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Critical Review

NANOMATERIALS IN THE ENVIRONMENT: BEHAVIOR, FATE, BIOAVAILABILITY, AND EFFECTS

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Abstract—The recent advances in nanotechnology and the corresponding increase in the use of nanomaterials in products in every sector of society have resulted in uncertainties regarding environmental impacts. The objectives of this review are to introduce the key aspects pertaining to nanomaterials in the environment and to discuss what is known concerning their fate, behavior, disposition, and toxicity, with a particular focus on those that make up manufactured nanomaterials. This review critiques existing nanomaterial research in freshwater, marine, and soil environments. It illustrates the paucity of existing research and demonstrates the need for additional research. Environmental scientists are encouraged to base this research on existing studies on colloidal behavior and toxicology. The need for standard reference and testing materials as well as methodology for suspension preparation and testing is also discussed.

Keywords—Nanoparticles Ecotoxicity Colloids Plant uptake Ecological risk

INTRODUCTION

Nanomaterials in the environment

Much debate still exists regarding the nomenclature associated with nanoscience and nanotechnology, although a few documents have been published in recent years [1–4]. For the purpose of this review, the definition of a nanomaterial (NM), as adopted by the British Standards Institution [1], the American Society for Testing Materials [2], and the Scientific Committee on Emerging and Newly-Identified Health Risks [4], is a material with one dimension under 100 nm. Within this group of materials, nanoparticles (NP), defined as materials with at least two dimensions between 1 and 100 nm [2], are particularly important [4]. Nanoparticles have always existed in our environment, from both natural and anthropogenic sources. Nanoparticles in air were traditionally referred to as ultrafine particles, while in soil and water they were colloids, with a slightly different size range [5]. The objectives of this review are to introduce the key aspects pertaining to nanomaterials in the environment and to discuss what is known concerning their fate, behavior, disposition, and toxicity, with a particular focus on those that make up manufactured nanomaterials.

Natural and adventitious nanomaterials

In urban atmospheres, diesel- and gasoline-fueled vehicles and stationary combustion sources have for many years contributed particulate material throughout a wide size range, including NPs, amounting to more than 36% of the total particulate number concentrations [6]. In addition, there is a natural background of NPs in the atmosphere, although the total concentration is low in comparison to potential releases of manufactured NPs. The health effects of such particles are still being investigated with regulatory concerns moving from the traditional PM10 (particles less than 10 μm in aerodynamic diameter) to PM5, PM2.5, and below, as the increased toxicity of the finer particles has been identified. Most research carried out in this area to date has focused on ultrafine particulate material, including nanoscale particles, and their effects on human health, especially on respiratory systems [7]. Comparatively little work has been done on ecological systems. Human health research has focused on a number of effects, including oxidative stress [8] and inflammatory and fibrotic reactions [9].

In aquatic systems, colloid is the generic term applied to particles in the 1-nm to 1-μm size range. The natural NM fraction has been identified as being of particular concern because of the changes that occur in this size range, although the most important size range in terms of environmental processes is not well defined. Aquatic colloids comprise macromolecular organic materials, such as humic and fulvic acids, proteins, and peptides, as well as colloidal inorganic species, typically hydrous iron and manganese oxides. Their small size and large surface area per unit mass make them important binding phases for both organic and inorganic contaminants.

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Additionally, high surface energy, quantum confinement, and conformational behavior are likely to be important, although discussion of these parameters currently remains qualitative because of the complexity of colloids or NPs. Although dissolved species are operationally defined as those that pass through a 0.45-μm filter, this fraction also includes colloidal species whose bioavailability is quite different from truly soluble organic or ionic metal species [10], and more recent work has stressed the necessary separation of dissolved, colloidal, and particulate species [11].

In soils, natural NPs include clays, organic matter, iron oxides, and other minerals that play an important role in biogeochemical processes. Soil colloids have been studied for decades in relation to their influence on soil development (pedogenesis) and their effect on soil structural behavior (dispersion and crusting) [12]. Of particular relevance to manufactured NMs, soil colloids and other porous media may facilitate the movement of contaminants in soils and other porous media. Contaminants sorbed to or incorporated into colloids can be transported when conditions for colloidal transport are favorable. For example, natural soil colloids have been found to be vectors for transport of metals through soil profiles (discussed further here).

