish embryo test could be a potential biomarker or bioindicator to assess the effects of exposure of the fish to nFe2O3. Removal of manufactured nanomaterials (MNMs) from water was investigated by coagulation, flocculation, sedimentation, and membrane ultrafiltration of nFe_2O_3 ranging in size from 53 nm to 240 nm. The coagulation, flocculation, or sedimentation process alone or in combination, could not remove all the nFe2O3 at a very high alum dose (60 mg/L). A lower alum concentration of 20 mg/L resulted in removal of ca. 90% of nFe_2O_3 after 24 hours treatment. However, the ultrafiltation process completely removed nFe_2O_3 and the resulting permeate was essentially free of nanoparticles (NPs), suggesting that ultrafiltration can be used as an effective way to remove nFe_2O_3.

Introduction

The rapid growth of nanotechnology is stimulating the research on potential environmental impacts of manufactured nanomaterials (MNMs). Unlike larger particles, MNMs can probably accumulate or even penetrate the cell membrane which is the last protection barrier of living cells from the exotic intrusion. Our project is to focus on evaluation of the potential toxicity of MNMs, and investigation of potential treatment technologies that effectively remove MNMs from water. This paper investigated the potential effect of nFe_2O_3 (nanoparticles of Fe_2O_3) on zebra fish embryo and the removal efficiency by water treatment processes.

Materials and methods

Preparation of n Fe_2O_3

Stock solutions (100 mg/L) of nFe_2O_3 were prepared by stirring nFe_2O_3 vigorously in ISO standard culture medium (consisting of 64.75 mg/L NaHCO3, 5.75 mg/L KCl, 123.25 mg/L $MgSO_4 \cdot 7H_2O$, and 294 mg/L $CaCl_2 \cdot 2H_2O$) using a magnetic agitator at room temperature for 2 h. The morphology and the actual size of nFe_2O_3 in the culture medium were determined using a scanning electron microscopy (SEM, Philips XL30, FEI Company). Test solutions were prepared immediately prior to use by diluting the stocks of nFe_2O_3 with culture medium. During

the preparation of diluted solution, the stock solution/mixture was continuously stirred with a magnetic stirrer to maintain the suspension at as stable of a concentration as possible.

Exposure test

Exposure test began as soon as the healthy eggs were selected. 24 eggs (blastula stage) were transferred to the test wells of a 24-well multi-plate (Costar® 24Well Cell Culture Cluster, Corning Incorporated, USA) at 1 embryo/well, in which twenty wells contain 2 mL nFe_2O_3 test solution and four wells contain 2 ml of culture medium per well. The concentration gradients of nFe_2O_3 solution tested in this study were 100, 50, 10, 5, 1, 0.5, 0.1 mg/L and water control. The experiment was performed in triplicate (i.e., a total of 12 embryos were used in the water control and 60 embryos in the exposure group) for each treatment. The plates containing experimental embryos were placed in fish room with a controlled light and temperature (i.e., 28±0.5 °C with a 14h/10h light/dark cycle). At the end of the experiment, water samples were collected immediately for measuring the concentration of nFe_2O_3.

NPs removal

nFe_2O_3 solution was prepared using nano pure water for studying the removal of NPs with conventional coagulation-flocculation and sedimentation. The experiments were evaluated using standard jar test. Aluminum sulfate with a concentration of 20 and 60 mg/L were used and pH of the water was adjusted into 6.5. A PVC UF membrane was employed to evaluate the removal efficiency of NPs by membrane process. Molecular weight cut off (MWCO) of the Ultra filtration (UF) membrane is 50000 Dalton and the filtration area of the membrane is 0.125 m2. Water samples was taken and dried and digested by concentrated nitric acid. GF-AAS was used to analyze the nFe_2O_3 concentration.

Results

The state of nFe_2O_3 in water phase

The addition of nFe_2O_3 to the culture medium resulted in a formation of aggregates that settled down in the water column very quickly. Being observed by SEM, the nFe_2O_3 aggregates look like floccules with variable sizes from a few hundred nanometers to several microns in diameter (Figure 1). In this study, nFe_2O_3 with a primary particle size of 205 nm was observed to form large aggregates with average sizes more than 1 μm in diameter (Figure 1). This aggregation phenomenon has also been reported in other NPs, including Cu, TiO_2, NiO, fullerene NPs and SWCNTs [1, 2, 3]. These findings revealed that aggregates or agglomerates of NPs are likely to settle out of the solution and sink into sediments rather than remain in a suspension. Thus, the highest concentrations of such MNMs in the environment could be found in sediments.

The toxic effect on Zebra fish embryo

These nFe_2O_3 aggregates were found to be toxic to zebrafish embryos and larvae, causing a dose-dependent mortality and hatching inhibition, as shown in Figure 2. The survival rate of Zebra fish embryos were reduced when dosed 50 mg/L or higher nFe_2O_3. The hatch time was significantly delayed by the exposure of high concentrations of nFe_2O_3. The development of

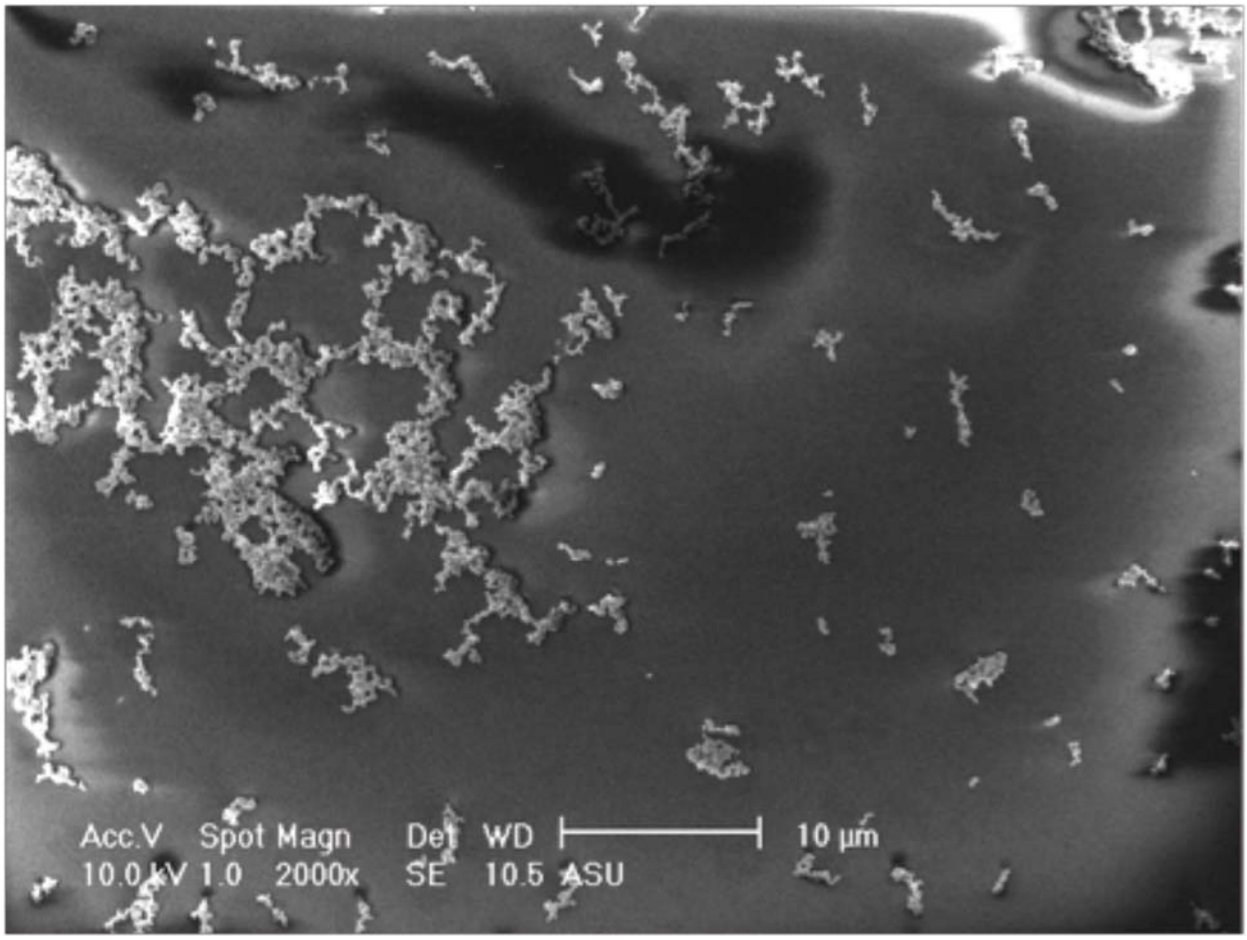

Figure 1. SEM images of nFe2O3 aggregates (10 mg/L) in zebrafish culture medium.

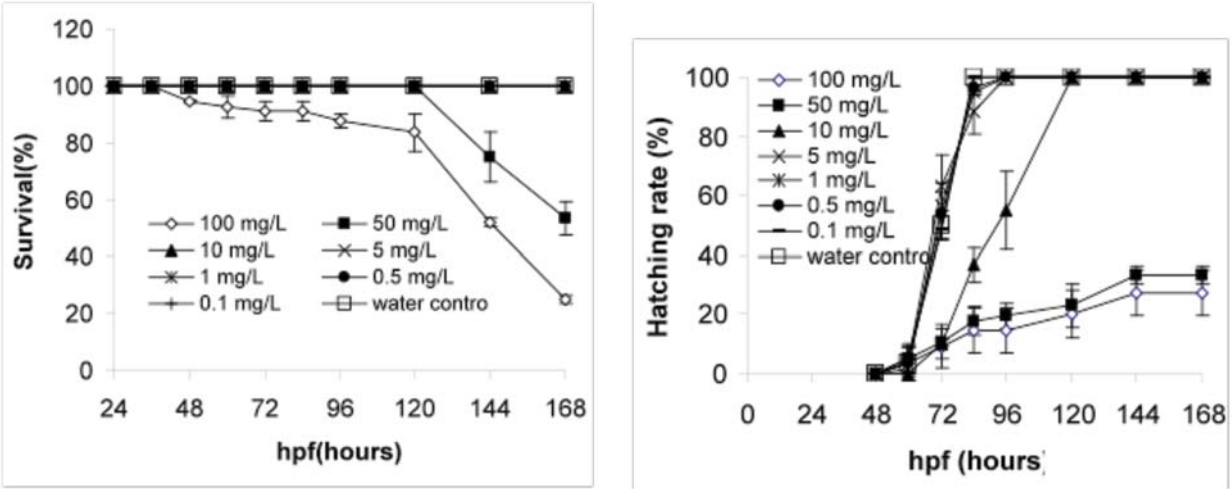

Figure 2. Survival and Hatching rate (%) of zebrafish embryos exposed to nFe2O3 over 168 h.

the zebrafish embryos and larvae was observed with an inverse microscope (Olympus, Japan) equipped with a digital camera and was documented photographically at specified time points (t =6, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144 and 168 hours post fertilization (hpf)). The endpoints used to assess developmental toxicity included embryo/larvae survival and embryo hatching rate. Malformations and pericardial edema were described and documented among the embryos and larvae from both control and treated groups. Developmental abnormality, e.g., pericardial edema, malformation and tissue ulceration, was found in 50 mg/L and 100 mg/L nFe_2O_3 exposure group (Figure 3), affecting more than 13% of the surviving fish by 168 hpf.

Interactions between nFe_2O_3 and embryo surface

The interactions between nFe_2O_3 and embryo surface were studied through Atomic force microscopy (AFM) to explore the possible underlying cause of toxicity of nFe_2O_3 to embryos. Adhesion force results indicated that the chorion of the embryo at an early stage had adhesion forces distributed from 2000 to 2800 pN. There was also a lower level of adhesion force domain from 800 to 1600 pN. After 8 hours the force distribution moved slightly to the low force range which indicated the average adhesion force decreased slightly and the chorion became less adhesive and more hardened.

Evaluation of NPs removal using conventional water treatment technology

Removal of nFe_2O_3 using conventional coagulation processes is shown in Figure 4. When alum concentration was 20 mg/L, concentrations of hematite decreased gradually with time. 80% of NPs was removed after 12 h sedimentation. There were still about 7% of hematite NPs remaining in the solution even after 24 h sedimentation. When alum concentration increased to 60 mg/L, the concentrations of hematite decreased sharply. More than 90% removal was achieved after 3 hour's sedimentation. However, it still took 12 hours to removal 97% of the NPs.

Evaluation of NPs removal using UF membrane

Figure 5 shows the removal efficiency of nFe_2O3 NPs using membrane. The concentration in permeate is less than the detection limit (0.005 mg/L) and their removal rate is more than 99.95%. Figure 5 also shows the EDX mapping images of the fiber after filtration. NPs deposited on the filter layer of the hollow fiber and there were no particles in the inner pores of membrane, indicating that no NPs can penetrate the active filtration layer.

Conclusion

The environmental behaviors and the toxicity of MNMs learned from this study provided the insight information that may help scientists and manufacturers to design and manufacture more environmentally benign MNMs and avoid environmental disasters such as DDT and PCB occurred in the past. The survival rate of Zebra fish embryos reduced when dosed 50 mg/L or higher nFe_2O_3. The hatch time was significantly delayed by the exposure of high concentrations of nFe_2O_3. Conventional treatment process is not efficient for nFe2O3 removal while UF is very effective for nFe_2O_3 NPs removal. No nFe_2O_3 NPs in permeate were detected and their removal efficiency was more than 99.95%.

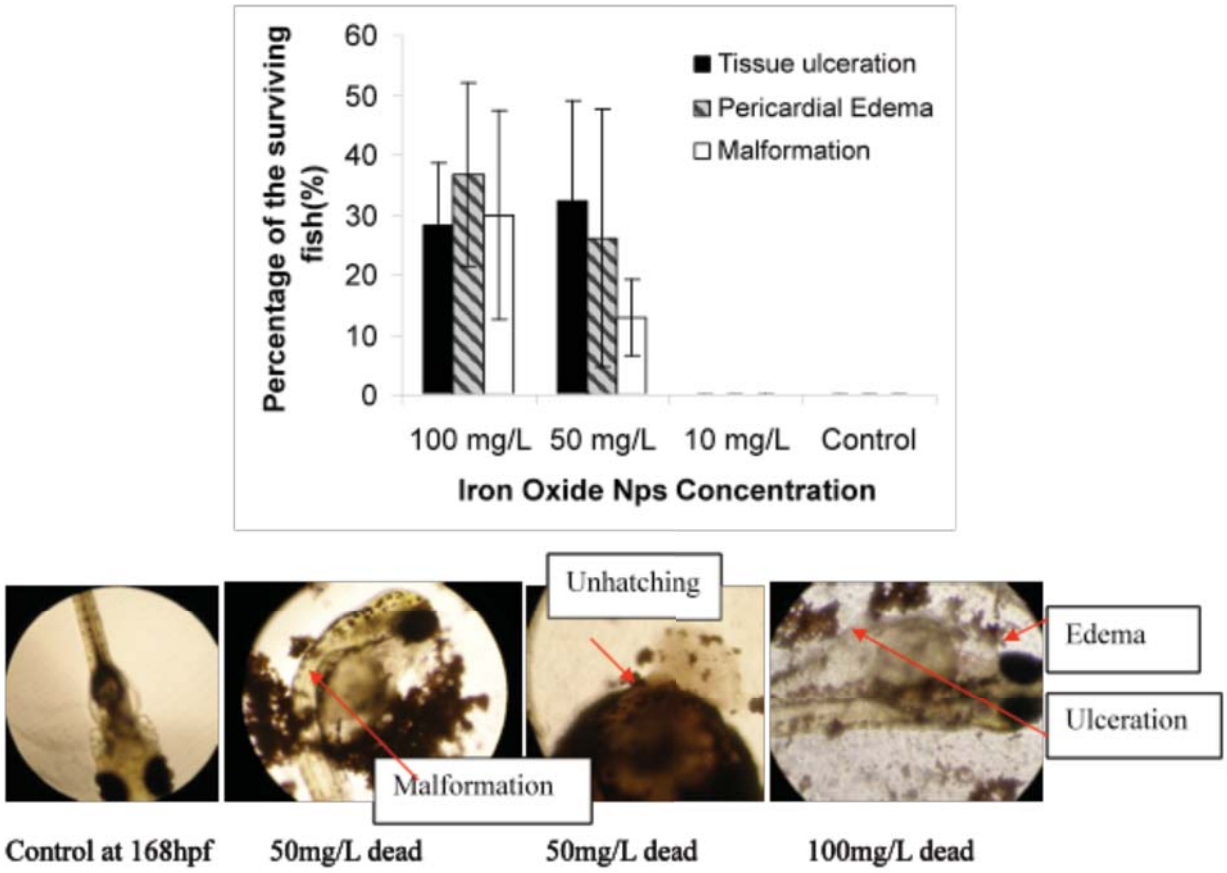

Figure 3. Developmental abnormality induced by nFe2O3 exposure at 168 hpf.

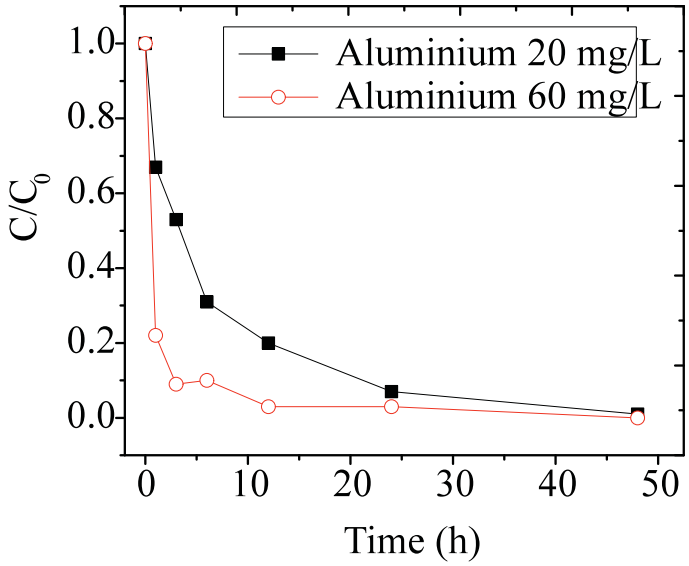

Figure 4. Removal of nFe2O3 using conventional coagulation processes.

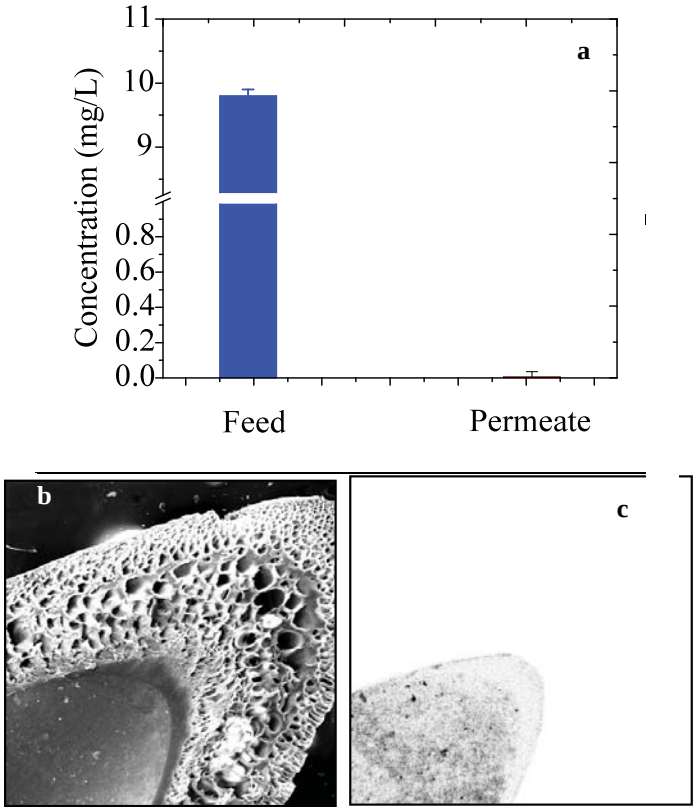

Figure 5. Removal efficiency of nFe_2O_3 by UF membrane (a) and deposition of NPs on membrane fibers.

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Conference Questions and Answers

Comment:
Other studies have shown that, if silver nanoparticles are removed from the water column, they may be left in the sediment. This may limit the use of the sediment if it is intended for soil amendment.

Comment:
It would be interesting to consider the flow rate that can be achieved with an ultra filtration (UF) filter.

Endotoxin Contamination of Engineered Nanomaterials

R. Keith Esch, Li Han, David Ensor and Karin Foarde, RTI International, U.S.A.

Abstract

Endotoxins are bacterial cell wall components that occur naturally in soil, water and air; and routinely contaminate other materials. Endotoxin exposure is associated with respiratory symptoms as well as fever, septic shock, impaired organ function and death. Endotoxin is a potential confounding factor in engineered nanomaterial (ENM) toxicity studies as induction of inflammatory response and oxidative stress are observed for both endotoxin and ENM. The established method for quantifying endotoxin relies on its activity in a complex biochemical assay system. Because of their physical and chemical properties, examination of many ENM under these conditions presents nontrivial technical challenges. We have made progress in identifying and implementing methods for analysis of ENM with respect to endotoxin contamination. An examination of a series of carbon-based ENM reveals varying levels of endotoxin. The physical association of ENM and endotoxin and their shared physiological effects suggest the possibility that contaminating endotoxin may represent a health risk and contribute to the toxicity that is ascribed to ENM.

Introduction

The decreased size, and greatly increased surface area, of nanomaterials carries the potential for much more activity, for a given mass, compared to larger particles of the same chemical composition. These qualities, while highly desirable in industrial, analytical, consumer product and medical applications also raise concerns for impact on human health.

Endotoxins are components of Gram-negative bacterial cell walls that contain both lipid and polysaccharide components (Fig. 1) and vary somewhat depending upon the species of origin. Endotoxins may be released upon cell death as well as during growth and division. They are nearly ubiquitous in the environment, present on surfaces and in particles made up of diverse materials (Douwes et al, 1995). Exposure to endotoxin is associated with respiratory symptoms and pulmonary inflammation. Airflow restriction in those with allergic asthma can be exacerbated by airborne endotoxin (Michel et al 1996). Repeated wheeze in infants has been linked to low level exposure (Park et al 2001). Acute endotoxin exposure is also linked to systemic response resulting in fever, septic shock, impaired organ function and death (Danner et al, 1991; Wanderley et al, 1996).

Examination of a series of ENM reveals levels of endotoxin contamination that vary over nearly 4 orders of magnitude (Kayo Inaba, personal communication). An investigation by Vallhov et al (2006) aimed at addressing the use of gold particles in therapeutic approaches found that gold nanoparticles became contaminated with endotoxin, producing altered immune responses in cell-based assays. These findings suggest the possibility that contaminating endotoxin may be contributing to the toxicity that is ascribed to ENM.

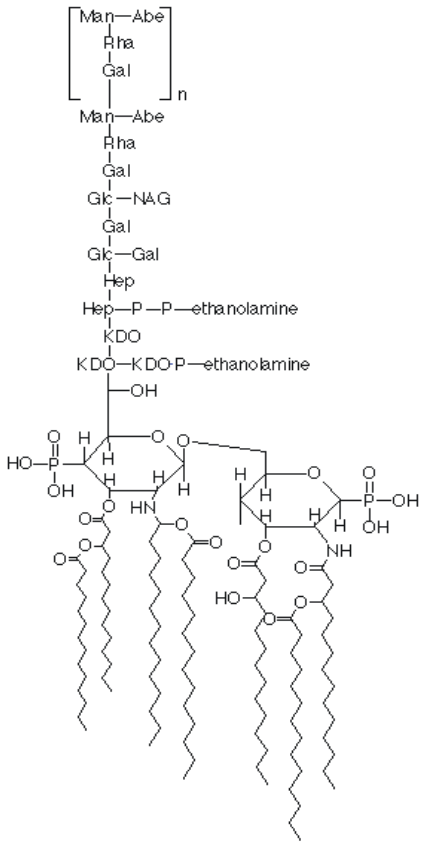

Figure 1. Endotoxin (LPS) is approx. 10kDa and has an amphiphilic structure consisting of a lipid portion, an anionic core polysaccharide and a variable polysaccharide-rich O-Antigen.

We sought to investigate ENM endotoxin contamination in order to characterize associated risks. Because of their hydrophobicity, many carbon-based ENM have limited dispersion in the aqueous suspensions in which endotoxin assays are conducted. Therefore, methodologies allowing accurate, representative assessments of endotoxin contamination need to be developed. We describe here the studies initiated to address this complex problem as well as endotoxin contamination findings for a set of carbon-based ENM from different sources.

Materials and Methods

We used Cryogenic gas sorption (BET) analysis to obtain ENM surface area information. In this analysis, Nitrogen served as the adsorptive gas with analysis bath temperature at 77.3K. It is known that the material diameter properties vary with different manufacturers and batch–to-batch production processes. We obtained diameter analysis results from the manufacturers of the carbon materials for individual production lots. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) verified the diameter information from vendors. As Carbon Nanotube/C_{60} synthesis requires metal catalyst materials such as Ni, Fe, Co, Y and Mo, metal residue remains in the final product. The weight percents of total metal composition were obtained from manufacture lot analysis results. Scanning electron microscopy (SEM) combined

with energy dispersive x-ray analysis (EDX) verified the information from vendors.

PyroCLEAN™ used for decontaminating endotoxin on surfaces was purchased from ALerCHECK Inc. and used according to the manufacturer's directions. Vitamin E d-α-tocopheryl polyethylene glycol – 1000 succinate (VETPGS), a water soluble vitamin E-based surfactant was acquired from Eastman Chemical. All glassware used to conduct the endotoxin assays was depyrogenated by exposure to 190 deg. C for at least 3 hours. All remaining labware used for extractions and assays was designated as pyrogen free by the manufacturer.

Liquid suspensions of each ENM were prepared for determination of endotoxin contamination. The mass of each sample was measured using a balance in a containment hood following decontamination with pyroCLEAN. Depyrogenated implements and containers were used in gravimetric procedures. Each preparation was agitated by a minimum of 30 seconds of vortexing and 40 minutes of sonication in a Branson 2200 bath sonicator.

Preparation A: All nanomaterials were transferred to depyrogenated glass tubes and endotoxin-free water was added to produce each at a concentration of 10 mg/ml. The nanomaterial particles were allowed to settle/separate from the liquid and the liquid extracts were removed for assay of endotoxin content.

Preparation B: Triethylamine (TEA) was added to each 10 mg/ml nanomaterial sample in water to a concentration of 0.01%. Each sample was then diluted 2-fold in 0.01% TEA, resulting in 5.0 mg/ml nanomaterial samples. The nanomaterial particles were allowed to settle/separate from the liquid and the liquid extracts were removed for assay of endotoxin content.

Preparation D: A 1% VETPGS solution and endotoxin free water were added to the preparation B samples to produce each nanomaterial sample at 1.0 mg/ml in 0.1% VETGPS. Samples of each mixture were removed without a settle/separation period such that representative amounts of nanomaterials remained in the portions removed for endotoxin testing.

Preparation E: Preparation D samples were centrifuged in a model 5415 D microcentrifuge (Eppendorf, Westbury, NY) at maximum speed for 5 minutes. Supernatants were transferred from the pelleted material, to minimize any ENM content, into endotoxin-free tubes for analysis.

Endotoxin levels were quantified using a kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (Associates of Cape Cod, Woods Hole Mass.) following the manufacturer's instructions. The level of endotoxin activity in a sample was determined by the reaction of endotoxins in the specimen with the lysate and a substrate, producing a color change over time, and comparing rate of color change to similar reactions of known endotoxin reference standards.

In preparation for endotoxin assay, the samples were put at R.T., vortexed for 30 seconds and sonicated for a minimum of 30 minutes. Dilutions of tests samples and standards were made using the solution used to generate the test samples. For samples that include endotoxin spikes, indicated known quantities of standard endotoxin were added directly to samples at the time the assay was conducted. VETGPS was included in a series of endotoxin standards of varying concentrations. Assay interference was assessed by comparing the resulting endotoxin values of these samples to those produced from the same endotoxin standards lacking this surfactant.

Results and Discussion

Visual observation indicated that the mixing of the ENM at 10 mg/ml in endotoxin-free water or 0.01% TEA generally failed to produce stable homogeneous suspensions. The ENM did not extensively disperse in the liquid, separating out by settling or clinging to the inside surface of the glass tubes. Accordingly, significant endotoxin was not detected in extracts from these preparations. Dilution in 0.01% TEA to 5 mg/ml (preparation B) produced minor increases in dispersion of the ENM. Assay of these suspensions produced detectable endotoxin for some ENM, but the values were generally far lower than later determined values, indicating significant interference by the ENM in the suspensions.

Because the ENM were largely refractory to dispersal in either water or TEA, the water soluble vitamin E surfactant (VETGPS) was considered for use as a dispersing reagent and accordingly was tested for interfering effects in the endotoxin assay. No significant interference was observed for the vitamin E surfactant up to 0.1%, the highest concentrations tested. Addition of VETGPS to 0.1% produced a readily visible increase in dispersion. These suspensions (preparation D) produced quantifiable levels in some cases. For ENM 2, the value obtained was reasonably close (within about two-fold) to the final estimates despite the presence of ENM in the assay mixtures. However, there was considerable variation in individual assay samples, perhaps due to particle interference with the colorimetry light path.

Since improved suspension had been realized by the addition of VETGPS, we reasoned that an increase in interaction between the ENM and the liquid likely facilitated more complete extraction of endotoxin from the ENM surfaces into the aqueous phase. Therefore, extracts were produced (preparation E) that employed centrifugation to remove the ENM. This extraction resulted in detection of quantifiable endotoxin for all five ENM (Fig. 2). The levels of endotoxin extracted from this set of ENM varied by more than an order of magnitude, depending on the material tested. Extracts from two of the ENM tested (#2 and 3) had considerably higher endotoxin contamination than the others.

The results from the physical characterization studies are summarized in Table 1. No striking similarities or commonalities are evident among the two ENM that were most contaminated with endotoxin. While ENM #2 is a multi-walled carbon nanotube (MWCNT), ENM #3 is a C60 fullerene. Whereas ENM #2 has relatively high metal composition and surface area values, those for ENM #3 are quite low. Each is manufactured by a different organization and thus in different environments.

Conclusions

Significant progress toward meaningful determinations of endotoxin contamination of carbon based ENM has been achieved. Once established, these methods can be employed to address the extent of ENM-endotoxin associations in commercial preparations, laying a foundation for assessing the risk presented by endotoxin contamination. The current work, in conjunction with the successful completion of further studies, will allow examination of relationships between specific ENM properties and the degree of endotoxin adsorption, producing critical information for understanding factors determining contamination. These studies will also illuminate our understanding of endotoxin as a confounding factor in nanoparticle toxicity investigations and as

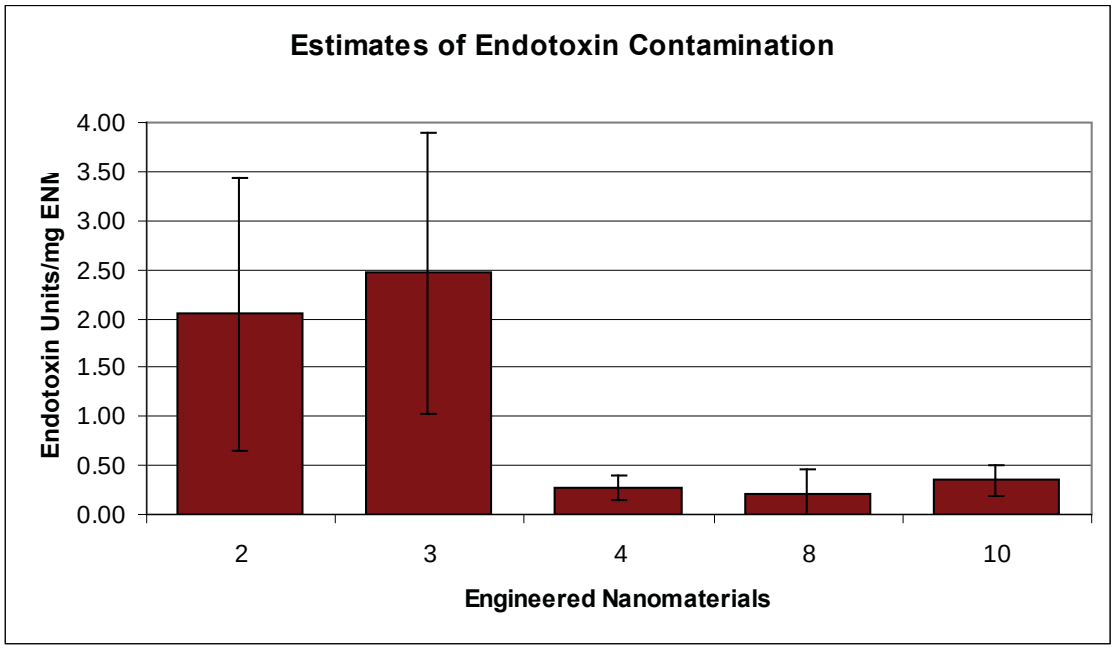

Figure 2. Determinations of extracted endotoxin from preparations of 5 commercially available carbon-based ENM. Error bars are based on standard deviations from multiple independent measurements.

Materials	Vendor information	Catalog #	Surface area (m^2/g)	Diameter (nm)	Total Metal composition wt %
2 MWCNTs	SES Research	900-119	194	< 10 nm	<5%
3 Fullerene	MER Corporation	MR6LP	0.07	20 nm	0.001%
4 MWCNTs	Sigma -Aldrich	636843	97	40-70 nm	>2.23%
8 Fullerene	Sigma -Aldrich	572500	0.22	ND	0.1%
10 Carbon Black	Cabot Corp	Vulcan XC 72R	221	30 nm- primary particle size; 250 nm – Aggregates size	<0.006%

Table 1. Physical characteristics of ENM used in endotoxin contamination studies. (ND signifies value was not determined). The surface areas of the ENMs are determined by BET measurement and the low surface area number for ENM#3 and ENM#8 is likely due to the large agglomeration of the fullerene particles, as indicated by TEM imaging of these samples (not shown).

a contributor to ENM-based adverse health effects.

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Effects of Ingested Engineered Carbon Nanomaterials on Zooplankton

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Abstract

Nanotechnology is a rapidly growing industry, and increased manufacturing and use of engineered nanoparticles will likely increase their deposition into aquatic ecosystems. However, relatively little is known about the potential impacts of engineered nanoparticles on aquatic biota. Particularly relevant to aquatic ecosystems are those particles which display increased solubility either through specialized coatings or through an ability to interact with water column constituents such as natural organic matter. Previous research indicated that grazing zooplankton (*Daphnia magna*) were able to ingest lipid-coated single walled carbon nanotubes (SWNTs) from the water column during their normal feeding behavior. While SWNTs were observed to fill the gut of the zooplankton, they were easily egested, and acute mortality was observed only at high concentrations (>5mg/L). The purpose of this research was to examine the potential for sublethal effects to occur at lower concentrations following ingestion of solubilized engineered carbon nanomaterials. *D. magna* and *C. dubia* were exposed to a range of concentrations of multiwalled carbon nanotubes (0.1 -1mg/L) suspended in water using natural organic matter. Survival was monitored in each species for the duration of the test period (7 days for *C. dubia* and 4 days for *D. magna*). In order to assess sublethal effects, reproduction was monitored in *C. dubia*. We hypothesized that the accumulation of nanotubes in the gut tract of zooplankton would decrease their ability to take up normal food (algae) and, thus, growth (dry mass per individual) was measured in both species using an electromicrobalance. No significant effect on survival of either species was observed at any of the concentrations tested. However, *C. dubia* reproduction was significantly decreased by 50% at concentrations > 0.25mg/L. Growth in both species was inhibited in a concentration dependent manner. Although we observed no evidence that the MWNTs were taken up across the gut membrane, we have shown that simply ingesting the materials can lead to significant toxic effects in zooplankton through the inhibition of normal feeding activity.

Introduction

Nanotechnology is a rapidly growing industry, and increased manufacturing and use of engineered nanoparticles will likely increase their deposition into aquatic ecosystems. However, while some authors have reported deleterious effects of nanomaterials on fish and plankton, relatively little is yet known about the potential impacts of engineered nanoparticles on aquatic biota (Oberdorster 2004, Lovern and Klaper 2006, Oberdorster et al. 2006, Cheng et al. 2007, Lovern et al. 2007, Roberts et al. 2007). Particularly relevant to aquatic ecosystems are those particles which display increased solubility either through specialized coatings (Wu et al. 2006,

Roberts et al. 2007) or through an ability to interact with water column constituents such as natural organic matter (NOM) (Hyung et al. 2007, Roberts et al. 2007). Previous research indicated that grazing zooplankton (*Daphnia magna*) were able to ingest lipid-coated single walled carbon nanotubes (SWNTs) from the water column during their normal feeding behavior (Roberts et al. 2007). While SWNTs were observed to fill the gut of the zooplankton, they were easily egested, and acute mortality was observed only at high concentrations (>5mg/L). While ingestion of nanotubes may not result in acute mortality, filter feeding or non-specific grazing organisms might accumulate large amounts of the materials in their guts and inhibit the uptake or digestion of normal food items resulting in toxicity following more chronic exposures. The purpose of this research was to examine the potential for sublethal effects to occur at lower concentrations following ingestion of solubilized engineered carbon nanomaterials, specifically multiwalled carbon nanotubes (MWNTs). We hypothesized that the accumulation of nanotubes in the gut tract of zooplankton would decrease their ability to take up normal food (algae) resulting in decreased growth and reproduction.

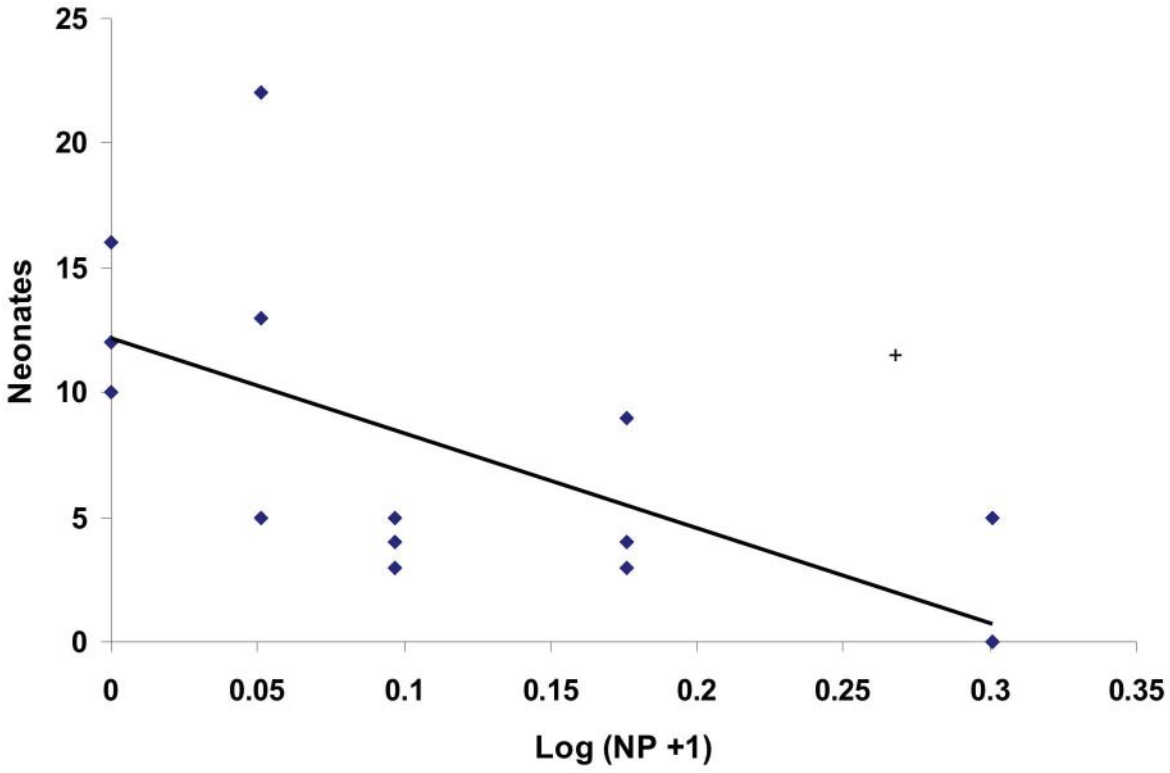

Figure 1. *C. dubia* reproduction**.**

Methods

MWNT-NOM Solutions

Suwannee River Natural Organic Matter (SR-NOM) was purchased from International Humic Substances Society (St. Paul, MN, USA) and used as the NOM source throughout this study. NOM solutions were made by weighing out the desired amount of SR-NOM and placing it in a volumetric flask filled with the appropriate amount of EPA Moderately Hard Water (MHW). The solution was then stirred with a Teflon stir bar on a mixing plate before being filtered with a 0.2 μm cellulose membrane filter prior to use.

Multi-walled nanotubes (MWNTs) were made by Dr. A. Rao's Laboratory at Clemson University (Clemson, SC, USA) using the thermal chemical vapor deposition method. MWNTs had an approximate diameter of 25 nm, length of approximately 50 μm, and a purity of >95%. In order to suspend the MWNTs in NOM, they were first weighed on waxed weigh paper and then placed in a 100 mL glass centrifuge tube. Twenty-five mLs of NOM solution was added to the centrifuge tube and the solution was sonicated with a Fisher model 300 dismembrenator with a 1/8" microtip for 15 min. Twenty-five mL aliquots of dilution water (containing NOM) were added and the solution sonicated for additional 15 min intervals after each aliquot until the solution reached a total volume of 100 mL and total sonication time of 1 hr. The solutions were allowed to settle for approximately 24 hrs before the supernatant (stable solution) was removed with a glass pipette. Test concentrations of NOM-MWNT were achieved by sonicating appropriate volumes of NOM-MWNT supernatant stock solution in dilution water containing NOM.

Bioassays

Bioassays were conducted according to US EPA methods (EPA 1993, 2002) with slight modification using a dilution series of MWNTs in NOM. NOM solution (without MWNTs) and MHW were used as controls. Fifteen mLs of stable MWNT solutions was added to 30 mL glass beakers that served as test chambers (six concentrations 0.625-20 mg/L; n = 3 replicates per concentration). Equal volumes of control waters were poured into 30 mL glass beakers to serve as test controls (n = 3 replicates per control). Test solutions were renewed daily.

D. magna and Ceriodaphnia dubia were obtained from existing cultures at the Dept. of Biology, University of North Texas (Denton, TX) maintained in MHW. Growth in *D. magna* neonates (< 24 hrs old) were measured following exposure to a range of MWNT concentrations for 96 hrs (0-1mg/L MWNT; n = 5 replicates per concentration). *C. dubia* reproduction was monitored over a seven day exposure period and growth was measured at the end of the 7 day test (n = 5 replicates per treatment). Organisms were fed a mixture of green algae-YTC and test solution renewed daily. Growth was measured as dry weight on a Kahn electromicrobalance.

Results

MWNTs were observed in the gut tract of *C. dubia* within hours of exposure initiation. However, MWNTs in NOM were not acutely toxic (lethal) to *C. dubia* at the tested concentrations (0-1mg MWNT/L). Mean survival was greater than 85% in all treatments, and no relationship was observed between MWNT concentration and mortality.

Chronic toxic effects at sublethal concentrations of MWNTs in NOM were observed in *C. dubia*. A negative relationship between reproduction and MWNT concentration between 0 and 1 mg/L was found ($p < 0.01$) (Fig. 1). Reproduction was significantly inhibited by approximately 50% at concentrations >0.25mg/L MWNTs. Growth was also inhibited at concentrations >0.25mg/L (Fig. 2).

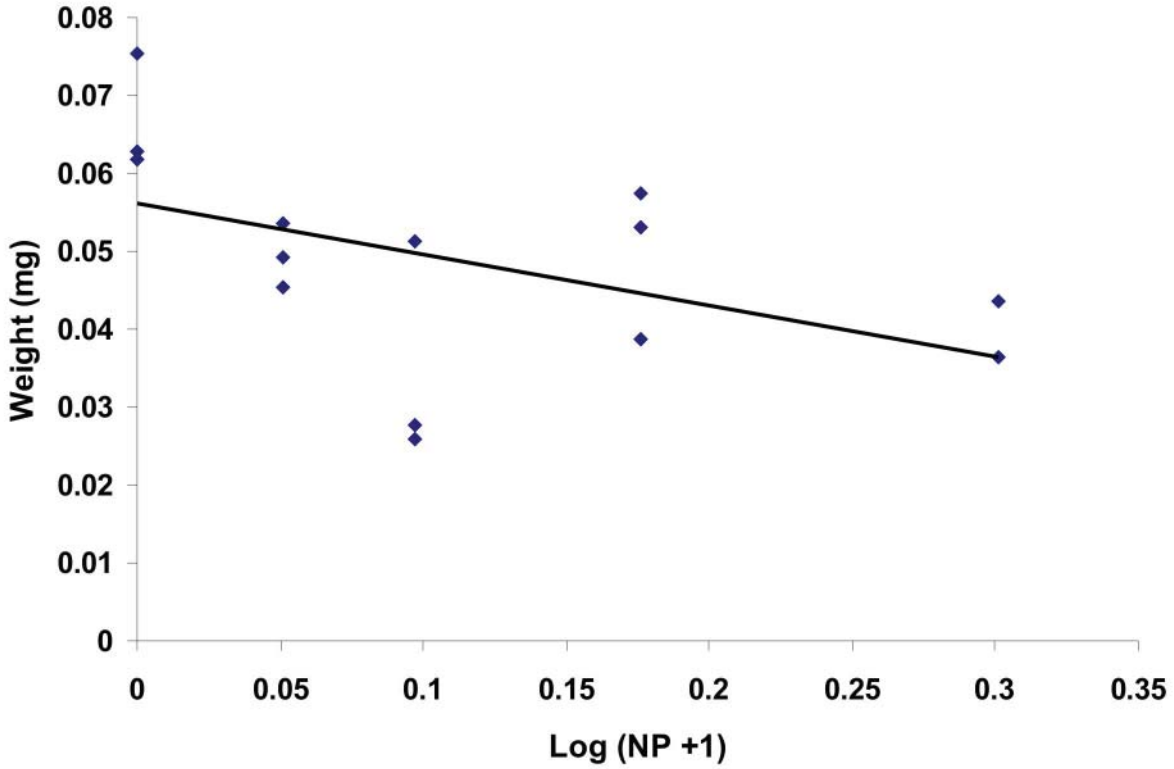

Figure 2. *C. dubia* growth.

A negative relationship was found between *D. magna* growth and MWNT concentrations between 0 and 1mg/L ($r^2 = 57\%$; $p < 0.01$) (Fig. 3). Mean dry weight was significantly reduced by 25% in organisms exposed to 0.25 mg/L MWNTs, a trend which increased in a dose-dependent manner. No acute mortality was observed.

Discussion and Conclusions

We were able to create relatively stable aqueous suspensions of MWNT using NOM, a constituent of all surface waters. We observed that grazing zooplankton were able to ingest the suspended MWNTs during their normal feeding behavior in a manner similar to previous reports of the ingestion of coated SWNTs (Roberts et al. 2007). MWNTs were visible in the guts of exposed animals at concentrations <1mg/L within hours of exposure initiation. These findings indicate that other filter feeding aquatic organisms including clams and mussels may also be at risk to dietary carbon nanomaterial exposure. We also found that, as in previous studies using coated SWNTs, MWNTs did not appear to be acutely toxic to zooplankton (Roberts et al. 2007).

Interestingly, although we observed no evidence that the MWNTs were taken up across the gut, it does appear that simply ingesting the materials can lead to significant toxic effects. Growth was reduced in both of the test species and reproduction was inhibited in the *C. dubia* model. We hypothesize that this is due to a decreased ability to take up normal food items such as algae as a result of the physical agglomeration of MWNTs in the gut rather than through increased oxidative stress or other biochemical mechanism. Future studies will examine these mechanisms more closely as well as the potential for the materials to be passed up the food chain to other planktivorous species.

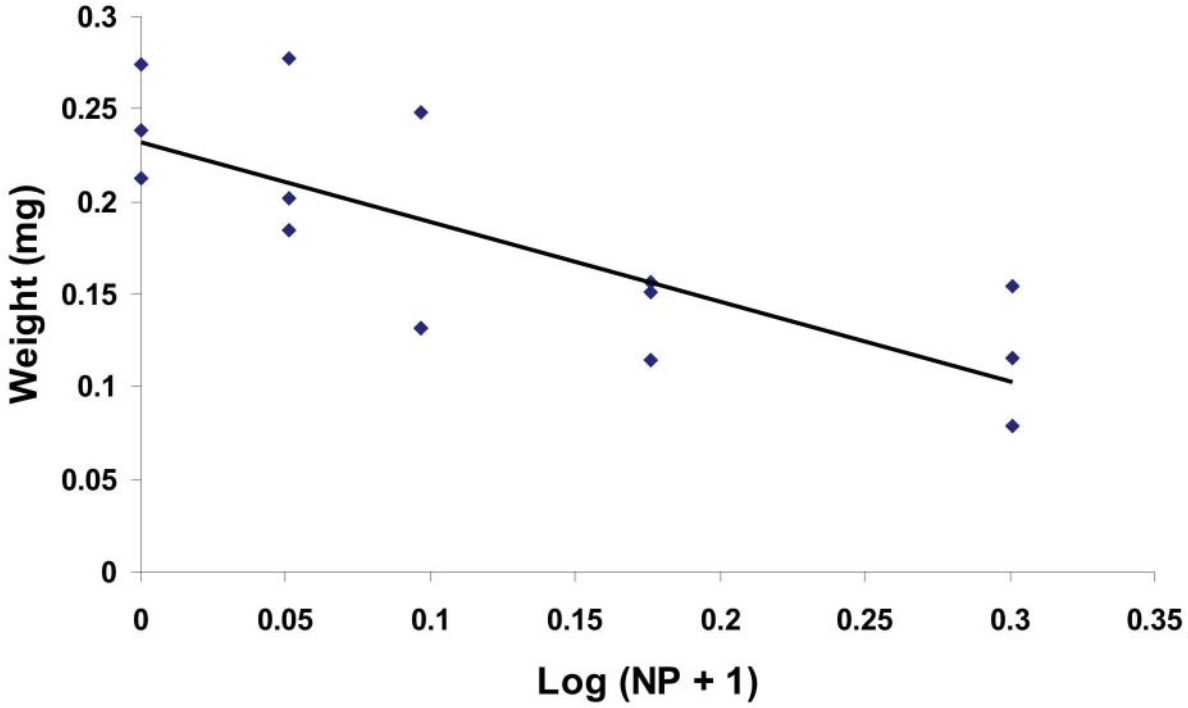

Figure 3. *D. magna* growth.

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Toxicity of CdSe/ZnS nanocrystals to D. magna.

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Abstract

With the rapid expansion of the nanotechnology industry, quantum dots (QDs) are situated to become a prominent new source of metal contamination. We are currently researching the acute toxicity of CdSe/ZnS QDs on *Daphnia magna* using 48hr exposure studies. Toxicity of QDs was hypothesized to be directly related to either one of two scenarios: 1) the solubilization of the QD and subsequent release of toxic metals (Zn, Cd) or 2) the physical or chemical impairment of key physiological functions by the nanoparticle itself. To test this hypothesis we investigated QDs with two different CdSe core diameters, 2nm green emitting QDs and 5nm red emitting QDs. To investigate potential particle effects we also examined two separate surface coatings, polyethylene oxide (PEO) and 11-mercaptoundecanoic acid (MUA), which are polar and anionic respectively (Evident Technologies, Troy NY and NN-Labs, Fayetteville AR). These coatings, which serve to render the QDs water stable, increase the hydrodynamic diameter of all QDs to approximately 25nm. Thus, while the metal content of the red and green emitting QDs (2nm vs. 5nm) was substantially different, the total particle size was the same. Using a fluorescence scan of the QDs (400-800nm) we monitored the QD concentrations during exposures. We found that PEO coated QDs remained well-dispersed throughout the 48hr exposures with no significant change in QD concentration whereas MUA coated QDs had a higher tendency to aggregate. In addition, we characterized the QDs before and after exposure via filtrations and ICP-OES metal analysis (unfiltered, 0.02µm and 3kDa filtrations). Finally, fluorescence microscopy and synchrotron micro-XRF showed accumulation of nanoparticles within exposed daphnids.

Introduction

Nanomaterials, due to their small size, differ from their bulk material counterparts. Accordingly, when evaluating potential toxicity of nanomaterials, one must address not only their chemical composition, but also the unique physiochemical properties associated with their extremely small size. Quantum dots (QDs) are nanoparticles that frequently contain toxic heavy metals. With numerous potential applications in the biological imaging industry as well as in optics and electronics, QDs are situated to become a new source of metal contamination. Originally, many reports indicated that QDs did not produce any significant cellular toxic effects [1-3]. However, it was recognized that these studies were designed to answer questions concerning the QD's novel physiochemical properties such as fluorescence, detectability and stability, and were not designed to specifically investigate QD toxicity [4]. Recently, a group at the University of California San Diego reported that CdSe-core quantum dots can produce cytotoxic effects under certain conditions [5]. It was found that these cytotoxic effects correlated with the release

of free Cd^{2+}, which arose from surface oxidation due to exposure to air or UV light. Various surface coatings were found to resist oxidation, thus producing reduced cytotoxic effects. This study suggests that it is the release of the metals from the nanoparticle that causes a toxic effect. While further investigating QDs, Kirchner et al. found that besides the release of Cd^{2+} ions, the aggregation and precipitation of the nanoparticles on the cell surface could impair cellular function [6]. These results suggest that the particle itself, independent of QD metal release, can play a role in QD toxicity. An additional study by Lovric et al. [7] found that green emitting CdTe-core QDs produced increased cytotoxic effects over that of red emitting QDs. Since emission wavelength of QDs is directly related to QD core diameter, these results further suggest that particle effects are playing a role in QD toxicity.

Much of the research done on QD toxicity has been related to cytotoxic or *in vivo* effects. However, questions concerning the environmental effects of QDs are also important to characterizing potential risks of these nanoparticles and these questions remain largely unanswered. Based on the results of previous studies, QD toxicity was hypothesized to be directly related to either one of two scenarios: 1) the solubilization of the QD and subsequent release of toxic metals (Zn, Cd) or 2) the physical or chemical impairment of key physiological functions by the nanoparticle itself. The goal of this study was to use the well-established framework of acute toxicology for dissolved metals to glean a basic understanding of the potential risks associated with metal-containing nanocrystals in aquatic environments.

Methods

Green (2nm core) and red (5nm core) polyethylene oxide (PEO) coated CdSe/ZnS QDs (Evident Technologies, Troy NY) as well as green and red mercaptoundecanoic acid (MUA) coated CdSe/ZnS QDs (NN-Labs, Fayetteville AR) were investigated during this study. The two surface coatings, which both serve to render the QDs water stable, increase the hydrodynamic diameter of all four QDs to approximately 25nm. Thus, while the metal content of the red and green emitting QDs (2nm vs. 5nm) was substantially different, the hydrodynamic diameter of all QDs was approximately the same.

We performed 48 hr acute toxicity tests for the four QDs using *Daphnia magna* according to USEPA Standard Test Protocol with a mortality (i.e. immobile) endpoint [8]. *Daphnia magna* were obtained from cultures maintained in USEPA hard water medium in an incubator at 20 °C with a 16:8 hr day: night cycle. QD solutions were made immediately preceding the test through simple dilution into hard water with minor stirring and no additional sonication. Synthetic natural waters were made from nanopure water and analytical grade chemicals according to USEPA guidelines for hard water. Statistical analysis on acute toxicity data was done with Priprobit (Ver 1.63, Kyoto Japan).

We characterized the nanoparticle concentrations during tests via fluorescence, which was measured at 0, 24 and 48 hours after exposure (FluoroMax 4, Joriba Yorn). In addition, we measured total metal concentrations suspended in solution (i.e. no stirring prior to sample collection) at 0 and 48 hours using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Finally, 3000 Dalton filtrations were done at 0 and 48 hours to separate dissolved metals from whole and partial nanoparticles (Millipore Bioseparation Spin Filters).

Micro X-ray fluorescence (µXRF) imaging for elemental mapping was performed at the National Synchrotron Light Source (Beamline X27A) at Brookhaven National Laboratory, Long Island NY. Daphnia were air dried and mounted on Kapton tap for image scans.

Results and Discussion

The decline of fluorescence along with the emergence of dissolved Zn and Cd in the 3kDa filtration samples indicate that the MUA coated QDs are unstable during a 48hr toxicity test. In addition, visible aggregation (Red MUA QDs specifically) and a significant drop in total metals in solution after the 48hr exposures suggests MUA QDs not only undergo dissolution, releasing dissolved metals, but they also fall out of solution as aggregates. We monitored actual concentrations of MUA QDs in solution using fluorescence. In contrast, the characterization data for the PEO coated QDs indicate that they remain stable in solution throughout the acute test.

Dose-response curves were established for each of the four QDs tested. The dose for each curve was represented four different ways: 1) particle number (nmol QDs/L), 2) mass basis (mg QDs/L), 3) equivalent Cd concentration (mg Cd/L), and 4) equivalent Zn concentration (mg Zn/L). See Figure 1.

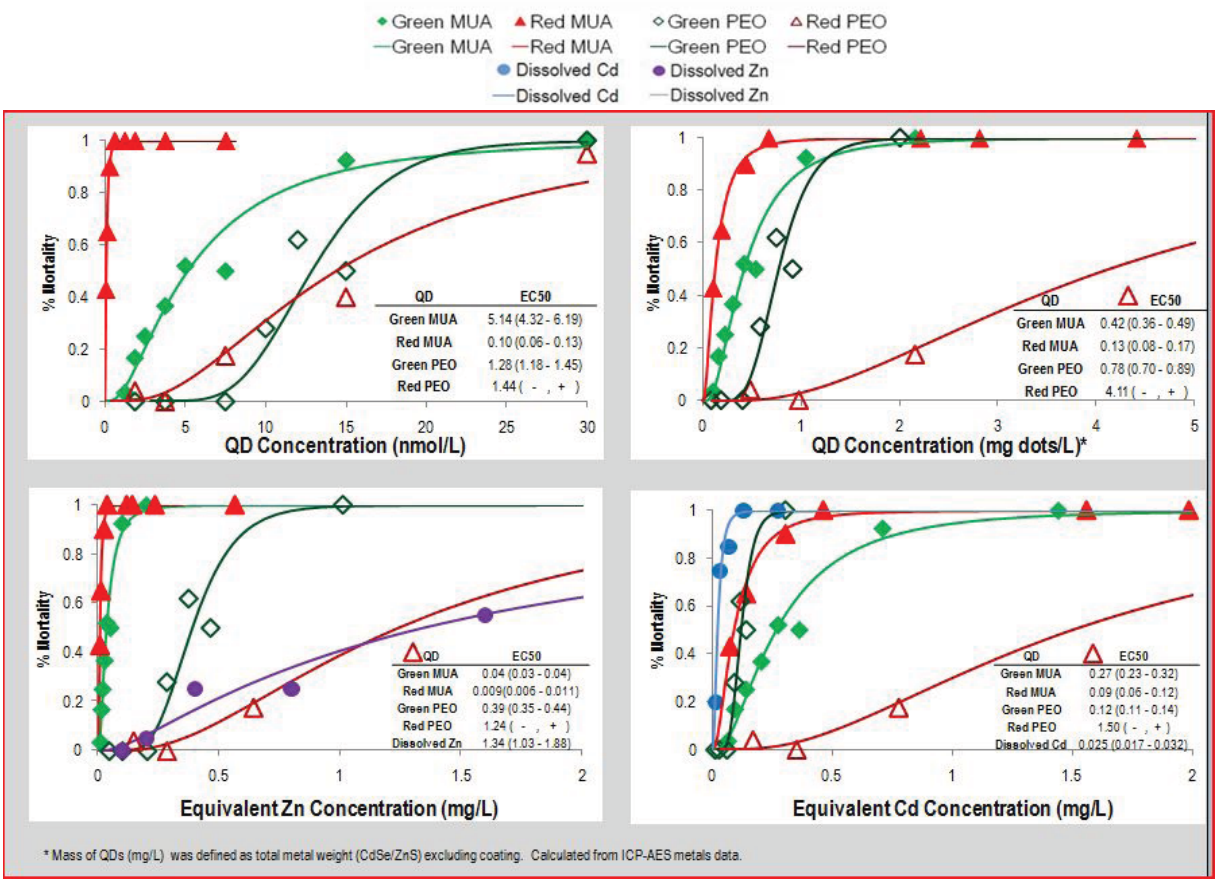

Figure 1. 48 hour acute toxicity dose-response curves for four QDs and dissolved Cd and Zn metals.

On a particle concentration basis (nmol/L): 1) MUA coated QDs are more toxic than PEO coated QDs, 2) Red (5nm) MUA QDs are more toxic than Green (2nm) MUA QDs, and 3) Green and Red (2 and 5 nm) PEO QDs are similar in toxicity. On a mass concentration basis (mg/L): 1) MUA QDs are still more toxic than PEO QDs, however there is a greater similarity in toxicity between MUA QDs and Green PEO QDs, and 2) Red PEO QDs are significantly less toxic than other QDs tested. On an equivalent Zn basis, QDs are more toxic than dissolved Zn, indicating that Zn is not solely responsible for QD toxicity. This excludes Red PEO where the toxicities are similar suggesting Zn as the cause of QD toxicity. Finally, on an equivalent Cd basis, all QDs are less toxic than dissolved Cd, indicating that the Cd in the CdSe core is not entirely bioavailable.

Finally, synchrotron images show presence of QDs in gut of the daphnid, however current images show no evidence of QD metals migrating to other daphnid organs within the 48hr exposure. Future work will include further synchrotron imaging, including 3D tomography to further explore potential exposure routes and target organs for QD toxicity.

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Acute and Chronic Toxicity of TiO_2 to Freshwater Aquatic Organisms

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Abstract

Preliminary testing of TiO_2 particles indicated the fathead minnow is much less sensitive to TiO_2 than *Ceriodaphnia dubia* and *Daphnia pulex.* The most chronically sensitive species tested was the green algae *Pseudokirchneriella subcapitata.* Total organic carbon (TOC) appears to decrease TiO_2 acute toxicity to *C. dubia*.

Introduction

The aquatic toxicity of metals-based nanoparticles is relatively unknown, but the limited data available indicate it is not highly toxic to aquatic life, with effects concentrations in the range of approximately 5 to above 100 mg/L (e.g., Velzeboer et al., 2008; Lovern and Klaper, 2006). Aquatic testing of nanoparticles has indicated concerns related to test material preparation techniques, and the general applicability of conventional toxicity test methods for evaluation of nanoparticles. The effects of water quality parameters on the toxicity of nanoparticles is unknown. This study evaluated the acute and chronic toxicity of TiO_2 nanopowder to freshwater aquatic organisms as determined in standard USEPA toxicity tests, and assessed the effects of organic carbon on TiO_2 acute toxicity.

Methods

The test material was 99 percent titanium dioxide nanopowder (10 nm) from American Elements. TiO_2 stock solutions were prepared by adding known masses to moderately hard water (USEPA, 2002a) or algae culture water (USEPA, 2002b) and stirring for a minimum of 30 minutes before addition to test waters. Test exposures were prepared by serial dilution of TiO_2 stocks with respective test waters: moderately hard water for fish and cladocerans, and algae culture medium for algal tests. Fish (fathead minnow, *Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia* and *Daphnia pulex*) were cultured in moderately hard or similar water, and green algae (*Pseudokirchneriella subcapitata,* formerly *Selenastrum capricornutum*) was cultured in USEPA culture water. Acute (48 hours for cladocerans, 48 and 96 hours for fathead minnow) and chronic (96 hours for *P. subcapitata*, 7 days for fathead minnow and *C. dubia*) toxicity tests followed methodologies outlined by USEPA (2002a, 2002b). Acute median lethal concentrations (LC50) and chronic 25 percent inhibition concentrations (IC25) were calculated as recommended by USEPA (2002a, 2002b). Nominal TiO_2 concentrations were used in the calculation of LC50 and IC25 values. A wheat grass food component of the cladoceran foods was used to establish

total organic carbon (TOC) levels of 1.5 mg/L in test waters to evaluate the effects of TOC on TiO_2 toxicity. Assessments of the forms of TiO_2 in toxicity test solutions were performed using powder x-ray diffraction (XRD) techniques (Klug and Alexander, 1974).

Results and Discussion

All toxicity tests met control and test acceptability criteria specified by USEPA for survival and growth (fathead minnow), survival and reproduction (*C. dubia*), and cell production (*P. subcapitata*). The addition of TiO_2 to test waters did not alter general test water chemistry (e.g., pH, conductivity). Table 1 summarizes the results of TiO_2 acute toxicity tests. For tests conducted in the absence of TOC additions, the cladocerans (*C. dubia* and *D. pulex*) demonstrated high sensitivity to TiO_2 relative to the fathead minnow: cladoceran LC50 values approximately 3 to 16 mg/L, fathead minnow LC50 values 500 mg/L and above. Although *C. dubia* at times demonstrated higher sensitivity to TiO_2 than *D. pulex*, the range of *C. dubia* and *D. pulex* LC50 values was similar: *C. dubia* 3.0 to 15.9 mg/L, and *D. pulex* 6.5 to 13.0 mg/L, and no between-species values were statistically different given that 95 percent confidence intervals overlapped. Fathead minnow LC50 values were consistently greater than 1,000 mg/L. These data confirm the relatively high sensitivity of the cladocerans (as compared to fish) as has been reported in the literature for conventionally-tested compounds such as salts and metals.

In an initial acute toxicity test conducted in the presence of approximately 1.5 mg/L TOC, *C. dubia* demonstrated much lower sensitivity to TiO_2 as compared to tests in the absence of TOC. The *C. dubia* LC50 value for TiO_2 in the presence of organic carbon was above 100 mg/L. This indicates that organic carbon decreases the bioavailability of TiO_2, likely by complexation (e.g., chelation or sorption) with the TOC and/or suspended solids in the wheat grass preparation added to increase the organic carbon content of the test water. Decreases in toxicant bioavailability due to complexation mechanisms have long been recognized for both metal and organic toxicants. It is unlikely that the decrease in TiO_2 toxicity observed in this study is due to enhanced nutritional status of *C. dubia* in test waters to which organic carbon was added. In tests in our laboratory with salts (e.g., NaCl), which should not be markedly complexed by organic carbon, toxicity decreases due to wheat grass additions were typically on the order of 20 percent, not upwards of 6-fold as observed for TiO_2.

Table 2 summarizes the results of TiO_2 chronic toxicity testing. Although the *C. dubia* IC25 value was among the lowest observed, *P. subcapitata* demonstrated the highest sensitivity to TiO_2, with an IC25 value of 1 mg/L TiO_2. These data indicate that algae and cladocerans are much more sensitive to TiO_2 than the fathead minnow on a chronic toxicity basis. It should also be noted that the ratio of the acute to chronic toxic effects levels for *C. dubia* and the fathead minnow are small (on the order of two to three). This indicates that acute and chronic toxicity to water column organisms occurs at similar concentrations of TiO_2. The food additions in chronic toxicity tests may play a role in this phenomenon given the results of the TOC-addition tests.

Powder XRD scans of test solutions indicated that the only detectable TiO_2 phase present was the *anatase* form. No traces of crystalline *rutile* or *brookite* were observed. The average particle size of the TiO_2 nanoparticles determined from XRD peak widths was 11.2 ± 3.0 nm, very similar to the nominal particle size of 10 nm listed by the manufacturer. TiO_2 samples isolated from the moderately hard test waters showed no significant changes in average particle size due

Table 1. Results of TiO_2 acute toxicity tests

Species	LC50 (mg/L TiO_2)	95% Confidence Intervals (mg/L)
Cladocerans		
Ceriodaphnia dubia	3.0	1.6 to 6.4
Ceriodaphnia dubia	13.4	9.5 to 18.4
Ceriodaphnia dubia	15.9	13.4 to 19.0
Ceriodaphnia dubia - + TOC	>100	NC
Daphnia pulex	6.5	4.4 to 12.9
Daphnia pulex	13.0	2.8 to 24.0
Fish		
Fathead Minnow- 48 hour	500	NC
Fathead Minnow- 48 hour	>1,000	NC

NC = Not calculable.

Table 2. Results of chronic toxicity tests with TiO_2

Species	IC25 (mg/L TiO_2)	95% Confidence Intervals (mg/L)
Cladoceran		
Ceriodaphnia dubia	2.5	NC
Ceriodaphnia dubia	9.4	2.8 to 15.3
Fish		
Fathead Minnow	342	283 to 430
Green Algae		
Pseudokirchneriella subcapitata	1.0	0.33 to 5.2

NC = Not calculable.

to agglomeration.

Conclusions

The green algae *P. subcapitata* was shown to be most sensitive to TiO_2, followed by cladocerans (daphnids), then the fathead minnow. In comparison to the acute and chronic toxicity of many environmental contaminants, TiO_2 is of moderate toxicity. This study also demonstrated that the widely-applied USEPA test protocols are appropriate for evaluation of the ecotoxicological effects of TiO_2. A key study finding was that TOC can decrease TiO_2 toxicity. Thus, toxicity tests utilizing reconstituted test waters likely overestimate TiO_2 toxicity in natural receiving streams containing TOC. The TiO_2 acute to chronic ratio was shown to low, indicating that acute and chronic toxicity occurs at similar concentrations of TiO_2.

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Toxicity of Multi-Walled Carbon Nanotubes in Water to Sediment-Dwelling Invertebrates

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Abstract

Carbon nanotubes (CNTs) are relatively insoluble in water and are likely to accumulate in sediments if released into the aquatic environment. The potential impacts of CNTs released into the environment are largely unknown. The objective of this study was to evaluate the potential toxicity of commercially available multi-walled carbon nanotubes (MWCNTs) to sediment-dwelling invertebrates. Short-term 14-d water-only tests were conducted by exposing the amphipod (Hyalella azteca), the midge (*Chironomus dilutus*), the oligochaete (*Lumbriculus variegates*), and rainbow mussels (*Villosa iris*) to a thin layer of two MWCNT samples with periodic replacement of water. The survival of the invertebrates was significantly reduced in both MWCNT samples relative to the control, and in most cases the growth of the test organisms was also significantly reduced. Photographs and light microscopy images of surviving organisms at the end of the tests showed presence of MWCNTs in the guts of the amphipods, midge and oligochaete. The MWCNTs appear capable of smothering the organisms and may interfere with their ability to feed. Other mechanisms may exist for the demonstrated toxicity such as by dissolution of toxic metals from the MWCNTs. Further tests are planned to evaluate the toxicity of the MWCNT in sediment to the sediment-dwelling invertebrates.

Introduction

Carbon nanotubes (CNTs) have high electro-optical, thermal conductivity, mechanical strength and large surface area. Applications of the CNTs include aerospace and fiber industries, electronics and semiconductors, hydrogen-based fuel cells, environmental sensors and medical fields. With increasing commercial interests, the supply and demand of CNTs is expected to grow rapidly (Hyung et al. 2007). The CNTs have shown toxicity to living biological cells and tissues (Panessa-Warren et al. 2006, Pulskamp et al. 2007) and to rainbow trout (*Oncorhynchus mykiss*) and cladoceran (*Daphnia magna*) (Nowack and Bucheli 2007) and to developing embryos of zebra fish (*Danio rerio*) (Chen et al. 2007). The potential impact of CNTs released into the environment however is largely unknown. Since the CNTs are relatively insoluble in water, the materials would likely be associated with sediment in the aquatic environment. The objective

of this preliminary screening study was to evaluate the potential toxicity of MWCNTs in water using four sediment-dwelling invertebrates.

Method

Two samples of MWCNTs for toxicity testing were obtained from Helix Material Solutions Inc., TX, USA (Sample 1) and Shenzhen Nanotech Port Inc., Shenzhen, China (Sample 2). The amphipod (*Hyalella Azteca*), the midge (*Chironomus dilutus*), the oligochaete (*Lumbriculus variegates*), and rainbow mussel (*Villosa iris*) were exposed to the two MWCNTs samples in water adjusted to 100 mg/L hardness with 4 replicates/treatment and 10 organisms/ replicate chamber. The treatments included 200 mg of sonicated or non-sonicated MWCNTs added to each 300-ml glass beaker containing 200 ml of water. The MWCNTs formed a thin layer at the bottom of the beakers. The control treatment received 200 ml of water and a 5-ml fine sand substrate except for mussel tests with no sand. The tests were conducted for 14 d in static conditions with aeration of overlying water and 50% water replacement every Monday, Wednesday and Friday. Juvenile mussels were fed 2 ml of a non-viable algal mixture twice daily, amphipods were fed 0.5 ml of Yeast-Cerophyl-Trout Chow (YCT) while oligochaete and midge were fed 1.0 ml of Tetrafin® flake fish food every Monday, Wednesday and Friday immediately after water replacement. Other test conditions generally followed the standard conditions outlined in ASTM (2007) and USEPA (2000) with test water maintained at 23±1ºC, ambient laboratory illumination, wide-spectrum fluorescent lights at about 200 lux, and photoperiod of 16L: 8D.

Results and Discussion

The survival or growth of all four species exposed to the MWCNT Sample 1 was significantly reduced relative to the control except for the growth of mussels in non sonicated or sonicated MWCNTs (Table 1). The survival of amphipods exposed to non sonicated or sonicated MWCNT Sample 2 was significantly reduced relative to the control. The survivals of oligochaete exposed to non-sonicated MWCNTs Sample 2 and mussel exposed to sonicated MWCNTs Sample 2 were significantly reduced relative to the control (Table 1). The midge survival of 63% in control from test with MWCNT Sample 2 was below the established acceptability criteria (≥70%) but a general decline in survival between the controls, sonicated or non sonicated MWCNT treatments was observed. These results indicate the two MWCNTs samples tested were toxic to the invertebrates.

The MWCNTs were observed in the gut of midge, oligochaetes and amphipods (e.g., Figure 1 for amphipods) and was adsorbed onto the surface of these invertebrates including the shell of mussels. Light Microscopy images illustrated MWCNTs clumped in the gut of midge and amphipods. The MWCNTs may obstruct the passage of food through the gut leading to starvation. The coating of the MWCNTs on the surface of the organisms may impair respiration through blockage of the gills or possibly offered an entry route of the MWCNTs into the body of the invertebrates. Other mechanisms may exist for the demonstrated toxicity such as by dissolution of toxic metals from the MWCNTs and will be documented. Toxicity studies are planned spiking MWCNTs and other nanomaterials into sediment under environmentally realistic exposure concentrations (perhaps up to 1% MWCNTs added to sediment by weight).

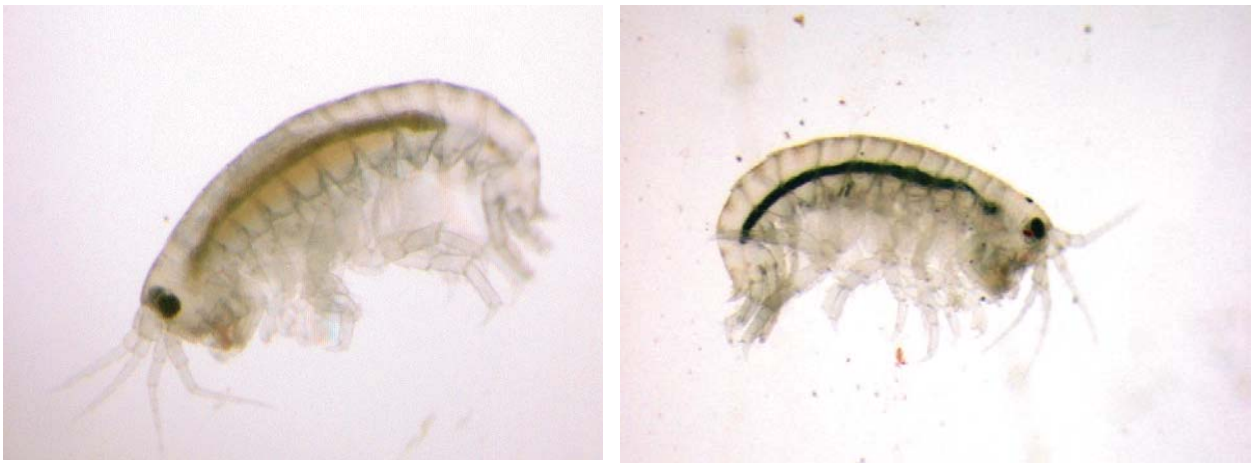

Figure 1. Amphipod *Hyalella azteca* (x18) on 6-d of exposures in: (A) control (water only), (B) non-sonicated MWCNT treatment, showing MWCNTs in the gut.

Acknowledgements

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Table 1. Mean (n=4) survival or growth of amphipods (*Hyalella azteca*), rainbow mussels (*Villosa iris*), midge (*Chironomus dilutus*), and oligochaetes (*Lumbriculus variegatus*) in 14-d water-only toxicity test with two multi-walled carbon nanotube (MWCNT) samples. Standard deviations in parenthesis.

Test organism	Treatment	MWCNT Sample 1		MWCNT Sample 2	
		Survival (%)	Biomass (mg) [a]	Survival (%)	Biomass (mg) [c]
Amphipod	Control	88 (5.0)	0.41 (0.07)	100 (0)	ND
	Non-sonicated MWCNT	5.0 (10)*	0.02 (0.03)*	7.5 (9.6)*	ND
	Sonicated MWCNT	2.5 (5.0)*	0.01 (0.01)*	5.0 (10)*	ND
Midge	Control	80 (8.2)	5.4 (0.2)	63 (15) [b]	2.1 (1.0)
	Non-sonicated MWCNT	60 (8.2)*	0.4 (0.1)*	55 (6)	1.2 (1.0)
	Sonicated MWCNT	43 (9.6)*	0.2 (0.1)*	7.5 (9.6)	ND
			Shell length (mm)		
Mussel	Control	98 (5.0)	2.2 (0.1)	80 (28)	ND
	Non-sonicated MWCNT	20 (14)*	1.6 (1.0)	35 (25)	ND
	Sonicated MWCNT	43 (19)*	2.4 (0.3)	5.0 (10)*	ND
			Biomass (mg) [c]		
Oligochaete	Control	16 (1.5)	15(1.3)	3.3 (1.8)	2.7 (1.8)
	Non-sonicated MWCNT	14 (1.0)*	13 (1.0)*	0.8 (0.2)*	0.2 (0.2)
	Sonicated MWCNT	13 (1.7)*	11 (1.7)*	2.8 (1.7)	2.2 (1.3)

[a] Dry weight of individual amphipods was estimated from the measurements of the lengths of individual amphipods using a length-weight relationship equation generated from a linear regression of amphipod length to the cube root of amphipod dry weight (unpublished data, Nile Kemble, USGS, Columbia, MO).
[b] Less than 70%, the test acceptability criteria established for the control and statistical analysis was not performed.
[c] Ash free biomass.
ND – Not determined because the surviving organisms were used for light microscopy imaging.
* Significantly different from control (t- test, $p<0.05$).

Chapter 7 – Introduction

Environmental Fate & Transport of Nanomaterials

Barbara Karn and Madeleine Nawar, United States Environmental Protection Agency

As more manufactured/engineered nanomaterials are produced, their release into the environment becomes more probable. Since some of these materials have exhibited intrinsic toxicity in laboratory tests, it is important to understand their transformation, pathways, and potential hazards of exposure to public health and the environment. Some nanomaterials may persist, bioaccumulate, or become mobile in the ecological matrices of air, water and land.

This understanding can lead to mitigating and minimizing exposure and possible adverse effects. For example, magnification and accumulation of nanomaterials through the food chain and other routes of exposure may lead to effects that are not evident in short-term toxicity tests. While the short-term effects of a toxic nanomaterial may result from a single exposure, the long-term effects due to bioaccumulation and persistency may be more severe, ranging from lasting health problems to organ damage.

The fate and transport session consisted of 17 presentations of multidisciplinary research papers. The plenary addressed the importance of using studies and techniques involving natural nanoparticles in order to inform research on manufactured nanomaterials. In particular, studies on ultrafines in air and natural colloids or nanoparticles in water and soil in the environment can help inform work on manufactured nanomaterials. Existing techniques may be interchangeable. However, because of the novel properties of nanomaterials, new or modified test methods for environmental fate and transport endpoints, and applications of new or existing air dispersion, soil transport, groundwater models, may be needed. Changes that nanoparticles undergo due to environmental factors such as pH, ionic strength, organic matter, or calcium need to be taken into account.

Characterization of the nanomaterials under study is essential to understand their fate, transport, toxicity and other factors. An integrated approach involving characterization of bulk and surface properties of nanoparticles, the effects of different environmental factors and combining both lab and field studies is necessary. Characterizing aggregation and agglomeration is also important.

Several talks focused on measuring the movement of nanomaterials in various media.

For example, quantum dot movement can be traced by their fluorescence; gold nanoparticles can be used to detect bacteria density using X-ray computed tomography.

Standard column tests showed that multi-walled carbon nanotubes, fullerenes, and aluminum nanoparticles behaved differently from normal chemical flows, indicating the need for different models. Columns were also used to compare movement of lactate-coated and uncoated particles of nanoscale zero valent iron.

Transformations and aging played a role in changing iron nanoparticles in water. Humic

acids caused aggregation of boron nanoparticles by different mechanisms dependent on the natural background. Natural organic matter was also studied as it affects quantum dot phase transfer, enhanced C60 dispersion, movement of iron oxide nanoparticles, and multiwall carbon nanotubes.

Models can be used to inform the movement of nanoparticles through the soil. A commercial model was used to predict nanoparticulate zero valent iron (nZVI) concentration and determine injection rates needed to clean up hexavalent chromium. Mesocosms can also be used to determine the movement, bioavailability, and toxicity of nanomaterials in various environmental compartments of water systems. For example, TiO2 has been studied as a sample nanomaterial in mesocosms. These mesocosm systems then can provide data for building models.

Fate, transport, and transformation could be affected by bacteria in the environment. For example, composition of quantum dots is not the only aspect of these materials that could change their toxicity. Bacteria can change the aggregation state which may change their environmental toxic effects, indicating that toxicity is controlled by aggregation as well as composition.

The papers in the session were indicative of the diverse approaches and the diverse nanomaterials being studied to determine fate and transport of nanoparticles in the environment. In general, the papers highlighted the differences in behavior of nanomaterials when using conventional environmental tests, the importance of characterization of the nanomaterials, and the different environmental interactions nanomaterials can undergo.

Interactions Between Natural and Manufactured Nanoparticles.

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Abstract

Manufactured nanoparticles have huge potential in a number of areas. However, there is also potentially large but unknown risk from their use both to human and environmental health. Understanding their potential exposure and hazard requires that their chemistry and transport in the environment is better understood. Some research has been performed in these areas, especially on the impacts of pH and ionic strength. A few studies have investigated the role of natural colloids and nanoparticles and these are reviewed in this study and the effects this interaction may have manufactured nanoparticle effects and behaviour.

Manufactured nanoparticles (NPs) are usually defined as being between 1-100 nm in size and are an important product of both nanoscience and nanotechnology i.e. the science and technology of the nanoscale (1-100 nm). There is huge current investment in nanotechnology and the expected market for nanoparticles is already large and set to grow massively. Evidence of the scientific interests and outputs is abundant in the literature (Ju-Nam and Lead, 2008). Nanotechnology has the potential to enable, support and develop current industries and has the potential to revolutionise fields such as environmental remediation, health care, computing and electronics. Nevertheless, despite this potential, there are concerns that nanotechnology and nanoparticles could pose serious risks to the environment, although both exposure and hazard are poorly understood; indeed a key problem is that our direct understanding is extremely poor. Innovation and the synthesis of new materials are currently expanding near-exponentially from a large base of current research mass and research spending. However, the communities investigating the possible effects of nanoparticles is small and relatively poorly funded. Greater funding in this area is starting to occur but is still inadequate given the development and changes in nanotechnology.

Understanding the environmental behaviour of NPs can be performed fully only if their behaviour at realistic conditions is considered. Such conditions include ionic strength, pH and, importantly, NP concentration. Another important consideration, often overlooked is the importance of natural organic colloids and particles. Naturally occurring colloids and particles are usually defined as materials between 1 nm – 1 μm and > 1 μm, respectively (Lead and Wilkinson, 2006). These materials are naturally produced by microbial action, weathering, hydrolysis and other processes and are important in manufactured NP fate and behaviour for two reasons:

1) As with the study of atmospheric ultrafine particles, natural aquatic and terrestrial colloids offer a large background of data and understanding which can be used to help understand what might happen to NPs in environmental systems.

2) Natural colloids/nanoparticles and manufactured nanoparticles directly interact in the environment and this results in changes in their dispersion, bioavailability and potential mobility.

Below, we will discuss briefly the two points raised above: what we can learn from the behaviour of natural colloids that is relevant to manufactured nanoparticle behaviour and the direct interactions which affect nanoparticle behaviour and effects.

Natural colloids and nanoparticles. There is a long history of research in aquatic and terrestrial systems which has developed for two reasons. Firstly, the development of often cheap, readily available and robust technology during the twentieth century enabling their study (membrane technology, humic substances, HS, extraction, electron microscopy etc) and the realisation that these solid-phase material were important in pollutant, particularly metal, transport and bioavailability. This area has been thoroughly reviewed recently (Lead and Wilkinson, 2006). Areas of particular relevance are:

1) The ability of some small nanoparticles such as HS to form nanoscale films on all surfaces (Lead et al, 2005) which stabilise surfaces by imparting a highly negative charge at environmental concentrations and also by steric mechanisms, giving further stability. HS of a few nm to tens of nm have also been shown to sorb onto biological cell surfaces (Lead and Wilkinson, 2006), changing cell membrane charge and permeability and also to act as an energy source for bacteria, indicating uptake through cell walls

2) The secondary role of fibril-like polysaccharides and proteins which result in increased aggregation via bridging.

3) The large specific surface area of natural colloids and nanoparticles allowing them to bind pollutants strongly while aggregating and settling over short time periods, removing pollutants from the water column and causing a build-up in the sediments, sometimes termed colloidal pumping.

4) In porous media, an increased pollutant transportation rate and distance due to binding with small colloids. This reduces pollutant uptake onto the immobile solid phase rock or soil, while enabling transport though small pores, the whole system being analogous to a chromatography system.

Colloid-nanoparticle interactions. In the absence of direct information of nanoparticles, this data is one of the main sources for consideration of nanoparticle effects on environmental human health. The points above have provided the background to generate the on-going debate on nanoparticle dispersion and aggregation, nanoparticle sedimentation or retention in the water column and nanoparticle transport in soils and groundwater. Nevertheless, most current research in this area has looked directly at point 1 and the ability of HS to stabilise nanoparticles (Baalousha et al, 2008; Diegoli et al, 2008). The general conclusion is that stabilisation is effected to a large degree, due to both charge and steric stabilisation. However, some studies have shown that ionic strength, Ca concentration and pH are all important and may result in further aggregation. Our own data (manuscript in prep) further shows that the dispersion of strongly bound, sterically stabilised NPs (PVP stabilised 7 nm old) are unaffected by any of these

conditions, although small changes in surface chemistry were apparent, indicating the importance of binding and stability mechanisms of capping agents to environmental behaviour.

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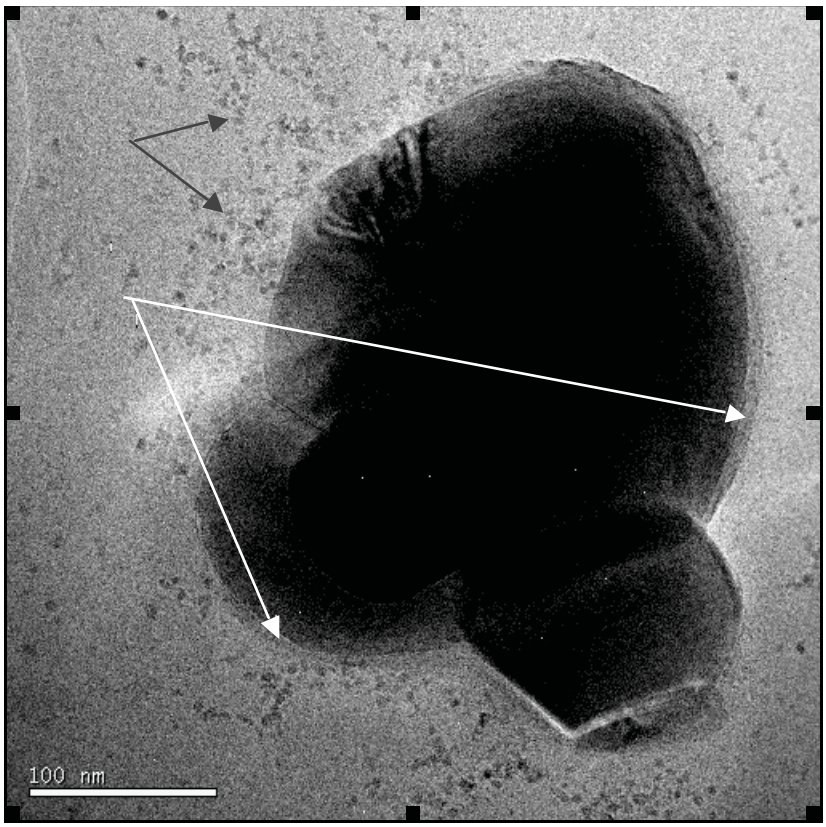

Figure 1.TEM micrograph showing iron oxide particles coated with a layer of HA (white arrows) and surrounded by a network of humic substances molecules (black arrows).

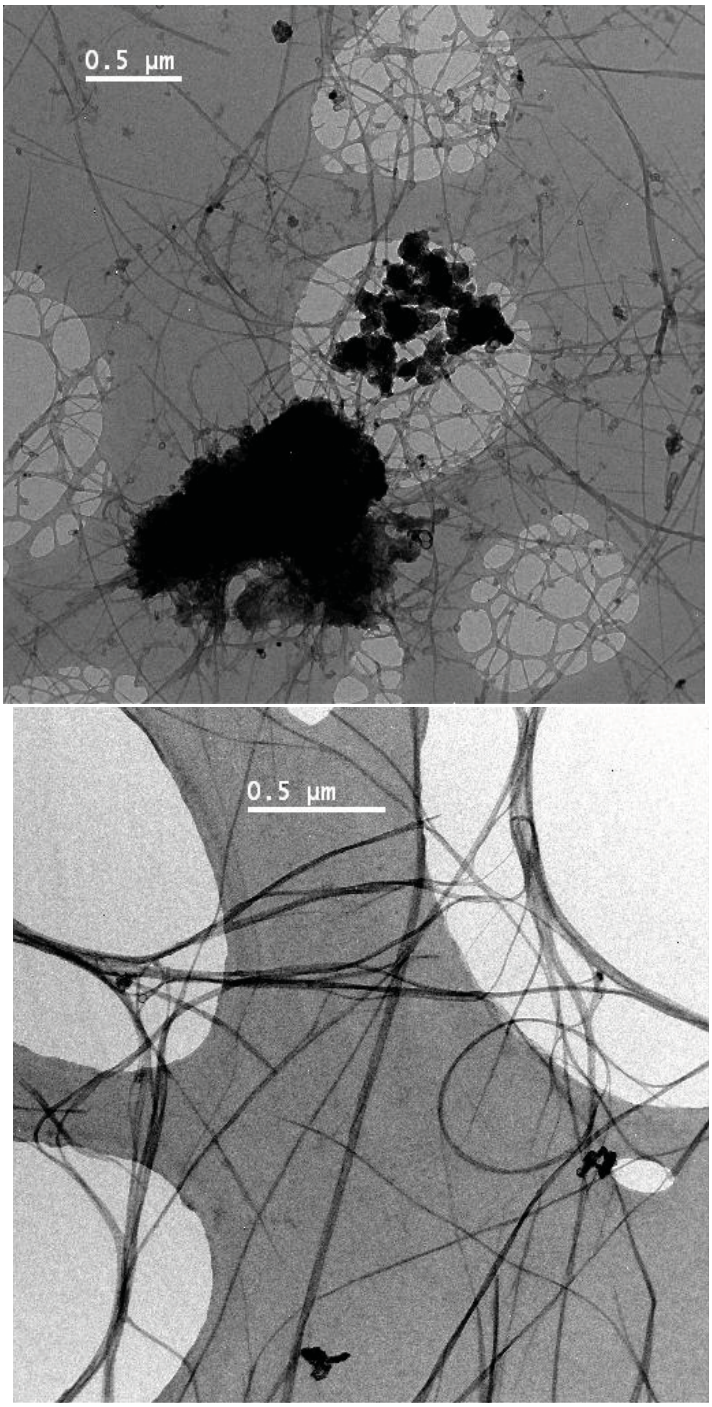

Figure 2. TEM micrograph showing single walled carbon nanotubes in the presence (top) and absence (bottom) of a filtered natural water (Vale Lake, Birmingham, UK).

An Integrated Approach Toward Understanding the Environmental Fate, Transport, Toxicity and Occupational Health Hazards of Metal and Metal Oxide Nanomaterials

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Abstract

There are many questions related to the environmental fate and transport of nanomaterials as well as the environmental health and safety of nanomaterials on living systems. Nanoparticles, the primary building blocks of many nanomaterials, may become suspended in air during production, distribution, use and disposal, or get into water systems, e.g. drinking water systems, ground water systems, estuaries and lakes. Therefore, manufactured nanoparticles can become a component of the air we breathe or the water we drink. One important issue in understanding the environmental fate, transport, toxicity and occupational health hazards of nanoparticles is the characterization of the nature and state of nanoparticles in air, water or in vivo.

Introduction

Studies directed toward understanding the environmental and biological fate of nanoparticles and the potential transformation of nanoparticles have just begun in recent years (Elzey et al. 2009). It is becoming increasingly clear that the environmental and biological fate as well as the toxicity of nanoparticles depends on nanoparticle size, shape, bulk composition and phase, and surface area and composition. Therefore, it is imperative that an integrated approach that combines extensive nanomaterial characterization along with any investigation of the environmental fate, transport, toxicity or environmental health and safety of nanomaterials be employed (Pettibone et al. 2008a).

For the nanomaterials of interest in these studies, metal oxide and metal nanoparticles, it can be asked: (i) will metal oxide and metal nanoparticles be present in air or water as isolated particles or in the form of aggregates?; (ii) will metal oxide and metal nanoparticles dissolve in aqueous solution or in vivo?; and (iii) under what conditions will metal oxide and metal nanoparticles aggregate or dissolve? As the size regime will be very different depending on the state of the nanoparticles, as dissolved ions, isolated nanoparticles or nanoparticle aggregates, these questions are important to address as it impacts the size regime that needs to be considered or modeled in, for example, environmental transport or lung deposition models. Furthermore, the effect on biological systems including nanoparticle-biological interactions and toxicity will depend on the state of nanoparticles *in vivo*.

In the studies discussed here, an integrated approach is used to address these questions and issues. The approach combines state-of-the-art characterization of the bulk and surface properties of nanoparticles, studies of the state of nanoparticles in different environments as determined by aggregation measurements and dissolution measurements in laboratory, field and

toxicity studies. The data from these studies are expected to provide for a better understanding of the environmental fate, transport, toxicity and occupational health hazards of metal and metal oxide nanomaterials.

Methods

Nanoparticle Characterization Methods. Unlike molecules with discreet molecular formulas, nanoparticles are often of varying size with size distributions that can be quite narrow and monodispersed or in some cases much more polydispersed with wider distributions. Since both the macroscopic and microscopic behaviors of metal and metal oxide nanomaterials depend on size, phase and surface properties, it is essential that these properties be characterized using a suite of complementary techniques that include X-ray diffraction, microscopy, surface area and surface composition measurements.

Aggregation in Air and Water. Since there is a propensity for nanoparticles to aggregate under certain conditions, measurements of aggregate size are important in the characterization of nanoparticles. Aggregation measurements in solution with two types of light scattering measurements are used to investigate nanoparticle aggregation size and nanoparticle aggregation kinetics, dynamic light scattering and sedimentation kinetics using light scattering. These techniques provide information about the size of the aggregates and the stability of the aggregates in solution. In air, a scanning mobility particle sizer is used to measure the size of nanoparticles formed once generated as an aerosol.

Surface Chemistry. Surfaces play an important role in the properties of nanomaterials. Surfaces of nanomaterials can be functionalized either in the manufacturing process or from the adsorption of molecules from the ambient environment. In these studies, spectroscopy is used to measure surface adsorption and surface chemistry of nanoparticles from both solution and gas-phase environments for nanoparticles of different size. In addition, since dissolution by its very nature is a surface phenomena, dissolution studies in various media provide essential data on the stability of metal oxide and metal nanoparticles.

Inhalation and Instillation Toxicity Studies. As inhalation is expected to be a major route of exposure, especially in occupational settings, a summary of some recent toxicity studies will be discussed. Details of the experimental protocols can be found in Grassian et al. (2007a,b) and Pettibone et al. (2008c).

Field Measurements. There is a great deal of interest in characterizing nanomaterials in outdoor and indoor environments, especially in occupational settings. Samples were collected on filter media in a manufacturing facility (Peters et al. 2008). These samples were then analyzed further with electron microscopy techniques including: transmission electron microscopy, scanning electron microscopy and energy dispersive X-ray analysis.

Results and Discussion

Metal Oxide and Metal Nanoparticles – Laboratory Studies. Laboratory studies of metal oxide nanoparticles have focused on metal oxide and metal nanoparticle aggregation and dissolution as well as the surface chemistry of these nanoparticles. One example is a study of the adsorption of oxalic acid and adipic acid, on TiO_2 nanoparticles (Pettibone et al. 2008b). Solution phase

measurements were used to quantify the extent and reversibility of oxalic acid and adipic acid adsorption on anatase with primary particle sizes of 5 and 32 nm. At all pH values considered, there were minimal differences in measured Langmuir adsorption constants between the two. Although macroscopic differences in the reactivity of these organic acids as a function of nanoparticle size were not observed, ATR-FTIR spectroscopy show some distinct differences in the infrared absorption bands present for oxalic acid adsorbed on 5 nm particles compared to 32 nm particles, suggesting different adsorption sites or a different distribution of adsorption sites for oxalic acid on the 5 nm particles. Furthermore, it is clear that particle aggregation occurs at all pH values and that organic acids can destabilize nanoparticle suspensions.

Although metal nanoparticle aggregation is evident in air and water, the focus of our most recent studies is on the propensity of metal nanoparticles to dissolve under different environmental conditions. We have investigated the dissolution of Ag, Fe and Cu nanoarticles using particle sizing measurements as well as more conventional solution phase studies. The results of these studies provide insights into the stability of nanoparticles in different environments and media.

Nanoparticle Toxicity – Size and Composition Comparisons. Using murine models for inflammation, size effects of inflammatory response in instillation and acute inhalation exposures of TiO_2 nanoparticles with manufacturers' average particles sizes of 5 and 21 nm were investigated. The properties of the primary nanoparticles, aerosol and instillation solution for both sized nanoparticles were evaluated. Results show the larger TiO_2 nanoparticles were found to be moderately, but significantly, more toxic. The nanoparticles had different agglomeration states which may be a factor as important as the surface and physical characteristics of the primary nanoparticles in determining toxicity. Furthermore, we recently investigated using similar methods copper and iron nanoparticles. Copper nanoparticle-exposed mice had significantly higher levels of inflammation and response. At biologically relevant pHs, *in vitro* studies showed that copper nanoparticles displayed a propensity for dissolution. We conclude that the presence of dissolved ions and the concomitant formation of smaller nanoparticles play major roles in copper nanoparticle toxicity.

Characterization of Manufactured Nanomaterials Collected in a Manufacturing Facility. Analysis of airborne particles collected from a manufacturing facility that produces titanium oxide-based nanomaterials provides insight into the potential exposure in an occupational setting (Peters et al. 2008). In particular, particles collected on filter media by electron microscopy and energy dispersive X-ray analysis were used to distinguish airborne engineered nanomaterials from incidental particles. The analysis showed that during the production of engineered nanomaterial relatively large particles are liberated into the air. In particular, microscopy shows that formation of several different types of particles including large 200-nm to 10-µm spheres. Further analysis showed that the large spheres were composed of smaller fused 10-80 nm nanoparticles. These large spherical aggregates contained titanium and were thus positively identified as related to the production of engineered nanoparticles and distinct from other particle types that were incidental to production.

Conclusions

A number of conclusions come about from these studies and include: (i) dissolution and aggregation of metal and metal oxide nanoparticles will play a large role in the environmental

fate, transport and toxicity of these nanomaterials; (ii) smaller TiO_2 nanoparticles below 10 nm in diameter are statistically less toxic than larger ones indicating that the nanoparticle surface area alone does not account for nanoparticle toxicity; (iii) characterization of nanoparticles in air and water in both laboratory and field studies should remain a high research priority if the environmental fate, transport, toxicity and occupational health hazards of nanomaterials are to be well understood.

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Conference Questions and Answers

Question:
In my research, we see that metal oxides in water tend to aggregate to a certain size, but we do not see aggregation behavior with the metals alone or the carbonaceous metal materials. We know it has to do with the surface charge, and maybe the smaller particles have more quantum effects than the larger particles. Could you comment on this?

Answer:
The steps necessary to get the nanomaterial to the chamber may have some effect on the aggregation process. I do not think I have the data to answer the question. These aggregates are really densely packed; I think it may be related to edge and corner effects. I have not done experiments to investigate quantum effects. The aggregates made tend to be about 125 nm, and I believe it may be due to the way they are made. A different process might yield a different size.

Question:
Are the chemical properties of, for example, titanium dioxide or copper different?

Answer:
Copper is different from titanium dioxide, and in terms of toxicity, copper is more toxic. Copper is used in different ways, for example as a catalyst, as compared with titanium dioxide. Copper tends to have a copper oxide overlayer. In that way, they are similar; there are no organic functional groups.

Save

Fate of Quantum Dots Nanomaterials in Unsaturated and Saturated Porous Media

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Introduction

Nanomaterials are at the leading edge of the rapidly growing field of nanotechnology. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity. Nanotechnology has considerable global socio-economic value, and is expected to have significant impacts on everyday life. Nanomaterials have numerous commercial and technological applications in chemical, biomedical, energy, electronics and space industries. A wide range of nanomaterials such as carbon nanotubes, fullerene derivatives, and quantum dots are used in almost all industries and all areas of society and the prevalence of these materials in society will be increasing, as will the likelihood of exposures (Roco and Bainbridge, 2001; Roco, 2003; Hardman, 2006). Once nanomaterials are released into the environment via manufacturing, use or disposal, their transport is the critical parameter in assessing their exposure and impact on the public health and the ecosystem, therefore understanding the fate of nanomaterials in the environment is critical (Colvin, 2003; Biswas and Wu, 2005; Weisner et al., 2006; Sayre, 2007; USEPA, 2007). Among the various types of nanomaterials, the semiconductors, quantum dots are key enablers in nanosciences, engineering and technology. Since they were discovered in early 1980's they have a longer impact on nanotechnology compared to the other nanomaterials such as carbon nanotubes and composites emerged in 1990's. Currently, the data and literature on the fate and transport of quantum dots, currently is sparse and there is a great need for knowledge and detailed information. Quantum dot nanomaterials are a potentially new source of contaminants, and because of the broad suite of physcial-chemical properties, could exhibit a wide range of transport properties. Futhermore, their unique fluorescence properties make them an excellent material to use for the investigation of the transport of nanomaterials in porous media as it greatly facilitate their detection and quantification through visualization. Therefore, our research goal aims at developing a visualization method and imaging process to investigate the fate and transport of quantum dot nanomaterials in variably saturated porous media using a non-intrusive high spatial and temporal visualization technique based on white light transmission and UV fluorescence detection.

Method

Visualization of quantum dot nanomaterials in variably saturated porous media

The visualization method was derived from a light transmission method developed by Darnault et al., 2001. The visualization technique selected to investigate transport of quantum dot nanomaterials in two-dimensional variably saturated porous media is a non-intrusive method

based on fluorescence resulting from the quantum dots optical properties. The visualization procedure consists of exciting fluorescent quantum dots in porous media by using a UV light on front side of the chamber and by using a light emitted devices (LEDs) as a light source in the back of the chamber and detecting the light transmitted through the porous media to characterize the water content. Images were acquired through a Q-IMAGING MicroPublisher RTV camera located in front of the chamber. The visualization, calibration and image analysis was performed using IPLab software.

To calibrate the fluorescence intensity to the quantum dots concentration in variably saturated sand, calibration cells were used. A stock solution of quantum dot nanomaterials was prepared and diluted by 4, 6.6 10, 13.3, 20, 50 and 100 to obatin a wide range of concentrations (corresponding to 25%, 15%, 10%, 7.5%, 5%, 2% and 1% respectively of the stock solution concentration). Calibration cells consist of plastic cuvettes (1.1 x 1.1 x 4.5 cm) filled with sand as porous media and with various degree of water saturations to obtain both saturated and unsaturated systems as well as a wide range of quantum dots concentrations. The saturated cells were filled by 5 steps. Dry sand was poured into the cuvette and then the quantum dots solution was added for saturation. 1.45 g of sand and 0.32 ml of quantum dots solution were used in each step. For the unsaturated cells, the quantum dots solution and the dry sand were first mixed in a beaker. Afterwards, the mixture was put into the cuvettes. Two calibration curves were obtained to determine the relationship between water saturation and intensity; as well as quantum dots concentration and hue. Each experiment includes a two-step process. In the first step, the light source placed behind the calibration cells is switched on in a dark room and the resulting transmitted light from the cells is recorded with the camera. In the second step, the UV light is placed in front of the calibration cells (about 25 cm away) and the fluorescence resulting from the cells is recorded. Both images are recorded in RGB format and processed with IPLab software as follow: the image resulting from the light transmission is converted in intensity format to relate the intensity parameter to water saturation, and the image resulting from the cell fluorescence is converted in hue format to relate the hue parameter to quantum dot nanomaterials concentration in variably saturated porous media.