Classes of manufactured nanomaterials

It has been recognized for some time that NMs possess enhanced or even unique mechanical, catalytic, and optical properties, and electrical conductivity primarily because of their nanosize. The result has been an exponential growth over the past decade in the development of new manufactured or engineered nanomaterials and their exploitation by burgeoning nanotechnology industries. The range of nanotechnology products is now extensive and can be broken down into a number of different compound classes, including carbonaceous nanomaterials; metal oxides; semiconductor materials, including quantum dots; zero-valent metals such as iron, silver, and gold; and nanopolymers, such as dendrimers. A variety of products are now being generated, including NPs as well as nanofibers, nanowires, and nanosheets, and the range and types of NMs are continually expanding.

The preparation of NPs typically involves either a direct synthetic route that yields particles in the nanosize range or the application of grinding or milling to reduce the size of a macroparticulate product. The difficult component is controlling the size and shape precisely. It has been shown that there is an equilibrium limit to the size of particle that could be achieved by mechanical grinding, such as ball milling, and this could be as high as 300 nm [13]. Wet grinding using fine ceramic beads below 30 μm in diameter was shown to be highly effective [14]. Where materials are highly crystalline, the size after milling could be as low as 1 to 10 nm.

The first class of NMs, carbon nanotubes (CNTs) and related materials, originated with the discovery in 1985 of the first fullerene, a 60-carbon atom hollow sphere, also known as the buckyball [15], produced by evaporating graphite. Subsequent research in 1991 produced a cylindrical fullerene derivative, the CNT, synthesized under defined conditions that can control the diameter and size of the tubes either from graphite using arc discharge or laser ablation or from carbon-containing gas using chemical vapor deposition. The first product was shown to be multiwalled carbon nanotubes with concentric cylinders up to 10 μm in length and 5 to 40 nm in diameter. It was later shown that it was also possible to produce single-walled CNTs in the presence of a cobalt-nickel catalyst. These fullerene structures were found to have excellent thermal and electrical conductivity. Single-walled carbon nanotubes (SWCNT) have a strength-to-weight ratio 460 times that of steel [16; http://www.wilsoncenter.org]. Despite being classified together in terms of composition, it is clear that fullerenes and CNTs may behave completely differently in the environment. For instance, in the human health area, CNTs are increasingly being grouped together with other rods and tubes as high-aspect-ratio nanomaterials, as there are concerns that they may behave similarly to asbestos [17]. In aqueous systems, carbon NPs are subject to precipitation and aggregation because of their inherent hydrophobicity. This limits their use in aqueous and biomedical applications. Much research has been done to surface modify these CNTs to improve stability of aqueous suspensions. Covalent modification, such as the attachment of polyethylene glycol to SWCNTs [18], and noncovalent modifications, such as the self-assembly of SWCNTs and the phospholipids lysophosphtidylcholine [19] or C_{60} and lysophosphotidylcholine [20], result in very stable carbon NP suspensions. These modifications have implications for their use in certain applications as well as repercussions for their fate and behavior in the environment.

Annual worldwide production of SWCNT is estimated to exceed 1,000 tonnes by 2011 [16]. Fullerenes and CNTs are produced in large quantities in factories with capacities as high as 1,500 tonnes/year (Frontier Carbon Corporation, Tokyo, Japan; Fullerene International Corporation, Tucson, AZ, USA). Carbon nanotubes and their derivatives are used in plastics, catalysts, battery and fuel cell electrodes, supercapacitors, water purification systems, orthopedic implants, conductive coatings, adhesives and composites, sensors, and components in the electronics, aircraft, aerospace, and automotive industries. Increased production results in an increased potential for release to the environment, either deliberately in discharges or accidentally in spillages, and a greater possibility of adverse environmental effects.

The second class of nanomaterials, composed of metal-containing materials, including metal oxides, has received considerable attention and has a variety of applications. The synthesis of metal and metal oxide nanoparticles is very common and within the capability of most chemical laboratories [21]. Grinding of bulk materials is a common procedure for producing metal oxide NPs. Titanium dioxide (TiO_2) and zinc oxide (ZnO) are widely exploited for their photolytic properties [22; http://www.nrdc.org]. Titanium dioxide is a photocatalyst that has been used in solar cells, paints, and coatings. Zinc oxide and TiO_2 are finding extensive application in sunscreens, cosmetics, and bottle coatings because of their ultraviolet-blocking ability and the visible transparency of nanoparticulate forms. Production of metal oxides for use in skin care products is estimated to be 1,000 tonnes/year in 2005–2010 [23]. The range of nanoparticulate metal oxides includes both individual (such as ZnO, TiO_2, cerium dioxide [CeO_2], chromium dioxide [CrO_2], molybdenum trioxide [MoO_3], bismuth trioxide [Bi_2O_3]) and binary oxides (such as $BaTiO_3$, lithium cobalt dioxide [$LiCoO_2$], or indium tin oxide [InSnO]). Cerium dioxide is finding major use as a combustion catalyst in diesel fuels to improve emission quality [24] as well as in solar cells, gas sensors, oxygen pumps and metallurgical and glass/ceramic applications [25].

The third class of NMs includes semiconductor nanocrystals, also known as quantum dots. Quantum dots (QDs) have

a reactive core which controls its optical properties, and these cores can be made out of metals or semiconductors such as cadmium selenide (CdSe), cadmium telluride (CdTe), CdSeTe, indium phosphide (InP), or zinc selenide (ZnSe). The reactive semiconductor cores are surrounded by a shell, such as silica, or a ZnS monolayer that protects the core from oxidation and enhances the photoluminescence yield [22,26]. The cores are produced from a nucleation reaction of the metal/semiconductor material, such as in a high-temperature solution phase synthesis, and subsequent growth of these crystals, with reaction conditions controlling the size [27]. Surfactants in the solution initially form an organic cap that stabilizes the particle; this cap can later be modified or exchanged with other materials. Although to date used largely in medical applications such as medical imaging and targeted therapeutics, the use of QDs is being extended to include solar cells and photovoltaics, security inks, and photonics and telecommunications [28].

Zero-valent metals make up the fourth class of nanomaterials. They are typically made by reduction of solutions of metal salts. Their physical properties can be controlled by varying the reductant type and the reduction conditions. For zero-valent iron, the easiest and most popular method of preparation is through the reduction of ferric (Fe[III]) or ferrous (Fe[II]) salts with sodium borohydride [29]. Nanoparticulate zero-valent iron has been used for some time for the remediation of waters, sediments, and soils to remove nitrates via reduction and has most recently for detoxifying organochlorine pesticides and polychlorinated biphenyls [30]. Use of nanoiron in the United States is widespread. However, there is effectively a (voluntary) moratorium on zero-valent iron being used in the United Kingdom for remediation purposes because of unknown potential effects of release of free NPs into the environment [31].

By far the greatest number of consumer product applications of NMs has involved nanoparticulate silver. As listed in the inventory developed by the Woodrow Wilson International Centre for Scholars Project on Emerging Nanotechnologies [32; http://www.wilsoncenter.org], these products range from wound dressings, socks, and other textiles; air filters; toothpaste; baby products; vacuum cleaners; and washing machines. In some cases these are metallic silver NPs, while in others they are electrochemically generated ionic silver. Ionic silver is highly reactive, is readily adsorbed by both macroparticles and colloidal particles such as iron oxyhydroxides or natural organic matter in natural waters, and ranges in size from <1 kDa to >0.45 μm [33]. Silver's antimicrobial activity is most often attributed to the dissolved cation rather than the high-surface-area, low-solubility nonionic metallic NP. The instability of the monovalent anion and the nonionic NP, however, resulted in extremely short half-lives of the desired form. This has resulted in research to stabilize silver NPs to make them useful in biological and other aqueous applications [34]. This has created ambiguity in how investigators describe test systems and manufacturers describe products. For example, it is common for manufacturers to describe colloidal silver as nanosilver. Colloidal elemental gold has been used for many years, especially in medical applications as vectors in tumor therapy. Newer applications of nanoparticulate gold include its use in electronics in flexible conducting inks or films and as catalysts.

The fifth class of NMs is dendrimers. These are multifunctional polymers whose size, topology, flexibility, and molecular weight can be controlled. Dendrimers can be used for many applications in different fields ranging from biology, material sciences, and surface modification to enantioselective catalysis. These include macrocapsules, nanolatex, colored glasses, chemical sensors, modified electrodes, DNA transfecting agents, therapeutic agents for prion diseases, hydrogels, drug delivery, and DNA chips.