Results

Water content of each calibration cell was obtained from the intensity image of the light transmitted and quantum dot concentration of each calibration cell was obtained from the hue image representing the fluorescence detected with the UV light (Fig. 1). Calibration curves where developed to establish relationships between intensity versus water content and hue

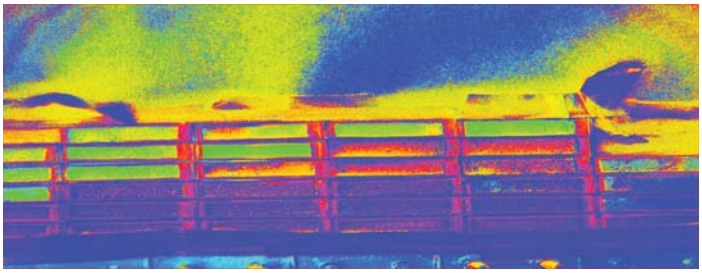

Figure 1. Hue image of the calibration cells under UV light.

versus quantum dots concentrations (Fig. 2 and 3). The procedure to quantify the quantum dots concentration in variably saturated porous media includes two steps: first the water content in porous media is quantified using intensity values resulting from the image obtained through light transmission through porous and then, once the water content is determined, the quantum dots concentration is obtained from hue values resulting from the image obtained through fluorescence using the UV light. A linear relationship is observed between hue values and quantum dots concentration for a constant water content (Fig. 3).

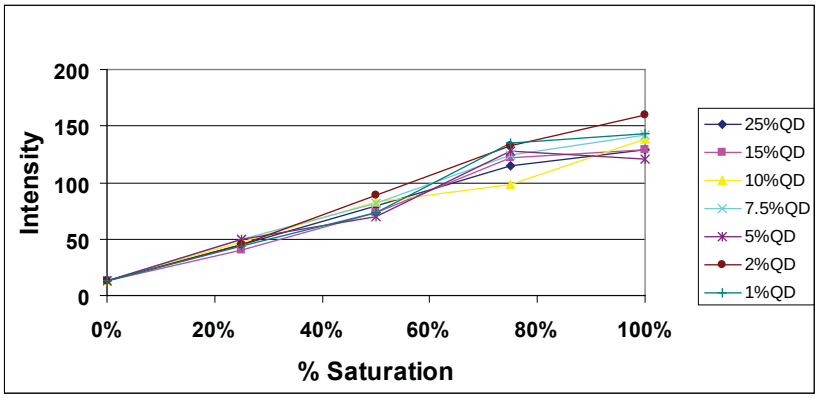

Figure 2. Water Saturation versus intensity values for various quantum dot concentrations.

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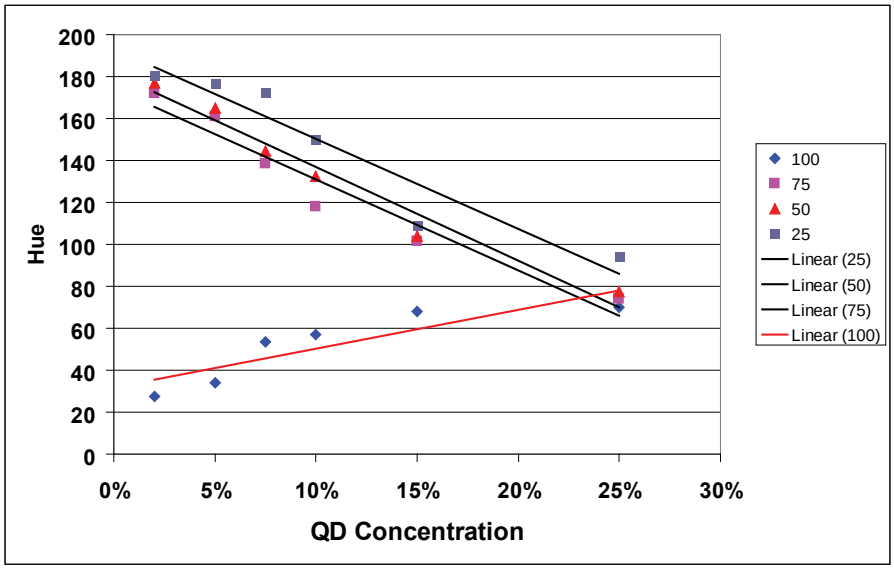

Figure 3. Quantum dots concentrations versus hue values for various degrees of saturation of porous media (25%, 50%, 75% and 100%).

Application

Quantum dots transport through variably saturated porous media

Vadose zone processes play a pivotal role in the fate and transport of subsurface contaminants as it is typically the first subsurface environment encountered by contaminants before reaching

the groundwater (Looney and Falta, 2000; Witherspoon, 2000). Groundwater contamination is influenced by the hydrodynamics of vadose zone system, and the two main processes controlling water in the vadose zone are gravity which moves water downward and capillary process that moves water in all directions, stores it and releases it (Looney and Falta, 2000; Faybishenko et al., 2000). In this context, a two-dimensional flow experiment in homogeneous sand was designed to assess the role of preferential flow – fingered flow - on the transport of quantum dot nanomaterials in vadose zone. This experiment was analyzed and processed by the visualization technique and imaging procedures. The experimental system consisted of a two-dimensional chamber - height: 30 cm, width: 20 cm - with 1 cm thick inner compartment that was filled with sand porous media and various degree of water saturation were achieved through saturation and drainage. The resulting initial experimental conditions simulated both vadose zone and aquifer system. A quantum dots solution was applied as a point source on the sand surface to simulate the release of nanomaterials in the subsurface environment. This simulation resulted in the formation of a fingered flow phenomena. The fate and transport of quantum dot nanomaterials in the vadose zone were observed and analyzed with the visualization method. The image obtained under the UV light exposure were converted to hue system to visualize and quantify the quantum dots nanomaterials in porous media (Fig. 4a, b). The mobility and transport of quantum dot nanomaterials through the vadose zone by preferential flow phenomena – fingered flow – were demonstrated (Fig 4a). The role of gas-water interfaces on the retention of quantum dot nanomaterials at the capillary fringe was also established (Fig. 4b).

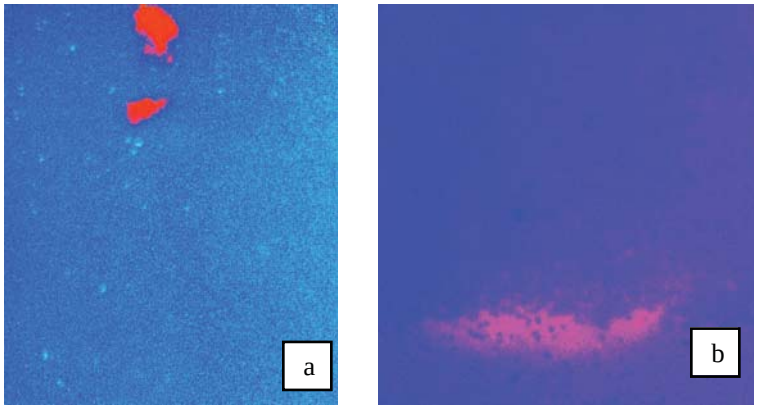

Figure 4. Fate and transport of quantum dots nanomaterials in vadose zone in Hue format (Quantum dots are visualized in red color). Transport of quantum dots by fingered flow in vadose zone (a). Retention of quantum dots nanomaterials by gas-water interface located at the capillary fringe (b).

Acknowledgements

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Conference Questions and Answers

Question:
In the cells that you homogenized, do you have any concerns that the air-water interface is different in comparison to the sand box where there is flowing water?

Answer:
The cells were 1 centimeter (cm) thick. We input that by matrix and container type. By doing that, you have a better distribution of the gas interface in the cell. As you fill the cells up the particles want to settle, which you want to avoid. We have done comparative experiments using the two techniques and have found no difference between them in results.

Question:
How do you dispose of waste?

Answer:
We follow EPA guidance but do not have a formal policy.

Question:
Do you have plans to conduct these experiments in soil (mixed texture as opposed to sand)?

Answer:
Yes.

Question:
Have you characterized the interactions between your nanoparticles and the porous media itself?

Answer:
No.

Assessing Transport of Gold Nanoparticles and Bacteria in Porous Media Using X-ray CT Scanning

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Abstract

X-ray computed tomography (CT) scanning is used in medical research for non-invasive tracking of nanoparticle tracers or drug delivery agents in animal and human bodies. We have recently demonstrated that a medical X-ray CT scanner can be used to quantitatively determine the spatial distribution of gold and other metallic nanoparticles in environmental porous media. The ability to non-invasively detect gold nanoparticles using X-ray CT was used to develop a technique for assessing bacterial density distributions in a saturated porous media column by labelling bacterial cells with gold nanoparticles.

Introduction

Traditionally, the transport of microbes and other colloids has been studied in experiments with packed columns filled with a porous medium, and the changes in effluent concentration are monitored as a function of time and compared to the influent concentration (Tufenkji, 2007). Non-invasive visualization of columns could potentially provide additional valuable data for improving our understanding of colloid transport and deposition behaviour in granular porous media.

Non-invasive characterization of bacterial density distributions in porous media has been performed using magnetic resonance imaging (MRI) (Olson et al., 2004). Those studies employed iron-oxide labelled bacteria. However, MRI cannot easily characterize environmental porous media, and thus it is difficult to correlate the bacterial distributions with respect to the porous media features. X-ray CT can be used to characterize porosity and provide 3-D images of the interior features of packed columns based on density differences. Colloid transport has been visualized in X-ray CT, but only at the microscale (Li et al., 2006), and, the use of X-ray CT for the imaging of bacterial density distributions in granular porous media has not been previously reported.

The overall objective of this study was to quantify the bacterial density distribution within a saturated sand column using X-ray CT. This was achieved by labelling bacterial cells with gold nanoparticles which served as an X-ray contrast agent for the detection of bacteria in CT scanning. Because bacterial cells have a density similar to that of water, they cannot be

identified by X-rays in aqueous phases.

Methods

The Gram-positive bacterium, *Bacillus subtilis* (ATCC 6633) was used in this study. Gold nanoparticles labelled on the *B. subtilis* cells served as a contrast agent to facilitate the determination of bacterial density distributions within saturated sand columns by X-ray computed tomography (CT). Methods for synthesis of the gold nanoparticles and its labelling on to *B. subtilis* cells have been described by Berry et al. (2005).

Quartz sand (Unimin #2040) was used as the granular porous medium in the column experiments. The sand was milled and fractionated into coarse and fine sands, and by sieving the sand the resulting mean grain size for coarse and fine sands was 775 μm and 200 μm, respectively. Traditional column experiments were carried out to analyze the deposition of the gold nanoparticles alone and gold-labelled bacteria using X-ray CT. These were conducted by pumping either a gold-labelled bacteria or gold nanoparticle suspension through a Plexiglas column (diameter 25 mm, height 40 mm) with adjustable Teflon end fittings (Ace Glass). Half the column (20 mm) was wet-packed with coarse sand, and half the column (20 mm) was wet-packed with fine sand. This created an interface where changes in the deposition behaviour could be observed.

The columns were pre-equilibrated with de-ionized (DI) water and placed horizontally on the bed of the CT scanner (Toshiba XVision medical scanner) and were scanned to provide the baseline scan for the CT data analysis. After scanning, the column was removed from the scanning bed and replaced in the horizontal position. Forty mL (~ 5 PV) of gold nanoparticles or gold-labelled cells in DI water were then pumped through the column followed by 40 mL of DI water. The effluent at the end of the outlet tube was collected every 2 min for a duration of 1 min. The effluent samples were then analyzed using a UV-Vis spectrophotometer (HP Model 8453) at a wavelength of 535 nm. At the end of the DI water injection, the column was returned to its horizontal position on the CT scanning bed and was scanned. Techniques for CT scanning have been discussed elsewhere (Goldstein et al, 2007; McKenna 2008).

Results and Discussion

Cell Labelling

Gold nanoparticles were successfully synthesized and labeled onto *B. subtilis* cells in suspension as shown in Figure 1. Gold nanoparticles capped with CTAB molecules were synthesized with a diameter of 94.3 ± 12 nm and a positive surface charge (ζ-potential ~ +40 mV) in DI water. These gold nanoparticles were attached to suspended *B. subtilis* cells (average length = 2.0 μm, dia = 0.75 μm, ζ-potential of approximately -10 mV in DI water) in a single step. The gold-labelled cells retained their structural integrity over time, remained viable, and suspended in solution, throughout the time period used for the column experiments.

The gold nanoparticles and labelled cells showed predictable X-ray attenuation properties that permitted quantification of their mass using X-ray CT. The CT scanning signal (CT number) for the gold nanoparticles and labelled cells increased linearly with particle and cell concentration. By measuring the CT number at various concentrations, the detection limits for the gold nanoparticles and labelled cells were determined to be 4.5×10^{10} particles/mL and 3.3

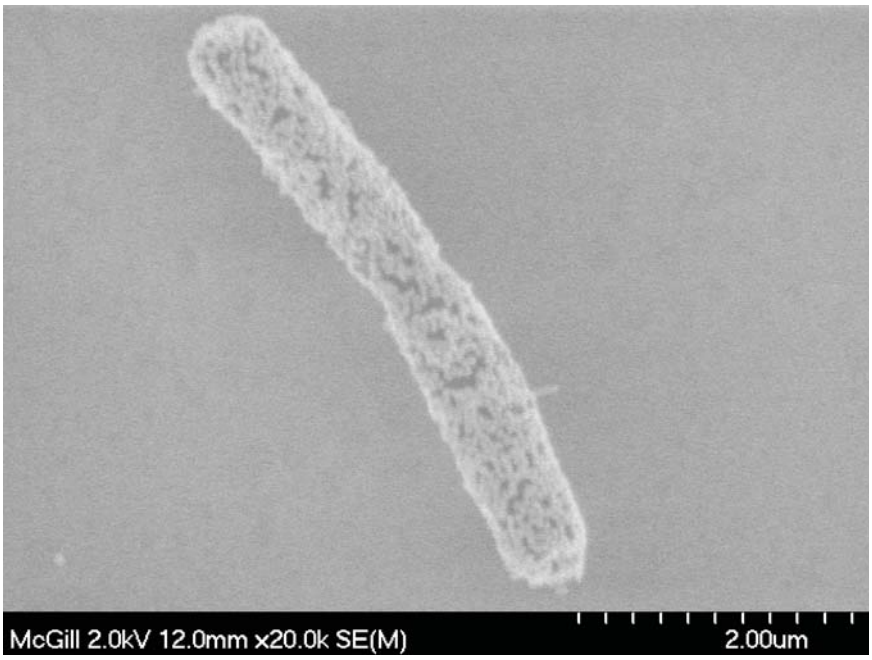

Figure 1. SEM image of a gold-labelled *B. Subtilis* cell.

$\times 10^7$ cells/mL, respectively. The detection limit for cell concentration is one order of magnitude less than the cell concentrations quantified by MRI in another study that employed iron oxide nanoparticles for cell-labelling (Sherwood et al., 2003).

Characterization of Cell Distribution in Sand Columns

Experiments were conducted in a packed column containing a 20 mm layer of fine quartz sand overlaid with a 20 mm layer of coarse grains of the same quartz sand. The gold-labelled cells were pumped in the direction from the coarse sand to the fine sand. A column breakthrough curve obtained for gold-labelled cells pumped through the layered sand column is shown in Figure 2 with the normalized effluent concentration, C/C_0, plotted as a function of time. The influent concentration of labeled cells was 1.0×10^8 cells/mL. The maximum effluent concentration for the gold-labelled cells was 0.19 and occurred after 14 min followed by a gradual decline suggesting that a high concentration of gold-labelled cells had been retained within the column. After the first forty min, the injection was switched to DI water for a further 40 min.

The profiles showing the spatial distribution of the retained gold-labelled cells for the same experiment were obtained from the X-ray CT data with the retained particle or cell concentration plotted as a function of distance (Figure 3). The concentration of retained gold-labelled cells shows a sharp increase in the retained concentration at the interface, followed by a region of very low retention. The data confirms that there was a relatively high concentration of gold-labelled cells retained in the column.

The average porosity for this experiment was 0.42. From the CT data, the porosity for each slice was found to be consistent with the average porosity. The porosity in the coarse and fine sand layers are not expected to be different because both layers are comprised of grains of relatively

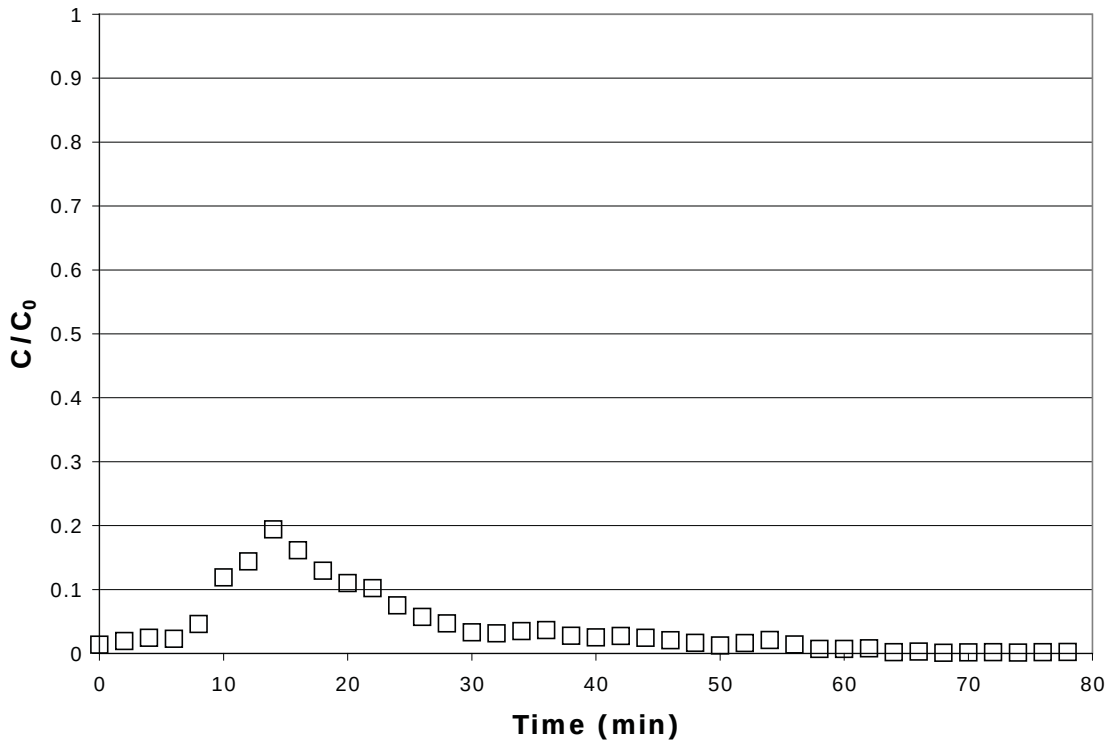

Figure 2. Representative breakthrough curve for gold-labelled cells in a sand column with coarse and fine sand layers. Key experimental conditions: (pH =6.5, flow rate = 1mL/min, porosity = 0.42, mean coarse grain diameter = 800 μm, mean fine grain diameter = 200 μm, interface at 20 mm.

uniform size.

At the interface of the two sand layers, the pore size is reduced due to a decreased grain size, which increased the effect of the straining on the gold-labelled cells. As the bacteria are removed from the aqueous phase by the collector grains in this region, the pore spaces become smaller and the labelled bacteria can no longer pass through (Dunmore et al., 2004). This resulted in a high concentration of gold-labelled cells being retained within the column. In the coarse sand region of the column, the particle to collector diameter (d_p/d_c) ratio is 0.002 for the gold-labelled cells. After the interface, in the fine sand region of the column, the mean grain diameter is reduced to 200 μm and the d_p/d_c ratio increases to 0.008 for the gold-labelled cells. Experimental evidence has shown that straining can be important at ratios above 0.002 (Bradford and Bettahar, 2006) and was most likely the governing mechanism in the removal of the gold-labelled cells. The breakthrough curves, as well as the spatial distribution of the retained CTAB-capped gold nanoparticles (with no cells attached) were significantly different from the gold-labelled cells, suggesting that gold nanoparticles did not detach from the cells during the column transport.

Conclusions

To the best of our knowledge, this study is the first to examine the transport and deposition

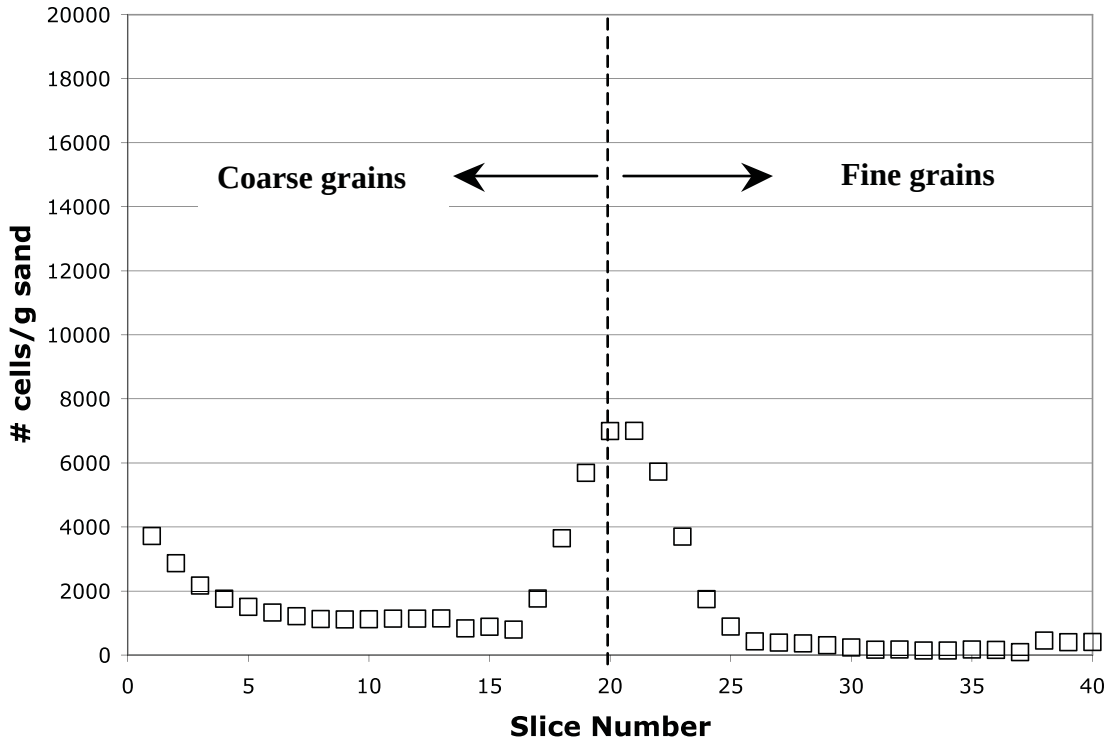

Figure 3. Profile for gold-labelled cells in a packed column with coarse and fine sand layers determined by X-ray CT scanning. Key experimental conditions: (pH =6.5, flow rate = 1mL/min, porosity = 0.42, mean coarse grain diameter = 800 μm, mean fine grain diameter = 200 μm, interface at 20 mm (slice 20).

behavior of bacteria in porous media using X-ray CT. In this study, the density distribution of gold nanoparticles and labelled cells was quantified at a spatial resolution of 1 mm. Furthermore, X-ray CT permitted the non-invasive calculation of porosity *in situ* as well as observing changes in deposition behavior due to sand heterogeneities such as the accumulation of gold-labelled bacteria at the interface between coarse and fine sands. This knowledge on spatial distribution of bacterial density distributions could not be otherwise determined from analyses of the effluent concentrations or without sampling the porous medium destructively. Ongoing work is aimed at labeling cells with gold nanoparticles inside the cell rather than on the cell surface. If successful, this technique will allow use of cells with relatively unaltered surfaces in studies aimed at characterizing their deposition behavior using X-ray CT scanning.

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Conference Questions and Answers

No questions.

Impact of Size on Carbon Nonotube Transport in Natural Media

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Abstract

Carbon nanotubes are becoming increasingly common in commercial applications. However, there is evidence that carbon-based nanoparticles could cause cell damage. As a result their impact to humans and the ecosystem has become a concern with the growing use of manufactured carbon nanotubes. Therefore, it is essential to understand the factors that control the transport of carbon nanotubes in the environment, and of particular interest to this study, their transport in porous media. An assessment of the mobility of carbon nanotubes in porous media would facilitate the determination of the ability of drinking water treatment facilities to remove them from source waters as well as assist in the prediction of their fate in subsurface aquifers. In this work, the transport behavior of multi-walled carbon nanotubes (MWCNTs) is investigated in sand packed column experiments. To determine the importance of MWCNT shape and aspect ratio experiments were conducted using four commercially available MWCNTs with differing diameters and lengths. MWCNT diameter was varied within one order of magnitude and MWCNT length was varied two orders of magnitude. Results suggest that under the experimental conditions tested smaller diameter MWCNTs are less mobile than larger diameter MWCNTs and that MWCNT length is less important than MWCNT diameter for the prediction of MWCNT mobility in porous media. Experiments were also conducted at ionic strengths typical of groundwater environments and at very low ionic strengths. Results from these column experiments suggest that the transport of MWCNTs is not solely governed by mechanisms traditionally associated with filtration theory (ie: sedimentation, diffusion and interception) and other transport mechanisms also control their transport.

Introduction

Carbon nanotubes have been the subject of intense research since their discovery in 1991 (Iijima, 1991). Their unique properties (e.g., light weight, significant strength, excellent conductivity, and outstanding chemical resistance) have lead to their application in a wide variety of industries such as composite material and electrical field emission applications (Andrews et al., 2002; Wang et al., 2001). However, there is concern that carbon-based nanoparticles could cause cell damage, and with the growing use of manufactured carbon nanotubes, their impact to humans and the ecosystem has become a concern to researchers, regulators, manufacturers, and consumers. It is therefore essential to understand the factors that control the transport of carbon

nanotubes in the environment, and of particular interest to this study, their transport in porous media. An assessment of the mobility of carbon nanotubes in porous media would facilitate a determination of the ability of drinking water treatment facilities to remove them from source waters as well as assist in the prediction of their fate in subsurface aquifers.

MATERIALS AND METHODS

Multi-Walled Carbon Nanotubes

Multi-walled carbon nanotubes (MWCNTs) were purchased from Cheap Tubes Inc. (Brattleboro, VT). These MWCNTs were synthesized using the chemical vapor deposition (CVD) method with metal catalysts. Energy dispersive X-ray spectroscopy found that these purified MWCNTs are over 97% by weight carbon (Cheap Tubes Inc.). Detailed dimension information is listed in Table 1. Purchased carbon nanotubes were further functionalized using a concentrated aggressive acid mixture containing a 3 to 1 ratio by volume of sulfuric and nitric acids (95-97% and 70%, respectively) to enhance the MWCNT hydrophilicity through the addition of carboxylic groups on the MWCNT surface (Liu et al., 1998). An ultrasonic probe was used to produce stable carbon nanotube suspensions in aqueous solutions at a pH of 10 and at two ionic strengths. The first aqueous phase solution (SS I) had an ionic strength of 10 mM and was buffered to pH 10. 1 mM NaBr or NaCl was added to SS I as a conservative tracer. For the low ionic strength aqueous phase solution sodium hydroxide (NaOH) was added to de-ionized water to make a 0.1 mM ionic strength solution with a pH around 10. 2 mg of carbon nanotubes were added to a 250 ml beaker containing 200 ml aqueous solution and sonicated at 210 watts in an ice-water bath for 45 minutes. These suspensions were stable in the aqueous phase for months. Concentrations of aqueous phase MWCNT suspensions were quantified using a UV-Vis spectrophotometer at a wavelength of 400 nm.

Table 1. Average Length and Diameter of theCommercial Multi-Walled Carbon Nanotubes

	CNT-a	CNT-b	CNT-c	CNT-d
Outer diameter (nm)	30-50	30-50	< 8	< 8
Length (µm)	0.5-2.0	10-20	0.5-2.0	10-30

Porous Media

Quartz sand (d_{50} = 476 µm, U_I = 1.5; Barco 32, BEI Pecal, Hamilton, ON, CA) was used as the representative porous medium. Purchased quartz sand was cleaned alternately with hydrochloric acid (0.1M) and hydrogen peroxide (5%) to remove all impurities and grease. De-ionized water was used to rinse the sand between steps and afterwards until a neutral pH was achieved. Washed sand was dried in an oven (105ºC) over night. Particles smaller than 152 µm were removed by sieving.

Aluminum columns, 5 cm in diameter and 10 cm in length, were dry packed with clean sand. A stainless steel mesh (150 µm openings) was placed at both ends of the column to support the sand as well as distribute the aqueous flow. Carbon dioxide gas was flushed through the column upwards for at least 15 minutes to displace air. De-ionized water was then pumped upwards through the column for at least 30 pore volumes to saturate the sand column. This packing

procedure yielded an average column porosity of 0.347 (ranging from 0.335 to 0.368). The stock solution and MWCNT suspensions were delivered by two or three 60-ml plastic syringes to the column via a syringe pump at a pore water velocity of 0.42 m/d. The MWCNT suspensions were injected downwards through the column.

Results and Discussion

A series of column experiments were conducted to investigate the impact of size on the transport of MWCNTs. The selected pore water velocity (0.42 m/d) represents typical natural groundwater conditions. Representative breakthrough curves are presented in Figures 1 to 4. In these figures the normalized effluent concentrations of MWCNTs are plotted as a function of pore volumes (Vp) flushed. For CNT-a the carbon nanotubes exited the column at the same time as the conservative tracer for the lower ionic strength solution and were retarded to a very small extent at the higher ionic strength solution. The maximum normalized effluent concentrations

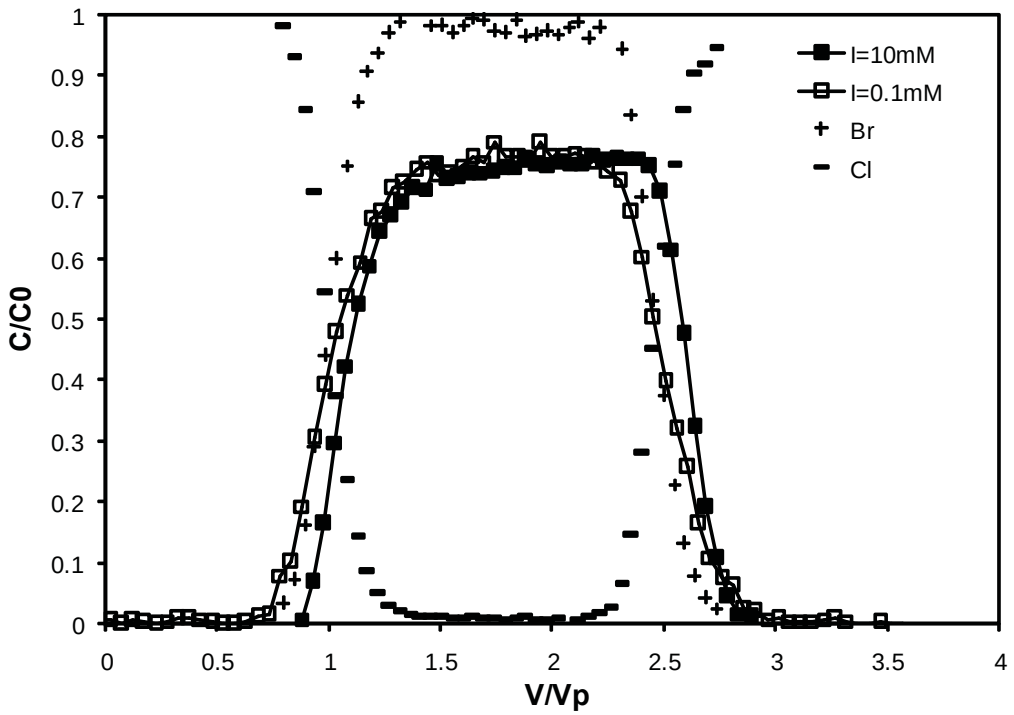

Figure 1. Breakthrough Curves of CNT-a.

were similar at both ionic strengths. CNT-b was retarded to a more significant extent at the higher ionic strength in comparison to CNT-a. Similar behaviour was observed for CNT-c and CNT-d but to a more significant extent. It would appear that for the experiments conducted with the higher ionic strength solution a similar maximum effluent concentration to the lower ionic strength solution would be achieved if sufficient pore volumes were flushed. Unfortunately not enough pore volumes were flushed for cases CNT c and d. These results indicate that the lower ionic strength aqueous solution impeded MWCNT deposition on the porous medium.

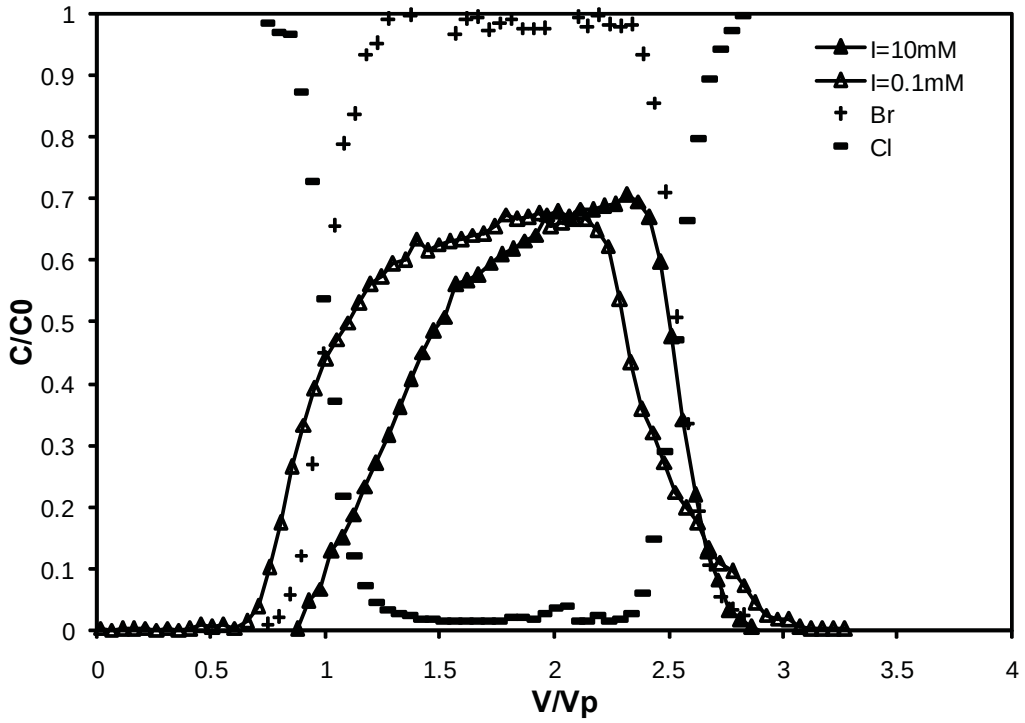

Figure 2. Breakthrough Curves of CNT-b.

In low ionic strength systems it is generally assumed that the energy barrier is too large for colloid removal due to deposition (i.e., interception, sedimentation or Brownian diffusion) (Tufenkji and Elimelech, 2004). At the lower ionic strength all of the carbon nanotubes generally exited the column with the conservative tracer. However they all achieved a maximum normalized effluent concentration of less than 1.0, with CNT-a achieving a maximum normalized effluent concentration of 0.77 and the other carbon nanotubes achieving a maximum normalized effluent concentration of approximately 0.7. Given that deposition on the sand surface should be minimal at the lower ionic strength this suggests that other removal mechanisms are also important in carbon nanotube transport. One removal mechanism that is achieving significant attention is straining, which is the removal of particles at grain to grain intersections (Bradford et al., 2006). If the carbon nanotube major axis is oriented perpendicular to the flow direction straining is more likely than if the minor axis is oriented perpendicular to the flow direction. This is due to the larger dimension attempting to pass through the pore space. Therefore, CNT-a & c, with shorter lengths, are expected to be less susceptible to straining. At the lower ionic strength the results from CNT-a would support this hypothesis as the maximum normalized effluent concentration is higher than the longer carbon nanotubes (CNT-b & d). The results from CNT-c, however, do not support this observation as the maximum normalized effluent concentration is similar to the longer carbon nanotubes.

Experiments at the higher ionic strength are used to evaluate carbon nanotube removal

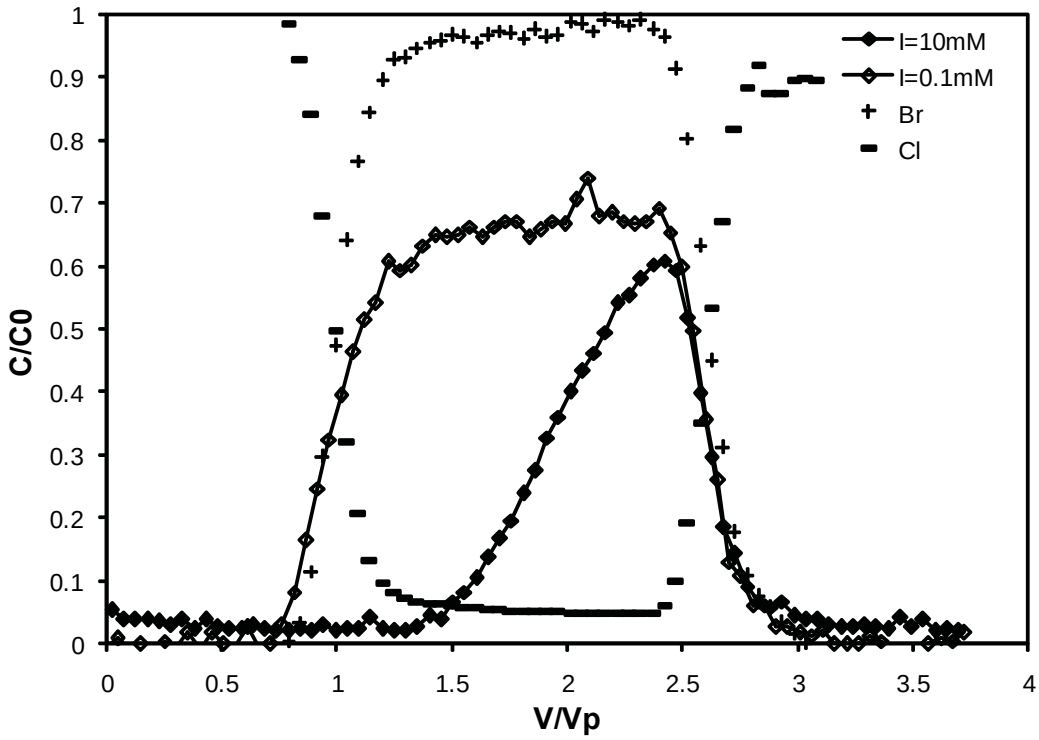

Figure 3. Breakthrough Curves of CNT-c.

mechanisms due to mechanisms associated with classical filtration theory (ie: interception, sedimentation and Brownian diffusion) and straining. At the higher ionic strength the pore volumes flushed prior to the normalized effluent concentration achieving 50% of its maximum value increases from CNT-a, to CNT-b, to CNT-d and finally CNT-c. For these experiments it would appear that the smaller diameter carbon nanotubes are retained to a greater extent than the larger diameter carbon nanotubes (ie: CNT-c & CNT-d have smaller diameters than CNT-a & CNT-b). It would appear that carbon nanotube length is less important than diameter, as the pairs CNT-a and CNT-c or CNT-b and CNT-d have similar lengths but very different retardation behaviour. Finally CNT-c was removed to the greatest extent in these experiments at the higher ionic strength. This carbon nanotube had the smallest diameter (< 8 nm) and shortest length (500 to 2000 nm). With decreasing particle size Brownian diffusion becomes a dominant classical filtration removal mechanism. Due to its smaller size classical filtration predicts that a larger fraction of these smaller carbon nanotubes that approach the sand grain would strike the sand grain. For example η_0 (both based on carbon nanotube diameter and surface area based equivalent diameter) is greatest for CNT-c. Based on η_0 alone it is unclear why CNT-a is more mobile than CNT-b as η_0 is equivalent to or smaller for CNT-b. At both ionic strengths CNT-a was removed to a lesser extent than the other carbon nanotubes. This carbon nanotube had a larger diameter than CNT-c and CNT-d and had a shorter length than CNT-b and CNT-d. Carbon

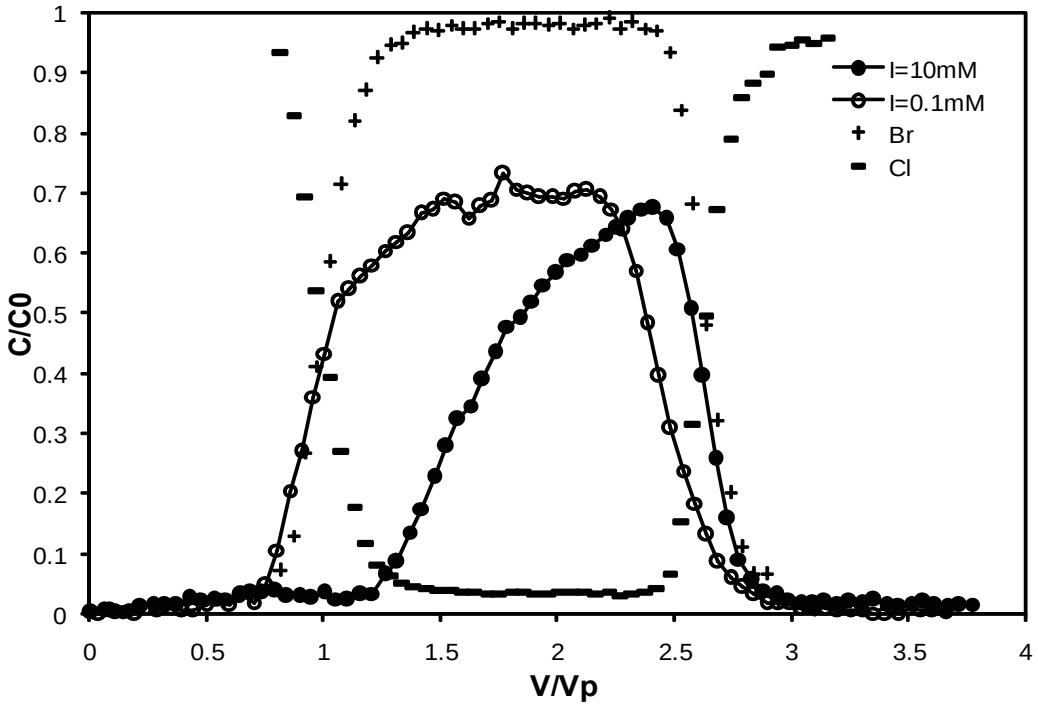

Figure 4. Breakthrough Curves of CNT-d.

nanotube removal, however, is not solely related to η_0 but also related to α, which is the fraction of particles that strike the sand surface that are removed and is generally treated as an empirical parameter. Even though the surface functional groups of these carbon nanotubes should be the same the differing carbon nanotube sizes could impact α. For example larger carbon nanotubes may have more difficulty finding appropriate deposition sites. Other factors that may influence α include the species and the concentrations of electrolyte in solution, pH value, surface characteristics of MWCNTs and the grain collectors (Lecoanet and Wiesner, 2004).

Summary

A series of column experiments were conducted to determine the impact of MWCNT dimensions on their transport in porous media. This work suggests that mechanisms associated with traditional colloid filtration theory cannot solely be used to predict MWCNT transport in porous media and other removal mechanisms are important. In addition MWCNT diameter appears to be more important than MWCNT length in the prediction of the fate of carbon nanotubes in porous media.

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Conference Questions and Answers

Question:
You are basing your experiment on the shape and size of individual carbon nanotubes. Would you care to comment on how your method affects bundling in suspension?

Answer:
We did transmission electron microscopy (TEM) images, which dry the sample and are not exactly representative of what is in the aqueous solution. When you do TEM you reduce the coiling environment of the carbon analysis.

Question:
Did you try standardized settling?

Answer:
We did, and we identified two peaks-one for the diameter, and one for the length.

Question:
How do they compare with particles in suspension?

Answer:
They compare reasonably well.

Question:
What is the reason for using a solution with a pH of 10?

Answer:
When the work began several years ago, there was a great deal of difficulty in getting nanotubes stable in suspension. We found that the carbon nanotubes were more stable at a higher pH. Since then, we have found other techniques to stabilize the nanotubes at a lower pH; however, we decided to continue to use a pH 10 solution for consistency with the earlier research. The governing principles would be the same for a pH of 7.

Question:
Are you going to conduct further experiments with a different soil matrix and nanoparticle?

Answer:
Yes, we plan on expanding the research. One interest is what happens in clay. We are considering an in-field experiment at Canadian Forces Base Borden.

Question:
In addition to your looking at the diameter and length of the carbon nanotubes and their effects on transport, have you looked at chirality?

Answer:
No.

Evaluating the Clean-Bed Filtration Theory for Modeling Transport of Fullerene C_{60} Aggregates in Saturated Porous Media

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Abstract

A coupled experimental and mathematical modeling investigation was undertaken to evaluate the applicability of the clean-bed filtration theory for modeling transport of fullerene aggregates (nC_{60}) in water-saturated porous media. nC_{60} transport experiments were conducted in the columns packed with 40-50 or 100-140 mesh Ottawa sand. The clean-bed filtration model failed to reproduce both the observed asymmetric breakthrough curves and flat retention profiles. A model that incorporated a maximum retention capacity term provided improved simulation of nC_{60} transport and retention. The collision efficiency factor values calculated based on the clean-bed filtration model were orders of magnitude smaller than the values calculated by the maximum retention capacity model.

Introduction

World wide production of fullerenes (C_{60}) is expected to exceed 300 tons/year in year 2010 (UNEP 2007). Widespread application and production of C_{60} will inevitably lead to its release into the environment. Once released, the bioavailability and potential exposure pathways of the fullerene nanoparticles will be strongly influenced by transport and retention processes. In aqueous systems, C_{60} is capable of forming stable nano-scale aggregates (nC_{60}). Although the transport of nC_{60} in porous media has typically been analyzed using the clean-bed filtration model (Yao, Habibian et al. 1971), its appropriateness for this application has not been carefully assessed. The goal of this research was to evaluate the applicability of the clean-bed filtration model for simulating nC_{60} transport and retention in water saturated quartz sand.

Experimental Methods

Aqueous suspensions of C_{60} were prepared following procedures in (Wang, Li et al. 2008). The resulting suspension contained 3.0 mg/L of nC_{60} in 1.0 mM $CaCl_2$ solution with an average diameter of 120 nm as determined by dynamic light scattering (DLS). Transport experiments were conducted in borosilicate glass columns packed with either 40-50 or 100-140 mesh water

saturated Ottawa sand (U.S. Silica, Berkeley Springs, WV). A 10 pore volume (PV) pulse of nC_{60} suspension was introduced at a flow rate of ca. 1.0 mL/min, followed by a 3 PV pulse of nC_{60}-free solution at the same flow rate and ionic strength. Column effluent samples were collected continuously and the nC_{60} concentration was monitored using UV spectroscopy. At the conclusion of each experiment, the column was dissected into 1.5-cm increments and retained nC_{60} aggregates were extracted by addition of deionized water, agitation for 3 h on an oscillating shaker (Labquake, Barnstead International, Dubuque, IA), and ultrasonication for 1 min (Model FS20H, Fisher Scientific). A summary of the conditions for each column experiment is provided in Table 1.

Table 1. Experiment conditions of nC60 column experiments.

Column	d_c[a] (mm)	v_p[b] (m/d)	C_0[c] (mg/L)	Retained (%)	MB[d] (%)	α_0	α_1	k_{att} (h^{-1})	S_{max} (μg/gsand)
OT1	0.335	1.04	3.05	27.0	91.7	0.00045	0.086	2.50	1.93
OT2	0.335	1.04	3.12	37.2	97.4	0.00048	0.084	2.44	2.44
OT3	0.125	1.03	3.07	99.8	99.7	--	0.074	10.10	13.7

[a] mean sand grain diameter. [b] pore water velocity. [c] influent n-C_{60} concentration. [d] mass balance.

Clean-bed Filtration Model

According to clean-bed filtration theory (Yao, Habibian et al. 1971), the transport of particles through a water-saturated homogeneous porous medium can be described by advection, hydrodynamic dispersion, and retention (filtration) processes. A one dimensional advection-dispersion equation with first-order particle retention kinetics is typically employed to simulate particle transport:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\theta_w}\frac{\partial S}{\partial t} = D_H \frac{\partial^2 C}{\partial x^2} - v_p \frac{\partial C}{\partial x} \tag{1}$$

$$\frac{\rho_b}{\theta_w}\frac{\partial S}{\partial t} = k_{att} C \tag{2}$$

where C is the concentration of suspended nC_{60} in solution, S is the concentration of retained nC_{60}, ρ_b is the solid-phase bulk density, θ_w is the volumetric water content, D_H is the hydrodynamic dispersion coefficient, v_p is the pore velocity, and k_{att} is the rate of particle attachment, which can be expressed as (Yao, Habibian et al. 1971):

$$k_{att} = \frac{3(1-\theta_w)v_p}{2d_c}\alpha\eta_0 \tag{3}$$

Here, the collision efficiency factor (α) represents the fraction of nC_{60} that remain attached after collision and the single collector efficiency (η_0) represents the frequency of nC_{60} collisions with the porous medium grain surfaces, which can be calculated using a dimensionless correlation that incorporates the contributions of diffusion, interception and sedimentation processes (Tufenkji and Elimelech 2004).

Results and Discussion

Effluent breakthrough curves (BTCs) and retention profiles for nC_{60} columns packed with either 40-50 or 100-140 mesh Ottawa sand are shown in Figures 1A and 1B, respectively. Transport of nC_{60} in columns packed with 40-50 mesh sand yielded asymmetrical BTCs that gradually increased to a maximum value before declining sharply to relative concentrations (C/C_0) approaching zero. The retention profiles of nC_{60} in 40-50 mesh Ottawa sand columns were relatively uniform over the entire length of the column. On the other hand, no breakthrough of nC_{60} was observed in the column packed with 100-140 mesh sand. Retained nC_{60} concentrations in this column were higher close to the column inlet and then declined to lower values near the column outlet. These results are consistent with those reported in (Wang, Li et al. 2008).

Steady State Analysis

The clean-bed filtration model is often applied by assuming steady-state conditions and negligible hydrodynamic dispersion effects. Under such conditions, the collision efficiency can be calculated as (Espinasse, Hotze et al. 2007):

$$\alpha_0 = -\frac{2d_c}{3(1-\theta_w)\eta_0 L}\ln(C/C_0) \tag{4}$$

Here, L is the length of the column. α_0 values were calculated as 0.00045 and 0.00048 (Table 1) for the two duplicate experiments conducted in Ottawa 40-50 sand, where a relatively

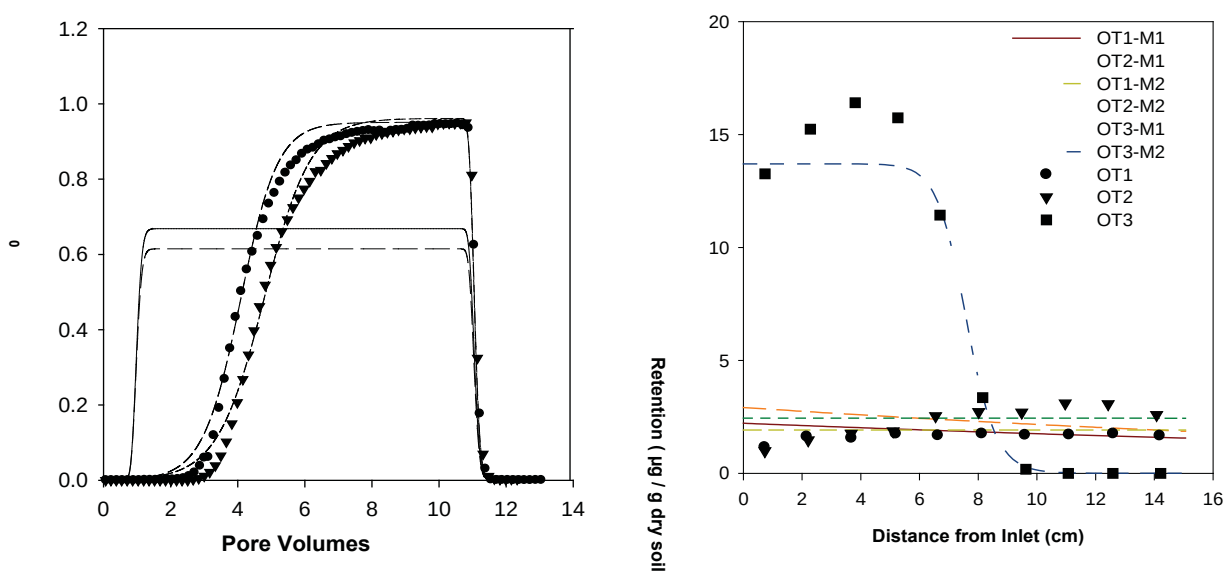

Figure 1. Measured and simulated breakthrough curves (A) and retention profiles (B). Symbols represent experimental data, and lines are modeling results. M1 refers to filtration model under transient conditions; M2 refers to maximum retention capacity model.

stable effluent concentration was achieved after injecting 10 PV of nC_{60} suspension. Since no breakthrough occurred in the column packed with 100-140 mesh sand, α_0 cannot be calculated for this experiment. Note that, at steady state, an exponential decay of retained particle concentration with distance is predicted, which obviously is not consistent with the observed flat retention profile for nC_{60} transport. Thus, the validity of these calculated α_0 values can be questioned. This contradiction is further discussed below.

Transient Analysis

Under transient conditions, we employed clean bed filtration model (eq. 1-2) to simulate nC_{60} transport. The attachment rate coefficient, k_{att} was obtained by minimizing the sum of the squares residuals between measured and modeled effluent concentration data and the retention profile data. Comparisons of the measured and simulated nC_{60} breakthrough curves and retention profiles are presented in Figure 1. As illustrated here, application of the clean-bed filtration model to simulate nC_{60} transport under these experimental conditions failed to reproduce both the observed asymmetric BTCs and flat retention profiles.