Entry of nanomaterials into the environment

Given the increasing production of NMs of all types, the potential for their release in the environment and subsequent effects on ecosystem health is becoming an increasing concern that needs to be addressed, especially by regulatory agencies. In doing so, it is necessary first to determine the fate and behavior of manufactured NMs in the environment. Do they retain their nominal nanoscale size and original structure and reactivity in aquatic and soil/sedimentary systems? Does an association exist with other colloidal and particulate constituents? What are the effects of solution and physical (e.g., flow) conditions? Is their effect on aquatic and sedimentary biota different from that of larger particles of the same material? Do biota, such as biofilms and invertebrates, modify the behavior of NMs? Answers to these and other questions will guide the setting of regulatory guidelines that will provide adequate protection to ecosystems while permitting the advantages that nanotechnology offers to be fully developed.

Manufactured NMs enter the environment through intentional releases as well as unintentional releases such as atmospheric emissions and solid or liquid waste streams from production facilities. Deliberate release of NMs includes their use to remediate contaminated soils including the use of iron NPs used to remediate groundwater (for a review of this field, see Zhang and Elliot [35]). Filtration of NPs from stack emissions requires a new generation of nanostructured sorbents for their effective removal. In addition, NPs in paints, fabrics, and personal health care products, including sunscreens and cosmetics, enter the environment proportional to their use [36]. Emitted particles will ultimately deposit on land and surface water bodies, although treatment to avoid aggregation may result in enhanced buoyancy of these NPs when compared with NPs from other sources, such as the ones arising from diesel emissions.

Nanoparticles reaching land have the potential to contaminate soil, migrate into surface and groundwaters, and interact with biota. Particles in solid wastes, wastewater effluents, direct discharges, or accidental spillages can be transported to aquatic systems by wind or rainwater runoff. With increasing control of fugitive releases arising within the manufacturing process, the biggest risks for environmental release come from spillages associated with the transportation of manufactured NPs from production facilities to other manufacturing sites, intentional releases for environmental applications, and diffuse releases associated with wear and erosion from general use. Hence, most of the research to date has focused on the nanomaterials in greatest production, CNTs, and metal oxides.

Risk assessment

Manufacture, use, and potential release of NMs have preceded evaluation of risk to ecosystems, including humans. Currently, there are no factual data on concentrations of NMs in the environment, and certainly none on their physicochemical forms or distribution, although models have been use to estimate potential releases and loads [37; http://www.defra.gov.uk]. The development of techniques to measure and char-

acterize NMs in atmospheric, aquatic, and terrestrial environments is an important immediate research priority in order to facilitate quantitative ecological risk assessment. This is particularly critical since manufactured NPs represent an intermediate supramolecular state of matter with a size between bulk and molecular [38]. Classic approaches used in aquatic ecological risk assessments [39] may be less applicable to NMs since exposure assessments have classically depended on predicting the soluble portion of the contaminant. Further, it has been assumed that the predominant bioavailable portion of the total contaminant was the soluble form [40]. These assumptions and approaches must be taken with caution and modified to deal with the issues of particle fate and behavior, bioavailability, and toxicity that are central to quantitative ecological risk assessment of NMs. In fact, environmental chemists, toxicologists, and risk assessors might be well served to preface research on NPs with a primer on colloid fate, behavior, and toxicity (for a comprehensive review of colloids and other particles, see Wilkinson and Lead [41]).

As noted previously, characterization of NP fate and behavior in the environment is needed to quantify exposure scenarios. Related to this, differences in speciation (between dissolved, colloidal, and particulate phases) due to dissolution and aggregation of NPs under environmental conditions are also important. It is worth noting here that these are characteristics usually unfamiliar to most environmental toxicologists, chemists, and risk assessors, although they have now been progressively addressed in the context of toxicology and ecotoxicology of nanomaterials [4]. Clearly, the need for interdisciplinary collaboration among biologists, chemists, physicists, and material scientists is essential. Once exposure scenarios are characterized, organisms potentially at risk must be identified. Quantitative assessments of organism response to NP exposure must be conducted to facilitate effects assessment. Once exposure and effects assessment are complete, risk characterization can be accomplished This review critically examines the current state of knowledge of the fate, behavior, and impacts of NMs in freshwater, marine, and terrestrial ecosystems.