Maximum Retention Capacity Model

The poor performance of the clean-bed filtration model demonstrated above could be attributed to the assumptions that attachment depends only on the aggregate concentration in the aqueous phase and follows a first-order kinetics. Relatively flat retention profiles of the nC_{60}, however, are indicative of a maximum retention capacity. The attachment rate, thus, may depend on the retained particle concentration. Therefore, a modification of Eq.2 was proposed (Li, Wang et al. 2008):

$$\frac{\rho_b}{\theta_w}\frac{\partial S}{\partial t} = k_{att}\frac{S_{max} - S}{S_{max}}C \qquad (5)$$

Here, S_{max} is the particle maximum retention capacity. Eqs. 1 and 5 were applied to simulate the BTCs and retention profile, with k_{att} and S_{max} as fitting parameters. As illustrated in Figure 1, this model provides a markedly improved simulation to the asymmetrical BTCs and relatively flat retention profiles observed in the column experiments. Collision efficiency factors α_1 calculated based on eq.3 and fitted values of k_{att}. ranged from 0.074 – 0.086 (Table 1), which is more than two orders-of-magnitude larger than α_0. Since the α_1 values were obtained from a model that more accurately captures the transport and retention of nC_{60} in porous media, these data provide a more accurate representation of nC_{60} collision efficiency.

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Conference Questions and Answers

Question:
What was the method for coating the sand with the surfactant?

Answer:
Ten pore volumes of surfactant solution were flushed through the sand column, and this was followed by a surfactant-free water flush.

Question:
Do you know how far nanoparticles might travel during a remediation, and how this work would inform that?

Answer:
Surfactants are being used to increase the mobility of the nanoparticles. The increase in mobility, however, is very site-specific.

Save

Aging of Iron Nanoparticles in Water: Effects on Structure and Reactivity

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Abstract

We studied the aging of Fe^0-core/oxide-shell nanoparticles in water with a focus on changes in (*i*) the composition and structure of the particles (by XRD, TEM, XPS, and bulk Fe^0 content), and (*ii*) the reactivity of the particles (by carbon tetrachloride reaction kinetics, electrochemical corrosion potentials, and H_2 production rates). The results show that nano-Fe^0 becomes more reactive between 0 and ~2 days exposure to water, and then gradually loses reactivity over the next few hundred days. The behavior of unaged nano-Fe^0 in our laboratory experiments may be similar to that in field-scale applications for source-zone treatment due to the short reaction times involved. However, nano-Fe^0 that has been aged in water for >3 days acquires properties that are relatively stable over weeks or even months.

Introduction

Most nanoparticles that are used in environmental applications are reactive in ways that alter the particle's properties over time. This process is responsible for the apparent aging of these materials and is a primary determinant of their longevity. Aging (or longevity) and transport in aqueous media are among the most important and potentially limiting factors in the use of nano-Fe^0 to reduce contaminants in groundwater remediation. However, while transport of nano-Fe^0 in the subsurface is now receiving a great deal of investigation, few studies have explicitly addressed the issue of aging.

From a priori considerations—and results from the few prior studies that have significant bearing on the aging issue (Liu and Lowry 2006; Sohn et al. 2006)—we anticipate the following fundamental processes will be responsible for nano-Fe^0 aging under environmental conditions: (*i*) breakdown of the oxide shell by hydration, autoreduction, etc.; (*ii*) oxidation of the exposed surface coupled with reduction of solutes; and (*iii*) aggregation of particles and subsequent cementation. Two additional considerations are (*iv*) potential feedbacks between the above primary effects (e.g., between *i* and *ii*, which alter solution chemistry of the medium, or between *ii*-*iii*, which are affected by solution chemistry) and (*v*) the relative timing (i.e., kinetics) of the above primary effects, which vary so widely that some processes are essentially independent of others.

The multiplicity of processes that contribute to aging—combined with uncertainties regarding their relative significance, relative timing, and interactions—makes the pursuit of a complete and balanced understanding of the problem quite challenging. To achieve this goal, we have employed expanded and novel set of complementary characterization methods for both particle structure and reactivity. The scope covers the whole range of aging regimes (concentrated slurry and dilute suspensions, with and without contaminants present), and therefore the results have implications for laboratory and field-scale applications of nano-Fe^0 in any aquatic media.

Methods

We used two preparations of Toda RNIP-10DS (Fe^{H2}): one that had never been exposed to water ($Fe^{H2(D)}$) to represent short-term aging effects, and one that had been in an aqueous slurry for approximately a year ($Fe^{H2(W)}$) and therefore reflected long-term aging. For both of these types of materials, we characterized the time dependent changes in (*i*) the structure of the iron particles, using spectroscopy and microscopy (XPS, XRD and TEM); (*ii*) the Fe^0-content and rate of H_2 production by reaction of Fe^0 with H_2O (using manometry of H_2 pressure); (*iii*) the kinetics and pathway of reaction (focusing on carbon tetrachloride, CT); and (*iv*) corrosion potential of the iron-oxide particles using electrochemical experiments. Details of the treatment regimes and analytical methods are given in (Sarathy et al. 2008).

Results and Discussion

While the Fe^0-content of the nanoparticles decreased with aging time, as has been reported previously by others (Liu and Lowry 2006; Sohn et al. 2006), most of the other properties show more complex behavior, with a period between 0 and a few days exposure to water where the $Fe^{H2(D)}$ becomes more reactive followed by a gradual decline in reactivity of the next few hundred days. In Figure 1, this can be seen in the kinetics of CT reduction, yield of chloroform from CT, corrosion potential, and hydrogen production rate (i.e., k_{CT}, Y_{CF}, E_{corr}, and R_{H2}, respectively).

Between Y_{CF} and R_{H2}, nearly all the data fall on a line because both properties peak at the same time (1 day) and the rates of change on the sides of the peaks are similar. In contrast, k_{CT} and R_{H2} gives a correlation with marked hysteresis, even though both of these parameters also peak at 1 day, because the rate of change in k_{CT} is less than that for R_{H2}. The time series are less complete for correlations involving E_{corr} because aging data were only collected up to 2 days. Nevertheless, the correlations between E_{corr} and k_{CT} or Y_{CF} have similar features to those involving H_2 production rate because the data for $Fe^{H2(W)}$ apparently are sufficient to represent the effect of long term aging. In this case, the correlation between k_{CT} and E_{corr} shows no hysteresis (and also is nonlinear); whereas the correlation between Y_{CF} and E_{corr} shows modest hysteresis. In general, correlations without hysteresis imply a more direct relationship between two variables. Therefore, it appears that R_{H2} is better at describing the effect of aging on Y_{CF}, whereas E_{corr} comes closer to explaining changes in k_{CT}. These relationships are mechanistically plausible given that (*i*) E_{corr} reflects the particle's potential to donate electrons (Nurmi and Tratnyek 2008), and accepting electrons is generally regarded as determining k_{CT}, and (*ii*) H_2 production involves formation of reduced forms of hydrogen (Reardon et al. 2008), and the availability of reduced forms of hydrogen probably control Y_{CF}.

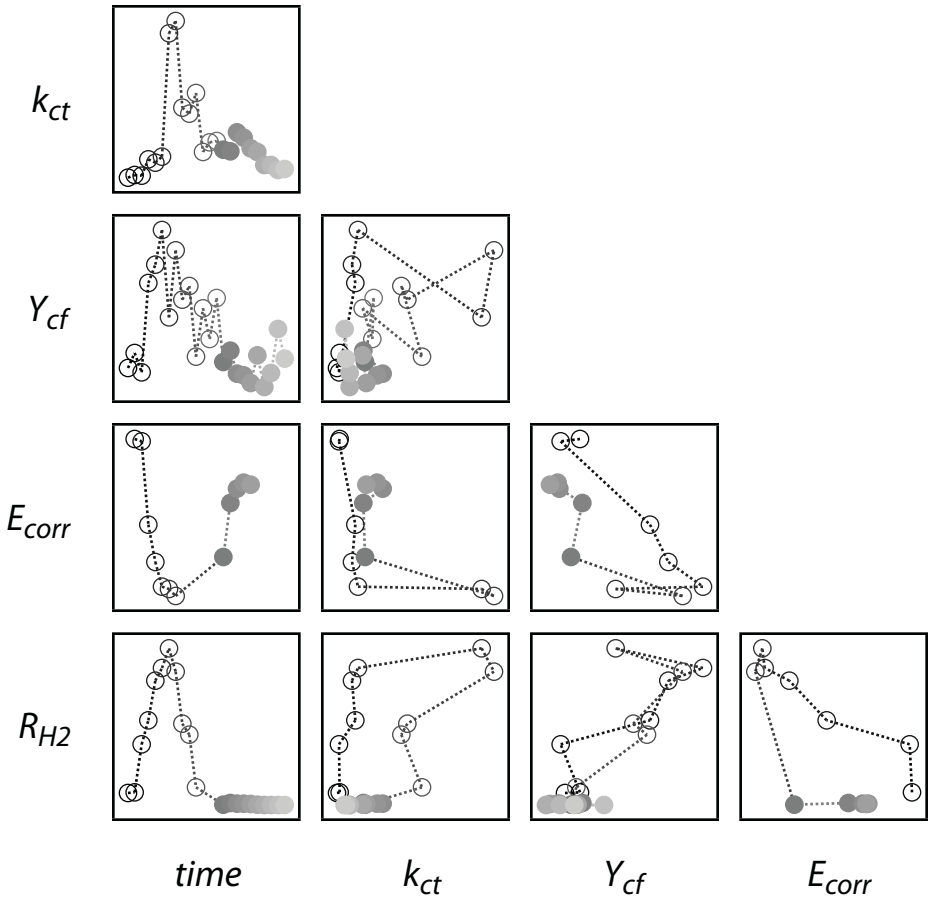

Figure 1. Matrix of scatter plots showing all combinations of four measured properties of Fe^0 (pseudo first order rate constants for carbon tetrachloride disappearance, k_{CT}; yield of chloroform from carbon tetrachloride, Y_{CF}, corrosion potential, E_{corr}; and the rate of hydrogen production due to reduction of water, R_{H2}) and aging time. Open symbols are for $Fe^{H2(D)}$ and solid symbols are for $Fe^{H2(W)}$. Symbol color scales from black to gray with increasing age. Data adapted from (Sarathy et al. 2008).

Conclusions

In general, decreasing Fe^0 content, and concomitant shrinking of the Fe^0 core, are the primary characteristics of aging nano-Fe^0. However, while these changes in structure eventually must result in the loss of reactivity, the short- and medium-term effects of aging on two types of reactivity—hydrogen production or contaminant degradation—seem to vary with the type of aging regime and other reaction conditions. These changes in reactivity correlate with evidence for rapid destruction of the original Fe(III) oxide film on Fe^{H2} during immersion and the subsequent formation of a new passivating mixed-valence Fe(II)-Fe(III) oxide shell. These dynamics have implications for in situ remediation applications of nano-Fe^0, because the oxide shell must mediate reaction of the core with all solution species, including contaminants (Scherer et al. 1998).

Acknowledgements

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Conference Questions and Answers

No questions.

Effects of Humic Acid on Aggregation of Boron Nanoparticles in Various Electrolyte Solutions

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Abstract

Nano boron is a promising new propellant and is being considered for military and civilian applications; however, the impact of its release on the environment is largely unknown. Aggregation studies help to assess the fate, transport, and exposure pathways of various nanopartcles in aquatic environment. The aggregation kinetics of boron nanoparticles was investigated in the presence of monovalent (NaCl) and divalent electrolytes ($CaCl_2$ and $MgCl_2$), and Suwannee River humic acid (SRHA) through time- resolved dynamic light scattering (DLS). In the presence of SRHA, the attachment efficiency of the boron nanoparticles decreased for the reaction- limited regime. The presence of SRHA caused the boron nanopartciles to stabilize and resulted in greater critical coagulation concentrations (CCC). It appeared that for the sodium, magnesium and calcium solutions, the surface charge became more negative due to the adsorption of SRHA on the surface of the boron nanoparticles.

Introduction

Studies on fate and toxicity of engineered nanomaterials are being reported more frequently due to the potential risk of these materials to human safety [1-7]. However, due to the different surface characteristics of nanomaterials, the potential impact of these particles is being evaluated on a case-by- case basis [8-10]. Due to its desirable heat of combustion and fast energy release rate, boron nanoparticle is being considered as a promising solid fuel for rocket and gun propellants [11-14]. Even though boron is beneficial to plants in small amounts, excessive amounts are injurious and even lethal [15]. Upon release to the environment, natural organic matters (NOMs) are expected to play a critical role in the stabilization and transport of these particles as has been recently reported [16]. The purpose of this paper is to investigate the influence Suwannee River humic acid (SRHA) on the aggregation of boron nanoparticles in three common electrolyte solutions.

Materials and Methods

The boron nanoparticles were obtained from Alfa Aesar with an average particle size of 10-20 nanometers. The boron nanoparticles were dispersed in DI water and ultrasonicated for 30 minutes, to break up aggregates before aggregation experiments. The particle size distribution and surface charge were measured by a Nano Zetasizer (Malvern, Worcestershire, UK). All

Dynamic light scattering (DLS) measurements were conducted at 25 ˚C at pH 5.6± 0.2 unless otherwise specified.

The electrolyte stock solutions (NaCl, $CaCl_2$, and $MgCl_2$) were prepared separately and filtered through 0.2 μm filters before use. SRHA (standard II, International Humic Substances Society) solutions were made by dissolving 22.9mg dry powder into 50 mL DI water and were stirred overnight. The solutions were then filtered through 0.2 μm filters and pH was adjusted from 3.4 to 10.2 by addition of NaOH. The total organic carbon content was measured at 232.76 mg/L.

For experiments in the absence of NOM, various electrolytes were added into 1 mL boron dispersion in cuvettes. The dispersions were then shaken by hands and were placed into the zeta-sizer immediately. For experiments in the presence of NOM, 70 μL SRHA stock solution was added to the nanoparticle dispersions following the addition of the electrolytes. The aggregation rate constant k_{11} is proportional to the slope of the hydrodynamic radius R_h versus time as $t \rightarrow 0$ at each salt concentration, divided by the initial nanoparticle number concentration N_0 [16-19]

$$k_{11} \propto \frac{1}{N_0}\left(\frac{dr_h(t,q)}{dt}\right)_{t\rightarrow 0} \quad (1)$$

The attachment efficiency α (the inverse of the stability ratio W) is defined as the aggregation rate constant of interest normalized by the rate constant derived under diffusion-controlled (fast) aggregation conditions (in the absence of an energy barrier).

$$\alpha = 1/W = \frac{k_{11}}{(k_{11})_{fast}} = \frac{\frac{1}{N_0}\left(\frac{dr_h(t)}{dt}\right)_{t\rightarrow 0}}{\frac{1}{(N_0)_{fast}}\left(\frac{dr_h(t)}{dt}\right)_{t\rightarrow 0,fast}} \quad (2)$$

Results and Discussion

The boron nanoparticles suspensions in various electrolytes displayed similar aggregation behavior as shown in Figure 1. There appears to be two regimes; a reaction controlled regime and a diffusion controlled one. The two regimes are separated by the critical coagulation concentration (CCC). When ionic strength (IS) is smaller than the CCC, the attachment efficiency increased with IS due to the screening of the electrostatic forces. As the IS increased and became greater than CCC, the attachment efficiency kept constant (diffusion- controlled regime) because electrostatic repulsion was screened completely. The CCC for the Na^+ ions was determined at ~0.2M much greater than those for the divalent ions— ~1mM for Ca^{2+} and Mg^{2+}.

Boron nanoparticles suspensions were stabilized in the presence of SRHA in various electrolyte solutions. As shown in Figure 2, the attachment efficiency in the presence of SRHA was smaller than that in the absence of SRHA in the reaction-controlled regime. However, in the diffusion-controlled regime, the aggregation rates were similar to those in the absence of SRHA. At the same time, the CCC for NaCl increased from 0.18 M in the absence of SRHA to 0.22 M in the presence of SRHA. The CCC for $CaCl_2$ increased from 1 mM in the absence of SRHA to 2.5 mM

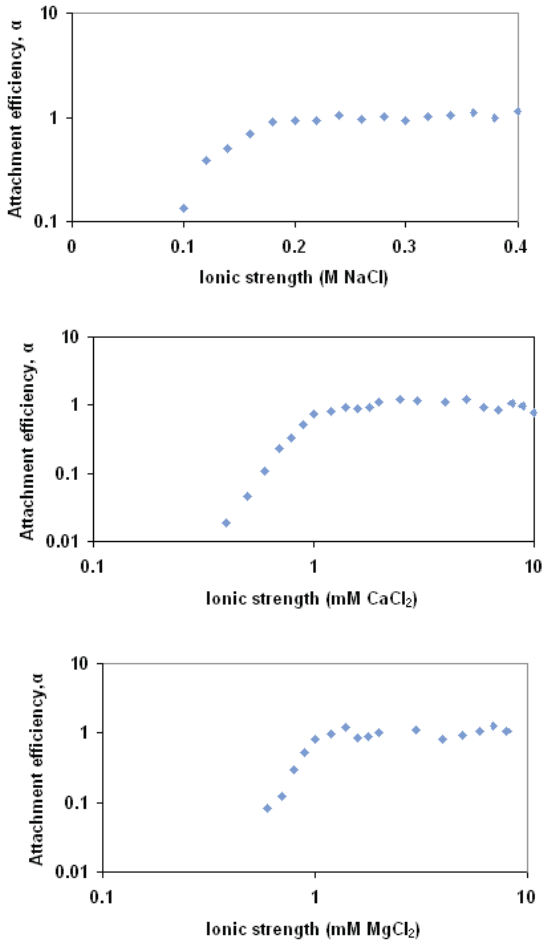

Figure 1. Attachment efficiencies as a function of (a) NaCl (b) $CaCl_2$ and (c) $MgCl_2$ concentration.

in the presence of SRHA. Although the CCCs for $MgCl_2$ did not changed greatly, the aggregation rates decreased ostensibly in the presence of SRHA. Therefore, it appears that SRHA stabilizes the boron nanoparticles suspensions.

The surface charge of boron nanoparticle suspensions was measured to delineate the mechanism of stabilization induced by SRHA. As shown in Figure 3, the surface charge was more negative in the presence than that in the absence of SRHA at low IS. This indicates that electrostatic repulsion increased probably due to the adsorption of SRHA on the surface of boron nanoparticles. With the increase of ionic strength surface charge became less negative, probably due to the neutralization effect [20]. Interestingly, it was also observed in Figure 3, that when IS was greater than 0.22 M, the surface charge was similar in the presence and absence of SRHA. It is consistent with the fact that attachment efficiencies were unity in the diffusion- controlled regime whether in the absence or presence of SRHA as shown in Figure 2- a.

For the $CaCl_2$ electrolyte, the surface charge in the presence of SRHA was greater (more negative) than those in the absence of SRHA. More interesting as shown in Figure 4, the zeta-

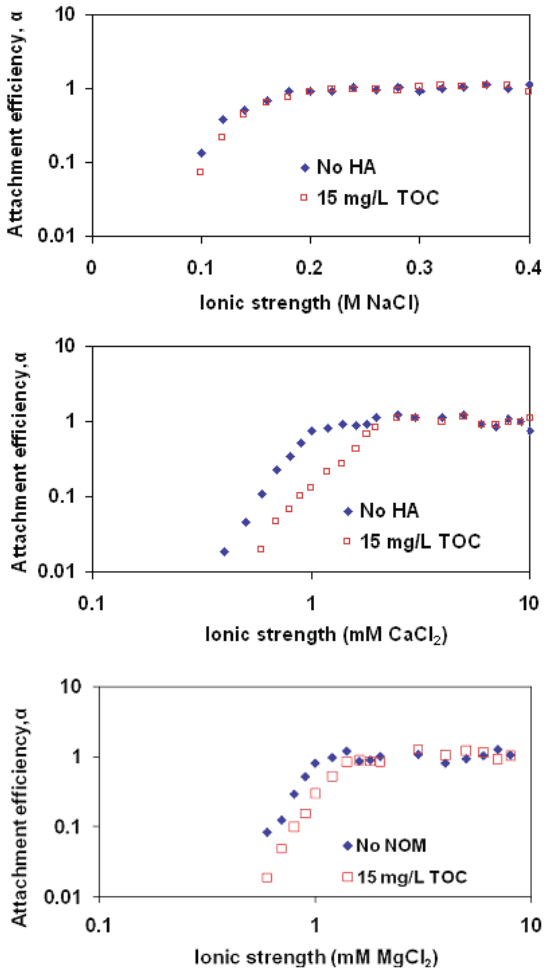

Figure 2. Attachment efficiencies as a function of (a) NaCl (b) $CaCl_2$ and (c) $MgCl_2$ concentration in the presence of SRHA compared with in the absence of SRHA.

potential in the absence of SRHA at CCC (16.3 mV at 1mM $CaCl_2$) was, almost the same as that in the presence of SRHA at CCC (2.5 mM $CaCl_2$). It reveals that at the CCC, boron nanoparticles and aggregates have similar surface charge in the presence and absence of SRHA. Therefore, it can be speculated that the increased electrostatic repulsion in presence of SRHA is the reason for the stabilization of boron nanoparticles in calcium or sodium electrolytes solutions. Similar phenomenon was also observed in the presence of SRHA and magnesium ions.

Conclusions

The behavior of boron nanoparticles aggregation changed in the presence SRHA. It appears that in the presence of SRHA boron nanoparticles suspensions were stabilized due to increased surface charges in the presence of sodium or calcium ions, however, more investigation is needed to confirm this observation. In natural aquatic environments, the aggregation process will be more complicated due to the presence of various ions and NOMs. It is therefore important to

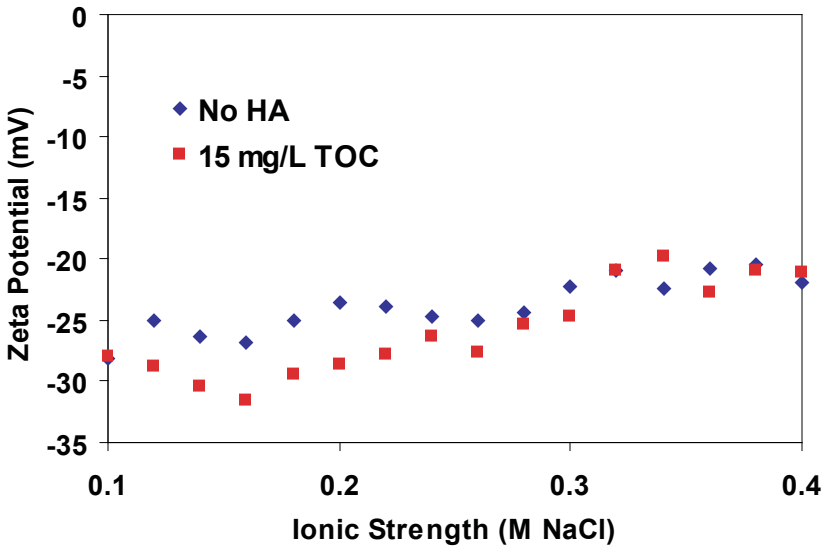

Figure 3. Study of surface charge of boron nanoparticles in the presence of SRHA and NaCl.

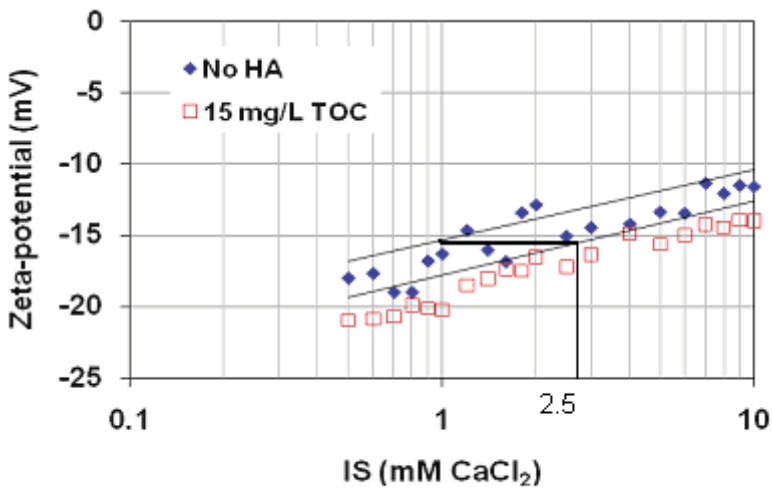

Figure 4. Study of surface charge of boron nanoparticles in the presence of SRHA $CaCl_2$.

conduct more studies to predict the fate and transport of emerging nanomaterials on a case- by-case basis.

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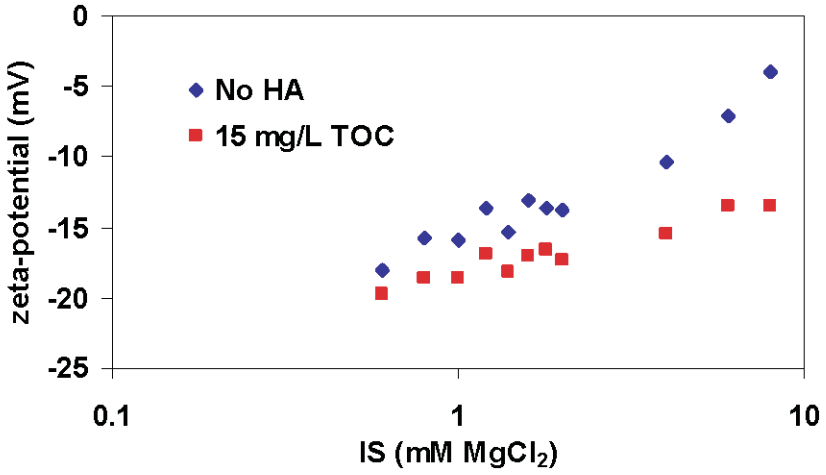

Figure 5. Study of surface charge of boron nanoparticles in the presence SRHA and $MgCl_2$.

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Conference Questions and Answers

Question:
If your hypothesis that steric tendency may play in the formation of aggregates with $MgCl_2$ is correct, could you say the same for sodium chloride?

Answer:
Maybe, but I cannot say for certain. For the steric tendency to take place you must have some absorption on the surface of the boron nanoparticle. Maybe you can do some experiments for these effects without calcium or magnesium to characterize the particle surface. Calcium and

magnesium are known to complex, so it is possible that, when you have a higher concentration of calcium with the same concentration of NOM, you show less effect for the humic acid. It is possible that the humic acid and the boron nanoparticle are competing for calcium, thus reducing its ionic strength and leading to less effectiveness.

Predictive Numerical Model of Post-Injection Distribution of Nano-Size ZVIin the Ringold Aquifer for Mending an Existing Permeable Reactive Barrier in the 100-D Area at the Hanford Site

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Akshai K Runchal, Analytic and Computational Research, Inc., Bell Air, California, U.S.A.

David Reichhardt, Montana Tech, Butte, Montana, U.S.A.

Scott Petersen, Fluor Hanford, Washington, U.S.A.

Abstract

MSE Technology Applications, Inc. has conducted investigations associated with the injection of nano-size zero-valent iron (nZVI) into the subsurface of the 100-D Area at the U.S. Department of Energy (DOE) Hanford Site in Washington State. The purpose of this work was to demonstrate the feasibility of using nZVI to repair portions of the In Situ Redox Manipulation (ISRM) installed at the site to intercept a hexavalent chromium plume moving towards the Columbia River. The investigation identified RNIP-M2 (RNIP), produced by Toda Kogyo Corporation, as most suitable for mending the ISRM barrier. Since Toda nZVI will be emplaced in the Ringold aquifer through injection wells, the PORFLOW™ computer model was used to optimize injection parameters and predict the post-injection distribution of nZVI material in the Ringold aquifer. The model used an empirically developed mathematical expression for deposited nZVI as a function of injection time, distance from the injection point, and nZVI-fluid velocity. Modeling results provided information on the predicted concentration of deposited nZVI within the model domain and optimized the injection rate. This work was conducted through the support of Fluor Hanford, a subcontractor to the DOE under Contract Number 30994.

Introduction

We have conducted investigations associated with the injection of nano-size zero-valent iron (nZVI) into the Ringold aquifer beneath the 100-D Area at the U.S. Department of Energy (DOE) Hanford Site in Washington State. The purpose of this work was to demonstrate the feasibility of using nZVI as a source of electrons to repair portions of the In Situ Redox Manipulation (ISRM) barrier. The ISRM barrier was installed at that site to intercept a hexavalent chromium (Cr^{6+}) plume moving towards the Columbia River. The barrier was installed from 1999 to 2002 (DOE, 2006) by injecting sodium dithionite to the Ringold Formation aquifer and creating persistent reducing conditions by converting native Fe^{3+} to Fe^{2+}. Although laboratory and field tests indicated the barrier would effectively treat Cr^{6+} for nearly 20 years, a few of the barrier wells exhibited signs of breakthrough after less than two years. The work reported here was performed to support testing an alternative technology to mend the ISRM barrier by injecting nZVI into the Ringold aquifer through the existing injecting well.

We conducted comprehensive investigations on commercial ZVI materials (Zaluski et al. 2008), and identified an nZVI manufactured by Toda as most suitable for mending the ISRM barrier. This investigation included geochemical and injectability lab studies and the computer modeling described in this paper.

Problem Definition

Hydrogeologic setting comprises an unconfined aquifer of 4.9 m (16 feet) saturated thickness and 26.2 m (86 ft) thick unsaturated zone. The aquifer is stratified with respect to hydraulic conductivity (K) that ranges from 7.1×10^{-5} m/s to 2.3×10^{-3} m/s (20 ft/d to 652 ft/d), with the highest K present in the bottom 0.3 m (1 ft) of the aquifer (Figure 1). At the location of injection (Well 199 D4-26) the aquifer is intercalated by a 0.6 m (2 ft) thick layer of very low K sediment. Because of proximity to the Columbia River the hydraulic gradient of the aquifer is controlled by river stage, and for the injection time (August 2008) is expected to be negligible in magnitude.

RNIP is provided by Toda as a solution containing 80 % water, 17-18 % solids, and 2-3 %

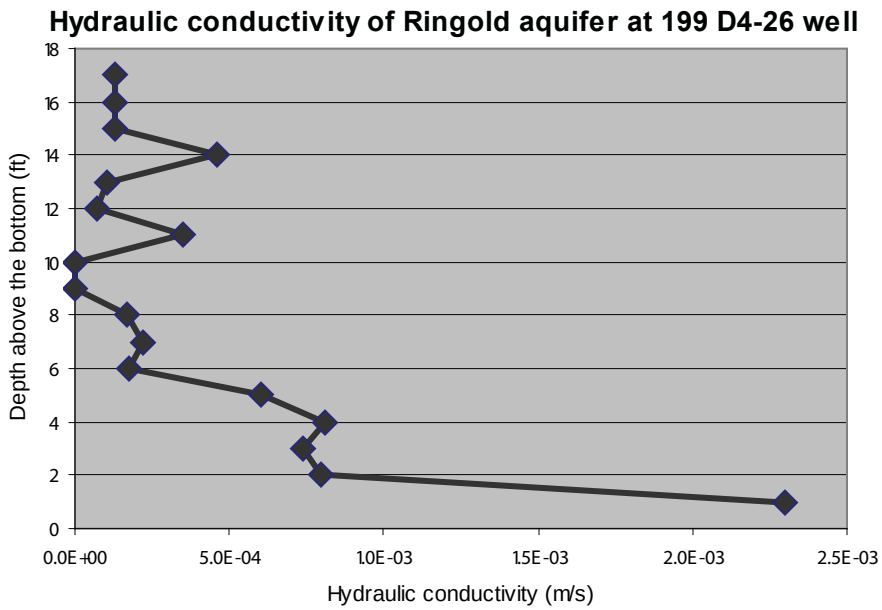

Figure 1. Hydraulic Conductivity of Ringold Aquifer.

olefin maleic copolymer (all by weight). The solids, which include 65 % ZVI and 35 % Fe_3O_4, come from the production line at an average particle size of 70 nanometers (nm), but promptly agglomerate to 2 micrometer particles (Jazdanian, 2008). Though the injected nZVI fluid contains 1 % (weight) of solids and only 0.14 % of maleic copolymer, the presence of the latter precludes use of Stokes' law to define deposition of the solids. This phenomenon was demonstrated in our laboratory experiments with 3-m long flow cells into which nZVI fluid was injected at four different flow velocities.

Amassing of nZVI particles, defined as an increase in concentration of suspended particles (nZVIs) above influent nZVI concentration, was observed during flow cell tests. The results

of our tests indicated that unlike transported solute, which partition to an adsorbed portion and that remaining in the solution, suspended particles may partition in three ways: (1) a significant portion of particles drops out of suspension and deposits within the medium, possibly due to gravity, electromagnetic forces, adsorption etc. – an equivalent of adsorption for the solute transport, (2) a small portion of particles remains suspended moving at the same velocity as the water, and (3) particles (nZVIs) that have some mobility, thus can be measured via aqueous sampling, but move at a velocity much slower than that of the water.

Our observation of nZVI amassing may be mathematically described by the following advection-dispersion-deposition-reentrainment equation (Johnson et al 2007):

$$(\partial C/\partial t)\theta = -v(\partial C/\partial x)\theta + D(\partial C^2/\partial^2 x)\theta - K_f C\theta + K_r C_s \rho_b \qquad (1)$$

Where C is the aqueous concentration of the constituent, C_s is the reversibly retained stationary phase concentration of the constituent, K_f and K_r are the forward (removal from the aqueous phase) and reverse (addition to the aqueous phase) coefficients, D is the dispersion coefficient, θ is volumetric water content, and ρ_b is a bulk density of the stationary phase. The amassing phenomenon that we observed in our flow cell experiments is related to the last components of Equation 1.

Since we were limited to conducting the flow cell experiment using only one concentration of the nZVI in influent, it was impossible to define the K_f and K_r coefficients. Instead, we used a statistical application of multiple linear regression (applied through MSExcel) to develop the following mathematical expression for deposited mZVI (nZVId) as a function of injection time, distance from the injection point, and nZVI-fluid velocity:

$$\text{nZVId} = 0.0322 + 3.77\text{E-}7 \times \text{Time} - 0.0192 \times \text{Distance} + 24.12 \times \text{Darcy Velocity} \qquad (2)$$

Where nZVId, time, distance, and Darcy velocity are expressed in Kg of nZVI per Kg of soil, seconds, meters, and meters per seconds, respectively.

The objective of the investigations was to deposit at least 0.001 Kg/Kg or 1 g/Kg of nZVI at the distance of 7m (23 ft) from an injection well, which is half of the ISRM barrier's width.

Modeling

For the computer modeling we used the PORFLOW™ model and focused on prediction of spatial distribution of nZVId emplaced in the Ringold aquifer by injecting nZVI fluid. PORFLOW™ is a software tool for solution of multi-phase fluid flow, heat transfer, and mass transport problems in variably saturated porous or fractured media. The PORFLOW™ model was developed by Analytic & Computational Research, Inc. (ACRI 2008).

Because PORFLOW™ is a highly flexible, modular, and user-oriented software package we were able to develop a special subroutine (Figure 2) for solving Equation 2 based on the simulated flow field, which resulted from the injection of nZVI fluid, and the distribution of K. While solving Equation 2 for a given element of the model domain flow velocity, elapsed injection time and distance of the given element from the injection point were used to calculate nZVIe i.e. the concentration of nZVId for each element. These values were then normalized with respect to K to obtain nZVIKe, i.e. the concentration of nZVIe in each element. Finally, for mass

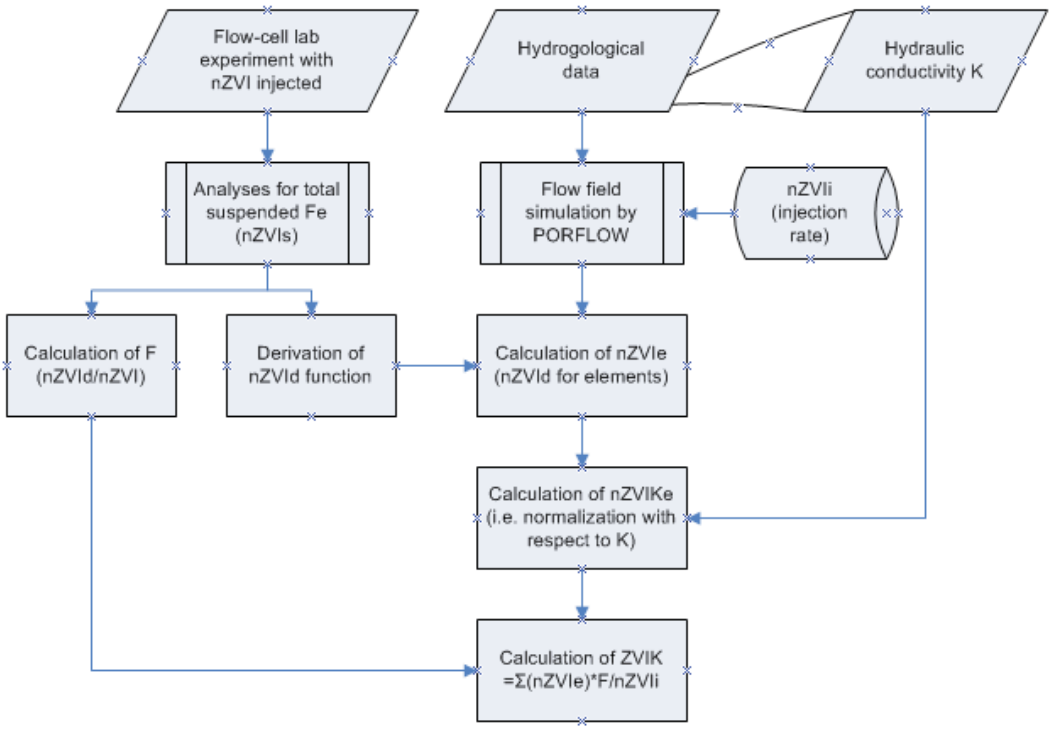

Figure 2. Modeling Logistics.

conservation, we calculated ZVIK, i.e. concentration of deposited nZVI for each element as the following:

$$ZVIK = nZVIKe * \Sigma(nZVIKe) * F / nZVIi \quad (3)$$

Where nZVIi is the total mass of injected nZVI through the injection well at the given time, and F is a dimensionless laboratory-defined factor calculated as a ratio of nZVId to nZVI.

We used a simplified 3D approach, i.e., cylindrical coordinates, with the injection well being the axis of the cylinder. The model domain encompasses a cylindrical block, 31.1 m (102 ft) of height (X-axis) and of 30 m (98.4 ft) radius set along the Y-axis. The domain was discretized to a 109x82-structured grid with irregular (progressively larger) spacing along the Y-axis.

By using different injection rates (nZVIi) of nZVI fluid we defined the optimal injection rate of 0.00089 m^3/s (14 gpm) that was related to nearly maximum concentration of ZVIK in the highest-K strata at the distance of 7 m (Figure 3). This nZVI concentration was 4.7 g/Kg as illustrated in Figure 4.

Conclusions

Because of the observed amassing phenomenon of colloidal nZVI, modeling of its transport and deposition can only be achieved by flexible modular computer codes like PORFLOW™.

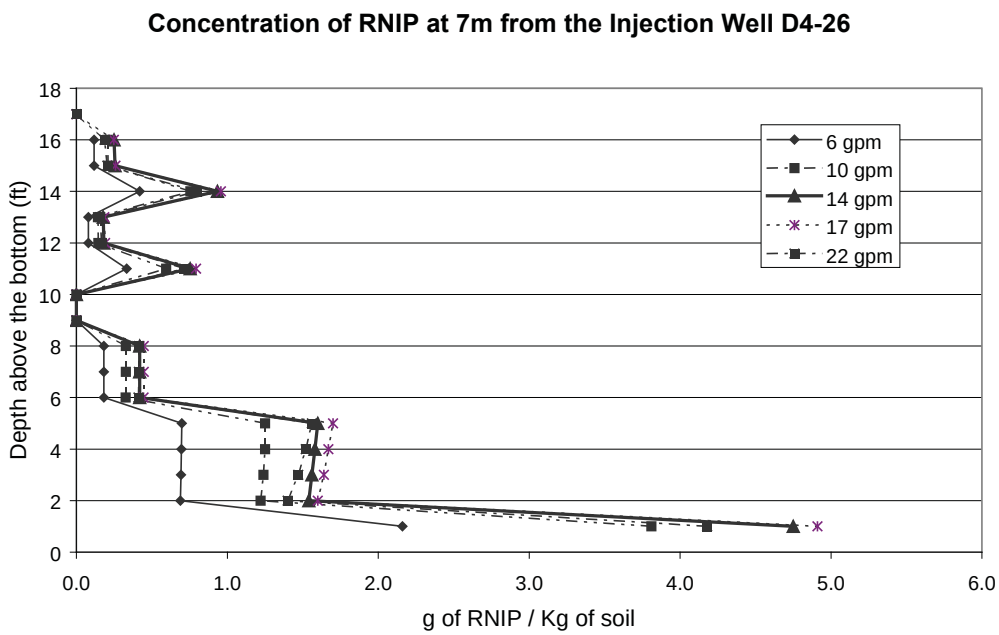

Figure 3. Concentration of RNIP at 7m from the Injection Well D4-26.

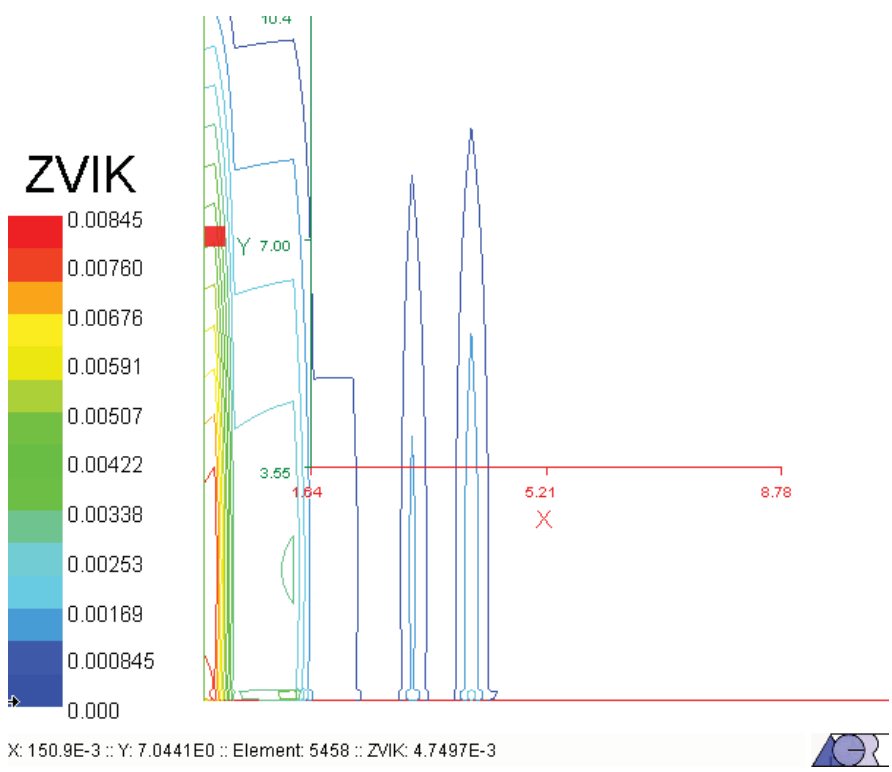

Figure 4. Distribution of ZVIK – Concentrations in Kg/Kg.

Intensive laboratory efforts are required to develop empirical constants needed for predicting nZVI deposition within the model domain.

To increase the reliability of the prediction, flow-cell experiments need to be conducted using materials of various K and several nZVI concentrations in the influent, so an isotherm for amassing of nZVI can be developed. This would allow for solving nZVI transport equation internally in the model, rather than using a deposition function in a post-processor manner.

Acknowledgements

The authors appreciate the insight of and helpful discussions with Drs. P. Tratnyek, G. Lowry, C. Palmer, and A. Jazdanian during execution of the investigations.

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Conference Questions and Answers

Question:
What do you think about this amassing function with different kinds of ZVI, and you said you were also testing the polymetals?

Answer:
We investigated six ZVIs. Some of them were micro, and some were nano. We observed advancement of the particles, and we collected samples and measured how much iron was forming. After a number of pour volumes, the iron in the effluent was greater than the iron in the influent. This build up of suspended iron particles was unexpected. The different iron types behaved in different fashions, but we only ran one of the ZVIs in the 3-meter column. We did, however, observe the "amassing" effect for other ZVIs in earlier experiments.

Question:
You said earlier that you would be using geophysics. What type?

Answer:
Electromagnetic for sure and maybe others.

Question:
There will be a lag between the injection time and the geophysics measurements. Will this lag affect the results?

Answer:
Yes.

Question:
Do you think the effective life of the ZVI will be longer than the lag time in performing the geophysics?

Answer:
Absolutely. Why inject the iron if it is only going to last a few months.

Save

Fate and Transport of Titania Nanoparticles in Freshwater Mesocosms

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Abstract

Titania nanoparticles are currently associated with air, soil, and water and with numerous products directed at human use and consumption (e.g., sunscreen, cosmetics, and food coatings). The environmental fate and transport of TiO_2, or any nanomaterials entering dynamic aquatic environments are largely unknown. Because the physical and chemical properties of TiO_2 are variable (size, surface chemistry, and composition), the movement, bioaccumulation, and toxicity of these materials are difficult to study in a complex ecosystem. Many metal oxide materials are durable and recalcitrant, and the accumulation of TiO_2 in the environment could be significant over time and cause unforeseen impacts on ecosystems. Fate and transport of TiO_2 nanomaterials in a bench-scale mesocosm system was assessed through nanomaterial partitioning and complexation in water, sediment, and tissue media characterized using inductively coupled plasma mass spectrometry and scanning electron microscopy with energy dispersive X-ray spectroscopy, respectively. Research data sets like these will build the foundation for future use in fate and transport of other nanomaterials in different water systems (fresh, estuarine, and marine) and in building empirical and process models that investigate environmental fate and transport and relevant freshwater ecological impacts of nanomaterials.

Introduction

The environmental fate and transport of TiO_2 nanoparticles (NPs) or any NPs entering dynamic aquatic environments has never been studied in a quantified manner (Maynard, 2006; USEPA, 2006). Because the properties of TiO_2 are dependent on size, surface chemistry, and composition, the movement, toxicity, and bioaccumulation of these materials are difficult to study in a complex ecosystem. Titania materials are likely to enter an aquatic environment through waste water treatment plants or directly into waters used for recreation. Because these materials are durable and recalcitrant, the accumulation of TiO_2 in the environment could be significant over time and cause unforeseen impacts on ecosystems. Research that demonstrates the capability to characterize and quantify TiO_2 distribution in different environmental compartments (i.e., water, sediment, and biota) will be relevant to setting guidelines for risk analysis.

Titania nanoparticles are currently associated with air, soil, and water and with numerous products directed at human use and consumption (e.g., sunscreen, cosmetics, and food coatings). What little research that has been done on TiO_2 has been primarily focused on *in vitro* exposure or air exposures (Bermudez et al. 2004; Gurr et al. 2005; Hussain et al. 2005; Peters et al. 2004; Ramires et al. 2001; Wamer et al. 1997; Warheit et al. 2005; Zhang and Sun 2004), all demonstrating the induction of oxidative stress. Recently, Lovern and Klaper (2006) reported the first study of TiO_2 on an environmental sentinel organism. In this study, the lethal concentration of TiO_2 (avg. size 30 nm) was only 10 ppm for *Daphnia magna* following a 48-hour water exposure. Federici et al (2007) investigated the toxicity of titania nanoparticles following exposures of 0.1, 0.5, and 1.0 mg/L to rainbow trout (*Oncorhynchus mykiss*) and while there was no acute toxicity to dispersed TiO_2 at the concentrations tested, there was a range of sub-lethal effects observed related to biochemical changes and respiratory distress. These aquatic exposure studies were carried out in artificial water systems used for organism culture and maintenance.

A gap remains in understanding the likely exposure scenarios or probabilities under real environmental conditions. For instance, pH, dissolved solids, flow, and other chemical/physical parameters exist in an environmental setting. NP properties and bioavailability will depend on the surrounding aquatic environment. This study investigates the partitioning of nano-sized titanium dioxide using Columbia River (WA) water and a constructed mesocosm with a homogenous sediment and biota that have life histories associated with the sediment or surface waters. A flow-through, benchtop, riverine mesocosm was used to examine the fate, transport, and association of titania NPs to determine relevant exposures for each of the examined compartments and biota.

Methods

Two different types of titanium dioxide were used for fate and transport experiments; pure anatase form, 5-30 nm in water dispersion (Nanostructured and Amorphous Material, Los Alamos, NM) and an anatase/rutile mixture, <75 nm in water dispersion (Sigma-Aldrich, St. Louis, MO). Dispersion and size were confirmed using scanning electron microscopy and ImageJ (Rasband, 2007) software analysis for particle sizing. The titania solutions were added to separate triplicate mesocosms with continual 50 mL/min flow rate of Columbia River water (CRW) at a concentration of 5 ppm for 12 hours, followed by an additional 36 hours of unamended CRW at the same flow rate. Water samples of the dosing solution, inlet, and outlet of the mesocosms were collected prior to titania addition, and at 1, 8, 12, 13, 24, 36, and 48 hours post titania addition for TiO_2 mass analysis.

Mesocosms were constructed using sterile Accusand at 2 cm deep sediment in 15 x 15 cm mesh baskets. Baskets were placed in chambers designed for flow-through aquatic exposures and flow rate was regulated by peristaltic pumps. Six Asiatic clams (*Corbicula fluminea*) and 25 amphipods (*Hyallela azteca*) were added to each of the 9 total mesocosms to provide 3 replicates per treatment (2 forms of titania and control). Mesocosm systems were allowed to run for biota acclimation for seven days prior to beginning exposures. Biota and sediment samples were collected following the conclusion of the 48 hour exposure and analyzed for TiO_2 uptake.

To assess mass of TiO_2 associated within a sample, inductively coupled plasma optical emissions spectroscopy (ICP-OES; Perkin Elmer 4300 DV) was used. All matrices (including water

samples) were lyophilized in trace metal cleaned polypropylene vials. The remaining dried material for the biota and river water samples were dissolved using a 70:30 mixture of nitric/ sulfuric acids followed by heating in a hot block at 95°C for 4 hours. The instrument was calibrated over a range of concentrations using certified Ti standards. Sediment samples required leaching using a dissolution method containing a mixture of hydrochloric, nitric acid, and hydrofluoric acids to solubilize all minerals (adapted from Wu et al. 1996). The experimental data generated from all compartments was used to parameterize and validate a physically based process model based on conservation of mass for each phase (i.e., water, sediment, biota).

Results and Discussion

The different titania solutions changed dispersion characteristics upon dilution in Columbia River water (CRW). The primarily single particle suspensions quickly formed large aggregates on the order of several microns (Figure 1). Water concentrations of titania were close to nominal dose through the first twelve hours of dosing, and then rapidly fell upon the change to depuration with unamended CRW (Table 1). The concentration of titania decreased 90% for the anatase material and 85% for the anatase/rutile mixture from the inlet to the outlet during the initial 12

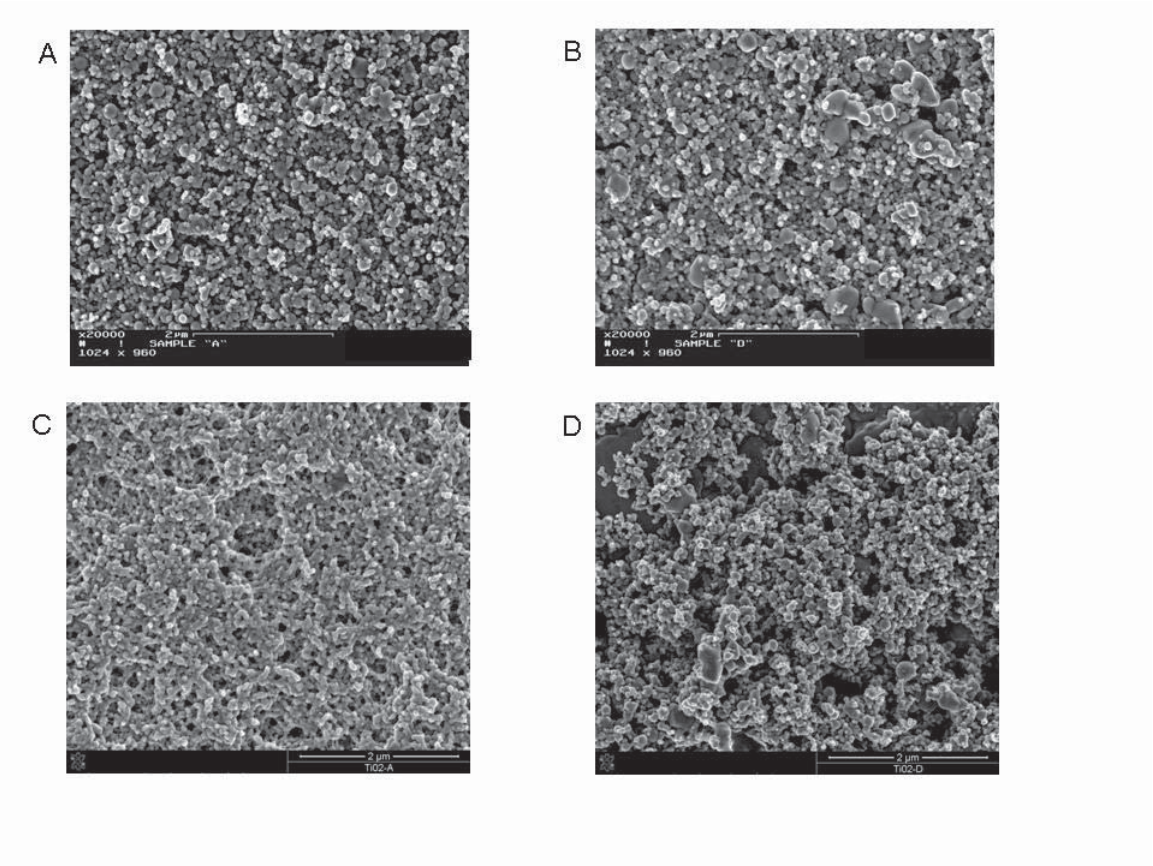

Figure 1. Scanning electron micrographs of titania solutions. A. Anatase titanium oxide, 5-30 nm, 15% w/v dispersion in water. B. Anatase-rutile titanium oxide, <75 nm, 10% w/v dispersion in water. C. Titanium oxide material shown in A following dilution to 5 ppm in Columbia River water. D. Titanium oxide material shown in B following dilution to 5 ppm in Columbia River water.

Table 1.

Titania Nanomaterial	1-12 hr exposure		13-48 hr depuration	
	inlet (ppm)	outlet (ppm)	inlet (ppm)	outlet (ppm)
Anatase	8.196 (±0.88)	0.833 (±0.05)	0.147 (±0.01)	0.037 (0)
Anatase/Rutile	5.138 (±0.27)	0.777 (±0.09)	0.013 (0)	0.078 (±0.01)
CRW	0.008 (0)	0.001 (0)	0.007 (0)	0.002 (0)

ICP-OES results of titanium dioxide concentrations in water samples. Concentrations are given as means in parts per million (ppm) with standard deviations in parentheses from triplicate samples. Two types of titanium oxide are listed as Anatase and Anatase/Rutile, and controls are listed as Columbia River water (CRW).

hours of continuous exposure. For both nanomaterials, there were visible flocs throughout the dosing portion of the experiment and the material was observed to settle out over the sediment, thus accounting for the rapid loss of titania from the inlet to the outlet of the mesocosms. The sediment analysis indicated that indeed the majority of sediment-associated titania was present in the inlet third, with a gradient in decreasing concentration for the middle and outlet thirds. However, more of the anatase titania nanomaterial solution associated with the sediment (0.5%) than the anatase/rutile titania nanomaterial (0.08%).

A greater percentage of anatase titania associated with the amphipods (48 mg/g) than the clams (0.55 mg/g). This difference was visible in that the amphipods appeared coated with the nanomaterials while the clams were observed to flush out visible titania flocs through their excurrent siphons and through deposition in fecal material. Although the same observations were also made with the anatase/rutile exposures, a greater concentration of titania nanomaterial was associated with amphipods (67.8 mg/g) and clams (1.2 mg/g). Mass measurements of the anatase/rutile mixture were more difficult to accomplish than the anatase material alone due to increase insolubility. The greater association of the anatase/rutile material with the biota may be attributed to lower overall solubility as well. Complexation of either material with dissolved solids in the CRW was not examined. The observations and mass concentration data are being explored with sedimentation rates for each titania nanomaterial for assessing association rates using a physically based process model.

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Conference Questions and Answers

Question:
How long did you let the titania sit in the river water before you exposed it? Did you vary that parameter? Did you see any settling out?

Answer:
The carboys had stir plates and stir bars to provide a uniform distribution. We took samples before and during the experiment. The material was mixed overnight before starting.

Question:
What was the pH of the water? Did you vary it?

Answer:
The pH of Columbia River is around 8, so it's basic. It was not changed over time. The pH of the titania material is around 4, so when it was added there was precipitation.

Natural Organic Matter-Mediated Phase Transfer of Quantum Dots in the Aquatic Environment

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Abstract

The increasing interest in quantum dots (QDs) raises a concern with regard to their environmental impact. With the eventual commercialization of these materials for applications such as in solar energy conversion and as fluorophores in biomedicine, their release in the environment is inevitable. One way by which the fate and transport of QDs will be influenced is through their interactions with Natural Organic Matter (NOM). This study examined the NOM-mediated phase transfer of TOPO-capped CdSe quantum dots in water. Results from our study indicates that humic and fulvic acids (HA and FA) could facilitate the solubilization of the organic QDs in water with kinetics that is measurable in less than 24 hours. Solution pH and Ca^{2+} ion concentration also influenced the rate of phase transfer, favoring lower pH and absence of Ca^{2+}. Dynamic light scattering, transmission electron microscopy and infrared spectroscopy studies showed that the interaction between HA/FA and the QD surface capping groups, as opposed to metal coordination, is the primary mechanism for transfer. Whether HA or FA forms aggregates with random coil conformations or as micelles when they facilitate QD transfer remains inconclusive and needs further investigation. The results observed with the Suwannee River HAs and FAs translated to the natural surface water samples collected from local creeks. This study presents the first evidence of stabilization of QDs in water by humic substances in real environmental samples, illustrating that NOM will have a significant role in the fate and transport of QDs in aquatic systems.

Introduction

Quantum dots (QDs) are semiconductor nanocrystals with diameters in the 2-100 nm size range that exhibit remarkable size-dependent optical properties.[1, 2] These properties, not present in their bulk counterparts, have made QDs ideal for applications like solar energy conversion,[3-6] and medical diagnostics. [7, 8] QDs are typically prepared as colloidal solutions by organometallic synthesis. These nanocrystals consist of an inorganic crystalline core surrounded by a shell of organic ligands; the ligands serve to passivate the nanocrystal surfaces, prevent the agglomeration of the particles, and impart solubility in various dispersion media. Typically, hydrophobic ligands and organic solvents are used. [9-11] However, for biological applications, water-soluble QDs are prepared and obtained by various methods involving exchange or modification of the initial hydrophobic capping ligands.[8] The most well characterized QDs synthesized to date are CdSe and CdS, [12, 13] owing to their bandgap tunability through the visible region of the electromagnetic spectrum. [2, 12, 14]

With increasing interest and their eventual large-scale production, release of these materials to the environment is inevitable and human exposure is likely from several sources including air

and the aquatic environment. This raises concern as to their potential harmful effects to humans and to the environment. Although several cell culture and animal studies of QDs have been reported in the literature, very little is known about the fate, transport, and bioavailability of these particles. Thus, there is an urgent need to evaluate the partitioning of QDs in air and water and to study the mobility and persistence of these materials in different phases.[15, 16]

In the environment, humic substances play a key role in the biogeochemical cycling of various metal species. Humic substances are ubiquitous NOM with highly complex molecular structures that allow metal chelation. Earlier studies have identified that metal-NOM complex formation involves coordination between the metal ions and the carboxylic or phenolic groups of NOM. [17, 18] In addition, humic acids are also able to change their aggregation states and act as amphiphilic systems in aquatic environments.

Our objectives in this study were to determine the mechanisms of interactions between TOPO-capped CdSe QDs and NOM, and to examine how NOM-mediated phase transfer of QDs between organic and aqueous phases is affected by pH and ionic strength. Finally, results based on the use of reference humic and fulvic acids have been compared with systems using natural water samples to demonstrate environmental relevance.

Methods

QD suspensions (in hexane) (3-nm TOPO-capped CdSe) were mixed with the same amount of 20 ppm HA, 20 ppm FA, deionized water, or Creek water in a clear vial. In between measurements, set-ups were continuously stirred and protected from light. This was stopped after 7 days by separating the different layers into individual vials. Phase transfer was also monitored as a function of the pH of the aqueous solutions, ionic strength and HA concentration. Absorption and emission spectroscopy were used to monitor the transfer of the QD particles from the organic solvent into the aqueous phase on an hourly/daily basis. Sample aggregation was monitored over time using dynamic light scattering (DLS) while transmission electron microscopy (TEM) and infrared spectroscopy (IR) were used to characterize the mechanisms of interactions. Approximately 1.0-10.0 μM of QD solutions were used in the experiments.

Results and Discussion

Evidence of phase transfer is clearly visible in the digital photographs shown in Figure 1 and is also apparent from the absorption spectra of the organic and aqueous phases. Figure 2 shows a strong diminution in the intensity of the QD optical absorptions in the organic phase alongside an increase in the optical absorbance of the aqueous phase after 24 hours of equilibration.
As seen from the increased absorption baseline and DLS results, this aqueous solubilization involves transfer of QD aggregates instead of individual QDs. The DLS correlation curves shows that the correlogram for the FA—QD composites decays to the baseline over a much longer period of time as compared to FA molecules alone, indicating slower diffusion and thus greater aggregation in these samples.[19] In addition, when transferred to the aqueous phase, the structural integrity of the QDs is still retained. This is supported by the photoluminescence spectra which show that the band edge emission is still clearly visible. This is significant because leaching of Cd^{2+} ions into the environment is a major concern.[20] However, the quantum yield of the transferred QDs is significantly diminished. This maybe a result of quenching from the

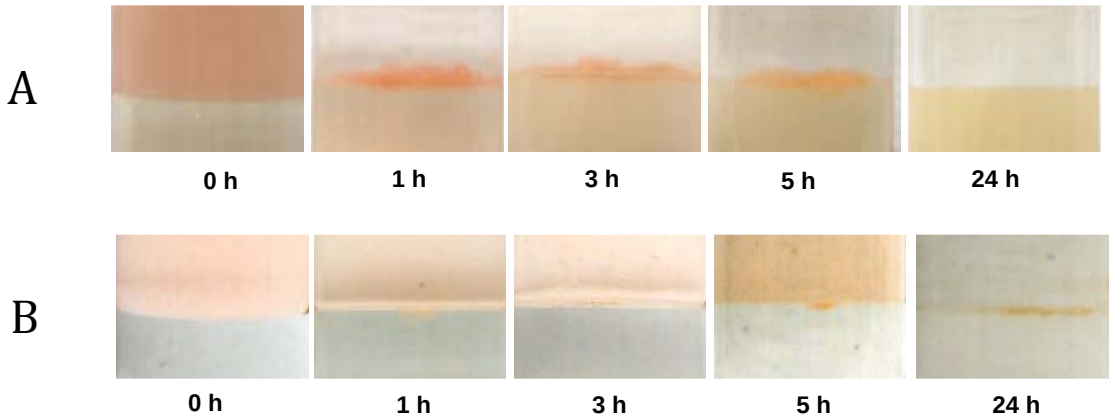

Figure 1. (A) Digital photographs illustrating transfer of 3-nm TOPO-capped CdSe in hexane (top layer) to the aqueous phase (bottom layer) containing 20 ppm HA. (B) Control set-ups for the phase transfer experiments with deionized water only.

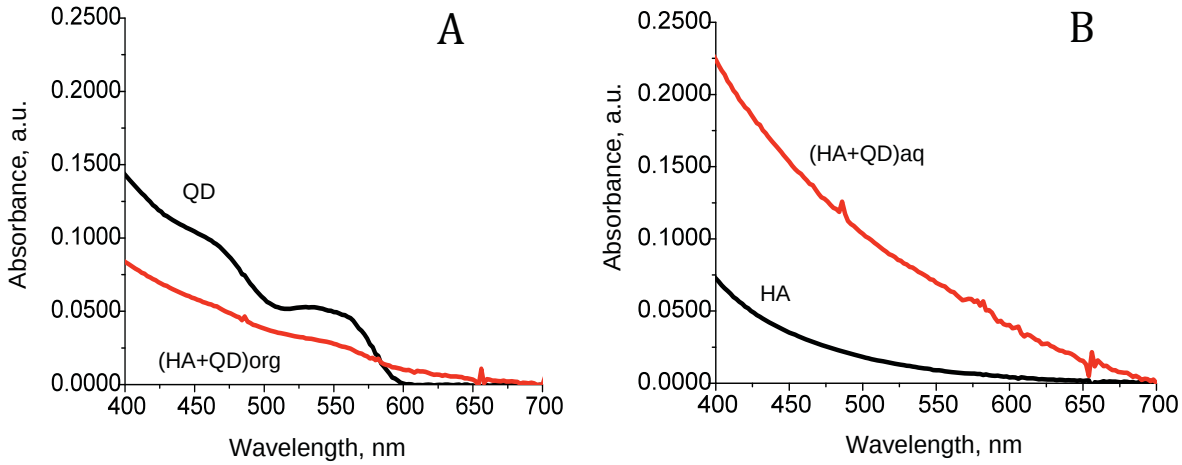

Figure 2. A) Hexane layer: UV-Vis absorption spectra of the 3-nm CdSe-TOPO after phase transfer, (HA+QD)org, in comparison with the QD spectra. B) Aqueous layer with 20 ppm HA solution: UV-Vis absorption spectra of the 3-nm CdSe-TOPO that transferred from the hexane layer, (HA+QD)aq, in comparison with the HA spectra.

NOM components and/or alternatively, a result of the immobilization of QDs within the solid humic matrix.

The size and morphology of aggregated and flocculated structures were characterized by TEM. TEM images of the QDs, HA, and the phase-transferred QDs are given in Figure 3. QD-HA aggregation, which is consistent with the increase in the absorption baseline and the DLS measurements discussed above, is shown clearly in Figure 3D with several embedded QDs demarcated in white clearly shows the lattice planes of several QDs residing within the amorphous humic matrix. Furthermore, surface-related interactions were determined by FTIR

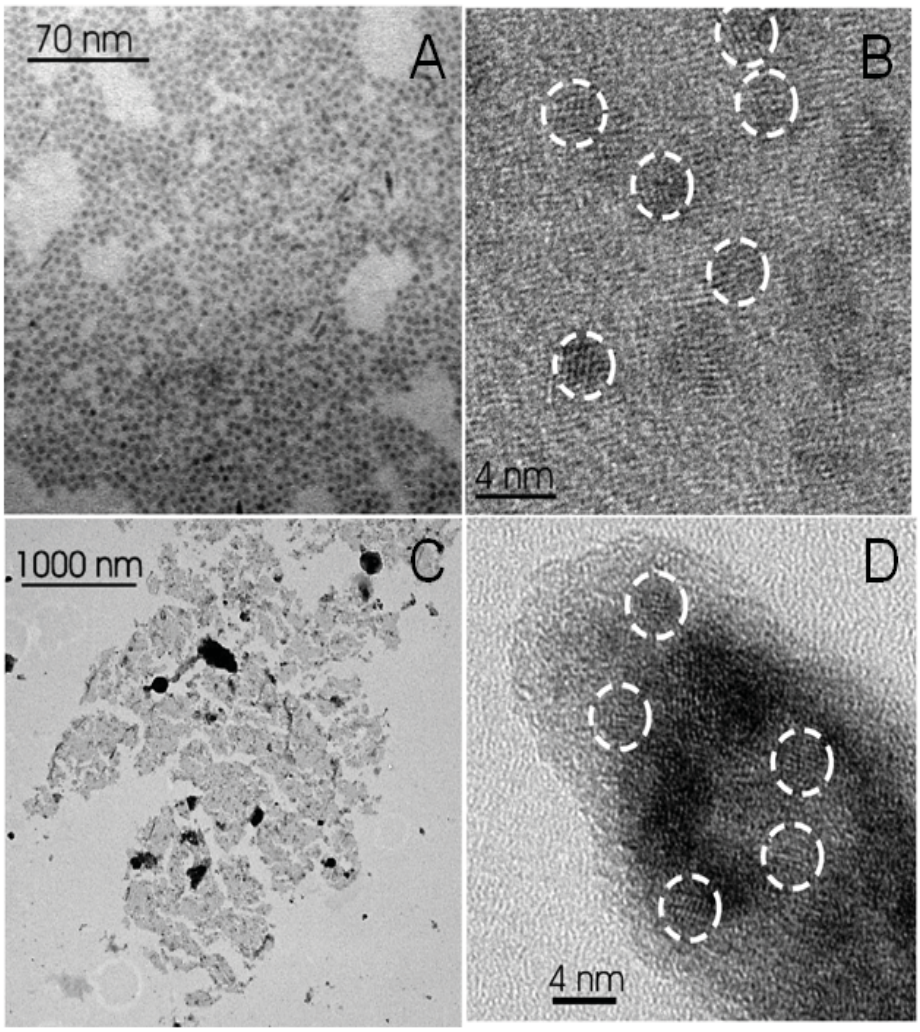

Figure 3. Low-resolution (A) and High-resolution (B) TEM image of the 3-nm CdSe-TOPO particles. Low-resolution (C) and High-resolution (D) TEM image showing phase-transferred QDs embedded in HA.

spectroscopy which provided more information corroborating the proposed model involving possible encapsulation of QDs by humic acid micelles. Considering the spectral signatures of the TOPO-capped CdSe and the HA, Figure 4, shows that the most noteworthy feature in the spectrum for the transferred QDs is that the TOPO peaks are essentially preserved, P=O stretch at ~1150 cm^{-1} and C-H stretch at ~2900 cm^{-1}. This indicates that TOPO ligands are definitely part of the phase-transferred QD which strongly suggests interaction between hydrophobic segments of the HA and hydrophobic alkyl chains of TOPO ligand on the QD. In addition, the asymmetric stretching bands of the carboxyl groups of HA undergo noteworthy changes which show possible coordination of the carboxylate groups of HA to Cd^{2+} surface sites that may play a role in the QD-NOM interaction.

Additional information on NOM-QD interactions comes from the phase transfer experiments performed by varying the solution pH or ionic strength of the aqueous phase. Both pH and ionic strength influence the structural conformation of the HA and FA moieties. Adjusting the pH of

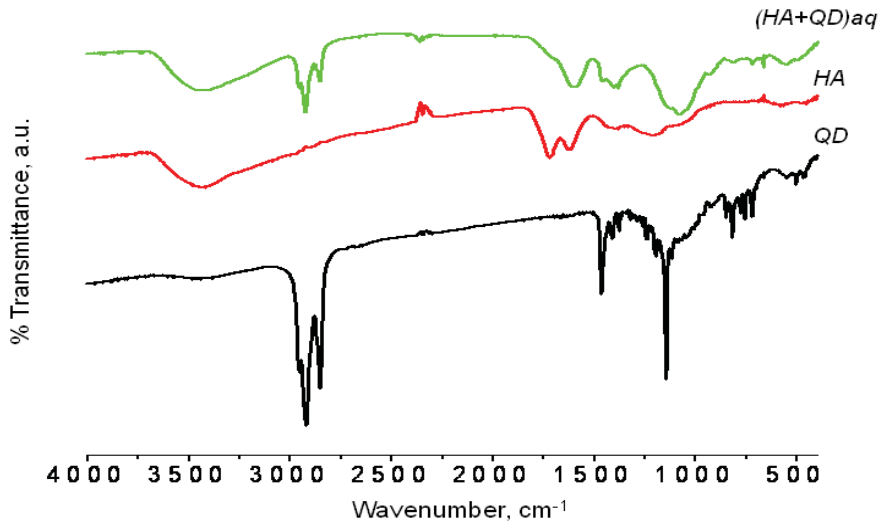

Figure 4. IR spectra fpr TOPO-capped CdSe QD, Suwannee River HA and the phase-transferred QD.

the aqueous solutions to low pH values results in HA and FA which are aggregated. At this range, the rate of phase transfer was observed to be fastest. In the same manner, the presence of cation, such as Ca (II), engenders aggregation of component HAs and FAs through its metal chelating ability. However, the phase transfer experiments using varying ionic strengths showed that the presence of $CaCl_2$ only increases the rate at which the QDs flocculate at the aqueous/organic interface and not the actual solubilization of the HA-QD composites.

To determine how results using the model NOM could translate into the behavior of QDs in the natural environment, experiments were performed using natural surface water samples collected from Tonawanda (TON) and Buffalo (BUF) Creeks, containing 11 and 5 ppm dissolved organic carbon (DOC), respectively. Results showed that only TON water sample was able to exhibit significant transfer of QDs into the aqueous phase in one day whereas in the BUF set-ups the QDs remain at the interface and do not transfer. This slow transfer of QDs settling at the hexane/water interface in the BUF set-up may indicate occurence of other factors affecting phase transfer which were not investigated in this paper.

Conclusions

This study presents the first evidence of NOM-QD interactions based on simple phase transfer experiments. HA and FA systems are able to engender the phase transfer of TOPO-capped QDs from hexane to water. Remarkably, NOM is able to stabilize in aqueous phase, hydrophobic systems that otherwise have very little tendency to dissolve in water. These results clearly illustrate that NOM present in the aqueous environment will have a strong influence on the partitioning and transport of these novel manufactured nanomaterials. Our spectroscopic measurements and control experiments point to the mechanism where the humic substances

essentially form micelles that can encapsulate the hydrophobic quantum dots with the polar functional groups on the humics engendering solubility in water.[21, 22] The precise structural conformations, however, are difficult to discern as it could essentially comprise of aggregates with random coil conformations, micelles with hydrophobic cavities, or a mixture of other such conformations. Furthermore, coordination of humic substances with QD surfaces following displacement of surface capping groups remains a possibility. The results here demonstrate the importance of the surface capping ligands on the QD surfaces and support the shift to biocompatible ligands based on poly(ethylene glycol).[23]

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Conference Questions and Answers

Question:
Did you differentiate the effect of capping agent interactions with the humic materials? You said there was displacement of the surface capping groups as well as interactions of the humic/fulvic acids with the capping groups themselves.

Answer:
We have not done this and have not determined what technique could be used.

Question:
Do you have any quantitative information on the partition coefficient? Did you do a mass balance to see exactly how much there is in each phase?

Answer:
We measured the cadmium concentration in the organic and aqueous phases by ICP/OES

(inductively coupled plasma/optical emission spectrometry), but we cannot associate this with the actual transfer, since there may have been leaching from the quantum dot.

Question:
On the transmission electron microscopy (TEM), you show aggregates; however, they are often seen due to evaporation. Did you do cryo-TEM to correct for this potential?

Answer:
We did not do cryo-TEM, but the overall evidence does point to aggregation.

Question:
When you showed images of quantum dots or aggregates at the interface between hexane and water you used the word "settling." When the dots or aggregates are in hexane, they are at 3 nm and unlikely to settle. Did you mean "settling" or something else?

Answer:
The solutions are continually stirred and the settlement is perhaps due to the interaction of the quantum dots with humic acids that allows them to aggregate and then settle out.

Natural Organic Matter Enhanced C_{60} Fullerene Dispersion in the Aqueous Phase

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Abstract

Assessing exposure and risk of engineered nanomaterials requires accurate prediction of their concentrations and physicochemical properties in the natural environment. Although C_{60} fullerene is virtually insoluble in water, stable aqueous suspension of C_{60} nanoparticles (nC_{60}) can form when C_{60} powder is mixed with water for an extended period of time. In this study, we investigate the effect of natural organic matter (NOM) on the dispersion of C_{60} in water as well as the properties of nC_{60} particles formed. We used Suwannee River humic acid (SRHA) and fulvic acid (SRFA) standards as model NOM compounds and tested a range of solution conditions (i.e., pH, total ionic strength and ionic composition) to simulate realistic natural aqueous environment. NOM was found to greatly increase C_{60} dispersion in water, and the dispersed C_{60} concentration increased with NOM concentration. Total ionic strength and calcium ion concentration also played a role in C_{60} dispersion. The effect of NOM was further enhanced under sunlight. UV/Vis spectra of the nC_{60} suspensions formed in the presence of NOM under sunlight suggest photochemical transformation of C_{60}. Further investigation is necessary to reveal the reaction mechanisms and to identify the products.

Introduction

Carbon-based nanomaterials have received increasing attention for their potential application in electronics, optics, and pharmaceuticals [1-3]. In particular, C_{60} fullerene is being produced at industrial-scale in tons per year [4]. Potential non-regulated discharge and incidental spill of C_{60} into the environment raises growing concern on its impact on the ecosystem as well as human health.

Although virtually insoluble in water[5], C_{60} can form stable aqueous suspensions of nanoparticles (nC_{60}) when mixed with water for an extended period of time [6-11]. The process typically took weeks to months[6, 7, 9, 10]. The amount of C_{60} that can be dispersed in water, i.e. "solubility" of nC_{60}, has not been quantified. In addition, almost all previous studies used organic-free water. The impact of natural organic matter (NOM), which has been shown to alter particle properties of preformed nC_{60}[12] and stabilize nC_{60} [13] as well as multi-walled carbon nanotubes [14], is unknown. In this study, we investigated the effect of NOM on direct dispersion of C_{60} in water under typical natural water conditions. The "solubility" of nC_{60} was quantified at different concentrations of NOM under systematically varied solution and light conditions. The physicochemical properties of the nC_{60} particles formed including particle size, morphology, and surface charge were carefully characterized.

Experimental Methods

Dry C_{60} powder was mixed with test solutions containing 0 to 20 mg/L of Suwannee River humic acid (SRHA) or fulvid acid (SRFA) in dark, with room light or sunlight. The total ionic strength of the test solutions ranged from 0.1 to 10 mM, adjusted using NaCl and $CaCl_2$. Concentration of C_{60} in all nC_{60} suspensions was determined by total organic carbon (TOC) measurement using a high-sensitivity TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD). Experiments in sunlight were performed on 10 consecutive sunny days during the summer. Mixing in dark or room light went on for up to 29 days. Samples were retrieved from each test suspension at predetermined times, filtered through 2-µm and 0.45-µm- pore-size membrane filters, and stored in darkness at 4°C before analysis. All samples were analyzed shortly after collection.

Hydrodynamic diameter and electrophoretic mobility of nC_{60} were measured by dynamic light scattering and phase analysis light scattering, respectively, using ZetaPALS (Brookhaven Instruments, Holtsville, NY). nC_{60} particle morphology and structure were analyzed using a JEOL-2010 TEM (JEOL Inc., Peabody, MA). UV/Vis absorbance spectra of nC_{60} suspensions were obtained using a dual beam, high resolution UV/Vis spectrophotometer (UV-2550, Shimadzu Scientific Instruments, Columbia, MD).

Results and Discussion

C_{60} dispersion kinetics. NOM significantly increases the rate of C_{60} dispersion in water (Figure 1), and the amount of C_{60} dispersed in water increased with NOM concentration. The rate of dispersion strongly depends on the total ionic strength (Figure 2). The amount of C_{60} dispersed increases significantly with decreasing total ionic strength. Although Ca^{2+} usually reduces colloidal stability due to its more efficient charge screening compared to monovalent cations, the presence of Ca^{2+} did not seem to affect C_{60} dispersion. In the presence of sunlight, C_{60} dispersion is greatly enhanced (Figure 3): In the presence of 1 mM Ca^{2+} and 10 mg/L SRHA with a total ionic strength of 10 mM, 9.9 mg/L of C_{60} was found in the aqueous phase after only 72 hours of mixing. This nC_{60} concentration is well above the minimal inhibitory concentration (MIC) or median lethal dosage (LC_{50}) of several bacteria according to literatures[8, 15, 16]. Under

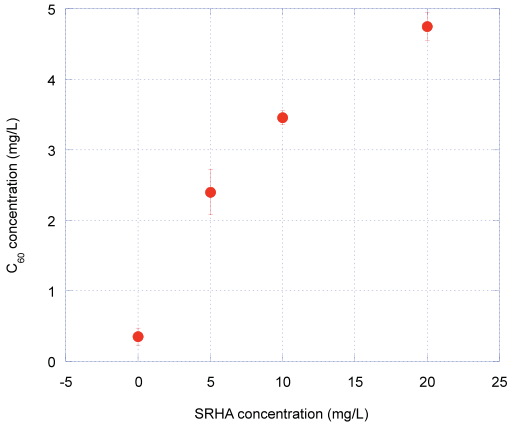

Figure 1. Effect of NOM concentration on fullerene dispersion. Samples were mixed for 72 hrs with sunlight; ionic strength = 1 mM.

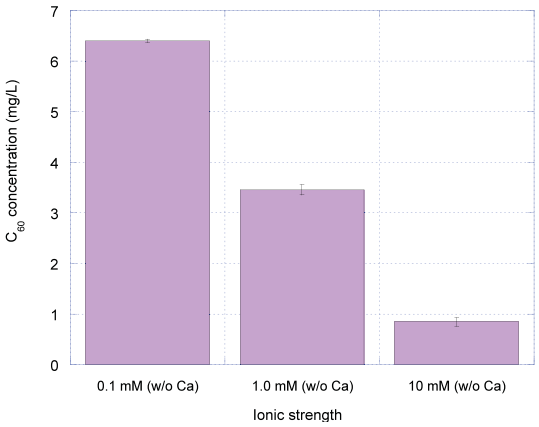

Figure 2. Effect of solution condition on fullerene dispersion. Samples were mixed for 72 hrs in 10 mg/L SRHA with sunlight

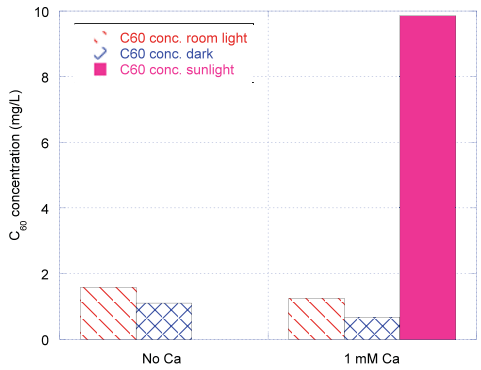

Figure 3. Fullerene dispersion under dark and fluorescent room light conditions. Samples were mixed for 72 hrs in 10 mg/L SRHA; ionic strength =

dark or fluorescent room light conditions, however, C_{60} dispersion rate was substantially lower. Increasing NOM concentration again increased C_{60} dispersion.

Dynamic light scattering measurement showed a rapid decrease in nC_{60} particle size during the dispersion process under sun light. At the same time, nC_{60} particle surface zeta potential became more negative with mixing time. Consistent with the classic colloidal theory, nC_{60} particle size increased with the total ionic strength and Ca^{2+} concentration. It is noteworthy that extremely small nC_{60} particles (less than 5 nm in diameter) formed after 72 hours of mixing at the lowest ionic strength tested (0.1 mM). These particles are much smaller than those previous reported in studies using organic free water.

Potential photochemical derivatization of C_{60}. Figure 4 compares the UV/Vis absorbance spectra of the nC_{60} suspensions prepared by direct dispersing in NOM solutions and NOM-free water, and nC_{60} formed through the solvent exchange method [13]. nC_{60} particles preformed using the solvent exchange method maintains C_{60} characteristic absorbance peaks at 266 nm and 343

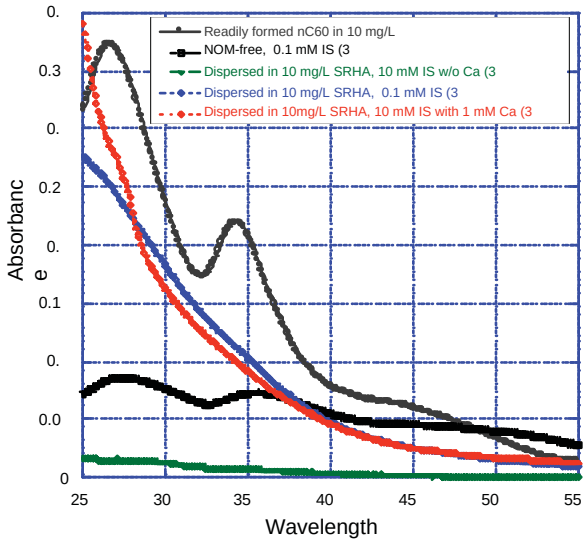

Figure 4. UV/Vis spectra of C_{60} aqueous suspensions prepared in NOM solutions.

nm in the presence and absence of NOM, indicating that adsorption of NOM on nC_{60} particle surface does not interfere with its UV absorbance. Similarly, nC_{60} dispersed in NOM-free water showed characteristic peaks at 273 nm and 355 nm. The slight red shift is probably the result of aggregate formation. These results are in consistency with previous reports [8, 9, 17-19]. However, nC_{60} directly dispersed in NOM solutions did not show any of the C_{60} characteristic absorbance peaks in all solution conditions tested. The absorbance spectra remained featureless after removal of free NOM molecules using a dialysis membrane with molecular weight cutoff of 5000 Dalton. These results indicate that C_{60} may have been photochemically derivatized when dispersed in NOM solutions under sun light.

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Conference Questions and Answers

Question:
What was the filter size used to measure the C60 in the aqueous solution?

Answer:
We used two filter sizes. For particle size analysis, we used a 2-micrometer (µm) filter followed by a .45-micrometer filter, because we wanted to include all non-separatable particles in the suspension. There was very little difference in the two methods, because most of the particles are smaller than .45 micrometers. Before sampling we let particulate in the sample water settle, because we found that particulate buildup (filtercake) on a filter had the potential to block the flow of particles smaller than .45 micrometers.

Question:
You used TEM. Could you describe how you sampled?

Answer:
At the beginning of the project we compared dried samples and ones prepared cryogenically and found that there was no real difference between the techniques. For sample preparation, we placed a 3-microliter (µL) sample on a grid in an evaporating environment and allowed it to dry

overnight.

Question:
For the dynamic light scattering (DLS) measurements, you had a choice between per-mass/per-number or scattered cross-section. Why did you choose one over the other?

Answer:
When using DLS you can get number-based averages, intensity-based averages, or mass-based averages. We chose to use number-based averages, because particle population was more important for our project. Intensity is proportional to the diameter of the particle to the power of 6, so if you have some larger particles the results are shifted towards the larger population.

Question:
With time mixing and the source of the light, are you saying that you have degradation in terms of carboxylation of materials?

Answer:
We are not sure it is carboxylation. We have some hypotheses about what is happening, but we have not done any work to identify the reaction.

Question:
Do you have increasing negative charge with time?

Answer:
There is increasing negative charge in the de-ionized water, but we have not identified why.

Interactions of Bacteria with Engineered Metal, Metalloid, and Metal Oxide Nanomaterials

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Abstract

Engineered nanomaterials in the environment can have many interactions with bacteria, including toxicity, breakdown, and accumulation. Such interactions will have consequences for the transport and fate of nanomaterials, and thus the broader environmental and societal outcomes. This talk presents laboratory research results regarding interactions of environmental strains of *Pseudomonas* with several metal, metalloid, and metal oxide nanomaterials. We ask: how do engineered nanomaterials affect bacteria, and how do bacteria affect the nanomaterials? The focus is on bacterial physiological effects and nanomaterials fates with follow-on to toxicity mechanisms where appropriate. Three projects are discussed: 1) comparative effects and fates of Cd(II) ions versus bare CdSe quantum dots with *Pseudomonas aeruginosa* PG201, a relatively cadmium-resistant bacterial strain, 2) effects of several industrial metal oxide nanomaterials on growing *P. aeruginosa* and bacterial effects on nanomaterials, and 3) effects of various TiO_2 on *P. putida* growth and bacterial effects on nanomaterial structures. Our research reveals several outcomes of interest, i.e. that nanoparticles can have specific particle effects on bacteria that are not explainable by the toxic metal content of the nanoparticles, that bacteria can change nanoparticle aggregation states, that aggregation may preclude toxicity of some nanoparticles to bacteria, and that photoactive nanoparticles can be toxic to bacteria in the dark. These results are applicable to envisioning possible outcomes of nanomaterial release into the natural environment where bacteria are abundant, readily colonize surfaces, and catalyze essential reactions.

Introduction

The fates of engineered nanomaterials (ENMs) in the environment could depend substantially on their interactions with bacteria. Laboratory research with bacterial cultures and introduced ENMs can reveal potential interactions that are environmentally-relevant such as ENM binding to the cell envelope [1-3], uptake of ENMs either nonspecifically [4] through damaged membranes [5, 6] or via specific receptors in the light [7], and dose-dependent reductions in population growth [1, 8-10]. In some cases, bacterial inhibition appears to arise from toxic metal ions released from metallic ENMs [11], yet there is also evidence for ENM-specific effects in that ENM shape altered inhibition patterns [9] and ENM capping with another material did not [7]. Extrapolating such findings to the environment is challenging, in part because ENMs can readily aggregate under environmental conditions and possibly lose ENM-specific characteristics. However, bacteria may in fact alter ENM aggregation states in, for example, wastewater treatment systems [12]. Our research regards bacterial interactions with pre-aggregated as well as dispersed ENMs, with particular interests in bacterial effects on aggregation states and the toxicity of ENMs that are either stable in

suspension or become dispersed.

Methods

Two gram negative bacterial species, both widely-distributed in nature and known to be metabolically-versatile and resistant to many toxic substances, were studied in separate pure culture experiments in the laboratory: *Pseudomonas aeruginosa* and *P. putida*. Rich media was used to simulate one type of environment, i.e. an environment rich in reduced organic nutrients and salts; rich media (Luria Bertani or LB broth) was also used to avoid starvation stress associated with nutrient-limiting conditions. Several metal oxides from industrial sources, including TiO_2, were amended to cultures at the inoculation stage. Separate experiments were also performed with CdSe quantum dots. As described elsewhere (Priester et al., manuscript in preparation; Horst et al., manuscript in preparation), growth was monitored by optical density and DNA analysis. Cell morphologies and associations with NMs were visualized with electron microscopy (EM), and in the case of CdSe QDs, the distribution of metals was quantitatively analyzed using various spectrometric methods. Evidence for intracellular oxidative stress was also acquired.

Results

As described elsewhere, (Priester et al., manuscript in preparation; Horst et al., manuscript in preparation), a range of observations were made including: 1) variations in bacterial colonization of NM aggregates, 3) variations in NM disaggregation in the presence of bacteria, 4) NM inhibition of bacterial growth concurrent with disaggregation, and 4) both ion and NM-specific growth inhibition. The observations are in the forms of EM images, quantitative growth data, and quantitative analyses of NM integrity as well as metal distribution.

Discussion and Conclusions

Our research reveals several outcomes of interest, i.e. that nanoparticles can have specific particle effects on bacteria that are not explainable by the toxic metal content of the nanoparticles, that bacteria can change nanoparticle aggregation states, that aggregation may relate to the toxicity of some nanoparticles to bacteria, and that photoactive nanoparticles can be toxic to bacteria in the dark. These results are applicable to envisioning possible outcomes of nanomaterial release into the natural environment where bacteria are abundant, readily colonize surfaces, and catalyze essential reactions.

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Conference Questions and Answers

Question:
How strongly does acetate have the ability to chelate cadmium (Cd)?

Answer:
I do not know.

Comment:
You might want to try the experiment again with a simple Cd salt that does not complex heavily. The difference in toxicity may be due to the Cd being removed from availability by complexation.

Question:

Do you know why the Cd appeared to be associated with specific areas of the bacteria membrane?

Answer:
These areas may have been where the efflux membrane pumps were.

Structure of Iron Oxide Nanoparticles; Influence of pH and Natural Organic Macromolecules

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University of Birmingham, Birmingham, United Kingdom

Abstract

Metal oxide nanoparticles (defined as material with at least one dimension is between 1 and 100 nm) are being exploited in a host of industrial and commercial products and processes remediation of ground and drinking water. Iron oxides in particular are used in vast quantities. However, due to the small size, increased surface area and related effects nanoparticles differ from their bulk counterparts in significant and unexpected ways. Studies have highlighted that there may also be environmental risks as the nanoparticle industry grows. With nanoparticles entering the environment, knowledge of fate and transportation in ground and surface waters is essential.

Synthesized iron oxide nanoparticles were mixed with an aquatic fulvic acid and a peat humic acid at different concentrations (0-25 mg L-1) and pH values (2-10). The suspensions were analysed by particle size using flow field-flow fractionation (FlFFF), dynamic light scattering (DLS) and electrophoresis. Primary particle size increased with both increased concentration of natural organic macromolecules (NOM) and with pH. Particle aggregation occurred at low pH and was extensive at pH 6 and higher. Some stabilisation with NOM occurred, although aggregation was found to increase at higher NOM concentrations. Aggregation occurred as surface charge approached zero, as no stabilising surface active agents (apart from NOM) were added in this system. NOM were found to form surface coatings on the iron oxide nanoparticles which were only 1-2 nm in thickness. The influence of pH and NOM concentration will affect the fate and bioavailability of nanoparticles in the aquatic environment due to these changes in surface properties, aggregation and subsequent sedimentation.

Introduction

In the past 20 years, there has been a marked increase in the application of nanotechnology in industry. Nanoparticles consisting of carbon, metal, metal oxides and other compounds, are defined as having one dimension measuring between 1 and 100 nm. They have unusual properties that are not observed in the bulk material, including large specific surface areas. At just a few nanometres, quantum effects begin to dominate affecting optical, conductive and semi-conductive properties (Alivisatos, 1996, Owen and Depledge, 2005), a characteristic which has been driving innovation in the electronics industry. Nanoparticles also have rheological, optical and adhesion properties that make nanoparticles applicable in metallic paints and thin films (Kendall, et al., 2004). Other applications include clear sun creams (Villalobos-Hernandez and Muller-Goymann, 2006), self cleaning windows, solar panel technology, car tyres, hydrogen fuel storage cells and anti-microbial laundry products (Colvin, 2003). The nanotechnology sector has created a multibillion US dollar market, and is expected to grow to 1 trillion US

dollars by 2015 (Aitken, et al., 2006). With huge investments and wide applications, release of products containing nanoparticles to the aquatic environment is inevitable. While there are clear benefits from the use of this technology, there is little understanding of their environmental fate, behaviour and ecotoxicology. A few toxicological studies have revealed that some nano-sized materials are more toxic than their bulk counter parts. Studies involving human and animal cells, plants and aquatic fauna have found varying degrees of toxicity ranging from irritation and aggression (Smith, et al., 2007), oxidative stress in fish (Oberdorster, 2004) and, ultimately, death. Interference and damage to DNA has been highlighted (Lewinski, et al., 2008).

Iron oxide (FeOx) nanoparticles are used in industry, particularly as pigments, catalysts, medical devices, sensors, recording media, and thin films (Cornell and Schwertmann, 2003, Jolivet, et al., 2006, Navrotsky, et al., 2008). Haematite is the most stable of all the nanoparticle iron oxides, with strong absorption to water (Navrotsky, et al., 2008).

Iron reactive barriers use zero-valent iron (ZVI) as a soil and ground water decontaminant. It is more effective at the nanoscale due to its greater reactivity and has shown to be an effective treatment where there is arsenic contamination (Giasuddin, et al., 2007), but rapidly forms an oxide layer resulting in a similar surface chemistry to the iron oxide (Sohn, et al., 2006). Iron oxides are more stable than nanoscale ZVI and therefore a more suitable material to study under laboratory conditions.

Despite the advantages that might be gained from using iron reactive barriers to remediate ground water, oxygen depletion (Zhang, 2003), an increase in hydrogen gas coupled with an increase in microbial population (Gu, et al., 2002) have been noted side effects. Moreover, cytotoxicity of iron oxides (Brunner, et al., 2006, Lewinski, et al., 2008), raises questions whether these releases of nanoparticle slurries is environmentally ethical.

The presence of humic and fulvic acids, a fraction of NOM, could stabilise nanoparticles in aquatic systems and increase the rate and distance of transportation in groundwaters and surface waters (Baalousha, et al., 2008, Chen and Elimelech, 2007, Diegoli, et al., 2008, Hyung, et al., 2007). By binding to the surfaces of nanoparticles, thereby creating a surface film, humics and fulvic acids may increase stability of the nanoparticles by charge and steric effects, increasing residence times in the water column. To assess the impact on aggregation behaviour of metal oxide nanoparticles of NOM, a number of laboratory experiments and measurements were conducted by looking at particle size under different pH and humic substance concentrations.

Methods

Experiments were conducted on charge-stabilised iron oxide (haematite) particles (formed by hydrolysis of iron chloride in dilute HCl at 100 °C) (Kendall and Kosseva, 2006, Matijevic and Scheiner, 1978). The resulting particles at pH 2 were used without further modification, and were mixed with varying concentrations (0-25 mg L^{-1}) of IHSS Suwannee river fulvic acid (FA) or peat humic acid (PHA), as a representation of NOM. The solutions were then analysed at different pH values (2-10). Particle sizes of the iron oxides and the iron oxides plus FA or PHA were determined by flow-field flow fractionation (FlFFF) (F1000 Universal Fractionator (Postnova Analytics, Germany) and dynamic light scattering (DLS) (HPPS, Malvern instruments). FlFFF is a chromatography-like technique which separates particles

across a 1 kDa membrane using a low salt concentration eluent and field force applied at right angles to the flow (Schimpf, et al., 2000). Particles are then detected with a UV spectrometer at 254 nm. DLS, in contrast, uses laser to detect Brownian motion in situ and converts the data to a Z-average particle size. Both techniques employ the Stokes- Einstein equation to convert diffusion coefficients into particle diameter. Iron oxide particle were also examined under a transmission electron microscope (TEM Tecnai F120) and characterised with FlFFF and was determined to be ca 7 nm ±2 nm. FeOx was used at concentrations of 200 mg L^{-1}. Electrophoretic mobilities (Zetamaster, Malvern instruments) (charge measurements) were also conducted on all the solutions and point of zero charge was determined to be at pH 7 for the FeOx, lowered by the presence of Cl^- ions. Measurements were carried out across the entire pH range, to represent pH in found in both natural and modified water bodies (such as acid mine drainage and acidified catchments) and to determine the size and aggregation properties at all pH range

Results and Discussion

Results are in agreement with Cromeries et al (Cromieres, et al., 2002) for hematite and Baalousha et al (Baalousha, et al., 2008) for FeOx with Suwannee River humic acid. Particle size increased with increase in pH from pH 2-7 and with an increase in organic matter from 0-25 mg L^{-1}. The particle size maxima from DLS coincided with the point of zero charge of the iron oxide, both in the presence and absence of NOM. Our previous results have had to invoke steric hindrance to explain stability of gold nanoparticles (Diegoli, et al., 2008) In this case, aggregation and stability could be explained purely by charge effects. Increased particle size with pH and NOM could be explained by: 1) a decrease in charge on the Fe oxide particles allowed particle growth by coagulation and aggregation, 2) hydrolysis and precipitation of dissolved iron as the pH rose and 3) by the humics forming a surface layer around the particle and thus shielding the charge. All three processes may be operative, but measurement of truly dissolved iron (using ultrafiltration) indicated that only (1) and (3) were important above pH 4.

FFF results at pH 2-6 allowed a greater discrimination between particles and for instance quantified a surface layer sorbed onto the iron oxide aggregates of ca 1 nm in thickness, which was responsible for charge (and potential steric) effects.

Conclusions

The addition of the fulvic or the peat humic acid and increase in pH caused an increase in hydrodynamic diameter of the nanoparticles. The influence of pH and NOM concentration will affect the fate and bioavailability of nanoparticles in the aquatic environment due to these changes in surface properties by altering aggregation and subsequent sedimentation. The increase in size with NOM may indicate that some stability may occur in the water column allowing some degree of transportation before sedimentation would occur. However, NOM only slowed aggregation and the production of large aggregates occurred rapidly. Implications for environmental transport are clear.

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Conference Questions and Answers

Question:
Did you change the pH of all the carrier solutions?
Answer:
Yes. Keeping everything in equilibrium is very important.

Question:
Is the change in aggregation size due to pH reversible? What happens if you change it one way and then change it back?

Answer:
It is likely that there will be some hysteresis. The extent of this may be due to the sample equilibrium, the advancement of the aggregation, and the final pH. Sample preparation may also have an influence-e.g., how quickly the pH was raised initially, and the order in which the nanoparticles were mixed with the natural organic matter. Irreversible effects may occur so that, under some conditions, fully aggregated samples may not disaggregate.

Surfactive Stabilization of Multi-Walled Carbon Nanotube Dispersions with Dissolved Humic Substances

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Pingheng Zhou, and Eizi Morikawa, J. Bennett Johnston Sr. CAMD Louisiana State University, Baton Rouge, Lousiana, U.S.A.

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Abstract

Soil humic substances (HS) stabilize carbon nanotube (CNT) dispersions, a mechanism we hypothesized arose from the surfactive nature of HS. Experiments dispersing multi-walled CNT in solutions of dissolved Aldrich humic acid (HA) or water-extractable Catlin soil HS demonstrated enhanced stability at 150 and 300 mg L^{-1} added Aldrich HA and Catlin HS, respectively, corresponding with decreased CNT mean particle diameter (MPD) and polydispersivity (PD) of 250 nm and 0.3 for Aldrich HA and 450 nm and 0.35 for Catlin HS. Analogous trends in MPD and PD were observed with addition of the surfactants Brij 35, Triton X-405, and SDS, corresponding to surfactant sorption behavior. NEXAFS characterization showed that Aldrich HA contained highly surfactive domains while Catlin soil possessed a mostly carbohydrate-based structure. This work demonstrates that the chemical structure of humic materials in natural waters is directly linked to their surfactive ability to disperse CNT released into the environment.

Introduction

Research over the past decade has elucidated much about the functionality of CNT and the many chemical derivatives possible, greatly expanding the potential uses of these materials. One potential use involves the environmental application of CNT for removing contaminants. Research was recently conducted in using CNT as a selective sorbent for organic/biological contaminants in water streams, such as carcogenic cyanobacterial microcystins (Yan et al., 2006), a variety of nitro- and chloro-substituted aromatics (Thomas, 1994), and methanol (Burghaus et al., 2007). CNTs also effectively adsorb dissolved heavy metals and actinides, including Cd(II), Cu(II), Ni(II), Pb(II), Zn(II), and Am(III) (Chen and Wang, 2006; Rao et al., 2007; Wang et al., 2005). However, little is actually known regarding how CNT will interact with soil-water systems once released into the environment. The poor water solubility of CNTs (unless

chemically derivitized) makes it difficult to disperse these materials in aqueous solution. Yet, CNT was successfully dispersed by the addition of ionic surfactants such as SDS, NaDDBS, and Dowfax (Vaisman et al., 2006, and references therein). Hyung et al (2007) found that natural organic matter served to stabilize CNT aqueous suspensions, yet there is no agreement on the mechanisms by which this behavior occurs. Thus, it is difficult to predict whether some forms of naturally occurring, biopolymeric substances may promote dispersion, while other may not. For example, polysaccharides do not apparently promote CNT dispersion (Lead, 2008).

The purpose of this work was to demonstrate the mechanism by which humic materials stabilize CNT dispersions in aqueous solution. Discerning this mechanism will facilitate a better understanding of how HS promote CNT dispersion, as well as provide a means for making qualitative assessments regarding the type of dissolved HS in the environment.

Materials and Methods

Aliquots of dissolved humic stock solutions were added to 50-mL test tubes containing 100 mg L^{-1} CNT suspension in 5 mM $NaNO_3$ solutions. In separate experiments, dissolved HS solutions were replaced with varying concentrations of the surfactants Brij 35, Triton X, or SDS. The tubes were capped and then shaken for 24 hours. Suspension settling was analyzed using a Varian Carey 50 UV-Vis-NIR spectrometer by reading the absorbance at 600 nm with time (Mathangwane et al., 2008). Suspension particle size was measured using a Brookhaven Instruments 90Plus/BI-MAS dynamic light scattering (DLS) spectrometer. Solution total organic carbon (TOC) was analyzed by a catalytic combustion technique.

Composition of carbon functional group was investigated by near-edge x-ray absorption spectroscopy (NEXAFS) at the carbon K edge. Measurements were carried out at the varied-line-space plane-grating-monochromator (VLSPGM) beamline at the J. Bennett Johnston Sr. Center for Advanced Microstructures and Devices (CAMD) synchrotron light facility, Louisiana State University. The photon energy scale was calibrated for the C 1s-π^* resonance peak using a polystyrene sample (Sigma-Aldrich) which was fixed at 285.4 eV. Sample spectrum were I_0 normalized using the total yield of clean gold mesh placed in the incident beam before sample. C-NEXAFS spectra was processed using the program Athena from the IFEFFIT software package (Newville, 2001). Linear combination fits of the C-NEXAFS spectra were compared to carbon reference standards also analyzed at VLSPGM beamline.

Results & Discussion

The settling behavior of CNT was studied in the presence of two different HS (Fig. 1). Settling data showed a rapid reduction in the solution optical density within the first 15 min. Afterwards, the suspension appeared to stabilize. Settling data showed that CNT suspensions demonstrated enhanced dispersion stability with Aldrich HA additions beginning at 150 mg L^{-1}Aldrich HA, with approx. twice the concentration of dissolved humics required for the Catlin soil HS. Data from DLS measurements showed that CNT MPD readily dropped to 600 nm with the addition of 5 mg L^{-1} Aldrich HA (Fig. 2). Further additions of Aldrich HA up to 150 mg L^{-1} and Catlin HS up to 300 mg L^{-1} resulted in a minimized MPD of approx. 250 and 420 nm, respectively. PD index also minimized to approx. 0.30 and 0.35 for the Aldrich HA and Catlin HS, respectively, along with the MPD. Both trends correspond to enhanced dispersion stability and particle size

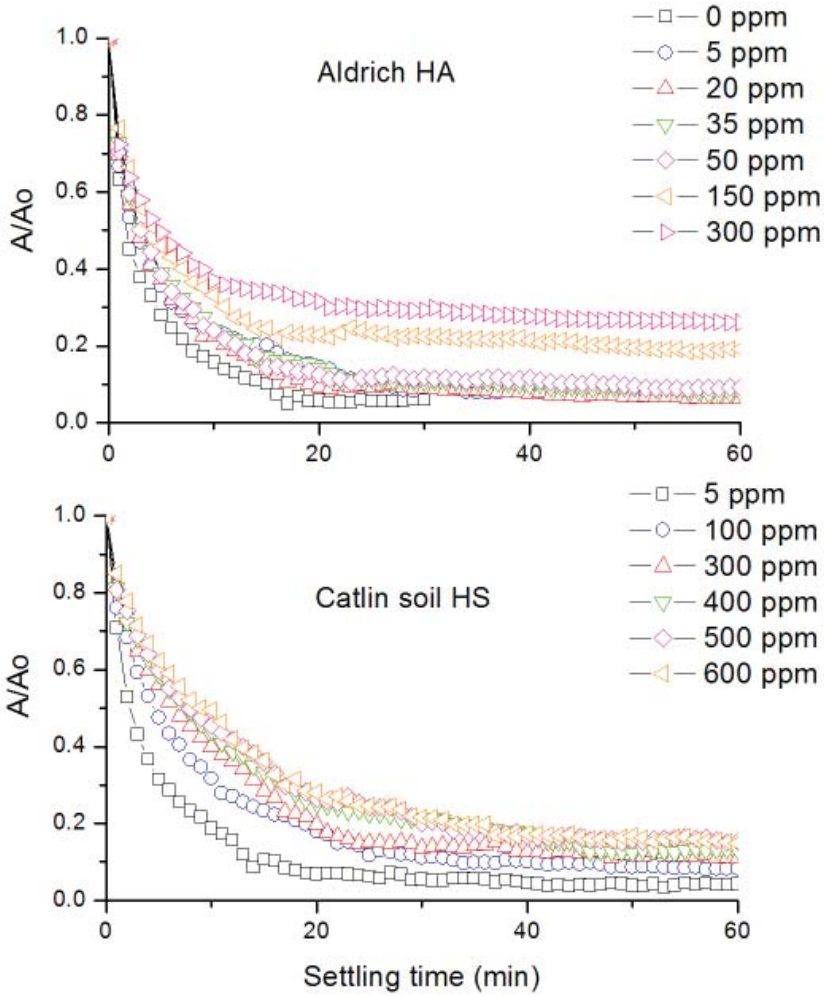

Figure 1. Settling data showing optical density (A/Ao for λ = 600 nm) of a 100 mg L^{-1} CNT dispersion, suspended in 5 mM $NaNO_3$ background solution and varying initial concentrations of dissolved humic substances (obtained from Aldrich humic acid and a Caitlin soil) with time.

homogeneity of CNT – a behavior particular to surfactive molecules.

To test this hypothesis, we conducted similar experiments investigating the effect of surfactants on CNT suspension particle size characteristics (Fig. 3). The data show that CNT MPD minimized to 210, 230, and 370 nm for SDS, Brij 35, and Triton X, respectively. Correspondingly, particle size PD minimized to 0.27, 0.26, and 0.32 for SDS, Brij 35, and Triton X, respectively. Note that CNT MPD and PD minimized in the presence of SDS and Brij 35 to values similar to the Aldrich HA system, indicating that the Aldrich HA exhibited strong surfactive ability. Following this reasoning, the surfactive ability of the Catlin soil HS (like the

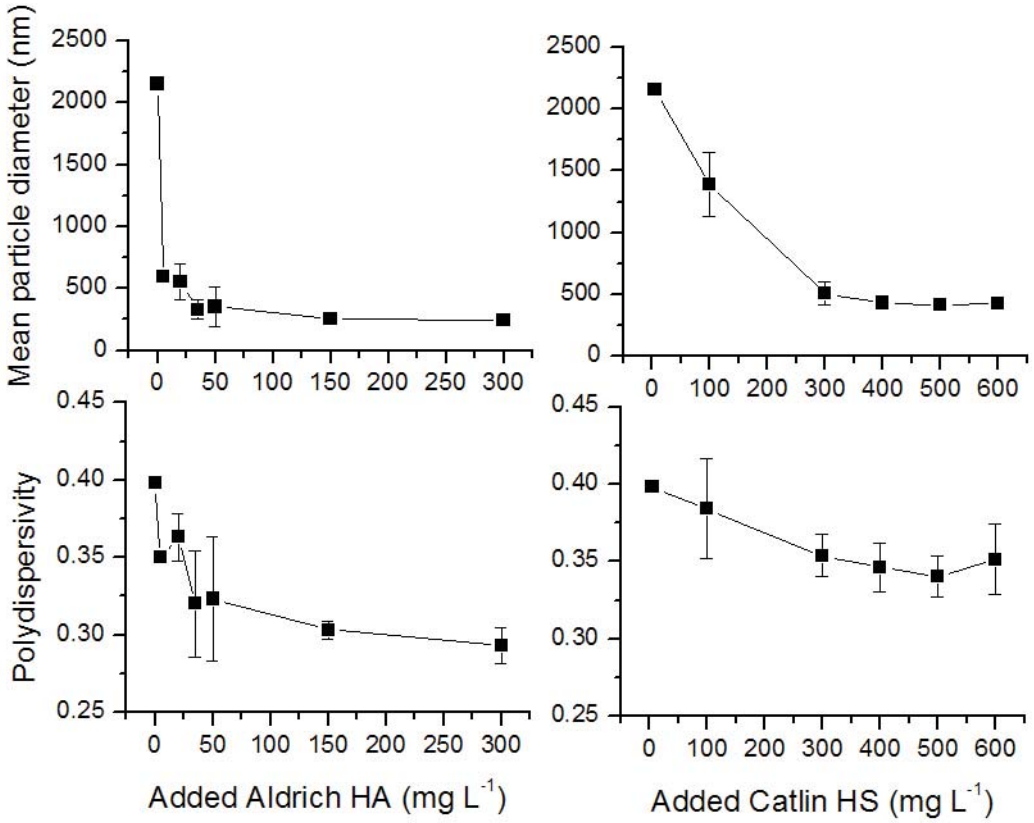

Figure 2. Effect of humic substances on the properties of CNT dispersions suspended in 5 mM $NaNO_3$ background solution. Mean particle diameter and polydispersivity measurements were obtained by dynamic light scattering. Sorption of humic substances to CNT was calculated by difference. Connecting lines are to guide the eye.

Triton X) was less capable of stabilizing CNT dispersions.

Minimization of MPD and PD values for CNT was compared to surfactant sorption isotherms (Fig. 3). All surfactants exhibited a high affinity of sorption for CNT, with individual differences in the sorption behavior. For Brij 35, minimization of CNT MPD and PD coincided with the surfactant saturation on the surface. This behavior is consistent with surfactant behavior in biphasic systems, where surfactant micelles tend to dissociate, and individual surfactant molecules adsorb to the surface, until the surface is saturated with surfactant (Chappell, 2004; Chappell et al., 2005). Surfactants tend to reach sorption maximum around its critical micelle concentration (Chappell et al., 2005, and references therein). Such a trend for the SDS and Triton X surfactants was more difficult to observe given the unexpected shapes of the sorption isotherms. However, for SDS, CNT MPD and PD does appear minimized with the first change in slope (perhaps an intermediate saturation point) of the biphasic sorption isotherm. Triton X sorption quickly maximized, then became negative, indicating reduction of Triton X surface coverage on CNT (supported by both TOC and MS measurements), but the relatively large error

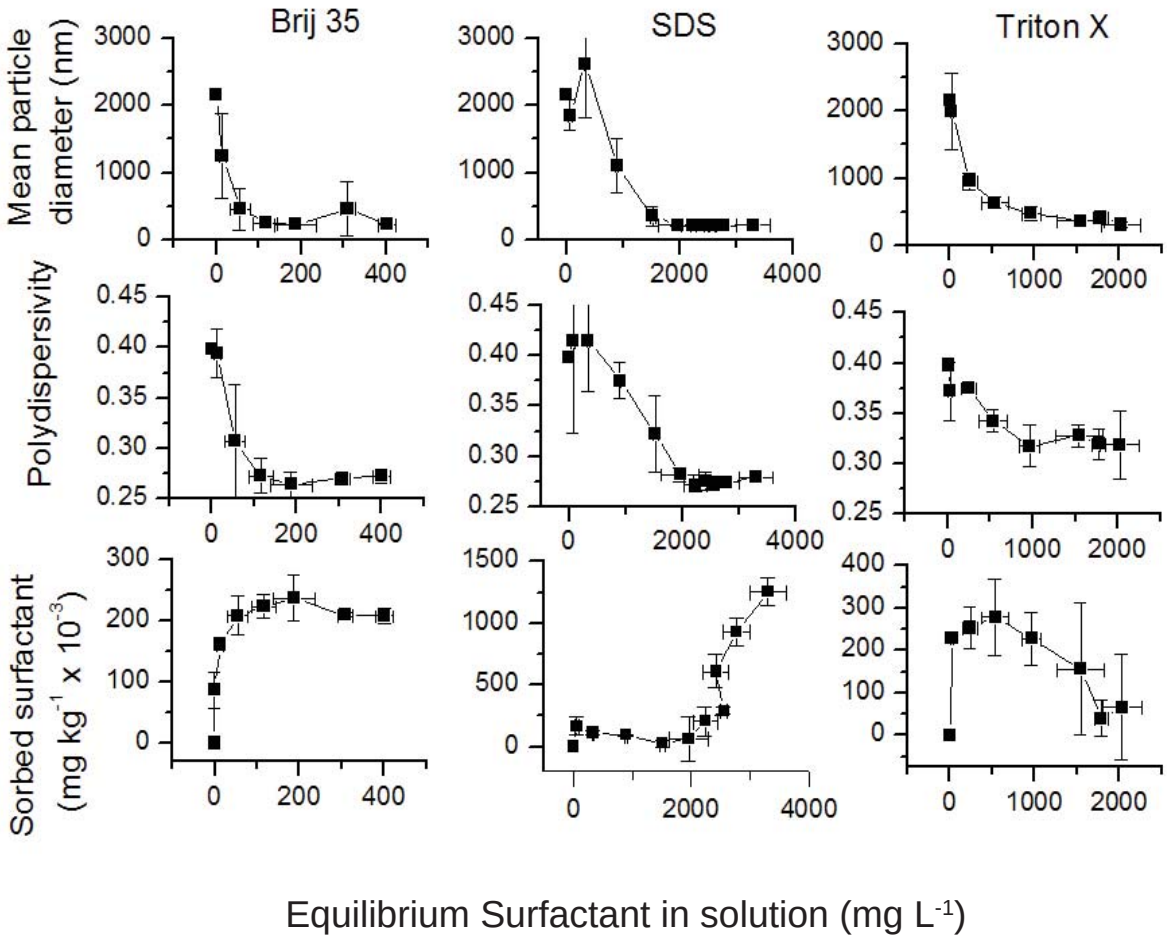

Figure 3. Effect of surfactants on the properties of CNT dispersions suspended in 5 mM $NaNO_3$ background solution. Mean particle diameter and polydispersivity measurements were obtained by dynamic light scattering. Surfactant sorption on CNT was calculated by difference. Connecting lines are to guide the eye.

associated with this data limits this interpretation.

Differences in surfactant sorption (and the resulting CNT MPD) are most likely attributed to differences in the surfactant's structure. For example, CNT exhibited a much higher sorption affinity for nonionic surfactants than the anionically charged SDS. However, the combination of both bulkier hydrophilic and lipophilic moieties of Triton X may have contributed to the poorer surfactive ability relative to Brij 35. Although anionic, SDS showed similar ability of Brij 35 to minimize CNT MPD. This ability may have been related to the simplicity in structure of the surfactant's hydrophilic/lipophilic moieties as well.

We investigated the structure of the Aldrich HA and Catlin soil HS using C-edge NEXAFS (Figure 4) to assess how the above relationships may influence their surfactive ability. Linear combination analysis of the NEXAFS data (Table 1) revealed that the Aldrich HA possessed a

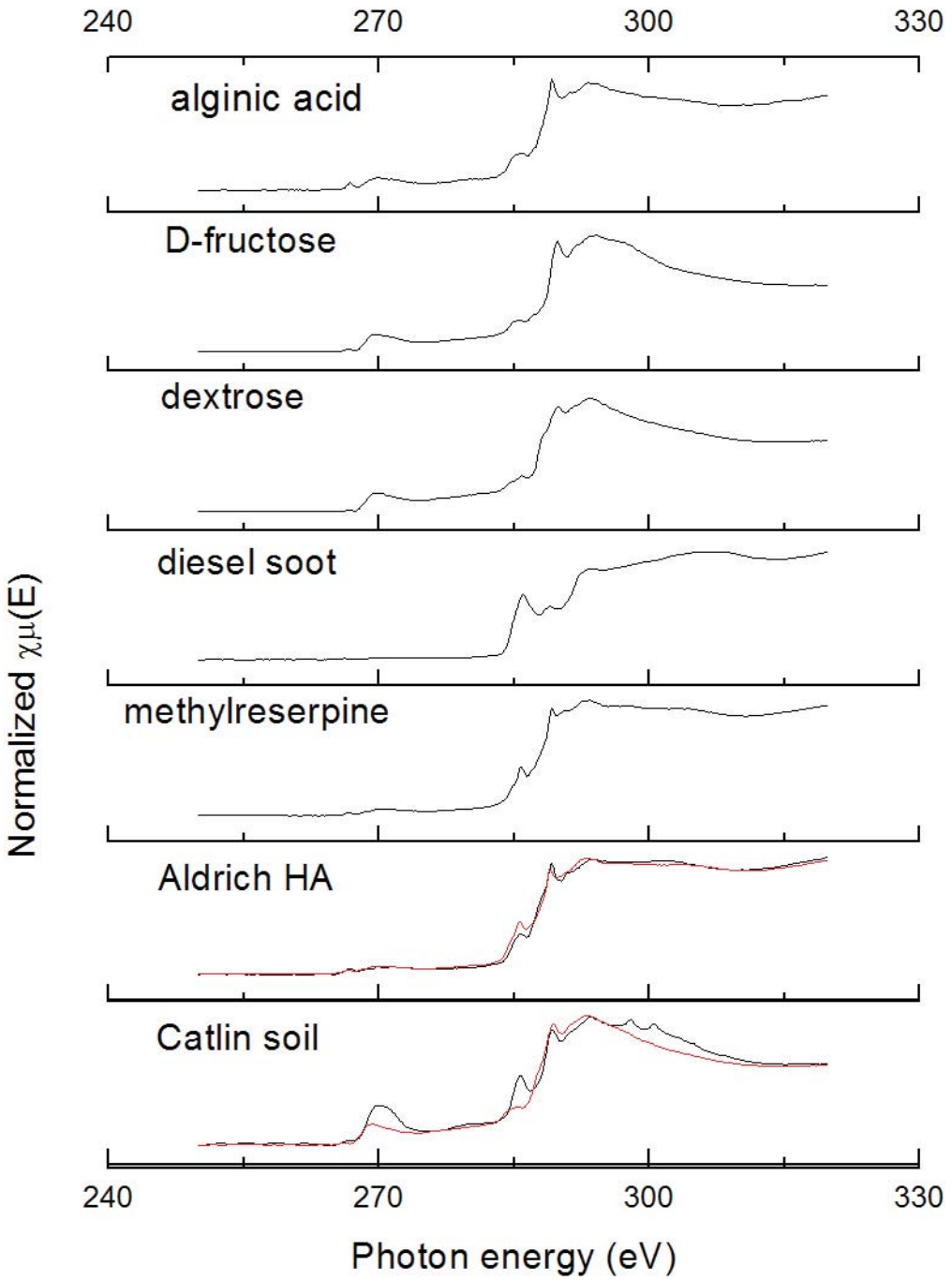

Figure 4. Carbon-edge NEXAFS for the Aldrich HA and Catlin soil HS compared to reference standards. Red lines demonstrate the linear combination fit of the spectra from standards to the Aldrich and Catlin samples. The peaks appearing at approx. 270 eV are due to 2nd order contribution from oxygen K edge absorption.

Table 1. Linear combination fits of the Aldrich HA and Catlin HS carbon-edge NEXAFS spectra.

sample	standard	weight	χ^2	R-factor
Aldrich HA	alginic acid	0.191	0.18776	0.001191
	diesel soot	0.179		
	methyl reserpine	0.629		
Catlin soil HS	D-fructose	0.359	1.070372	0.017806
	glucose	0.641		

structure that was highly aromatic: 63 % analogous to an alkaloid reserpine, 18 % analogous to a black carbon (diesel soot) material, and 19 % analogous to a polymeric polysaccharide (alginic acid). The Catlin soil HS structure was dominated by simple sugars, consisting of glucose and D-fructose-type analogs. Clearly, the superior surfactive ability of the Aldrich HA was linked to the high aromaticity of the black carbon phase (representing the material lipophile), the high polarity of the polymeric polysaccharide phase (representing the hydrophile), and "mixed" alkaloid phase containing oxygen-rich aromatic groups. The saccharide polymer-rich Catlin soil HS exhibited a limited ability to stabilize CNT dispersion because the material lacked a significant hydrophilic domain necessary for surfactive activity.

Conclusion

In this work, the potential of humic substances to stabilize CNT dispersions was demonstrated. This behavior was attributed to the surfactive nature of humics and their ability to promote the smallest CNT particle sizes and homogeneities. As demonstrated with well-defined surfactants, this stabilization is maximized when CNT is saturated with a monolayer of surfactant, which corresponds to the sorption maximum of the sorption isotherm and closeness of the equilibrium surfactant concentration in solution to the CMC value. The superior surfactive ability of the Aldrich HA appeared to be linked to the mixture of strong hydrophilic and lipophilic domains, compared to the Catlin soil HS, which appeared to be overwhelmingly hydrophilic. We conclude from this work that the most natural humic materials should exhibit at least some ability to stabilize CNT dispersions in aqueous environments.

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Conference Questions and Answers

Question:
With humic acids in soils, do you know what will happen with rain or irrigation?

Answer:
Rain or irrigation increases the ionic strength of the solvent and causes the humic acids to swell. This provides an ideal condition for dispersion of colloids in run off, whether they are engineered or natural.

Question:
You added very different concentrations of surfactants, for example, 400 milligrams per liter (mg/L) for Brij-35 (Polyoxyethyleneglycol dodecyl ether) and 4000 milligrams per liter for SDS

(sodium dodecyl sulfate). Why?

Answer:
We wanted to scan above and below the critical micelle concentration (CMC) for each surfactant. Brij-35 has a much lower CMC than the others and hence required a less concentrated solution.

Save

Evaluation of Nanoparticle and Matrix Characteristics Affecting Transport in the Environment

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Abstract

Concern is mounting over the potential for nanomaterials to enter the environment and cause adverse effects to biota and human health. While in vitro and in vivo toxicity research has progressed, there is a critical gap in the scientific literature linking release and exposure potential to the current body of toxicity evaluations on nanomaterials. Few studies have evaluated an ambient environment release scenario and attempted to determine how nanomaterials interact, are transported, and may change physically and chemically. Aluminum nanoparticles are being used in combination with metal oxides in propellants and have the potential to be released to the environment through aerosol deposition. Although aluminum is abundant naturally in the soil matrix, aluminum loading can lead to toxicity if it is transported to aquatic systems in soluble forms. Aluminum chemistry is complicated and the unique characteristics of aluminum at the nano-scale are not well understood. The objective of the study was to evaluate how aluminum nanoparticles changed physically and chemically in different environmentally relevant scenarios and how these changes affect transport. Aluminum nanoparticles were suspended in the different media by sonication and eluted by a forced up-flow system through the soil matrix over 17 hrs at a rate of 3 ml/hr. The type of media used to suspend the nanoparticles had a marked effect on the surface charge, stability, and aggregation state of the nanoparticles prior to introducing to the soil column. The properties of the suspension at the time of introduction and throughout the course of the experiment were important in determining transport. Additionally, the properties of the soil matrix including pore size, charge on the surface of the grains, salt content, and composition further impacted transport. Suspended aluminum nanoparticles with negatively charged surfaces had the highest rate of transport of the scenarios evaluated. Transport was also greater for matrices composed primarily of sand compared to those containing greater proportions of fine particulates. It is clear from these studies that many factors influence the transport of nanoparticles in the environment and transport cannot be reliably predicted from one factor alone, but evaluation should include many different physicochemical aspects of the nanoparticles and soil.

Introduction

Aluminum nanoparticles are incorporated into energetics, alloys, coatings, incendiary devices, and sensors by the Department of Defense (DoD) (Air Force Studies Board 2006). Their use in propellants is increasing because of the high energy release obtainable upon oxidation relative to micron-sized aluminum (Meda et al. 2007). In order to understand the impact of aluminum nanoparticles on the environment, the physical and chemical properties of these materials needs to be correlated to their fate and transport. To date, only studies on the transport of

uncharacterized aluminum nanomaterials has been performed (Doshi et al. 2008). The work presented here investigates the changes to aluminum nanoparticles that occur in different liquid media and the effect of these changes on the transport of aluminum nanoparticles through porous matrices.

Methods

Aluminum nanoparticle suspensions were prepared from powder (nominal 50 nm diameter aluminum, Novacentrix, Inc., Austin, TX) in different room temperature solutions by probe sonication (30 pulses for 1 s/cycle at 50% output [W-380 sonicator, CL4 375 watt converter head, Heat Systems Ultrasonics, Farmingdale, NY]) at a concentration of 1000 mg/L. Suspensions were then diluted to 50 mg/L in 50 mL conical vials. Aliquots were analyzed using laser doppler electrophoresis and dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS (Worcestershire, UK) DLS over a 168-hr time course.

Powdered particles were fixed on Formvar/carbon-coated copper transmission electron microscope (TEM) grids 300 mesh size (Ted Pella, Redding, CA) by aspiration in a vacuum chamber. Imaging was performed on a Jeol 1010 TEM and the diameters of randomly selected individual particles were quantified with Image J software. Statistical analysis of TEM size was conducted with SYSTAT statistical software package and Excel.

An automated system delivered nanoparticle suspensions by upflow to a borosilicate glass (6.6 mm bore size) liquid chromatography column (Biochem Valve, Inc., Boontown, NJ) containing sand or soil packed to a final column density of 1.68 ± 0.01 g/cm3 and 1.60 ± 0.01 g/cm3, respectively. Aluminum nanoparticle suspensions were delivered to the column at 3 ml/hr for 16.7 hr (50 ml total). Absorbance data from real time Ultraviolet-Visible spectrometry was collected under temperature controlled conditions (25 ± 2°C) every 100 s.

Results

The arithmetic mean diameter of individual particles as measured by TEM was 48 nm, which was comparable to the 50 nm nominal size stated by the manufacturer. The particle diameters are polydisperse with a wide distribution of sizes ranging from 7 to 126 nm. Figure 1 presents an image of the material in the powdered form and particle size distribution. When the size distribution is log-transformed to better approximate a normal distribution (Kolmogorov-Smirnov, n=433, p=0.044), the geometric mean of the sample is 41 nm, 18% less than the manufacturer quoted size.

Figure 2 presents a comparison of the hydrodynamic diameter of materials in different solutions. The hydrodynamic diameter of the particles is in all cases is larger (133 to 178% increase in size) than the measured size of the powdered materials due to extensive agglomeration of the nanoparticles. The rank order of particle hydrodynamic size immediately upon suspension is: phosphate treated particles, water, fetal bovine serum (FBS) < phosphate buffer saline (PBS) < salt solutions (RPMI, very soft reconstituted water [VSRW], moderately hard reconstituted water [MHRW], and NaCl). In water or reconstituted water solutions, the aluminum has a positive surface charge; however, in media that contain phosphate, the phosphate binds to the surface of the aluminum particle and imparts a negative surface charge to the nanoparticle (Figure 2B). In addition, solutions with higher ionic concentrations reduce the Zeta Potential of the nanoparticle

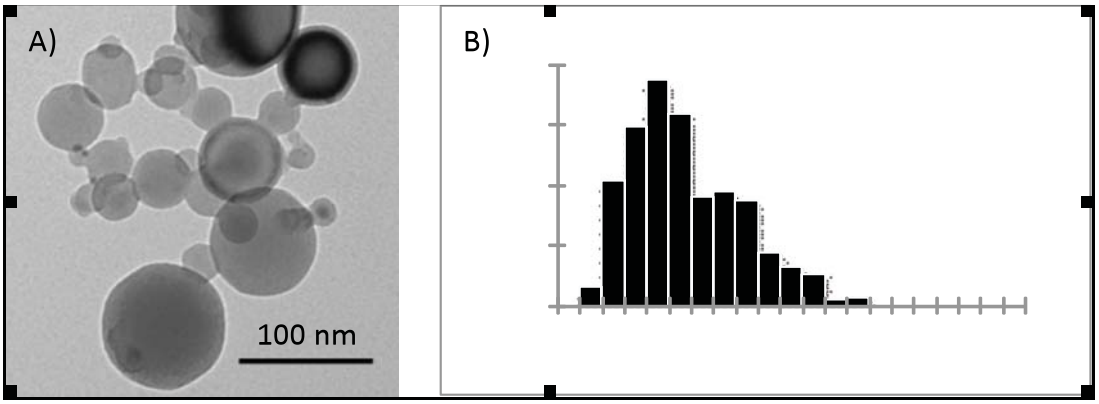

Figure 1. A) Transmission electron microscope image of powdered aluminum nanoparticles. B) Particle size distribution for each size class is based on the relative percent of the total particle diameters measured.

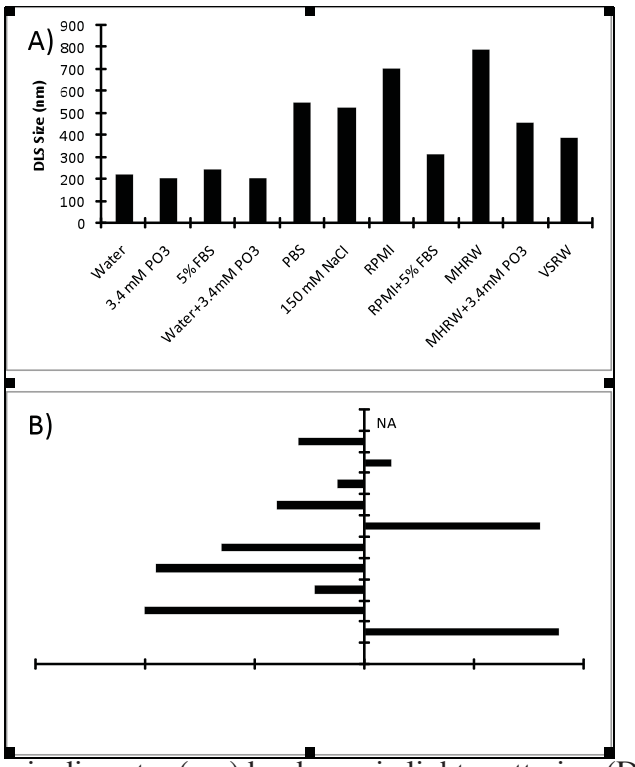

Figure 2. A) Hydrodynamic diameter (nm) by dynamic light scattering (DLS) of nominal 50 nm aluminum nanoparticles in different media. DLS measurements were performed in triplicate at 25°C with each size measurement being the average of 20 runs. Average of the triplicates is reported. Hydrodynamic diameter is based on the intensity weighted Z-average as calculated using a cumulative fit with Malvern Dispersion Technology Software. Water = distilled water was produced from a MilliQ-Plus filtration unit; PO3 = phosphate; FBS = fetal bovine serum; PBS = phosphate buffered saline; MHRW = moderately hard reconstituted water by EPA Method 600/4-91/002; and VSRW = very soft reconstituted water by EPA Method 600/4-91/002. B) Zeta potential by laser doppler electrophoresis of nominal 50 nm aluminum nanoparticles in different media. Zeta potential measurements were performed in triplicate at 25°C with each size measurement being the average of 10 runs. Average of the triplicates is reported.

that result in larger aggregate sizes in solution.

The agglomerate size of aluminum nanoparticles was dependant on the suspending fluid. In general, phosphate treated particles had not only the smallest starting diameter, but the diameter stayed consistent over the study period (Figure 3). Particles suspended in a salt solution (NaCl) had the largest diameter at the end of the experiment. Time course data is important as a change in the hydrodynamic size of the particle will affect the settling rate and pore-size related capture of the nanomaterials in the soil column.

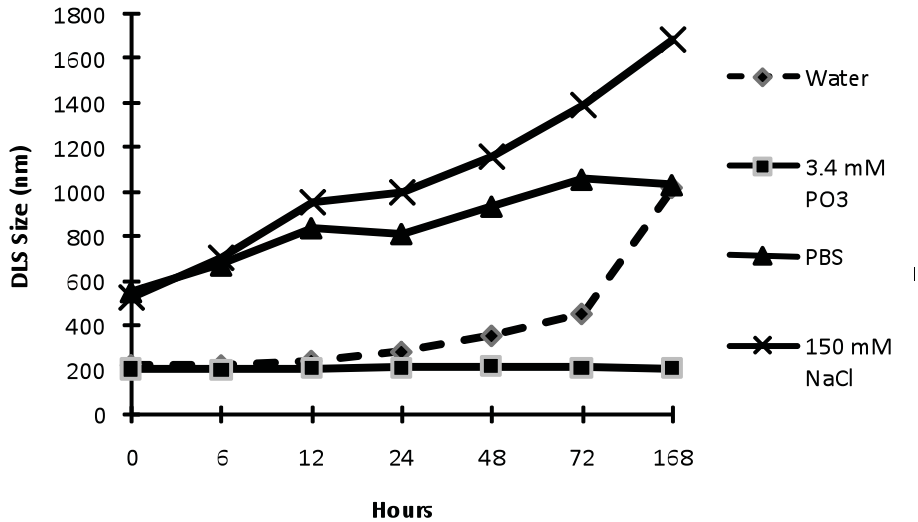

Figure 3. Hydrodynamic diameter (nm) by dynamic light scattering (DLS) of nominal 50 nm aluminum nanoparticles in different solutions over time. Water = distilled water; PO3 = phosphate; PBS = phosphate buffered saline.

When three different suspensions of differing properties were eluted through a sand column, phosphate treated particles had the greatest transportability compared to particles in water and those in a MHRW (Figure 4). Phosphate treated particles had >95% transportability and continued unimpeded through the column throughout the test. Particles in MHRW had less than 6% of the starting material transported through the column. Particles suspended in water had intermediate transportability; however, transport was delayed until approximately 27 pore volumes. Phosphate treated particles were also eluted through soil as a comparison of the effect of soil type on transport. Transportability was approximately 71% at breakthrough compared to 95% at breakthrough for the sand column.

Discussion

The size of nanoparticles relative to their bulk formulations have been implicated as a potential cause of toxicity (Colvin 2003, Hoet et al. 2004, Warheit 2004, Nel et al. 2006); therefore, describing size accurately of primary importance. However, for the transport of nanomaterials through porous media, it is important to differentiate between the primary size of the nanoparticles and the agglomerate size in the solution media. The vast majority of dry nanopowders are highly agglomerated in suspension. For the aluminum nanopowders investigated, the agglomerate size of the suspended material was over 133% larger than the

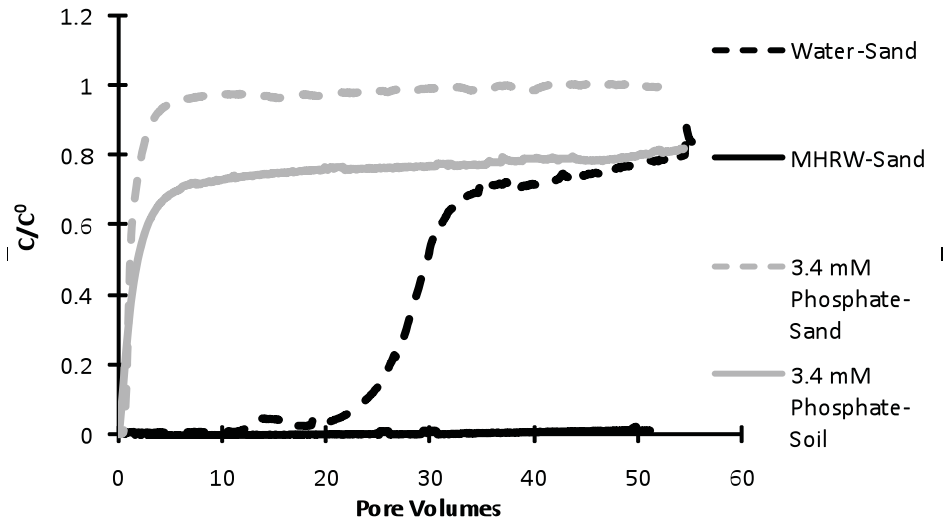

Figure 4. Transport of nominal 50 nm aluminum nanoparticles suspended in different solutions through a sand and soil column over time. Pore volumes (ml) were calculated as the difference between the total column volume and matrix volume and elution through the column was measured as the change in absorbance at 700 nm compared to the starting material (C/C0). Water = distilled water; MHRW = moderately hard reconstituted water.

primary size. The phenomenon of agglomeration is a critically important factor in nanomaterials research as it can change how the particles act in suspension. The formation of agglomerates will increase settling rates in suspension (Brant et al. 2007) and may impact deposition on solid surfaces from a colloidal suspension (Elimelech and O'Melia 1990). Since agglomerate size is dependent on the suspension media, monitoring the size and charge as a function of time is important. Additionally, the surface of the nanoparticle can be altered by the dispersion media. For aluminum nanoparticles, the surface charge is made negative when phosphate containing media is utilized. In water, the aluminum nanoparticles carry a positive charge and bind to the sand column for 27 pore volumes until the sand is saturated with positively charged aluminum after which transport occurs. For aluminum nanoparticles exposed to phosphate the surface charge is negative and the nanoparticles have a much higher initial transport rate.

Conclusions

This study demonstrates that understanding the properties of nanomaterials is important in predicting transport and that aluminum nanomaterials may be transported under certain but environmentally relevant conditions. In areas where high phosphate levels are present in surface waters, aluminum nanoparticles entering the system may be relatively small and stable, which may facilitate transport. Other organic materials found in surface water such as tannic acids may produce the similar results. However, if soils or surface water contain salts, the nanoparticles have high site fidelity, or in the case of surface waters settle quickly, due to agglomeration. It is therefore important to consider how other chemical components of the environment may interact

with nanomaterials. Only by understanding the characteristics of nanomaterial and how those characteristics change over time will researchers be able to develop meaningful models for fate and transport.

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Conference Questions and Answers

Question:
What were the dimensions of your column?

Answer:
It was a 6.6 micron bore, basically a chromatography column.

Question:
Did you look at the wall effect? The literature indicates that small diameter columns may affect the movement of the particles.

Answer:
I do not think there was a problem with wall effect in this experiment. The column setup is commonly used at Rice University.

Question:
Did you look at the particle size distribution as particles came out of the column?

Answer:
Yes.

Question:
Did you monitor the pH? What was the column flow rate? Did you notice any generation of hydrogen? Were there any changes?

Answer:
The flow rate was approximately 3 milliliters per hour. We did monitor the pH and did see some hydrogen production.

Save

Transport and Reactivity of Lactate-Modified Nanoscale Iron Particles in PCP-Contaminated Field Sand

Krishna R. Reddy, Amid P. Khodadoust and Kenneth Darko-Kagya, University of Illinois at Chicago, Department of Civil and Materials Engineering, Chicago, Illinois, U.S.A.

Abstract

In this study, the transport and reactivity of nanoscale iron particles (NIP) was investigated in horizontal column experiments using field sand contaminated with pentachlorophenol (PCP). Bare NIP and modified NIP with 10% aluminum lactate were investigated at two different slurry concentrations of 1 g/L and 4 g/L. Lactate was found to prevent or slow agglomeration and settlement of NIP. NIP slurry was introduced at the inlet of the soil column under a constant hydraulic gradient. Visual observations revealed that the distribution of NIP was uniform in the 4 g/L modified-NIP experiment compared to all other experiments. Hydraulic conductivity of the soil was measured during the course of each experiment and it remained approximately the same in all the experiments except it reduced in the experiment with bare NIP at 4 g/L concentration. Transport of NIP in experiments with bare NIP was not uniform and most of the PCP degradation occurred near the inlet where NIP could be transported during the initial stages of testing. The transport of NIP is enhanced by lactate, but the reactivity of NIP with PCP was decreased as compared to the bare NIP experiments. Degradation and the removal of the PCP were found higher (61.2% and 9.7%, respectively) for the 1 g/L lactate-modified NIP; while the degradation and removal were lower (51.6% and 6.4%, respectively) for the 4 g/L lactate-modified NIP. Overall, the results showed that 4 g/L lactate-modified NIP favors relatively uniform distribution of NIP in the soil, but the extent of PCP reduction is lowered by the surface modification. Further research is being performed to optimize the lactate-modified NIP that provides both efficient delivery as well as enhanced reduction of PCP in the soil.

Introduction

Nanoscale iron particles (NIP) have the potential to effectively treat PCP contaminated soils (Reddy and Karri, 2008). Despite the good reactivity, mobility of NIP in soils becomes restricted due to their aggregation and settlement. Delivery of NIP uniformly in required amounts is essential for successful in-situ remediation of soils. Schrick et al. (2004) revealed that the transport of NIP through most environmental media such as soil is difficult or not possible if the surface of iron particles is not modified. If the NIPs are modified with polyelectrolytes and polymers, their mobility increases through media such as soils (Saleh et al., 2007).

The objective of this study was to determine the transport of NIP and consequent PCP degradation in a natural sand. Two column experiments were conducted on PCP-contaminated sand using bare NIP slurry at two different concentrations (1 g/L and 4 g/L). Additional two column experiments were conducted with the same NIP slurry concentrations, but modified with 10% (NIP w/w) aluminum lactate to investigate enhanced transport and corresponding effects on degradation of PCP.

Materials and Methods

NIP used in this study was obtained from Toda Kogyo (Japan). The particles had an average diameter of 70 nm (with a range of 50-300 nm), pH of 10.7, and BET surface area of 37.1 m^2/g. Natural sand was used for this study and PCP was used to spike the sand at an initial target concentration of 100 mg/kg. PCP was chosen as the contaminant due to its toxicity and presence at numerous sites, including the Superfund sites. 98% purity PCP was obtained from Aldrich Company, CAS 87-86-5. The aluminum lactate used for surface modification of NIP was obtained from Aldrich CAS-18917-91-4. Electrolyte was used to simulate groundwater conditions. The electrolyte contained 0.006 M of sodium bicarbonate, 0.002 M of calcium chloride and 0.002 M of magnesium chloride. The pH, total dissolved solids and electrical conductivity of the electrolyte solution were 7.76, 500 mg/L and 1020 μS/cm, respectively.

For spiking of the soil, about 600 mL hexane was used to dissolve 100 mg of solid PCP. To ensure all the PCP solids are dissolved, the PCP-hexane mixture was mixed on a magnetic stirrer for about 45 minutes. Approximately one kilogram sand was weighed in a large glass beaker. The PCP-hexane solution was added to the soil in the beaker and mixed well with a stainless steel spoon continuously for about 30 minutes to ensure the PCP is distributed uniformly. The soil–hexane–PCP mixture was placed in a ventilation hood nearly seven days for the mixture to dry. During the drying period, the soil was mixed regularly to ensure uniform spiking and drying.

A horizontal column was used for this study. The column had an inside diameter of 3.81 cm and a length of 14 cm. The column was made of Plexiglas. One end of the column was connected to a reservoir made of Plexiglas with an inside diameter of 2 cm using a Tygon tube. The height of the reservoir could be adjusted to apply desired constant hydraulic head conditions. Two different concentrations (1 and 4 g/L) of bare NIP slurries were prepared using electrolyte and additional two slurries were prepared with 1 and 4 g/L NIP containing 10% aluminum lactate (w/w NIP). The spiked sand was placed in the cell in uniform layers and compacted using a tamper to ensure uniform density. The initial hydraulic conductivity was calculated by measuring the outflow volume in a given specified time interval under a constant hydraulic gradient. The electrolyte in the reservoir was replaced with the selected NIP slurry and allowed to flush through the sand. The effluent samples were collected in 120 mL bottles (i.e. approximately every 3 pore volumes) for analysis. At the end of each experiment, the soil was extruded from the column and sectioned into four parts. Soil sample from each section was visually observed and photographed, and the pH, and iron and PCP concentrations were measured. The aqueous effluent samples were analyzed for pH, total dissolved solids, electrical conductivity, and iron and PCP concentrations.

Results and Discussion

Figure 1 shows the residual PCP distribution within the soil at the end of testing. It can be observed that the amount of PCP remaining in the soil of 4 g/L NIP with lactate experiment was uniformly distributed, but at higher concentration levels as compared to the other three experiments. This indicates that the presence of lactate provides uniform delivery of the NIP, but reduces reactivity. In the experiments with bare NIP as well as with 1 g/L NIP with lactate, PCP concentrations increased from the inlet to the outlet. This indicates that the NIP was not

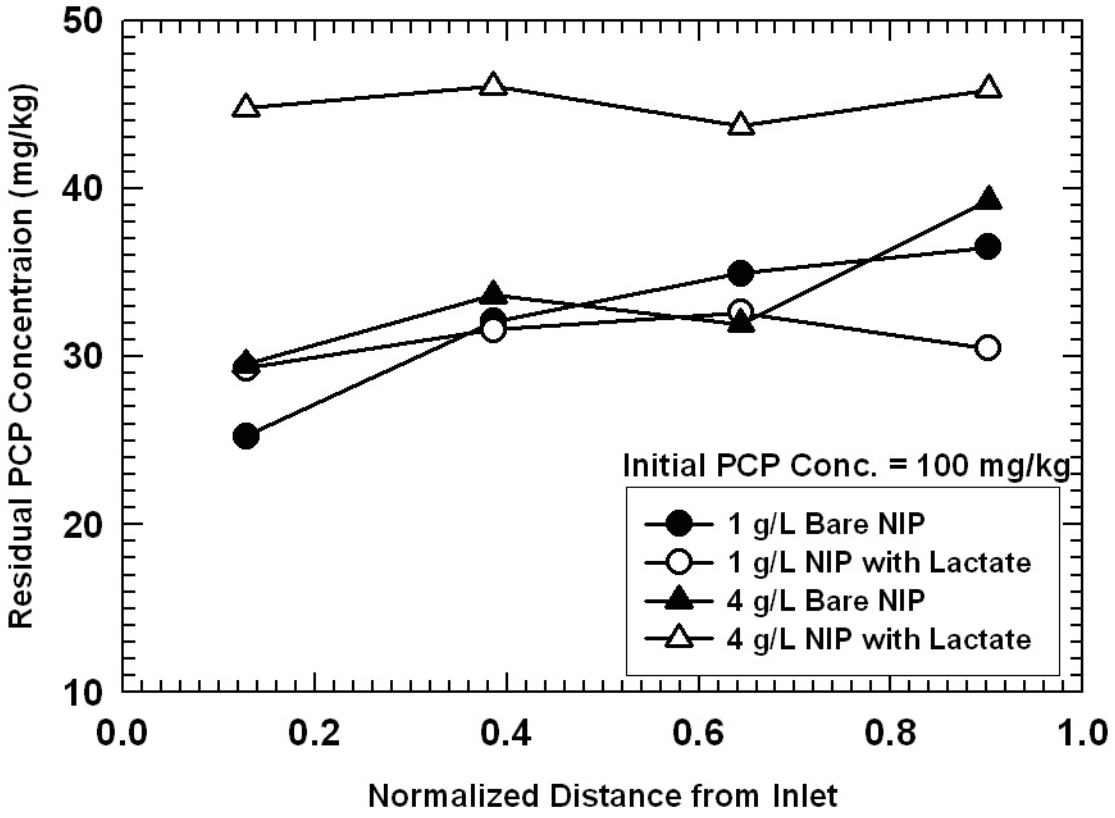

Figure 1. PCP remaining in soil

uniformly distributed. The presence of higher amount of NIP near the inlet caused higher reduction of PCP. The presence of lactate at low concentration of 1 g/L NIP contributed slightly better transport than that found in bare NIP tests, but the presence of small amount of lactate slightly reduced reactivity of NIP.

The amount of PCP found in the effluent is shown in Figure 2 and it demonstrates that the removal of PCP in the bare NIP experiments is approximately similar, while a decrease in removal of PCP is observed in the experiments with lactate. The presence of higher lactate has led to lower PCP removal. Based on the mass balance analysis, the amount of PCP reduced due to the presence of NIP is shown in Figure 3. These results show that maximum PCP reduction is observed in the experiment with 1 g/L NIP with lactate. This demonstrates that the presence of low amount of lactate caused slightly enhanced transport of NIP through the soil without significantly reducing the reactivity. The presence of higher amount of lactate caused enhanced transport, but reduced the reactivity, thus causing the lower reduction of PCP. The bare NIP at both concentrations was effective in reducing the PCP, but the transport of the NIP was limited and most of the reduction occurred near the inlet region of the soil before the NIP agglomerated and settled in the soil.

The outflow was carefully monitored during each experiment in order to determine changes in

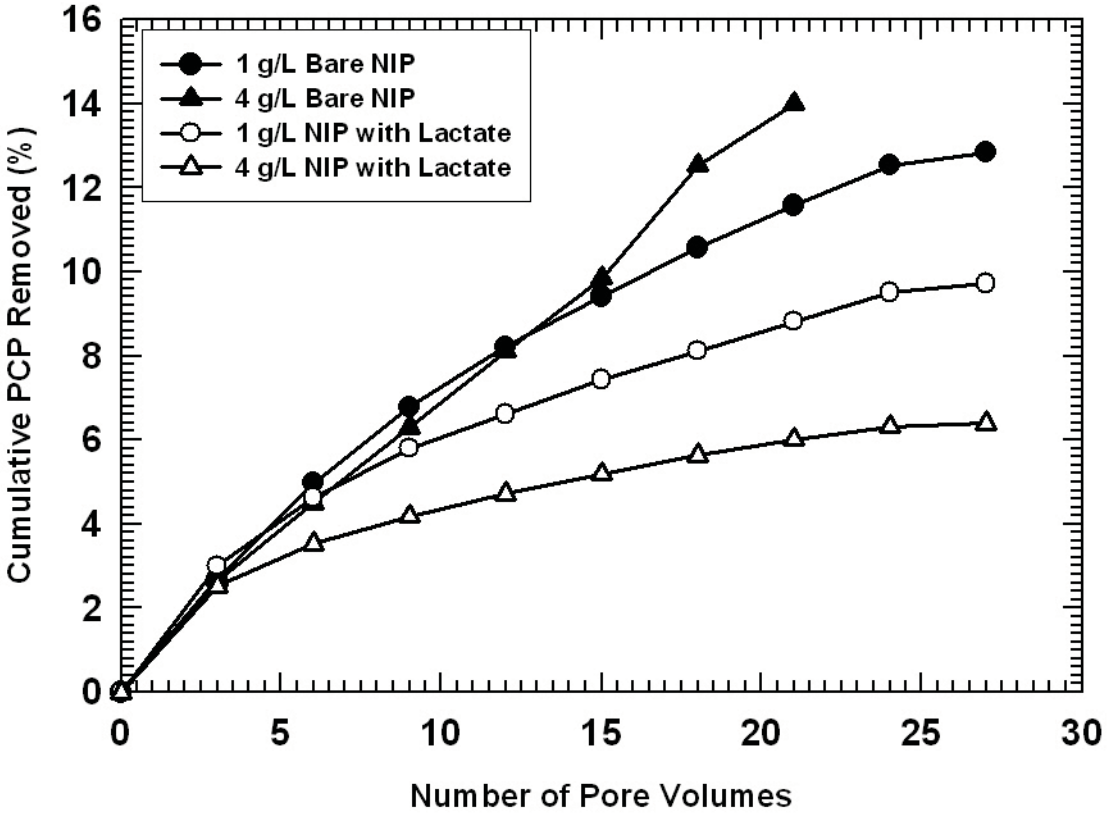

Figure 2. PCP removed in effluent

the hydraulic conductivity of sand as NIP is transported through the soil. As shown in Figure 4, hydraulic conductivity was not impacted in the experiment with 4 g/L NIP with lactate; however, it slightly reduced after 15 pore volumes in the experiments with 1 g/L NIP with or without lactate and it reduced significantly in the experiment with 4 g/L bare NIP. Therefore, the results suggest that higher amounts of bare NIP has potential to clog the pores and reduce the hydraulic conductivity, while low concentration of NIP may not have significant affect on hydraulic conductivity of sand. The use of lactate modified NIP prevents clogging and maintains the hydraulic conductivity of the sand. This study shows that lactate enhances the transport of NIP in the soil, but optimization of lactate and NIP concentrations is essential in order to achieve both adequate transport as well as high reactivity of NIP.

Conclusions

The objective of this study was to determine the transport and reactivity of bare and lactate-modified NIP for the remediation of PCP in field sand. Hydraulic conductivity was unaffected in the 4g/L NIP with lactate experiment; however, it slightly reduced after 15 pore volumes of flow and reduced in bare NIP concentration. Significant decrease in hydraulic conductivity was found in the experiment with 4 g/L bare NIP. Degradation and the removal of the PCP were found higher (61.2% and 9.7%, respectively) for the 1 g/L lactate-modified NIP; while the degradation and removal were lower (51.6% and 6.4%, respectively) for the 4 g/L lactate-modified NIP.

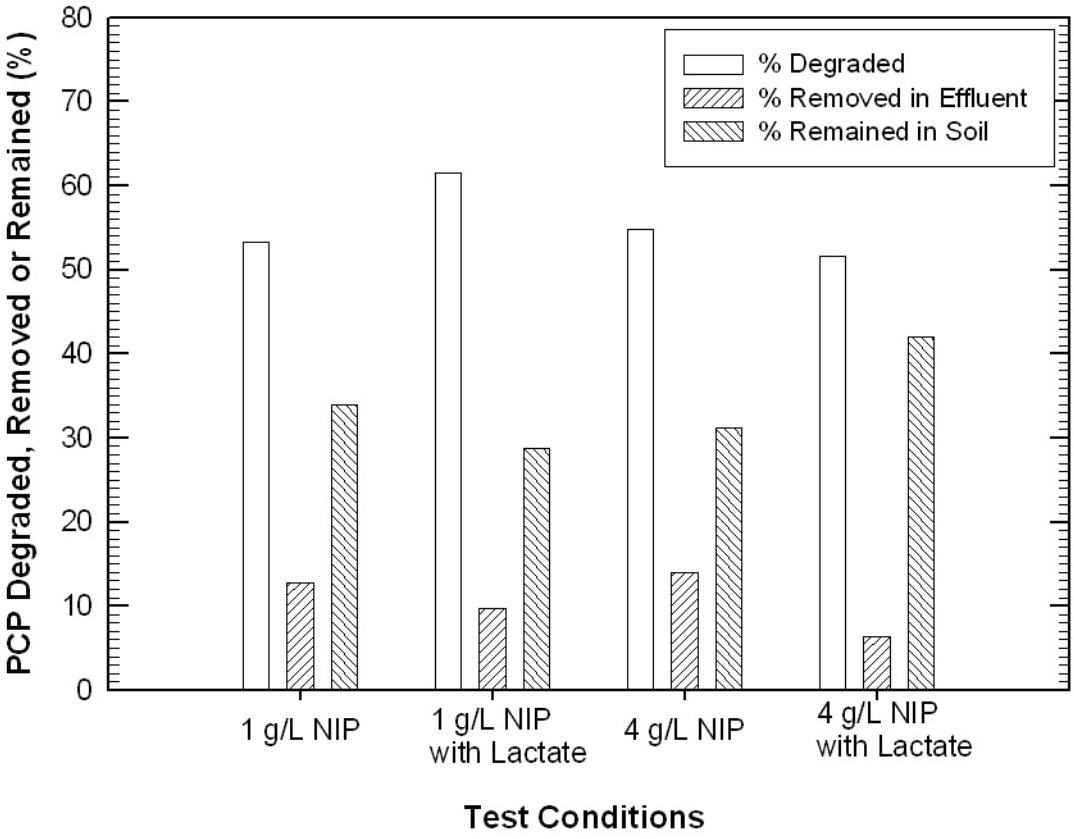

Figure 3. Degradation of PCP in the soil

Overall, this study shows that lactate-modified NIP can be effective for in-situ remediation.

Acknowledgements

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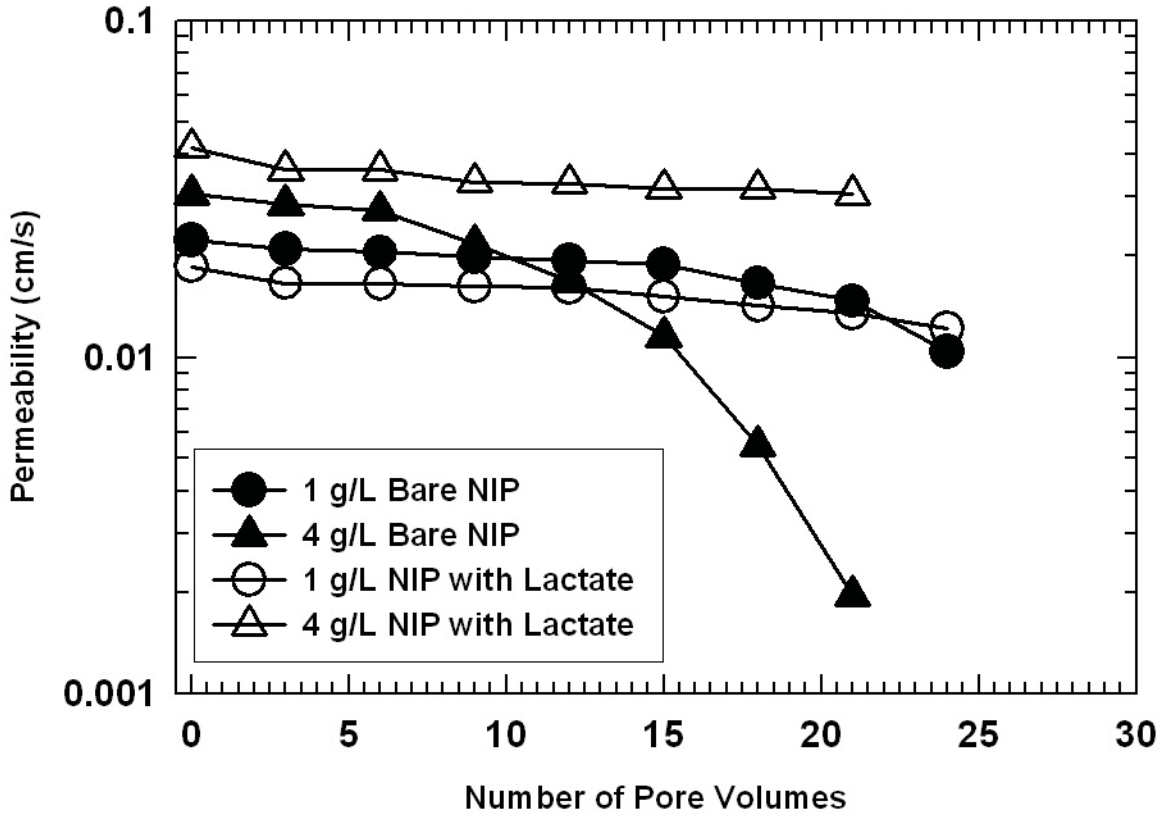

Figure 4. Effect of NIP transport on permeability of the soil

Conference Questions and Answers

Question:
Did you measure the concentrations of the iron in the influent and the effluent? Did you use the same method?

Answer:
We measured the effluent iron concentrations but not the influent.

Question:
You coated the nano-iron with lactate and this improved its ability to stay in suspension. Did you measure the activity of the coated iron?

Answer:
Yes. That was what the reactivity experiment did, and it showed that coated iron was less reactive than uncoated iron. As time passes the lactate degrades, and the iron reactivity increases. The coating does not affect the iron's innate activity.

Question:
In the permeability experiment, why does the 1-gram-per-liter solution initially exhibit less permeability than the 4- gram-per-liter solution?

Answer:
This is due to the initial packing of the cell.

Question:
Could you comment on the batch study for removal of pentachlorophenol (PCP) by bare iron and coated iron?

Answer:
The study showed that the bare iron removed more PCP than the coated iron, and the higher concentration bare iron (4 grams per liter) performed much better than the higher concentration coated iron.

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Chapter 8

Report Backs and Panel Discussion: Implications

Session Report Backs

Fate & Transport

Reported by Michele Conlon, U.S. EPA Office of Research and Development

Dr. Conlon reported that the underlying goal of fate and transport research is to understand nanoparticle interactions with their environment. One theme that came from the fate and transport researchers was the need to verify that the material received is in fact what was ordered. In many illustrations, a manufacturer specified nanoparticles to be within a given diameter range when, in fact, they were not, or the particles were heterogeneous in shape and size due to manufacturing procedures when they were supposed to be uniform.

All of the characterization and detection experiments reported on were in laboratory settings. While the findings from these studies are important, they may not reflect what can be achieved in a natural setting or predict the behavior of nanoparticles in that environment. The characterization of nanomaterials is key to fate and transport. One cannot determine migration unless the material found at point b can be identified as the same material found at point a.

As would be expected, the physical, chemical, and biological environmental context can strongly affect the fate and transport of nanoparticles. Nanoparticle aging and environmental factors such as water presence, ionic strength, and pH; humic and fulvic acid presence and concentrations; sunlight presence, quality, and intensity; and microbe presence and types, all can influence nanoparticle transformation and migration. Promotion or retardation of nanoparticle aggregation is one of many important mechanisms by which environmental factors can influence nanoparticle fate and transport. Analyzing the risks of nanoparticles in the environment are further complicated by chemical transformations of nanoparticles in the environment, which can greatly affect their bioreactivity and toxicity.

The laboratory studies have identified many challenges to understanding underlying principles in nanoparticle fate and transport that will only increase when the studies are taken into the field. This emphasizes the need for working together and sharing insights. The effort has to be a multi-disciplinary one. We need to develop predictive tools and models, which we do not have now. Mitigation of risk is not possible if the fate and transport of a material cannot be determined.

Toxicity & Risk Assessment

Reported by Barbara Walton, U.S. EPA Office of Research and Development

Dr. Walton reminded the audience that at the start of the meeting they had been challenged by Dr. George Gray to “characterize and collaborate.” Forty-four

organizations were represented in the 23 platform presentations and nine posters related to toxicity, which clearly showed inter-organizational collaboration. In addition, the

plenary session speakers posed challenges for nanomaterial toxicity. Jeff Morris asked what properties and characteristics of nanomaterials contribute to toxicity, and questioned whether toxic doses of nanomaterials are environmentally relevant. The numerous gaps in the knowledge about nanomaterial toxicity to the health of human and ecological receptors were presented by Dr. Anne Fairbrother. Dr. Jo Ann Shatkin discussed what constitutes realistic exposures to nanomaterials, the need for cross-disciplinary collaboration, sources of uncertainty in risk assessment, and the issues for risk assessment in an environment that is not highly regulated. Dr. Martin Philbert called for adherence to the fundamental principles of rigorous scholarship and scientific rigor.

Some overarching concerns emerged from the toxicity presentations and posters at the conference. A heavy emphasis was placed on the characterization of nanomaterials, and on life-cycle assessment of potential exposure. The relevance of the currently used test species and endpoints to existing test guidelines for human health and ecological receptors also was emphasized.

Many nanomaterials, including nanosilver, gold nanoparticles, a wide range of metals and metal oxides, quantum dots, and the carbon-based nanomaterials such as the fullerenes and carbon nanotubes, were evaluated in the presentations. The presentations indicated that ecotoxicological risk is being assessed using a wide variety of test species, including micro-organisms, fish, mollusks, macro-invertebrates, and plants. In vitro test systems discussed included mouse renal and liver cells, rat liver cells and human lung epithelial cells, and dermal fibroblasts. Twenty variables affecting toxicity and 28 endpoints were examined. Careful qualifications were given to the findings of the toxicological investigations; for examples, that multi-walled carbon nanotubes do not appear to be lethal to fish but are associated with histopathology of the gill, and that the toxicity of nanosilver is dependant on size and related to reactive oxygen species. The presenters were mindful of what is known and unknown, and were rigorous in their approach.

Dr. Walton distilled four nanotoxicity principles from the presentations:

- Toxicity is often associated with reactive oxygen species.
- Characterization of nanomaterials under investigation is essential for data interpretation.
- Aggregation of nanoparticles typically reduces toxicity.
- Aggregation and agglomeration are dynamic processes, so dissociation will occur over time, changing the initial associated toxicity.

Areas of concern noted by Dr. Walton:

- Nanomaterials - too many, too fast and too complex for conventional approaches.
- Material data safety sheets do not adequately communicate the hazards of nanomaterials to

workers - hazard communication must be improved.

- How to balance risks and benefits in hazard communication.
- Environmentally relevant concentrations and metrics for dose response are unknown.
- The validity of cross-species extrapolation (fish to mammal) is questionable for nanomaterial toxicity.
- Current studies and data are not standardized and thus are frequently incompatible.

Finally, broad areas were identified during the toxicity and risk assessment sessions, where improvements can and should be made in approaches to nanotoxicology. For example, in the absence of information, best practices including an honest appraisal of what is known and unknown should be employed to manage potential hazards. Nanomaterials need to be better characterized under a variety of conditions, in wet and dry states, in vitro, in vivo, and ex vivo. Nanomaterial characterization should be hypothesis-driven. It was recommended that toxicity assessment should be incorporated early in the research and development process of nanomaterial applications in order to prevent a potentially costly rework at the end of a project. Finally, in the absence of complete data, tools to enable decision-making are needed; these may include expert judgment and ad hoc processes.

Panel Discussion

Moderator:
Charles Maurice

Panelists:
Michele Conlon, Steve Diamond, Mark Johnson,
Jamie Lead, Martin Philbert, Barbara Walton

Charles Maurice: I will ask the same question that my colleague Dr. Layne asked the previous panel. Describe an important finding from your session and how you feel it has been affected by discussions amongst the international audience here.

Michele Conlon: Although laboratory methods are available to detect nanomaterials, there are no field portable/transportable instruments that can be employed. We need to detect them in the environment, and will need another approach using a secondary indicator. Biota (daphnia, fairy shrimp) can be collected and analyzed for nanoparticles. Results can be inferred back to a location. There is a need for research and collaboration to find engineered nanoparticles in the environment. At present they cannot be distinguished from natural materials.

Jamie Lead: This area is relatively new, 5 or 6 years old, and there are many research needs. There is a need for validated methods to quantify and determine their physico-chemical form to facilitate further understanding nanomaterials in the environment. We do not have information on the current situation, and modeling gives us the best guess available. We need to look to the future and try to see which nanomaterials will be important over the next 5-10 years and materials that will be more active than the passive materials, such as titanium dioxide, currently available.

Stephen Diamond: Dr. Philbert said that current data will be of little or no use, and this may be true in some cases. Why did he say this? There is an analogy with PCBs. When concern about PCBs first arose, we were looking at them at the Aroclor level. Much later we realized that we needed to understand what constitutes an Aroclor and the

concentrations of those constituents, so the early data were not useful. We need this vision for nanomaterials. The sessions were representative of a highly scatter-gun approach to the understanding of nanomaterials in the environment and nanotoxicity as a whole. Experimental material selection ranged from those prepared in small academic laboratories, to those produced by specialist laboratories making high quality products for research, to manufacturers producing them in ton quantities. This makes it hard to generalize across materials and results, and generalizations may not support risk assessment. Efforts to characterize nanomaterials face difficulties. There is a growing awareness of the need to characterize nanomaterials, yet media preparation is again a scatter-gun approach. There is an immense variety of preparation methods and characterization in specific media. Food chain transfer is a gap in understanding. Tissue uptake is probably limited, but guts of daphnia are full of C60 and metal nanoparticles. This enables them to act as vectors for the sediment accumulation of nanomaterials. If information was available from the producers of nanomaterials about what is being bought, used, or amended, we would have a better understanding.

Mark Johnson: Jo Ann Shatkin gave an excellent overview of the challenges we face, and endorsed the retention of the basic risk assessment framework for exposure to chemicals. It may be futile to do this in most respects, due to the rapid evolution of the field and complexity of the materials. In the face of such uncertainty it is daunting to translate and communicate technical information to the public. Dr. Linkov's suggested characterization approach may be one way to determine hazards semi-quantitatively and divide materials into low, medium and high hazards. Manufacturers may use information to decide against the development of a material. Worker exposure could also be appropriately managed. Christie Saye's approach could be used to predict toxicity for industry and the public. This approach utilizes the physical properties of nanomaterials and gives a score to a particular attribute (e.g., charge, mass, reactive oxygen species, or concentration) to derive a composite score indicative of toxicity.

Martin Philbert: We are at the gateway of a new and potentially disruptive technology. We have seen the first applications, but there are block-buster applications in development. There is a need to think critically now. Nanotechnology will be applied to many pressing social and environmental problems, but we are at the stage where the azo dye prontosil rubrum was in World War II. This drug saved lives but was replaced by penicillin. Penicillin was in turn replaced by safer and more useful derivatives, and by newer classes of antibiotics. The point is that we do the best we can, but keep a vigilant eye towards re-inventing. Another

underlying assumption is that the United States would be the world leader in the production of nanomaterials. We should be challenged, however, by the idea that the major producers of nanomaterials will be countries with lax regulatory frameworks, and we will be importing these materials. There is a need for detection methods for these materials.

Question:

In addition to conducting research, I work with ASTM (American Society for Testing and Materials) and ISO committees, and we need to recommend good practices for these materials and communicate best practices to the community. How do you envision this?

Diamond:

I served on an OECD working party for manufactured nanomaterials, doing extensive reviews of existing test guidelines used for regulatory purposes. Existing biotic effects test guidelines are framed in terms of concentration and solutions, for example, and do not work for nanomaterials. A guideline is in preparation for how to prepare a nanomaterial for one aspect of characterization, particle size. Rather than using a prescriptive approach, a range of parameters for any aspect of nanomaterial preparation should be devised, and results should be required to be reported. This is no real answer, but much attention is being given to this by working parties associated with the OECD.

Question:

Are state, local and national/international government doing enough to protect worker and public health and the environment from nanomaterials?

Philbert:

Perhaps, but there are not enough data. Some nanotoxicity is going to take a long time to develop. The current testing paradigm, loading a rat for two years, may not reflect what happens with low level exposures over the long haul.

Johnson:

We are always exposed to naturally occurring nanoparticles. It is difficult to know if we are doing enough if we do not know what is in the environment. Agnes Kane's work has shown similarity of nanotubes to asbestos fibers. This is an important analogy as there are similar mechanisms of action, but there are many aspects of these materials that we are just beginning to understand.

Question:

Is it safe to use a precautionary principle, i.e., to list all nanomaterials as hazardous until they are considered or known to be safe, especially on the MSDS data sheets?

Philbert:

"Do not deploy until safe" is not a realistic position. There are countries that do not respect intellectual property, but are going ahead with the technology and manufacturing nanomaterials.

Question:

Considering the current use of sunscreen, should it be labeled to show that it contains nanoparticles?

Philbert:

Sunscreen is needed by the fair-skinned, as exposure to sun can cause skin cancers.

Comment:

Nanomaterials should be listed to give the user a choice.

Conlon:

From the regulatory perspective, EPA has authority through statutes (Clean Water Act, Resource Conservation and Recovery Act), but does not have the authority to label materials as hazardous without evidence. We cannot stifle innovation and useful products in production.

Comment:

The EPA is not the only regulatory agency.

Question:

We have seen a lot of researchers, regulatory agencies, and universities at this meeting, but no speakers from industry are here. What are they using for best management practices to protect their workers?

Answer:

When we were advertising this conference, we tried to advertise in as many venues as possible; nobody was excluded.

Comment:

Two keynote speakers from the private sector cancelled, but for good reasons. Another thing to consider is that when companies develop products they are very reluctant to disclose what might be proprietary information. We have to understand the commercial side and the implications of a competitive market.

Question:

Is EPA collecting information from industry?

Answer:

EPA's Nanoscale Materials Stewardship Program is voluntary and is intended for the early collection of data. The program is about a year old. You can find a description of it on the OPPT website at http://www.epa.gov/oppt/nano/stewardship.htm.

Diamond:

For the OECD working party, industry was invited to participate in working/steering groups. Some companies are generous with their participation, information, time, and, in the case of Evonik Industries (formerly Degussa), with providing titanium dioxide for research.

Question:

Many nanotechnology companies are in the start-up phase. Many are very small and do not have deep pockets for nanotoxicology. What is being done on the national government level to help them?

Answer:

There are federal dollars for small business support, which includes support for research and development. EPA's Office of Research and Development also offers Cooperative Research and Development Agreements (CRADAs) that support collaboration between EPA and non-federal organizations. This is a good program for all small businesses concerned about health effects on their workers and waste streams that are emitted, and that are willing to collaborate.

Closing Comments

Charles Maurice

This conference has drawn 185 registrants from five continents and speakers from government, the private sector and non-government organizations (NGOs). The fundamental goal has been to get applications and implications people together, because a holistic, multidisciplinary approach is needed to this new area. We wish to thank the University of Illinois at Chicago for registration and catering this event. Credit is also given to the Hyatt Regency. Proceedings from the conference will be published and PDF-format files will be posted on EPA websites. Participants will be notified of the link. Thank you all for participating.

Presenter Biographies

A

Dr. Linda Abriola is a Professor of Civil and Environmental Engineering and Dean of the School of Engineering at Tufts University, and is a member of the National Academy of Engineering. Dr. Abriola is a leading researcher in the area of flow and transport in porous media, and has developed several numerical models to describe the contaminant fate and transport in heterogeneous soils and aquifer materials.

Diana S. Aga is an Associate Professor at the Chemistry Department of the University at Buffalo, The State University of New York. She obtained her degree in B.S. Agricultural Chemistry at the University of the Philippines at Los Baños, Philippines, and her Ph.D. degree in Analytical and Environmental Chemistry at the University of Kansas, Lawrence, KS. Dr. Aga was a postdoctoral fellow at the Swiss Federal Institute of Science and Technology (ETH)/ Institute for Environmental Science and Technology (EAWAG), Switzerland, and at the U.S. Geological Survey, Water Resources Division, in Lawrence, KS. Recently, she received a research fellowship from the Alexander von Humboldt Foundation to conduct research at the Bundesanstalt für Materialforschung und –prüfung, Berlin, Germany. Her research interests include investigation of the fate and transport of contaminants in the environment, such as persistent organic pollutants, pesticides, pharmaceuticals, and engineered nanomaterials.

Dr. Souhail Al-Abed is a Research Chemist at the National Risk Management Research Laboratory of the U.S. Environmental Protection Agency in Cincinnati Ohio. His research activities include using electrochemical methods and bimetallic nano-materials in the remediation of contaminated soils and sediments, removal of heavy metals from aqueous waste streams, and development of methodical leach tests for waste evaluation. His research contributed to the understanding of many challenging environmental problems and developing cleanup strategies based on sound science. He authored and coauthored more than 42 peer-review journal articles and five book chapters. He is member of the American Chemical Society and the American Geophysical Union and serves in many national and international research committees.

Clare Allocca is the Chief of The United States Measurement System (USMS) Office at NIST. This office is building upon a NIST-led assessment of the state of the USMS to transform the approaches taken into an increasingly more effective and efficient USMS. Her responsibilities include leadership, strategic planning, customer engagement, process development and program implementation. Ms. Allocca previously served as Senior Scientific Advisor to the Director of the NIST Materials Science and Engineering Laboratory; Senior Technical Advisor for the Automotive Sector in the NIST Industrial Liaison Office; Program Analyst in the NIST Program Office, as advisory staff to the NIST Director; and Program Manager for Materials in the Advanced Technology Program (ATP). Before joining NIST, she was a Senior Materials Engineer for Pratt & Whitney engaged in the development of advanced ceramic composites for jet engines. Ms. Allocca holds Bachelor of Science Degrees in Materials Science and Engineering and Geochemistry from the Massachusetts Institute of Technology; a Master of Science Degree in Ceramic Engineering from the University of Illinois at Urbana-Champaign; and an Executive Master of Science Degree in the Management of Technology from the University of Pennsylvania (Wharton Business School/School of Engineering).

Alla L. Alpatova received a M.Sc. degree (2004) in environmental diagnosis from Imperial College, University of London, UK. After graduation, he worked for Anglian Water, UK, where he was responsible for coordination of plumbsolvency trials and monitoring lead levels across domestic pipelines to establish the most cost-effective strategy of lead control in portable water. He is currently a Ph.D. student at the

Department of Civil and Environmental Engineering at Michigan State University, where he works within the National Science Foundation-funded project "Self-cleaning ceramic membranes for the removal of natural and synthetic nanomaterials from drinking water using hybrid ozonation-nanofiltration." His research interests include: (1) advanced membrane processes such as combination of membrane filtration with oxidation processes; (2) fate and transport of nanomaterials in environment; (3) toxicity of engineered nanomaterials.

Publications:

Alpatova A.L; Shan W; Rogensues, A.R; Masten, S.J; Alocilja, E.A. and Tarabara, V.V. Biocompatibility of single wall carbon nanotubes solubized by non-covalent functionalization technique. In preparation

Alpatova, A.L; Babica, P; Hashsham, S.A; Upham, B.L; Masten, S.J. and Tarabara, V.V. In vitro toxicity evaluations of fullerene nC60 derivatives formed in conditions that simulate disinfection processes at water treatment plant. In preparation

Dr. Pedro J. Alvarez is the George R. Brown Professor of Engineering at Rice University. He previously taught at the University of Iowa, where he also served as Associate Director for the Center for Biocatalysis and Bioprocessing and as Honorary Consul for Nicaragua. Prof. Alvarez's research focuses on the environmental applications and implications of biotechnology and nanotechnology, including bioremediation of contaminated aquifers, phytoremediation, fate and transport of hazardous substances, and nanomaterial-bacterial interactions and related disinfection approaches. Dr. Alvarez received a B. Eng. degree in Civil Engineering from McGill University and MS and Ph.D. degrees in Environmental Engineering from the University of Michigan, and was a visiting professor at the Swiss Federal Institute of Technology (EAWAG). Dr. Alvarez is a P.E., a Diplomate of the American Academy of Environmental Engineers and a Fellow of ASCE. Dr. Alvarez currently serves on the editorial boards of Environmental Science and Technology, Biodegradation, and the European Journal of Soil Biology. He is also an honorary professor at Nankai University in China and adjunct professor at the Universidade Federal de Santa Catarina in Florianopolis, Brazil, and UNAM in Mexico City.

James E. Amonette is a senior research scientist in the Fundamental and Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland WA.

Beth Anderson hails from the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health where she shepherds research translation for the Superfund Basic Research Program (SBRP). She began her long career at NIEHS in the molecular sciences studying prostaglandin synthesis and later switched to science administration where she worked for the National Toxicology Program (NTP). After a decade with the NTP, she joined the extramural program and SBRP. Here she pursues her professional passion of advancing SBRP research findings with the goals of improving human health and identifying better, faster and cheaper clean-up strategies for hazardous waste sites. Ms. Anderson has an undergraduate degree from the University of North Carolina at Chapel Hill and a masters degree from Duke University.

Dr. Anthony Andrady has more than 25 years of research and development experience in polymer science and engineering, having served as program manager on numerous research programs funded by US government agencies. Dr. Andrady is a polymer scientist with specialized research experience in degradation of polymers in the environment. His main areas of research interest are fabrication of electrospun nanofibers, biomedical applications of nanofibers and characterization of nanoscale particles (particularly carbon nanotube materials). He has authored or co-authored about 100 peer-reviewed publications including book chapters and two books.

B

Donald R. Baer is the lead scientist interfacial chemistry for the Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland WA

Dr. Sarbajit Banerjee is an Assistant Professor at the Chemistry Department of the University at Buffalo, The State University of New York. He received his undergraduate degree in B.S. Chemistry at St. Stephen's College, University of Delhi, in 2000 and his Ph.D. degree at the State University of New York at Stony Brook and Brookhaven National Laboratory under the supervision of Prof. Stanislaus S. Wong. His graduate work was focused on the surface chemistry of carbon nanotubes and the use of X-ray absorption spectroscopy to study nanostructures. From 2004 to 2007, he was a post-doctoral research scientist in the group of Professor Irving P. Herman in the Department of Applied Physics and Applied Mathematics and the Nanoscale Science and Engineering Center at Columbia University. Professor Banerjee and his research group are interested in the broad areas of carbon and metal oxide nanostructures for electronics and energy conversion.

Dr. Melissa Baumann is an associate professor in Department of Materials Science and Chemical Engineering at Michigan State University. She obtained her Ph.D. from Case Western Reserve University in 1988, after which she was postdoctoral fellow in the UKAEA Harwell Laboratories, Great Britain.

Dr. Neppolian Bernaurdshaw is a Research Scientist in the Department of Environmental Science and Engineering at Gwangju Institute of Science and Technology in Korea. Dr. Bernaurdshaw earned his bachelor's and master's degrees in chemistry at Government College, Kumbakonam, Bharathidasan University, India, and his Ph.D. in chemistry from Anna University in India. Prior to joining Gwangju Institute of Science and Technology, Dr. Bernaurdshaw served as a doctoral researcher at Osaka Prefecture University in Osaka, Japan. His work involves preparation of semiconductor photocatalysts (nanotubes, nanorods, etc.) using novel methods, as well as pollution abatement studies both in gas and liquid phases. Dr. Bernaurdshaw works with visible light responsive photocatalysts and synthesized hybrid PLEDs gold capped TiO2 polymer nanocomposites. He also studies Advanced Oxidation Techniques (AOTs) for complete degradation of organic and inorganic pollutants.

Dr. Dibakar Bhattacharyya is the University of Kentucky Alumni Professor of Chemical Engineering and a Fellow of the American Institute of Chemical Engineers. He received is B.S. in chemical engineering from Jadavpur University, M.S. in chemical engineering from Northwestern University, and Ph.D. in environmental engineering from the Illinois Institute of Technology. He has published 167 (mostly in water related area) refereed journal articles and 21 book chapters, and has recently received five U.S. Patents (Functionalized Materials/Membranes for toxic metals capture from water at ultrahigh capacity, and one on hazardous waste destruction technology). Dr. Bhattacharyya has mentored many graduate and undergraduate students in the area of environmental research, membranes, and separation/reactions. He and his graduate students pioneered the development of poly-ligand functionalized material development for toxic metal capture, and synthesis of nanostructured metals in polymers (nanocomposites) for toxic organic dechlorination from wastewater at room temperature. He has worked with several industries in projects dealing with wastewater, material recovery, and important separation problems. Dr. Bhattacharyya has received a number of awards for his research and educational accomplishments, including the 2004 Kirwan Prize for Outstanding Research, Larry K. Cecil AIChE Environmental Division Award, the Kentucky Academy of Sciences Distinguished Scientist Award, Henry M. Lutes Award for Outstanding Undergraduate Engineering Educator, AIChE Outstanding Student Chapter Counselor Awards, and the University of Kentucky Great Teacher (1984, 1996, and 2008) Awards. For his highly significant technical contributions in the area of environmental separation (particularly water treatment) and polymer-nanoparticle composite materials development for toxic organics degradation, he was recently honored (plenary/keynote lectures) at the NAMS meeting (Orlando), European Chemical Engineering Meeting (Copenhagen), Inter-Federation Chemical Engineering Congress (Buenos Aires), and in Indian Chemical Engineering Congress (Calcutta). In

Feb 2007 he was the Technical Program coordinator and Chair of the ECI Water Treatment and Reuse Conference in Tomar, Portugal.

Dr. Pratim Biswas is the Stifel and Quinette Jens Professor and Chair, Department of Energy, Environmental and Chemical Engineering, Washington University, USA. He received his Ph.D. from California Institute of Technology, and a M.S. from the University of California.

C

Claudio Cameselle is an Associate Professor in the Department of Chemical Engineering at the University of Vigo, Spain. He teaches advanced chemical reactors, waste water treatment, and solid waste management and treatment. His research expertise includes electrokinetic remediation of polluted soils and wastes, and bio-production of organic acids and other metabolites of industrial interest. He was awarded the fellowship from Xunta de Galicia (Spain) to perform research at the University of Illinois at Chicago during 2007-2008.

Dr. Tom Campbell of ADA Technologies is an active researcher in the nanotechnology/ environmental, health, and safety sector. He is currently under contract with NIST to support a national assessment of measurement needs for determining the effects of nanomaterials on environmental health and safety. Dr. Campbell received his B.E. in Mechanical Engineering from Vanderbilt University and his M.S./Ph.D. in Aerospace Engineering Sciences from the University of Colorado at Boulder. Most recently, he has worked as a Senior Research Scientist/Nanotechnology Program Manager within ADA Technologies, Inc., in Littleton, CO. He recently successfully completed a National Science Foundation (NSF) Phase I STTR project, A Carbon Nanotube Metrology System for Industry and Research Environments, in which he demonstrated the world's first quantitative, low cost, reproducible, and rapid means to characterize single wall carbon nanotubes (SWNTs). Prior to joining ADA, he worked for six years researching advanced materials at Saint-Gobain Crystals. This research had as its focus optical materials (CaF2, BaF2, MgF2) for the 157nm and 193nm microlithography laser markets. Dr. Campbell has also held a post-doctoral fellowship in Germany through the Alexander von Humboldt Foundation. His self-proposed, independent research project was to study Ge1-xSix crystal growth.

Barbara J. Carter is the Director of Research and Development for EcoArray, Inc. She is the Principal Investigator on two Phase 2 SBIR grants awarded by NIEHS, "Microarrays in fathead minnows and bass," in the process of completion, and "Developing and using sheepshead minnow microarrays for ecotoxicology" which began August 2007. She is also P.I. of a Phase 1 SBIR awarded by the EPA in March 2008, "Using fathead minnow microarrays to test toxicity of nanoparticles." She was hired in 2002 at the inception of EcoArray; providing laboratory expertise on two NIEHS Phase 1 SBIR grants, a CRADA with the EPA, and a grant from Project Wild Dolphin. Ms. Carter graduated from Northwestern University with a dual major in biological sciences and anthropology, and received her M.A. in anthropology (archaeology) from the University of Washington. A career military officer, she retired as a Captain, U.S. Navy Reserve.

Evrim Celik is a doctoral candidate in the Department of Environmental Science and Engineering at Gwangju Institute of Science and Technology in Korea. Mr. Celik received his bachelor's degree from Middle East Technical University in Ankara, Turkey, and his master's degree from Akdeniz University in Antalya, Turkey. His areas of interest include reactive membrane synthesis, membrane filtration processes, advanced oxidation processes, and water and wastewater treatment.

Maryam Zarei Chaleshtori holds a B.S. and a M.S. from Isfahan University of Technology, Iran. After her bachelor's degree, she worked with the Textile Department of Isfahan University of Technology, Iran, for almost 8 years as an expert and teacher of textile laboratories in dyeing and printing techniques, natural fibers chemistry, and textile fibers and material identification labs. During her employment, she

also continued her study toward an M.S. at the same university. As an undergraduate, she studied the dyeing of wool with natural dyes. A paper was published from her work in the 6th National Conference of Rug, Tehran, Iran, 1999, on which she received an award. Also, in her post-graduate research work, she studied the treatment of wool and nylon with the sulfamic acid to improve their dyeability. A paper was published from her work in 3rd National Conference on Textile Engineering in Isfahan University of Technology, Isfahan, Iran, 1999. She came to the United States in 1999, attended El Paso Community College, and then she started her Ph.D. studies in 2004 at the University of Texas at El Paso. Since then she has been doing research with Professor G. Saupe on photochemical water decontamination. Also she published a paper in Renewable Energy magazine in 2007.

Dr. Sylvia Chan-Remillard is an Alberta Ingenuity Industry R&D Associate awarded an Industrial Post Doctoral Fellowship through the Alberta Ingenuity Fund. She is an Environmental Scientist within the Strategic Risk Group in the Contaminated Sites Management Division of Golder Associates Ltd., Calgary and the Applied Sciences Group at HydroQual Laboratories Ltd. Calgary. Sylvia obtained her undergraduate degree in Food Sciences and Nutrition and a Ph.D. in Food Science and Technology from the University of Alberta. Her Ph.D. examined the ability of dairy derived probiotics and bioactive peptides in altering intestinal microbial ecology in the treatment of gastrointestinal disorders such as inflammatory bowel disease and colon cancer. She was nominated for the Governor General Gold Medal Award for her Ph.D. research. Sylvia is currently examining the fate and effects of nanoscale particles on ecological receptors and involved in developing a risk-based framework to assess the impact of nanotechnology on the environment. She has presented her current and previous work at numerous international and local conferences. Sylvia is a member of various ad-hoc nanotechnology working groups (SETAC, ASTM and SRA) and is a fellow of the International College of Nutrition.

Dr. Mark Chappell is a Research Physical Scientist at the Engineer Research and Development Center, US Army Corps of Engineers in Vicksburg, MS. He received his Ph.D. in Soil and Science in 2004 from Iowa State University, a M.S. in Plant & Soil Science in 1998 from the University of Kentucky, and a B.S. in Agronomy in 1995 from Brigham Young University. He was the ORISE Postdoctoral Research Fellow, US Environmental Protection Agency, Cincinnati, OH, in 2005 and a Postdoctoral Research Associate at Iowa State University in 2004. His research interests include metal-organic complexes in soil, solid-phase in-situ speciation of metals and organics, and chemistry of formulations in soil

Dr. Sandip Chattopadhyay, TetraTech/EM, Inc., has more than 18 years of experience in environmental fate and transport of emerging contaminants, sampling, handling, preservation techniques of samples in various matrices, development of analytical methodologies, treatment and monitoring of contaminated sediment, soil and groundwater and air. He has more than 10 years experience in managing numerous task orders for U.S. EPA. In the past, he has collaborated with different national laboratories and universities, such as Lawrence Berkeley Laboratory, Argonne National Laboratory, Stanford Synchrotron Radiation Laboratory and Purdue University. He is organizing, presenting and chairing a session on "Nanoscale ZVI" at the Sixth International Conference on Remediation of Chlorinated and Recalcitrant Compounds at Monterey, California. He has participated as an Expert Panel Member on Water Security Workshop organized by U.S. EPA and other federal agencies. He is a member of the Interstate Technology Regulatory Council's (ITRC) technical team, and has prepared technical guidance documents for scientists, engineers, regulators. Dr. Chattopadhyay published more than 50 peer-reviewed journal articles and reports. He led various R&D effort on dispersion, aggregation and sampling of anthropogenic (manufactured) nanoparticles (iron oxides and titanium oxides and other manufactured nanomaterials) in treatment of groundwater. These studies resulted in successful application of dispersed nanoparticles and control of aggregation in subsurface systems, and several reports for U.S. Navy and U.S. EPA. He is interested in application of nanomaterials (natural or man-made) as decontamination agent for chemical/biological/radiological- contaminated systems. He has received his Masters degree in Chemical

Engineering from Ohio University and Ph.D. from the Ohio State University. Presently, he is Tetra Tech's National Program Manager under the company's STREAMS contracts with ORD. Previously, he worked at Battelle Memorial Institute, Columbus, Ohio; ManTech Environmental Research Services Corp., and U.S. EPA's Kerr Environmental Research Center in Ada, Oklahoma. He has authored over 60 peer-reviewed publications and reports.

Dr. Heechul Choi is an Assistant Professor, Associate Professor, and Professor in the Department of Environmental Science and Engineering at Gwangju Institute of Science and Technology in Korea. Dr. Choi received his bachelor's degree in environmental engineering from National Fisheries University in Busan, Korea, his master's degree from Asian Institute of Technology in Bangkok, Thailand, and his Ph.D. in environmental engineering from Texas A&M University in the United States. Prior to joining Gwangju Institute of Science and Technology, he worked as a senior researcher in the Department of Environmental Engineering at the Korea Institute of Construction Technology. Dr. Choi's areas of interest include using nanomaterials (e.g., metal oxides, mesoporous materials, carbon nanotubes, etc.) for water purification and fate and transport of nanomaterials in ecosystems, advanced oxidation technologies for water and wastewater, contaminant transport and modeling through porous media, remediation of contaminated soil and groundwater, and water reuse and reclamation by natural purification.

Dr. Hyeok Choi is currently an Oak Ridge Institute for Science and Education research fellow at the U.S. Environmental Protection Agency National Risk Management Research Laboratory in Cincinnati, Ohio, USA. He obtained his Ph.D. degree at the Department of Civil and Environmental Engineering of the University of Cincinnati in 2007. His general research area includes environmental nanotechnologies with emphasis on the novel synthesis and environmental applications of nanostructured TiO2 photocatalysts and reactive metallic nanoparticles, advanced oxidation technologies, and membrane separation processes.

Okkyoung Choi is currently a Ph.D. student at the University of Missouri. She previously studied in the Department of Botany and Microbiology, University of Oklahoma, USA. She earned her B.S. and M.S. Degrees in environmental engineering from Korea in 2000 and 2002, respectively. Ms. Choi worked as a Research Associate, Research Institute of Biological and Environmental Technology, Biosaint Co., Seoul for one year before coming to the U.S. to pursue her Ph.D. degree. She has published several papers related to silver nanoparticle research in Water Research and Environmental Science & Technology.

Chanlan Chun was a Postdoctoral Fellow in the Department of Chemistry, University of Minnesota,

Sue Cumberland is currently a third year PhD student at the University of Birmingham UK under the supervision of Dr Jamie Lead. Her study area is the fate, transport and behaviour of manufactured nanoparticles within the aquatic environment. To date she has investigated the aggregation behaviour of synthetic iron oxide nanoparticles under conditions of pH and natural organic matter. Techniques include light scattering, electrophoresis, TEM and flow-field flow fractionation separation techniques. In addition she is also investigating in-house synthesized silver nanoparticles and bonding properties to natural organic matter and trace metals. Her background as a research assistant has involved working in areas of soil science, hydrology, water quality, and catchment studies of upland agricultural pollution in Scotland and lowland ground water recharge and wetland sytems in the Midlands. Her research interests include the role of humic substances in aquatic environment particularly with nanoparticles and pollution pathways through riparian systems. She holds degrees from Plymouth and Reading University, UK.

D

Kenneth Darko-Kagya: Kenneth Darko-Kagya is a doctoral graduate student in the Department of Civil

and Materials Engineering at the University of Illinois at Chicago. His research focus is on the fate and transport of nanoscale iron particles in soils and the remediation of contaminated sites.

Dr. Christophe Darnault is an Assistant Professor and the Director of the Burke Endowed Hydrology and Hydraulic Laboratory in the Department of Civil and Materials Engineering at the University of Illinois at Chicago since 2004. He is specialized in the hydrological, biochemical and environmental processes impacting water dynamic, water quality and quantity, the fate and transport of contaminants in the subsurface environment as well as water resources engineering and management. He obtained his PhD in Environmental and Water Resources Engineering from Cornell University in 2000. He is the editor of the book titled "Overexploitation and Contamination of Shared Groundwater Resources: Management, (Bio)Technological, and Political Approaches to Avoid Conflicts" published by Springer in collaboration with NATO in 2008. He is the author or co-author of more than 30 peer-reviewed book chapters and journal articles and presented more than 50 conferences papers at national and international meetings.

Dr. Simon Davies is a research specialist in Department of Civil and Environmental Engineering at Michigan State University. He obtained his Ph.D. from California Institute of Technology in 1985, after which he was a post-doctoral fellow at the Swiss Federal Institute of Technology (ETH) and Department of Physical Chemistry, University of Melbourne, Australia.

Dr. Dermot Diamond received his Ph.D. and D.Sc. from Queen's University Belfast (Chemical Sensors, 1987, Internet Scale Sensing, 2002), and was Vice president for Research at Dublin City University (DCU), Ireland (2002-2004). He has published over 160 peer reviewed papers in international science journals, is a named inventor in 13 patents, and is co-author and editor of three books 'Spreadsheet Applications in Chemistry using Microsoft Excel' (1997), 'Principles of Chemical and Biological Sensors', (1998) both published by Wiley, and 'Smart NanoTextiles', (Materials Research Society Symposium Proceedings, Volume 20, (2006). Professor Diamond is currently director of the National Centre for Sensor Research at DCU (www.ncsr.ie) which is one of the largest sensor research efforts world-wide (>260 researchers) and a Principal Investigator with the Adaptive Information Cluster (AIC), a major research initiative in the area of wireless sensor networks founded by Science Foundation Ireland (see www.adaptiveinformation.ie). He was also formerly the director of the Centre for Bioanalytical Sciences (www.cbas.ie). He is a member of the editorial advisory boards of the international journals 'The Analyst' and 'Talanta'. In 2002 he was awarded the inaugural silver medal for Sensor Research by the Royal Society of Chemistry, London. Details of his research can be found at http://www.dcu.ie/chemistry/asg/.

Dr. Steve Diamond is a Research Biologist with the US EPA's National Health and Environmental Effects Research Laboratory, within the Office of Research and Development. He currently coordinates the EPA's nanomaterials ecological toxicology research. He is a contributing author of EPA's ORD Nanotechnology Research Strategy, has coordinated reviews of standard test guidelines for their adequacy for testing nanomaterials for both the EPA and the Organization for Economic and Cooperative Development (OECD), and plays a leading role in the development of the OECD's nanomaterials research program (OECD Sponsorship Program). He earned his Ph.D. at Miami University (Ohio) and has worked in the area of Natural Resource Damage Assessments and phototoxicity of polycyclic aromatic hydrocarbons.

Dr. Baolin Deng is currently C. W. LaPierre Associate Professor in the Department of Civil and Environmental Engineering at the University of Missouri (MU). He completed his Ph.D. training from the Johns Hopkins University in 1996. After a year of postdoctoral research as a National Research Council research associate at the Air Force Research Laboratory, he began his academic career at New Mexico Tech as an assistant professor in 1996 and moved to MU in 2001. His research concerns with important environmental and geochemical processes relevant to contaminated site remediation, drinking water

treatment, and chemical and biological transformation of contaminants in aquatic systems. More recently, he has been exploring environmental applications of nanotechnologies and examining the aquatic toxicity of nanomaterials. He has obtained funds from the Department of Energy, National Science Foundation, and Environmental Protection Agency to support the research activities, and has authored ~50 journal articles and book chapters. Dr. Deng teaches several undergraduate and graduate courses, including Fundamentals of Environmental Engineering, Water Treatment Process Design, Water and Wastewater Laboratory, Aquatic Chemistry, Environmental Chemical Kinetics, Physicochemical and Biological Processes, and Hazardous Waste Management.

E

Aaron E. Edgington is a Ph.D. candidate at Clemson University in South Carolina. Aaron is a graduate research assistant in Dr. Stephen Klaine's lab.

Debbie Elcock is a policy analyst with the Environmental Science Division of Argonne National Laboratory in Washington, D.C. Among other things, she evaluates environmental regulatory approaches and helps develop cost-effective alternatives. She helped develop a strategy for establishing a laboratory-wide ES&H program for nanotechnology and has examined potential applications for nanotechnologies in areas ranging from groundwater remediation to energy transmission corridors. She has also made presentations on the ES&H concerns of nanotechnologies and the consequent challenges for regulation. Ms. Elcock has taught courses on environmental management standards, conducted workshops with various stakeholder groups on improved environmental regulatory approaches, authored more than 50 reports on environmental and energy topics, and spoken at numerous national and international conferences. Her education includes a masters degree in Business Administration from Dartmouth College and a bachelor's degree in mathematics from Connecticut College.

Dr. Daniel W. Elliott has more than 15 years of experience in the environmental industry 11 of which were spent in industry and that past 4 in consulting. In industry, Dr. Elliott focused on environmental due diligence assessments, the quantification of environmental liability, and internal compliance audits at two Fortune 500 multinational industrial firms. In the consulting arena, he has significant experience in leading and conducting environmental due diligence assessments as well as the management of various complex remediation projects in accordance with NJDEP's Industrial Site Recovery Act. He also led or supported several remediation projects, including one in NJ utilizing the innovative nanoscale zero-valent iron (nZVI) technology. Dr. Elliott is a recognized expert in the application of the emerging nZVI technology and has co-led implementation of numerous bench-scale and pilot scale assessments. He has co-authored several articles on the nZVI technology and applications in peer-reviewed journals. Dr. Elliott has significant experience in negotiating with regulators at all levels and has worked on technical, regulatory, and technology-transfer aspects of environmental projects in the United States, Mexico, and the Peoples Republic of China. Dr. Elliott holds a Ph.D. in Environmental Engineering, an M.S. in Environmental Science and Engineering, and an A.B. in Chemistry.

Robert J. Ellis, L.G., is a Senior Scientist, based in the ARCADIS Novi, Michigan office. He received a B.S. in Geology and a M.S. in Environmental Geosciences, both from Michigan State University. Mr. Ellis has been in the environmental consulting industry since 1998 and has managed remedial investigations and remedy selection/implementation at Resource Conservation and Recovery Act (RCRA), Toxic Substances Control Act (TSCA), and state-lead project sites with soil, sediment, and/or groundwater impacted with metals, chlorinated solvents, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Mr. Ellis is currently focused on performing geochemical evaluations that enhance conceptual site models, design and management of effective bench scale and field pilot studies for technology demonstrations, and development of remediation strategies that complement ARCADIS' innovative in-situ remediation techniques and lead to effective site closure strategies for

industrial and federal government clients.

Dr. David Ensor has 30 years of experience in aerosol and air pollution research as Director of the Center for Aerosol Technology (CAT), Senior Program Director, and Department Manager at RTI and as Manager of the Aerosol Science Department at Meteorology Research, Inc. Dr. Ensor has managed programs in nanotechnology, aerosol research, filtration, air pollution control technology, particle sampling and characterization, indoor air quality, pollution prevention, exposure research, surface cleaning, protective garments, microcontamination control, instrumentation development, and test methods development. These projects have been for the U.S. Environmental Protection Agency (EPA), the U.S. Department of Energy (DOE), the U.S. Army, SEMATECH, Semiconductor Research Corporation, universities, and numerous private organizations. Recently Dr. Ensor has been shifting his research interests to nanotechnology.

Dr. R. Keith Esch is a research microbiologist now serving in RTI's Microbiology Department and as adjunct faculty member in the Biochemistry and Biophysics department of the University of North Carolina, Chapel Hill. Dr. Esch received his B.S. degree in genetics from the University of California, Davis, and his Ph.D. in biology from the University of California, San Diego. He designs, and conducts applied and basic research in environmental biotechnology and bioaerosol science. Some areas of expertise include: method development, system evaluation, environmental monitoring, and exposure assessment. He conducts research in environmental microbial assessment, biological particulate matter analysis and antimicrobial/biocide efficacy evaluations. He supervises the sampling, isolation, quantitation, identification, and inactivation of microorganisms (bacteria, fungi, viruses) and their components in air, water, soils, industrial fluids and materials. His background in biochemistry and molecular biology is applied to bacteriology; mycology; sampling and analysis of microbiological agents, components or by-products; study design; quality assurance and quality control; and exposure assessment.

F

Dr. Anne Fairbrother, DVM, leads the Risk Assessment and Toxicology program at Parametrix, Inc. in Seattle, WA. She provides services in ecological risk assessment and ecotoxicology, with an emphasis on wildlife toxicology and terrestrial systems. Anne works in the areas of contaminated site assessment, pesticide regulatory science and similar needs of the chemical and metals industries. A recent addition to her practice has been regulatory support for companies that now need to comply with the European REACH legislation. She also supports state or national agencies through development of guidance documents e.g., for metals risk assessments and through technical support for site-specific soil and water criteria development for wildlife protection. Anne received her D.V.M. from Univ. California, Davis and her Ph.D. from Univ. Wisconsin. She has been the recipient of several honors and awards from professional societies, and holds a courtesy appointment on faculty at Oregon State University. She has authored more than 75 scientific papers and has delivered over 100 seminars, workshops, or other technical presentations.

Karin Foarde is a Senior Research Microbiologist with 30 years of experience and is the Director of RTI International's (RTI's) Microbial and Molecular Biology Department. She designs, directs, and conducts applied and basic research in microbiology and aerobiology. Her research interests focus on bioterrorism associated biological aerosols (bioaerosols) and the environmental causes of allergy and asthma. Her bioterrorism research experience includes detection, decontamination, and protection from biowarfare agents. Her asthma/allergy work focuses on researching the biological contaminants isolated from the environment to identify environmental causes of illness and to recommend methods for preventing such biological contamination and its associated adverse health effects. Some areas of expertise include

isolating and characterizing environmental microorganisms, airborne allergens, and pathogens. She directs analysis of samples for microorganisms, endotoxins, β-(1-3) glucans, and a variety of antigens.

Dr. Glenn E. Fryxell is a member of the Materials Chemistry and Surface Research Group within the Materials Division of ESTD, and has been a member of Materials since 1990. For the last 15 years, his research has focused on organic synthesis, surface chemistry, silane chemistry and the interfacial elaboration of self-assembled monolayers. He is a co-inventor of self-assembled monolayers on mesoporous supports (SAMMS) and has developed these materials for a wide variety of environmental applications, such as the sequestration of toxic heavy metals, radionuclides and oxometallate anions. Dr. Fryxell is named as inventor in 11 patents, and has over 100 publications and 60 invited presentations. He obtained his B. Sc. from the University of Texas in 1982, where he worked for two years in the laboratories of Prof. Marye Anne Fox studying the photochemistry of enolates and carbanions. His Ph. D. was award in 1986 from the University of North Carolina, where he worked with Prof. Paul J. Kropp studying the photochemistry of phenylthio ethers. A two-year postdoctoral appointment with Prof. Albert Padwa at Emory University was dedicated to the study of intramolecular dipolar cycloaddition and heterocyclic synthesis.

G

Florin Gheorghiu, C.P.G. is Project Director and Principal in the Philadelphia Office of Golder Associates. He is an expert in hydrogeologic testing, modeling and hydrogeologic designs and has over 29 years of experience in engineering geology and hydrogeology. He has directed numerous environmental projects at CERCLA and RCRA sites that required numerical groundwater flow and solute transport modeling using computer codes such as MODFLOW, MODPATH and MT3D. Mr. Gheorghiu served as Project Director and technical manager for the design and implementation of a large bedrock remedial system at Modern Landfill that involved extensive hydrogeologic testing of fractured bedrock, numerical modeling and deep bedrock blasting. This project received the Year 2000 Outstanding Groundwater Remediation Award from the National Groundwater Association. His publications include: "Hydrogeologic Characterization of Blasted Rock Mass," (2001 Key Note to the Geological Society of Philadelphia, Philadelphia, Pennsylvania); "Enhanced Western Groundwater Control System," (2000 Key Note to the Regional Hydrogeologists Meeting of the Department of Environmental Protection, Harrisburg, Pennsylvania); "Application of Analytical and Numerical Models for Natural Attenuation Characterization," (1997 Presentation at the Golder Associates Natural Attenuation Seminar, Princeton, New Jersey); "Advanced Test Analysis Methods for the Hydrogeological Characterization of Potential Nuclear Waste Repositories in Switzerland and Germany," (1996 Presentation to the technical staff of the U.S.EPA Region 3, Philadelphia, Pennsylvania); "Use of Derivative for Hydrogeologic Test Flow Model Identification with Application in Deep Borehole Testing," (1995 Presentation to the technical staff of the U.S.Geological Survey, Trenton, New Jersey).

Dr. Subhasis Ghoshal is an Associate Professor in the Department of Civil Engineering & Applied Mechanics. He joined McGill as an Assistant Professor in 1997 after completing his Ph.D. at Carnegie Mellon University and a postdoctoral fellowship at the University of Michigan at Ann Arbor. His research is in the area of Environmental Engineering and currently focuses on bioremediation of polluted sites and groundwater, and on carbon dioxide sequestration technologies for greenhouse gas mitigation. Prof. Ghoshal has contributed substantially to the understanding of NAPL-water interfacial mass transport processes and its impacts on remediation performance and groundwater quality. He has worked extensively on NAPL dissolution, biodegradation and interphase mass transfer in NAPL-surfactant systems. He has recently developed techniques for imaging of NAPL contamination in porous media using a medical X-ray scanner which allows non-invasive, quantitative contaminant mass characterization in soil columns and cores. Prof. Ghoshal received the PetroCanada Young Innnovator Award in 1998 and was named as a Dawson Scholar in 2005. He is a founding member of the CT Scanning Laboratory for

Agricultural and Environmental Research at McGill.

Michael Gill received his B.S. degree in electrical engineering from Northeastern University in Boston and his MSEE from Renssalear Polytechnic Institute in Troy, NY. He practiced electrical engineering in the 1980's with the U.S. Navy and government contractors until he made a career change to the environmental field in 1992.

He is currently the EPA Office of Research and Development (ORD) Superfund and Technology Liaison to EPA's San Francisco office (Region 9). This position is one of technical support and information brokering. His customers are for the most part Remedial Project Managers in the Superfund Program, but may include RCRA and other Regional EPA staff, state environmental staff, industry, and the public. In this position since 1998, he provides hazardous waste technical support to his customers and he also participates in research planning, environmental technology demonstrations, and workshop planning. He has been with EPA since 1992, when he took a position as a Remedial Project Manager in Region 9's Superfund program.

Dr. Vicki Grassian is currently a Full Professor in the Department of Chemistry and has secondary appointments in the Departments of Chemical and Biochemical Engineering and Occupational and Environmental Health. At the University of Iowa, Professor Grassian has been the recipient of a Faculty Scholar Award (1999-2001) a Distinguished Achievement Award (2002), a James Van Allen Natural Sciences Faculty Fellowship (2004), the Regents Award for Faculty Excellence (2006) and was named a Collegiate Fellow in the College of Liberal Arts and Sciences in 2007. In 2006, she became the Director of the Nanoscience and Nanotechnology Institute at the University of Iowa. She also serves as an Associate Director for the Institute of Clinical and Translational Science. For the past several years, a major research focus in her group has been on the applications and implications of nanoscience and nanotechnology in environmental processes. Professor Grassian has edited three books including the most recent one published by John Wiley and Sons entitled Nanoscience and Nanotechnology: Environmental and Health Impacts. She has over 140 peer-reviewed publications. In 2003, Professor Grassian received a US-National Science Foundation Creativity Award and in 2005, she was elected as a Fellow of the American Association for the Advancement of Science.

Dr. George M. Gray was sworn in on November 1, 2005, to serve as the Assistant Administrator for the Office of Research and Development, which is the 1,900-person, $600 million science and technology arm of the Environmental Protection Agency. Dr. Gray was appointed to this position by President George W. Bush and confirmed – by unanimous consent – by the U.S. Senate.

Prior to joining EPA, Dr. Gray was Executive Director of the Harvard Center for Risk Analysis and a Lecturer in Risk Analysis at the Harvard School of Public Health (HSPH). In 16 years at HSPH, his research focused on scientific bases of human health risk assessment and its application to risk policy with a focus on trade-offs in risk management. Dr. Gray taught toxicology and risk assessment to both graduate students and participants in the School´s Continuing Professional Education program.

Dr. Gray holds a B.S. degree in biology from the University of Michigan, and M.S. and Ph.D. degrees in toxicology from the University of Rochester.

Kimberly Guzan is an aerosol engineer at RTI. She earned a Masters of Science at University of Akron School of Polymer Science and a Bachelor of Engineering Degree in Chemical Engineering. Her research interests include polymer nanotechnology and spectroscopic characterization in materials fabrication, aerosol filtration, chemical sensors and bio-materials research. Subsequently, her research work has included metal/polymer interfacial adhesion in biomedical devises and organometallic-crystal synthesis.

H

Scott Hall manages ENVIRON's Ecotoxicology Group and toxicity testing facilities in Nashville, Tennessee. He serves on ENVIRON's Nanto-technology Task Force, and is conducting research related to the effects of titanium dioxide on aquatic life. Mr. Hall received a bachelor's degree in Environmental Protection from West Virginia University and a master's degree in Aquatic Toxicology from North Texas State University. He has been a consultant to industry for over 20 years.

Dr. Li Han has more than eight years of research and development experience in nanoscience and nanotechnology. Her original research findings have been published in 30 peer-reviewed journals, and resulted in two patents and six RTI International invention disclosures. Dr. Han's research interests include fabricating nanoscale materials, developing novel microscopic and spectroscopic characterization techniques for nanoparticles and nanofibers, and exploring the application of nanoscale materials in chemo- and biosensors, catalysis, and biomedical devices.

Dr. Stacey Harper leads the Nanotoxicology Division of the Tanguay laboratory at OSU where she employs in vivo approaches to provide feedback on the biological activity and toxic potential of nanomaterials. She has established a collaborative research group to develop the knowledgebase of Nanomaterial-Biological Interactions (NBI). She received her B.S. in natural sciences and mathematics from Mesa State College, Colorado in 1990; and earned her M.S. and Ph.D. in biological sciences from University of Nevada Las Vegas in 1998 and 2003. From 2003 to 2005, she held a biology postdoctoral position with the Exposure and Dose Research Branch of the EPA.

Dr. Ted Henry is a Research Assistant Professor in the Center for Environmental Biotechnology at The University of Tennessee (Knoxville, TN) and a Research Council of the United Kingdom (RC-UK) Academic Fellow at the University of Plymouth (Plymouth, UK). Investigating the characteristics and toxicity of nanoparticles is major part of his research program at both institutions and presently his work is supported by a U.S. EPA STAR grant to investigate the ecotoxicology of fullerenes in fish. A primary objective is to link nanoparticle characteristics with toxic effects and his research aims to clarify mechanisms at lower levels of biological organization with higher order effects at tissue and whole organism levels. His role at The University of Tennessee and the University of Plymouth provides a unique opportunity to integrate research in nanotoxicology among laboratories in the U.S. and the UK.

Mbhuti Hlophe is Head of the Department of Chemistry at North-West University (Mafikeng campus) in South Africa. His major research area is water treatment, particularly for the provision of potable water to rural communities. He is one of the principal researchers in water purification in the India, Brazil and South Africa (IBSA) trilateral cooperation agreement on nanotechnology. He has authored relevant conference papers, including a case study on a nanofiltration method for water treatment in South Africa background paper for Meridian`s workshop in Chennai (India), membrane nanotechnology in water treatment (IBSA workshop in Kalpakkam, India), and the role of nanotechnology in the provision of potable water to rural communities (IBSA workshop in Pretoria, South Africa). Papers that have been published include: "Nanotechnology, Water and Development" (http://www.merid.org/nano/waterpaper); "Nanotechnology and the challenge of clean water" (Nature Nanotechnology, 2 (11), 663 – 664); "Nitrogenous pollution in borehole water due to pit latrines and fertilizers" (submitted to Water SA review for possible publication); and a chapter in a book for the U.S. EPA titled "Nanotechnology Applications: Solutions for improving water quality.". He also has performed consulting work, the most important of which was the development of water safety and security plans for the Department of Water Affairs and Forestry in South Africa.

Dr. Patricia Holden is a Professor at the University of California, Santa Barbara in the Donald Bren School of Environmental Science & Management. The Holden lab researches environmental microbiology, focusing on questions in water, soil science and emerging pollutants. Holden's education is in Civil & Environmental Engineering (B.S., M.S.) with 8 years of professional engineering experience

followed by her Ph.D. and Postdoctoral Research in Soil Microbiology (U.C. Berkeley). Holden has been on the faculty at UC Santa Barbara since 1997. Current projects in the Holden group are in coastal bacteriological water quality with an emphasis on watershed processes, bacterial interactions with engineered nanomaterials, and vadose zone microbial ecology. Her co-authors for this talk include Allison Horst (doctoral student), John Priester (postgraduate researcher) and Dr. Andrea Neal (postdoctoral researcher) who are all actively researching nanomaterials interactions with bacteria.

Dr. Zhiqiang Hu is an Assistant Professor of environmental engineering at the University of Missouri. Dr. Hu has been studying biochemical processes for wastewater treatment and nutrient removal for more than ten years. His recent research interest includes bioavailability and toxicity of nanoparticles in wastewater treatment systems. One of his ongoing research projects entitled "Nitrification inhibition by silver nanoparticles" was financially supported by the National Science Foundation. Dr. Hu has published some of the nanotoxicity research findings by working with her Ph.D. student, Okkyoung Choi. The Water Environment Research Foundation recently awarded Dr. Hu $150,000 to determine more precisely when silver nanoparticles start to impair wastewater treatment. In that project, his research team will determine how silver nanoparticles affect representative wastewater treatment processes by gradually releasing as well as injecting a shock load of the nanomaterial into wastewater and sludge. Measuring subsequent microbial growth will allow MU researchers to determine the nanosilver levels that will harm wastewater treatment and sludge digestion.

De-Huang Huang is a senior environmental engineer in the Chinese Petroleum Corporation, Kaohsiung, Taiwan, ROC. He received his M.S. degree in Environmental Engineering from the Graduate Institute of Environmental Engineering at National Taiwan University. He is interested in applying novel technologies for groundwater remediation including iron nanoparticles, chemical oxidation and thermal technology.

Dr. William D. Hunt is Professor of Electrical Computer Engineering at the Georgia Institute of Technology and is Adjunct Professor in the Department of Hematology and Oncology at the Emory University School of Medicine. He runs the Microelectronics Acoustics Group at Georgia Tech and has a diverse collection of graduate students

Dr. Robert Hurt is Professor of Engineering at Brown University and Director of Brown University's Institute for Molecular and Nanoscale Innovation (IMNI). Dr. Hurt has a hybrid technical background in nanomaterials science and energy/environment. He has devoted the last four years to understanding the fundamental biological interactions of nanomaterials and in developing new nanostructures for environmental and biological applications. He has been involved in discussion of nanotechnology environmental safety and health policy and regulation through talks at the National Research Council of Canada (2007), the Environmental Business Council of New England (2006), the World Technology Evaluation Center (2006), and participation in the 2007 NanoBusiness Alliance Public Policy Tour in Washington D.C. Dr. Hurt received a Sc.B. from Michigan Technological University and a Ph.D. from the Massachusetts Institute of Technology, both in chemical engineering. Prior to joining Brown in 1994 he held posts at Bayer AG in Leverkusen, Germany, and Sandia National Laboratories in Livermore California. Professor Hurt is an Editor of the materials science and nanotechnology journal CARBON, has served as the Graffin Lecturer of the American Carbon Society, and has won the Silver Medal of the Combustion Institute for work on the high-temperature reactions of carbon materials. His current research interests are in the applications and implications of nanotechnology for human health and the environment, including nanosorbents for pollution abatement and the intelligent design and formulation of nanomaterials to minimize health risks. He is a member of the scientific advisory board for the company Nanotox.

Dr. Jim Hutchison is Professor of Chemistry and Director of the Materials Science Institute at the University of Oregon. He also directs the Safer Nanomaterials and Nanomanufacturing Initiative of

the Oregon Nanoscience and Microtechnologies Institute and has pioneered the University's Green Organic Chemistry Laboratory program. A native of Oregon, he received his B.S. in Chemistry from the University of Oregon in 1986 and a Ph.D. from Stanford University in 1991 (with James P. Collman). He then did postdoctoral work with Royce W. Murray at University of North Carolina, Chapel Hill. He has won numerous awards including a Postdoctoral Fellowship and a CAREER award from the National Science Foundation, as well as awards from the Sloan and Dreyfus Foundations. His current research interests include the design, synthesis and study of functional organic and inorganic materials, including functionalized surfaces and nanoparticles, green chemistry and green nanoscience.

J

Nick Jaynes is a Geotechnical Engineer for MSE Technology Applications in Butte, Montana. Mr. Jaynes holds degrees in Environmental Engineering and Civil/Geotechnical Engineering from Texas A&M University. His previous experience includes consulting in the environmental and geotechnical fields in Wyoming and Montana.

Dr. Gautham Jegadeesan is an Environmental Engineer with Pegasus Technical Services, Inc at Cincinnati. A graduate in Chemical Engineering and a Ph.D in Engineering Science, Dr. Jegadeesan has worked on diverse water remediation projects including the use of bimetallic nanoparticles for trace metal remediation and electrolytic processes for contaminant reduction. He is currently working on the fate and transport of engineered nanoparticles in the environment, speciation of trace metals in coal combustion residues and mining wastes.

Vijay T. John is Professor and Chair of the Department of Chemical and Biomolecular Engineering at Tulane University. He works on self-assembled nanoscale materials for environmental applications and in targeted drug delivery. He has published 130 Journal articles and has supervised 19 Ph.D. dissertations. He is funded by U.S. EPA, the NSF, U.S. Department of Energy, and NIH.

Jon Josephs' academic background includes degrees in chemical engineering from Rutgers University (1971) and Stevens Institute of Technology (1973). He was selected for membership in Tau Beta Pi, the national engineering honor society. In 1973, Jon joined the EPA Region 2 office in New York City where he was employed in permitting industrial wastewater discharges, regulating hazardous-waste management facilities and as a Superfund Remedial Project Manager. In 1994 Jon was reassigned from Region 2 to the Office of Research and Development as the Superfund and Technology Liaison (STL) assigned to EPA Region 2.

As an STL, Jon's activities have included: organizing a workshop on the natural attenuation of chlorinated solvents in groundwater, managing the development of a compendium of methods for monitoring the remediation of contaminated sediments, serving on Science Advisory Committees for the Northeast Hazardous Substance Research Center and for the Center for Hazardous Substances in Urban Environments, participating in the workgroup that developed the Office of Solid Waste and Emergency Response's directive on monitored natural attenuation and serving as EPA project coordinator for a research project on biodegradation of polychlorinated-dibenzo-p-dioxins. More routine activities include coordinating technical support for Region 2 Superfund projects, identifying EPA Region 2 research needs, serving on the EPA Region 2 Science Council and organizing technical presentations for Region 2 staff.

K

Dr. Agnes Kane is Professor and Chair of the Department of Pathology and Laboratory Medicine at Brown University, and has devoted her career to the study of biological responses to particulate and fibrous toxicants. She has served on scientific panels in environmental health sciences, including current membership of the EPA Science Advisory Board and the ICON working group on nanomaterial safety.

She has devoted the last four years to understanding the biological impacts of new nanomaterials. She has served as scientific advisor and invited participant in workshops on fiber toxicology and nanotechnology for NIOSH, US EPA, NAS, IOM, NTP and IARC.

Dr. Barbara Karn, a U.S. EPA scientist, built and managed a research grant program in nanotechnology and the environment at EPA. She formed and sustained a community of researchers in nanotechnology and the environment-both applications and implications-and brought nanotechnology into EPA's programs and mission. Through the interagency Nanoscale Science and Technology subcommittee of the Office of Science and Technology Policy, she led workshops to build consideration of the environment and human health in other government agency research programs related to nanotechnology. She helped provide leadership in international activities involving nanotechnology in the environment and human health. Currently, she is the nanotechnology scholar at Georgetown University's Program for Science in the Public Interest and recently returned from a detail at the Woodrow Wilson International Center for Scholars Program in Emerging Nanotechnologies. Dr. Karn holds the Ph.D. from Florida International University and a B.S. in chemistry from Ohio State.

Dr. Ian M. Kennedy joined the Department of Mechanical and Aeronautical Engineering at the University of California Davis in 1986 after a period as a Research Staff member at Princeton University and several years at the Aeronautical Research Laboratories in Australia. He has developed a major aerosol research facility at the University of California Davis in which efforts are directed at varied problems related to ultrafine particle synthesis and applications in technology. A major thrust of Dr. Kennedy's efforts is directed towards understanding the impact of ultrafine aerosol particles on human health. This interest is pursued via extensive multidisciplinary collaborations with colleagues in Environmental Toxicology, Land Air Water Resources, Veterinary Medicine, Chemistry and Civil and Environmental Engineering. He is also involved in applying nanoscale particles to detection technologies in biology and biophotonics e.g., using nanoscale phosphors as labels of bio-molecules. This work involves collaborative research with colleagues in the Departments of Entomology, Internal Medicine and Land Air Water Resources.

Alan J. Kennedy is a Research Biologist with the U.S. Army Engineer Research and Development Center in Vicksburg, Mississippi. His responsibilities include serving as project manager/principal investigator for ecotoxicological exposure and effects assessment; conducting water column and whole sediment toxicity and bioaccumulation testing in support of research, dredged material assessments, and other client needs; writing manuscripts, proposals, technical reports and laboratory SOPs; and managing laboratory technicians. His research has involved chemicals such as DDTs, PCBs, PAHs, explosives, metals and nanoparticles. Mr. Kennedy received a M.S. in Aquatic Ecotoxicology in 2002 from Virginia Polytechnic Institute and State University. His thesis work involved risk assessment methodologies to gauge multiple levels biotic impairment caused by the total dissolved solids (TDS) toxicity of a treated coal-mining effluent in southeastern Ohio. He received a B.S., with high honors, in Environmental Biology/Zoology in 1999 from Michigan State University.

Dr. Amid P. Khodadoust is an Associate Professor of Environmental Engineering at the University of Illinois at Chicago. He teaches environmental engineering, physico-chemical processes, waste water treatment, and pollution prevention. His research expertise includes bioavailability of contaminants in sediments, remediation of contaminated soils and sediments, and environmental nanotechnology.

Dr. Jeonghwan Kim is a research associate in Department of Civil and Environmental Engineering at Michigan State University. He received his Ph.D. degree in Environmental Sciences and Engineering from University of North Carolina at Chapel Hill in 2005.

Ayla Kiser received her bachelor of science in mechanical engineering and her master of science in

environmental engineering from the University of Nevada, Las Vegas. In August 2006, she began the environmental engineering Ph.D. program at Arizona State University. Under the guidance of her advisors, Dr. Paul Westerhoff and Dr. Bruce Rittmann, Kiser is currently doing research on the biological removal, environmental fate, and detection of engineered nanoparticles from wastewater. She is expected to graduate in 2010.

Dr. Stephen J. Klaine is a professor in the Department of Biological Sciences at Clemson University in Pendleton, SC. Dr. Klaine received his bachelor's degree in biology from University of Cincinnati and his master's degree and Ph.D in environmental science from Rice University in Texas. Dr. Klaine's research focuses on the fate and effects of contaminants in the environment. Specifically, he is interested in contaminants that migrate from various land uses into aquatic ecosystems and their effects on aquatic plants and animals. His laboratory studies contaminant effects on fish, aquatic invertebrates, plants, and algae. Current research on nanomaterials includes work on their behavior in aquatic systems, bioavailability, and food chain transport.

Dr. Rebecca Klaper received her Ph.D. in Ecology from the Institute of Ecology, University of Georgia. She is currently a Shaw Scientist at the Great Lakes WATER Institute, an organization dedicated to providing basic and applied research to inform policy decisions involving our freshwater resources. Dr. Klaper studies the potential impact of emerging contaminants, such as nanoparticles and pharmaceuticals, on aquatic organisms using traditional toxicology methods as well as investigations using genomic technologies. Dr. Klaper has served as an American Association for the Advancement of Science-Science and Technology Policy Fellow where she worked in the National Center for Environmental Assessment at the US Environmental Protection Agency. She has served as an invited scientific expert to the Organization for Economic and Cooperative Development panel on nanotechnology where she testified on the potential impact of nanoparticles on the environment. She also was involved in writing the EPA White Paper on the use of genomic technologies in risk assessment. She belongs to several scientific societies including the Ecological Society of America, The Society for Environmental Toxicology and Chemistry and the American Fisheries Society.

L

Dr. Sarah C. Larsen, is a Professor of Chemistry and the Associate Director of the Nanoscience and Nanotechology Institute at the University of Iowa. Professor Larsen has research interests in the applications of nanocrystalline zeolites to environmental remediation, decontamination and drug delivery. Professor Larsen has expertise in the synthesis, characterization and functionalization of nanocrystalline zeolites and hollow zeolite structures. Her research has been funded by the National Science Foundation (NSF), the Environmental Protection Agency, the Army Research Office, the Department of Energy and the Petroleum Research Fund. Professor Larsen has also been involved with educational efforts in nanoscience and nanotechnology. Currently, she is the Director of an NSF Research Experiences for Undergraduates (REU) program focused on nanoscience and nanotechnology. Professor Larsen is also a senior editor for the Journal of Physical Chemistry.

Dr. Warren Layne has a BA in chemistry from Boston University, MS in inorganic analytical chemistry from University of Massachusetts, and Ph. D. in medicinal chemistry from Northeastern University in Boston, with postdoctoral training at Harvard School of Public Health in nuclear medicine. He also has additional years of industrial experience in radiopharmaceutical research as an Assistant Professor at University of Connecticut Medical Center, University of Texas at Galveston, and Baylor University in Houston. Dr. Layne joined the EPA in 1991 as the Toxic Release Inventory (TRI) coordinator for Region 6 (Dallas, TX) and is the currently serves as a Quality Assurance Project Plan (QAPP) reviewer and Regional Sample Coordinator for the Superfund Division as well as Nanotechnology expert in Region 5 (Chicago, IL). He was a coauthor of Nanotechnology White Paper, participated in EPA-sponsored

National Nanotechnology Conferences, and is a member of Nanometers, the current co-chair of the EPA National Nanotechnology Workgroup. He is co-chair of the multi-agency steering committee for the International Environmental Nanotechnology Conference: Applications and Implications scheduled for Chicago, October 7-9, 2008.

Dr. James M. Lazorchak is an aquatic biologist/toxicologist for the U.S. EPA National Exposure Research Laboratory, Ecological Exposure Research Division, where he is Acting Chief of the Molecular Indicators Research Branch. He received a B.S. in biology (1987) from Southeast Missouri State University, a M.S. in aquatic ecology (1974) from Wright State University, and a M.S. in environmental sciences (1978) from the University of Texas at Dallas. He received his Ph.D. in ecotoxicology (1986) from the University of Texas at Dallas.

Research in Dr. Lazorchak's early career centered around developing fish, invertebrate, and plant bioasssessment and ecotoxicology methods to assess the biological integrity of lakes, streams, rivers, and estuaries. My current research activities are to bring genomic tools to bioassessments and ecotoxicity tests to assess ecosystem health and develop water quality criteria and water quality standards and limits that can be used in regulatory programs of emerging contaminants (i.e., EDCs and pharmaceuticals).

He has written 36 peer reviewed papers, 13 EPA manuals, 4 book chapters.

Dr. Qilin Li is an Assistant Professor in the Department of Civil and Environmental Engineering at Rice University. Dr. Li obtained her B.E. in Environmental Engineering from Tsinghua University in China. She received her M.S. and Ph.D. degrees in Environmental Engineering from University of Illinois at Urbana-Champaign in 1999 and 2002, respectively. Before joining the faculty at Rice University, she worked as a post-doctoral research associate at Yale University from 2002 to 2003 and an assistant professor at Oregon State University from 2004 to 2005. Dr. Li's current research focuses on advanced technologies for water quality control including adsorption and membrane separation, and environmental application and impact of nanotechnology.

Dr. Yusong Li is currently a postdoctoral associate in the Department of Civil and Environmental Engineering at Tufts University. She received her Ph.D. in Environmental Engineering from Vanderbilt University in 2005. She will start as an Assistant Professor in the Department of Civil Engineering at University of Nebraska-Lincoln. Her research area is numerical simulation of fate and transport of contaminants in the subsurface system.

Dr. Hsing-Lung Lien is an associate professor in the Department of Civil and Environmental Engineering at the National University of Kaohsiung in Taiwan. He received his Ph.D. in Environmental Engineering in 2000 from Lehigh University, under the guidance of Dr. Wei-xian Zhang. He worked as a research associate at the Ground Water and Ecosystems Restoration Division, an USEPA research laboratory, in Ada, Oklahoma from 2000 to 2002. Dr. Lien has joined the National University of Kaohsiung since 2002. His research interests include environmental nanotechnologies and physicochemical processes for water treatments. He has published over 10 peer-reviewed papers on the use of iron nanoparticles for groundwater remediation.

Dr. Igor Linkov is a Research Scientist at the US Army Engineer Research and Development Center and Adjunct Professor of Engineering and Public Policy at Carnegie Mellon University. Dr. Linkov has managed multiple risk assessments and risk management projects. Many of his projects have included application of the state-of-the-science modeling and software tools (e.g., probabilistic and Bayesian Monte-Carlo, spatially-explicit modeling) to highly complex sites and engineering problems (e.g., Hudson River, Dow Midland, Natick Soldier Systems Command, Elizabeth Mine, etc.) and projects (e.g., insuring emerging risks, risk-based prioritization of remedial projects, developing performance metrics for oil spill response). He was instrumental in developing an integrated risk assessment and

multi-criteria decision analysis framework that is now being widely applied by the US Army Corps of Engineers, including restoration planning for coastal Louisiana and Mississippi affected by the hurricane Katrina where a multi-billion dollar budget is at stake. Dr. Linkov is currently involved in several projects that examine factors responsible for nanotoxicology and nanomaterials risks. These projects investigate fate and transport of nanoparticles in the environment, ecotoxicology, assessment of nano-enabled product life cycle and risks. Dr. Linkov have organized three continuing education workshops in the area of nanomaterials health and safety and an international conference on "Nanomaterials: Environmental Risks and Benefits" (Portugal, April 2008). Dr. Linkov was part of international and national panels on nanotechnology, including: EPA Nanotechnology White Paper Peer Review Panel (2006), Nanotechnology Research Strategy (2008), and Nanotechnology Grants Review Panel (2007); Environment Canada Nanotechnology Expert Panel (2007); and the City of Cambridge Nanotechnology Ordinance Advisory Panel (2007-2008). The Governor of Massachusetts has appointed Dr. Linkov as a Scientific Advisor to the Massachusetts Toxic Use Reduction Institute. He is the recipient of the prestigious Chauncey Starr Award for exceptional contribution to Risk Analysis. Dr. Linkov has a BS and MSc in Physics and Mathematics (Polytechnic Institute, Russia) and a Ph.D. in Environmental, Occupational and Radiation Health (University of Pittsburgh). He completed his post doctoral training in Biostatistics and Toxicology and Risk Assessment at Harvard University.

Dr. Bruce Lippy has a Ph.D. in policy from the University of Maryland, with coursework concentrated in regulatory economics and quantitative measures of management. His doctoral research was on communicating the hazards of operating and maintaining innovative environmental technologies for cleaning up the Department of Energy's nuclear weapons complex. His work led to the development of over 150 Technology Safety Data Sheets for the Department of Energy. His undergraduate degree is a B.A. summa cum laude in biology from Western Maryland College. He is a Certified Industrial Hygienist and Certified Safety Professional. While with the University of Maryland School of Medicine, he co-authored an extensive review of the hazard communication literature on MSDSs, labels and warnings. He has participated in the White House Office of Science and Technology Policy's Nanoscale Environment and Health Initiative. Dr. Lippy has spoken on the worker health and safety issues of nanotechnologies at the Mount Sinai School of Medicine, the University of Massachusetts at Lowell, the Society for Chemical Hazard Communication, the American Society of Safety Engineers and the Community Colleges of Baltimore.

Dr. Tom Long is a staff scientist in the U.S. Environmental Protection Agency's (US EPA) National Center for Environmental Assessment (NCEA). Here, he prepares science assessments that evaluate the scientific evidence that relates to the health effects of criteria air pollutants. Prior to joining NCEA, he conducted research in the laboratory of Dr. Bellina Veronesi on the biological effects of titanium dioxide and nZVI nanoparticles used in environmental remediation. He has published these findings in Environmental Science & Technology and Environmental Health Perspectives. He recently received his Ph.D. from the Department of Environmental Sciences and Engineering, School of Public Health University of North Carolina at Chapel Hill (2007).

Dr. Gregory Lowry is an associate professor in the department of Civil and Environmental Engineering at Carnegie Mellon University. He teaches Environmental Engineering, Water Quality Engineering, Environmental Fate and Transport of Organic Compounds in Aquatic Systems, and Environmental Sampling and Sample Characterization. His research interest is broadly defined as transport and reaction in porous media, with a focus on the fundamental physical/geochemical processes affecting the fate of inorganic and synthetic organic contaminants and engineered nanomaterials in the environment. He is primarily an experimentalist and works on a variety of application-oriented research projects developing novel environmental technologies for restoring contaminated sediments and groundwater. His current projects include in situ sediment management using innovative sediment caps, DNAPL source zone

remediation through delivery of reactive nanoparticles to the NAPL-water interface, and CO2 capture, sequestration, and monitoring. The primary goal of most projects is to provide economical engineering solutions to specific relatively well-defined environmental problems, but each step of engineering development also provides the opportunity to make fundamental scientific contributions in the areas of contaminant transport and fate.

M

Dr. Bettye L.S. Maddux is the assistant director of the Safer Nanomaterials and Nanomanufacturing Initiative, a major research thrust of the Oregon Nanoscience and Microtechnologies Institute and a member of the Materials Science Institute at the University of Oregon. In 1992, she earned her Ph.D. in biological sciences with an emphasis in chemical carcinogenesis from the University of Texas at Austin. Her postdoctoral work at the University of California, Santa Barbara involved elucidating nature's mechanisms for creating environmentally benign nanomaterials. Previously, she has published peer-reviewed research articles as 'Bettye L. Smith' in the fields of nanotechnology, biophysics and chemical carcinogenesis.

Dr. Shaily Mahendra is CBEN Research Associate in the Department of Civil and Environmental Engineering at Rice University. Dr. Mahendra earned her B.Tech. degree from Indian Institute of Technology, Delhi, M.S. from Syracuse University, and Ph.D. from University of California, Berkeley. Her research areas are environmental toxicology and applications of nanomaterials, applications of molecular and isotopic tools in environmental microbiology, and biodegradation of emerging groundwater contaminants.

Dr. Susan Masten is a professor in Department of Civil Engineering at McMaster University, Canada. She obtained her Ph.D. in Environmental Engineering from Harvard University in 1986.

Bharat Mathur was appointed Deputy Regional Administrator of U.S. Environmental Protection Agency Region 5 in 2002. In this role, he assists the Regional Administrator in implementing federal environmental programs in the Great Lakes states of Illinois, Indiana, Michigan, Minnesota, Ohio and Wisconsin.

Mr. Mathur served as Acting Regional Administrator twice — for 16 months, beginning in April 2004, and again for six months, beginning in April 2006. During his second stint as the Region's acting leader, he assumed the additional responsibilities of Acting Manager of the Great Lakes National Program. In this role, he oversaw EPA's continued efforts to protect and clean up the Great Lakes, including advancing the efforts of the Great Lakes Regional Collaboration and pushing forward with Great Lakes Legacy Act cleanups.

Mr. Mathur came to EPA in January 2000 as director of the Air and Radiation Division after a lengthy career with the state of Illinois, where he managed Illinois EPA offices dealing with air pollution, hazardous and solid waste, and Clean Water Act programs.

He has served on numerous state and national committees to develop environmental policies and programs, and has consulted with government agencies in India, China, Indonesia, Korea and Mexico

Dr. Charles Maurice has served as the U.S. EPA Office of Research and Development (ORD) Superfund & Technology Liaison (STL) to Region 5 (Chicago, IL) since April 2004. As such, he holds a joint appointment with the Office of Science Policy in ORD and with the Innovative Systems & Technology Branch in the Region 5 Superfund Division. Chuck provides technical support regarding hazardous substances both through his own expertise as an ecological risk assessor and by coordinating with other scientists in the technical support centers and laboratories throughout ORD. He also communicates Regional research priorities and needs to ORD.

From 1995 to 2004, Chuck was an ecologist and ecological risk assessor the Region 5 Office of Strategic Environmental Analysis (OSEA), both in the immediate office and on the Critical Ecosystems Team. Chuck was an ecological risk expert, corrective action manager, and permit writer in the RCRA Permitting Branch, Region 5 Waste Management Division from 1993 to 1995. Before joining EPA, Chuck was a senior ecologist and ecological risk assessor for Ecology & Environment, Inc., a Superfund contractor.

Chuck holds a B.S. degree (1980) in environmental biology from Eastern Illinois University, a M.S. degree (1982) in biological sciences from Bowling Green State University, and a Ph.D. (1989) in plant biology from the University of Illinois at Urbana-Champaign.

Dr. Ann Miracle is currently involved in research incorporating environmental biomarkers into relevant remediation, monitoring, and risk assessment guidelines; and the environmental exposure of nanomaterials to aquatic organisms. Dr. Miracle leads a team of scientists addressing anthropogenic impacts to complex, ecological assemblages in freshwater communities using system biology approaches. In previous employment with the US EPA, Dr. Miracle led a team of scientists in linkages of chemical exposure and effects using 'omics technologies in small fish models as a part of that agency's Computational Toxicology Initiative.

Jeff Morris is EPA's National Program Director for Nanotechnology, and is responsible for managing EPA's Nanomaterials Research Program. Mr. Morris leads the U.S. delegation to the Organization of Economic Cooperation and Development's Working Party on Manufactured Nanomaterials, and co-chairs the Working Party's test guidelines steering group. He also co-chaired EPA's Nanotechnology Coordinating Committee, which issued EPA's Nanotechnology White Paper in February 2007. Prior to becoming National Program Director for Nanotechnology, Mr. Morris served as acting director of EPA's Office of Science Policy. His academic training is in economics and environmental policy, and all of the several positions he has held during his 16 years at EPA have focused on either regulatory issues or science policy.

N

Divina Angela G. Navarro is a graduate student at the Chemistry Department of the University at Buffalo, The State University of New York, working towards her Ph.D. in Analytical Chemistry. She obtained her undergraduate degree in B.S. Chemistry at the University of the Philippines at Los Banos, Philippines. Currently, she is working on studying the fate and transport of quantum dots in the environment, under the supervision of Dr. Diana Aga and Dr. Sarbajit Banerjee.

Dr. Arianne M. Neigh received her Ph.D. from Michigan State University in Environmental Toxicology and Zoology. Her work focused on ecosystem-level studies to identify exposure and effects of polychlorinated biphenyls to wildlife in a riverine system. This work is to date the most detailed evaluation of congener pattern changes of organochlorines in aquatic and terrestrial food webs. Dr. Neigh then joined CDM Federal Programs Corporation where she conducted human health and ecological risk assessments, biological evaluations, remedial investigations, and feasibility studies at hazardous waste sites for military and industrial clients. In 2007, she joined nanoComposix as a research scientist to apply her knowledge in risk assessment, environmental fate and transport, and toxicity evaluations to nanomaterials. Her work with nanomaterials includes evaluating assays for compatibility, high-level characterization during the course of experiments, and detecting and evaluating nanomaterials in the environment. Dr. Neigh's work is also focused on developing collaborations with a diverse group of researches in the US and in Europe in a multi-disciplinary approach to understand nanomaterials and the environment. She has authored or co-authored nine papers in the area of toxicology and risk assessment, in addition to presenting her work at national and international scientific meetings.

Dr. James T. Nurmi is a Senior Research Associate in the Department of Environmental and Biomolecular Systems, Oregon Health & Science University, Portland, OR.

O

Dr. Denis O'Carroll is an Assistant Professor in Civil and Environmental Engineering at the University of Western Ontario. Dr. O'Carroll completed his Ph.D. at the University of Michigan where he was awarded the 2004 Walter J. Weber, Jr. Student Prize. Upon completion of his Ph.D. Dr. O'Carroll completed one postdoctoral fellowship at the University of Michigan and was awarded a Government of Canada NSERC postdoctoral award to complete a postdoctoral fellowship at the University of Toronto. He was recently awarded the Province of Ontario 2007 Early Researcher Award for his work in the "Development of Nanomaterials and Hot Water Flooding for Enhanced Groundwater Remediation". The goal of this award is to attract and retain the best and brightest research talent in the Province of Ontario. Dr. O'Carroll has significant experience in laboratory studies developing innovative remediation schemes in addition to site remediation consulting experience. His work has investigated the utility of nanotechnology for contaminated site remediation, the impact of soil surface chemistry on NAPL migration and remediation and the utility of hot water flooding for NAPL remediation. He has ongoing research projects developing nanometals for contaminated site remediation and investigating the fate of carbon based nanoparticles in the environment.

P

Pankaj J. Parikh has been with U.S.EPA over 25 Years. He is an environmental scientist. He worked in EPA's Chicago Regional laboratory as a team leader/chemist, Asian Pacific Program manager, and as a project officer for Superfund contracts. He also has been a commissioner on the Village of Mount Prospect Solid Waste Commission and has served on the Village's Community Relations Commission for over five years. Prior to joining, EPA, he worked in private industry as a qualty control manager.

Dr. R. Lee Penn is an Associate Professor in the Department of Chemistry, University of Minnesota, Minneapolis, MN.

Dr. Kurt Pennell is a professor in the School of Civil and Environmental Engineering (CEE) at Georgia Tech and an adjunct professor in the Department of Neurology at Emory University School of Medicine. His expertise is in the areas of soil physics, contaminant fate and transport, and multiphase flow.

Tanapon Phenrat is a Civil and Environmental Engineering PhD candidate at Carnegie Mellon University. His PhD research involves the application of nanoscale zerovalent iron (nZVI) particles for groundwater and soil remediation. He has published multiple original papers on nanoparticle characterization in peer-reviewed journals including Environmental Science & Technology, Nano Letters, and Journal of Nanoparticle Research. In addition, he is involved in an EPA study on the fate, risk, and toxicity of nanomaterials in the environment.

Dr. Jonathan D. Posner earned his Ph.D. degree in Mechanical Engineering at the University of California, Irvine in 2001. In addition, he spent 18 months as a fellowship student at the von Karman Institute for Fluid Mechanics in Rhode Saint Genese, Belgium and two years as a postdoctoral fellow in the Stanford Microfluidics Laboratory. Dr. Posner is currently an assistant professor at Arizona State University in the Department of Mechanical and Aerospace engineering and director of the ASU Micro/Nanofluidics Lab. His interests include manipulation and self-assembly of nanomaterials, the physics of nanoparticles at interfaces, and transport and fate of nanomaterials in the environment and within animals. Dr. Posner was honored with a 2008 NSF CAREER award for his work on the physics of self-assembly of nanoparticles at fluid-solid and fluid-fluid interfaces. He has also been recognized for his Excellence in Experimental Research by the von Karman Institute for Fluid Dynamics.

Q

You Qiang is an Associate Professor of Physics, University of Idaho, Moscow ID.

R

Eric J. Reardon is a Professor in the Department of Geology, University of Waterloo, Waterloo, ON.

Dr. Krishna R. Reddy is a Professor of Civil and Environmental Engineering at the University of Illinois at Chicago. He teaches courses on environmental remediation, solid waste management and landfill engineering, and groundwater flow and contaminant transport modeling. His research expertise is remediation of contaminated soils, sediments and groundwater, environmental nanotechnologies, waste containment and landfills, and beneficial reuse of waste materials.

Dr. Bruce Rittmann is a professor of the Department of Civil and Environmental Engineering and director of the Center for Environmental Biotechnology at Arizona State University. Rittmann is an international leader in the fields of biofilm kinetics, biological treatment of drinking water, detoxification of hazardous organic chemicals, nitrification, the use of molecular techniques to study microbial communities in natural and engineered processes, bioremediation, and mathematical modeling that couples microbial kinetics to geochemical processes. His professional standing is evidenced by the numerous research prizes he received and his selection to be a chairman of two National Research Council committees (Water, Science, and Technology Board and Committee on Intrinsic Remediaion). Rittmann was elected to the National Academy of Engineering in 2004, cited for pioneering the development of biofilm fundamentals and contributing to their widespread use in the cleanup of contaminated waters, soils and ecosystems. Other honors and awards include: Founders Award, USA National Committee of IAWQ (1998), Fellow, American Association for the Advancement of Sciences (1996), A.R.I Clarke Prize, National Water Research Institute (1994), Engineering-Science Award, AEEP (1979, 1993), Montgomery-Watson Award, AEEP (1992, 1995).

Dr. Aaron P. Roberts is an Assistant Professor in the Department of Biological Sciences at University of North Texas in Denton, TX. Dr. Roberts received his bachelor's degree in biological sciences from the University of Missouri and his master's degree and Ph.D. in zoology from Miami University. His laboratory studies the interactive effects of non-chemical and chemical stressors on aquatic organisms including fish and zooplankton. He is primarily interested in the mechanisms by which these stresses elicit effects as well as the adaptations organisms use to ameliorate those effects. Work conducted in his laboratory on carbon nanomaterials has focused on dietary uptake, food chain transport, and biomodification.

Anna Ryu has research experience in water purification using nanoscale zero-valent iron, DNAPL, and water treatment of nitrate. She received a M.S. in environmental engineering, Gwangju Institute of Science and Technology, Gwangju, Korea, and a B.X. in construction, urban, and environmental engineering from Handong Global University, Pohang, Korea.

S

Dr. Vaishnavi Sarathy received her Ph.D.from the Department of Environmental and Biomolecular Systems, Oregon Health & Science University, Portland, OR.

Dr. Christie M. Sayes is currently an Assistant Professor in the Department of Veterinary Physiology and Pharmacology at Texas A&M University. Before her appointment at A&M, she held a post-doctoral fellowship at DuPont Haskell Global Centers for Health and Environmental Sciences under the direction of Dr. David Warheit. She is actively studying the health effects of various nanomaterials in animals, tissues, and cultured cells. She has made significant correlations between in vitro and in vivo studies,

which in turn have the potential to shape the landscape of nanotoxicology. Dr. Sayes earned her Doctorate of Philosophy in Chemistry, specializing in nanotechnology, from Rice University in Dr. Vicki Colvin's research group and earned her Bachelor's of Science in Chemistry from Louisiana State University, magna cum laude. Dr. Sayes has authored numerous research publications, reviews, and book chapters. She has ongoing collaborations and funding with academic, industry, and government. She has received awards including a Welch Fellowship, the Harry B. Weiser Graduate Student Award for Research, the Houston Livestock and Rodeo Endowed Scholarship, the International Toxicology of Nanomaterials: Young Investigator Award, a Society of Toxicology Post-doctoral Award, and a Society of Toxicology Best Publication Award. She is currently an active member of Texas A&M's Intercollegiate Faculty of Toxicology as well as the Faculty of Material Sciences & Engineering.

Dr. Kirk Scheckel is a Research Soil Scientist in the Waste Management Branch of the National Risk Management Research Laboratory at the US Environmental Protection Agency in Cincinnati, OH. Dr. Scheckel received his Ph.D. from the University of Delaware in Soil Science and a BS in Agronomy from Iowa State University. Dr. Scheckel professionally serves as an Associate Editor of the Journal of Environmental Quality, as an Adjunct Assistant Professor of Soil Chemistry at the Ohio State University, and as Chair-elect of the Division of Environmental Quality for the Soil Science Society of America as well as other committee assignments. Kirk Scheckel actively participates in laboratory and field research projects with the assistance of Postdoctoral Fellows and collaborators. The focus of his research program is solving fundamental problems regarding metal speciation in soils, sediments, and water via advanced, molecular-level spectroscopic techniques coupled with macroscopic kinetic and thermodynamic laboratory studies to elucidate reaction mechanisms that influence fate, transport, reactivity, mobility, bioavailability, and toxicity of metals in the natural environment leading to effective and economic remediation strategies.

Hatice Şengül is an Environmental Manufacturing Management fellow at the Institute for Environmental Science and Policy (IESP) and a Ph.D. student at the Department of Civil and Materials Engineering at University of Illinois at Chicago (UIC). She has a B.S. degree in environmental engineering from Middle East Technical University and an M.Sc. degree in environmental engineering from Tulane University. She worked at TUBITAK (Scientific and Technical Research Council of Turkey) and Simas Engineering (a private engineering firm based in Ankara) as an intern engineer. At UIC, she has been involved in an NSF funded research project under the direction of Thomas L. Theis concerning life cycle impacts of nanomanufacturing techniques. Her research interests include nanotechnology, clean energy, sustainable technology development, and natechs.

Dr. Virendra Sethi is Professor of Centre for Environmental Science and Engineering, Indian Institute of Technology Bombay in Mumbai, India. She received her PhD in Environmental Engineering in 1996 and a M.S. in Environemtal Engineering in 1990 from the University of Cincinnati in Cincinnati, Ohio.

Dr. Jo Anne Shatkin is Managing Director of CLF Ventures, a non-profit affiliate of the Conservation Law Foundation, New England's most influential environmental advocacy organization. CLF Ventures works at the intersection of business, stakeholder, and environmental issues to optimize environmental and economic gain. Dr. Shatkin is a recognized expert in strategic environmental initiatives, human health risk assessment, technical communications, and environmental aspects of nanotechnology. She leads and provides expertise on projects and manages the day to day operations of CLF Ventures.

Her work focuses on approaches for evaluating new and emerging contaminants in the environment, particularly on assessments of chemical and microbial concerns that inform policy development. She recently developed NANO LCRA, an adaptive life cycle framework for identifying and managing the risks of nanomaterials, described in her book, Nanotechnology Health and Environmental Risks, published in 2008 (CRC Press). Dr Shatkin recently founded the Emerging Nanoscale Materials Specialty

Group of the Society for Risk Analysis, with 130 international members from public and private organizations. A Research Fellow at the George Perkins Marsh Institute at Clark University, she received her Ph.D. in Environmental Health Science and Policy in 1994 and her MA in Risk Management and Technology Assessment, both from Clark University, Worcester, Massachusetts and possesses a Bachelor of Science degree from Worcester Polytechnic University in molecular biology.

Dr. Weiguo Song is Professor of Chemistry, Key Laboratory for Molecular Nanostructures and Nanotechnologies, Institute of Chemistry, the Chinese Academy of Sciences (ICCAS) in Beijing, Chinia. He received a Ph.D. in Physical Chemistry in 2001 from the University of Southern California, Los Angeles, California, a B. Sc. In Chemistry in 1992 from Beijing University, Beijing, China. Prior to joining ICCAS, he was a Postdoctoral Research Associate at the University of Iowa, Iowa City, Iowa, from 2003 to 2005, and at the Loker Hydrocarbon Research Institute, University of Southern California in Los Angeles, California, from 2001 to 2003. He was named to National Science Fund for Distinguished Young Scholars (2007) and the Chinese Academy of Sciences One Hundred Talented Program (2005)

Dr. Desmond Dion Stubbs received a B.S. degree in Chemistry from Morris Brown College, Atlanta GA in 1997. He later received his M.S. in Chemistry from Georgia Tech in 1999. After working in Georgia Tech's School of Chemistry as a Demonstrations Teacher for two years he later returned to Georgia Tech and received his doctoral degree in May, 2006. One of the highlights of his graduate career was a publication in Analytical Chemistry entitled "Investigation of a Cocaine Plume Using Surface Acoustic Wave Immunoassay Sensors". The paper was then flagged by the American Chemical Society interest and later featured as a press release on their website. The story led to numerous media interviews including an appearance on Fox News (cable service) and a feature in Time Magazine's new series Innovators highlighting the "dog-on-a-chip" a chemical sensing electronic device. Desmond currently holds joint positions at Oak Ridge Associated Universities (ORAU) and Battelle as a Senior Project manager and a Scientist in Residence respectively.

Dr. Chunming Su is a Soil Scientist in the Subsurface Remediation Branch in the Ground Water and Ecosystems Restoration Division (GWERD) of the USEPA's National Risk Management Research Laboratory, Ada, Oklahoma. He received a B.S. degree from China Agricultural University, China, an M.S. degree from University of Guelph, Canada, and a Ph.D. degree from Washington State University, all in Soil Science. His former work experience includes a term soil scientist position with the U.S. Department of Agriculture, a National Research Council Resident Research Associateship, and a project scientist position with ManTech Environmental Research and Services Corporation. Dr. Su conducts laboratory and field investigations in environmental geochemistry and nanotechnology. He is interested in studying: (1) applications and implications of environmental nanotechnology with respect to fate and transport of nanomaterials in the subsurface, (2) in situ treatment of organic (chlorinated solvents) and inorganic (chromate, arsenic, nitrate, etc) contaminants in ground water and soils using permeable reactive barrier technologies and monitored natural attenuation approaches, (3) arsenic sorption and redox transformation processes using specimen iron minerals including green rusts and iron oxides, and (4) organic contaminant degradation pathways using stable isotopes. Dr. Su is the principal author of more than 30 peer-reviewed journal articles and book chapters, and a co-recipient of a US patent. He also has served as a technical reviewer for numerous scientific journals including Environmental Science and Technology, Geochimica et Cosmochimica Acta, Chemistry of Materials, and Soil Science Society of America Journal; and on proposal review panels for the Department of Commerce, EPA, USDA, and USGS. Dr. Su has received several EPA awards for his research and technical support activities (including EPA Scientific and Technological Achievement Awards and an ORD Honor Award for Exceptional/ Outstanding Technical Assistance to the Regions and Program Offices).

Dr. Rao Y. Surampalli is the Engineer Director with United States Environmental Protection Agency (USEPA, Region 7). He received M.S and Ph.D. degrees in Environmental Engineering from Oklahoma

State and Iowa State Universities, respectively. He is a Registered Professional Engineer and has authored more than 370 technical publications, including five books, 31 book chapters, 136 refereed (peer-reviewed) journal articles, presented at more than 180 national and international conferences, and given over 30 plenary, keynote or invited presentations worldwide. Currently, he serves on 39 national and international committees, review panels, or advisory boards including the ASCE's National Energy, Environmental and Water Resources Policy Committee. He is Editor of two well-known refereed journals - the Water Environment Research Journal published by the Water Environment Federation (WEF), and the Hazardous, Toxic, and Radioactive Waste Management Journal published by the American Society of Civil Engineers (ASCE). He also serves on the Editorial Boards of three other Environmental Journals. His main expertise includes, but is not limited to emerging contaminants including nanomatetrials, water/wastewater treatment, hazardous/solid waste management, soil and groundwater treatment, and sludge treatment/disposal.

T

Dr. Robert Tanguay received a B.A. degree in biology from California State University, San Bernardino in 1988 and his Ph.D. degree in biochemistry from the University of California, Riverside in 1995. He received postdoctoral training in molecular and developmental toxicology with Richard E. Peterson at the University of Wisconsin between 1996 and 1999. He is currently an Associate Professor in the Department of Environmental and Molecular Toxicology at Oregon State University and is the director of the Sinnhuber Aquatic Research Laboratory. His current research interests include developmental biology, nanotoxicology, developmental toxicology, regenerative medicine, and chemical genetics.

Dr. Volodymyr Tarabara is an assistant professor in Department of Civil and Environmental Engineering at Michigan State University. He obtained his Ph.D. in Environmental Engineering and Computational Science and Engineering from Rice University in 2004.

Leigh M. Taylor is an undergraduate student at the University of North Texas in Denton, TX. Leigh is an undergraduate research assistant in Dr. Aaron Roberts' lab.

Dr. Thomas L. Theis is the director of the Institute for Environmental Science and Policy and a full professor at the Department of Civil and Materials Engineering, University of Illinois at Chicago. Professor Theis' areas of expertise include the mathematical modeling and systems analysis of environmental processes, the environmental chemistry of trace organic and inorganic substances, interfacial reactions, subsurface contaminant transport, hazardous waste management, industrial pollution prevention, and industrial ecology. He has been principal or co-principal investigator on over forty funded research projects totaling in excess of eight million dollars, and has authored or co-authored over one hundred papers in peer reviewed research journals, books, and reports. He is a member of the USEPA Science Advisory Board (Environmental Engineering Committee), is erstwhile editor of the Journal of Environmental Engineering, and serves on the editorial boards of The Journal of Contaminant Transport, and Issues in Environmental Science and Technology. From 1980-1985 he was the co-director of the Industrial Waste Elimination Research Center (a collaboration of Illinois Institute of Technology and University of Notre Dame), one of the first Centers of Excellence established by the USEPA. He is currently Principal Investigator on the Environmental Manufacturing Management Program, one of the Integrated Graduate Education Research and Training (IGERT) grants of the National Science Foundation.

Dr. Vinay Tiwari is a Research Scholar at the Centre for Environmental Science and Engineering, Indian Institute of Technology Bombay, Mumbai, India. He receive a M.E. in Chemical Engineering in 2004 from the Nirma Institute of Technology, Gujarat, India

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V

Dr. Ashok K. Vaseashta is Professor of physics and physical sciences in the Graduate Program in physical sciences at Marshall University, Huntington, WV. Presently, he is on detail to the U.S. Government. He received a B.S. and M.S. in Physics Honors from the University of Delhi, M.Tech. from the Indian Institute of Technology, Delhi, and a Ph.D. from Virginia Polytechnic Institute and State University, Blacksburg, VA. He directs research at the Nanomaterials Processing and Characterization Laboratories at Marshall University. His current research interests include nanostructured materials for energy generation and storage; development of chemical-bio sensors; and use of nanomaterials for monitoring, detecting and remediation of environmental pollution. He is one of the leading researchers in the field of green nanotechnology. He has authored over 170 research publications, edited/authored two books on nanotechnology, presented many keynote and invited lectures worldwide, served as Director of two NATO Advanced Study Institutes, and co-chair of an International Symposium on Nanotechnology in Environmental Protection and Pollution in Ft. Lauderdale, FL. He is an active member of several national and international professional organizations. He has earned several awards for his meritorious service including 2004/2005 Marshall University Distinguished Artist and Scholar (MU DASA) award. His experience spans the spectrum of academic and industrial positions. He has visiting positions at several national laboratories and universities in Eastern Europe. He also serves on the Nanotechnology Standard Committee of ISO/ANSI TAG-TC 229 and ASTM.

Dr. Bellina Veronesi is a senior scientist at the US EPA Division of Neurotoxicology (NHEERL). She is an in vitro and in vivo experimentalist and has published extensively in the areas of in vitro modeling, pesticide neuropathology and air pollution neurotoxicity. More recently, she has documented the oxidative stress-mediated neurotoxicity of various nanomaterials used in environmental remediation. Currently, she is developing in vitro models to examine how the physical properties of nanoparticles influence their movement through biological barriers such as the intestines and blood brain barrier.

W

Dr. T. David Waite is Director, Centre for Water and Waste Technology, and Director of Research, School of Civil and Environmental Engineering, The University of New South Wales. He hold a Ph.D. in environmental engineering from the Massachusetts Institute of Technology, Cambridge, MA (USA); a M.App.Sci. from Monash University, Melbourne, Victoria ,(Australia); a Grad. Diploma of Electronic Instrumentation from the Royal Melbourne Institute of Technology, Melbourne, Victoria (Australia); and a Bachelor of Science (Honours) from the University of Tasmania, Hobart, Tasmania (Australia). His research interests include chemical processes involving colloids and particles in aquatic systems; redox chemistry at the solid-solution interface; photochemistry in aquatic systems; water and wastewater treatment processes; hydrometallurgical techniques involving redox processes; hydrogeochemistry; theoretical and experimental studies on the fate and effects of chemical pollutants; and interactions between trace elements and microbiota in aquatic systems.

Barbara T. Walton is Assistant Laboratory Director for Emerging Programs, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency. She has responsibility for EPA's research on the health and ecological effects of nanomaterials.

Walton's 20-year career at the U.S. Department of Energy's Oak Ridge National Laboratory focused on the ecotoxicology of organic, inorganic, and radioactive contaminants in terrestrial and aquatic ecosystems. Before joining EPA, Barbara was Senior Policy Analyst for Environment, White House Office of Science and Technology Policy, Washington, DC. She's a board-certified toxicologist (American Board of Toxicology) and Adjunct Professor, Department of Environmental Science and

Engineering, University of North Carolina-Chapel Hill. Walton is a former President of the Society of Environmental Toxicology and Chemistry.

Yonggang Wang is currently a Ph.D. candidate in the School of Civil and Environmental Engineering at Georgia Tech. His research area is experimental investigation of fullerene nanoparticle aggregates transport in saturated and unsaturated soils.

Dr. Mahmoud Wazne is Assistant Professor, Department of Civil, Environmental and Ocean Engineering, Stevens Institute of Technology in Hoboken, New Jersey. He received his Ph.D. in Environmental Engineering in 2003 from Stevens Institute of Technology, a M.S. in 1991 and a B.S.in 1990 in Civil Engineering from Columbia University in New York. He was a Research Assistant Professor, Department of Civil, Environmental and Ocean Engineering., Stevens Institute of Technology from 2004 to 2005; a Postdoctoral Research Associate, Stevens Institute of Technology from 2003 to 2004; a Graduate Research Assistant, Stevens Institute of Technology from 2000 to 2003; a Licensed Civil Engineer, Sands Contractors from 1992 to 1998; Lecturer, City University of New York from 1991to 1992; and a Graduate Research Assistant, Columbia University from 1990 to 1991.

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