ENVIRONMENTAL FATE AND BEHAVIOR OF NANOPARTICLES

Behavior and deposition

Almost no direct data relevant to the fate and behavior of manufactured NMs in aquatic or terrestrial systems currently exist. Nevertheless, this is a rapidly developing area, and in the next few years a significant knowledge base will emerge in the scientific literature. It is instructive, however, to discuss the related and more mature field of natural colloids in aquatic and terrestrial systems. Aquatic colloids have a formal International Union of Pure and Applied Chemistry definition [5,41] based on size, with colloids defined as material with at least one dimension <1 μm. Clearly this material also contains (natural) NPs, which can be defined as material with at least two dimensions between 1 and 100 nm. The 100-nm mark is largely arbitrary and, unlike the colloidal definition, has little theoretical or environmental significance. In addition, there are indications from the literature [42] that the smallest fraction of perhaps <10 nm is the most environmentally relevant fraction, where properties change dramatically.

Figure 1 shows the major components of natural colloids, along with their possible methods for separation and analysis, and for our purposes, the fraction smaller than 100 nm is the one of most interest. Colloids may also be defined by environmental processes where they are dominated by aggregation behavior [43]. In practice, they may also be studied using cross-flow ultrafiltration membranes with nominal pore sizes of 1 nm and 0.2 to 0.45 μm in general. Nevertheless, the definition of colloids clearly includes both organic (primarily humic substances [HS] and fibrillar material, usually protein and polysaccharide exudates from microbes) and inorganic matter (e.g., oxides of Fe, Mn, Al, and Si and thus analogous to manufactured metal oxide NPs), as well as biological material such as viruses and some bacteria. Figure 2 shows representative electron microscopy images of the smallest fraction of selected natural aquatic colloids, with sizes of a few nanometers.

The value in considering the smallest fraction of natural aquatic colloids in this context is twofold. First, colloidal fate and behavior is dominated by aggregation [43,44], and colloids will eventually aggregate to particles (>1 μm) that are sufficiently large that their transport is dominated by sedimentation. This process has been well characterized to understand trace metal behavior, where it is sometimes termed colloidal pumping [45]. Metals in general tend to sorb to high-specific-surface-area small colloids that aggregate and settle out, resulting in a transfer of metals from the water column to the sediments. This process is important in the self-purification of water bodies and results in pollutant loss from surface waters and accumulation in the sediments [46] and is analogous to the likely behavior of manufactured NPs, with aggregation and subsequent sedimentation an important process in their ultimate fate. While other factors, such as turbulent motion in the benthos and bioturbation of the sediments, may complicate this simple picture [47], sediments and therefore benthic organisms are expected to be the main sinks and receptors of NPs in surface waters.

The second reason for examining natural colloids is that NPs and natural colloids will interact and this will affect NP behavior. Indeed, it is likely that NP aggregation and sedimentation will be dominated by natural aquatic colloids behavior, given the likely concentration differences between the two. No direct published data are available on the concentrations of NPs in natural waters, but a recent report using a simplified box model and known current uses [37] suggested environmental concentrations of approximately 1 to 100 μg/L, whereas typical dissolved and colloidal organic matter in freshwaters may be found at 1- to 10-mg/L concentrations. In estuarine and marine waters, natural colloids have much lower concentrations, but NP concentrations would likely be much lower also because of increased aggregation and sedimentation at higher ionic strengths.

The difficulties in studying any nanoscale material are exacerbated in natural systems because of their polydispersity, complexity, and spatial and temporal variability. Therefore, aggregation of NPs in the laboratory is made even more complicated by aquatic colloids in natural systems. A useful but simplified conceptual model of colloid aggregation and sedimentation is shown in Figure 3, where colloids are envisaged as small ($\sim$1 nm), HS, slightly larger inorganic colloids and fibrilar polysaccharide-type material. Interestingly, the different organic matter has different aggregation properties. Neihof and Loeb [48] reported that submicron- to micron-sized particles in seawater had negative electrophoretic mobility that became less negative with increasing salinity. They found that the electrophoretic mobilities of other organic and inorganic

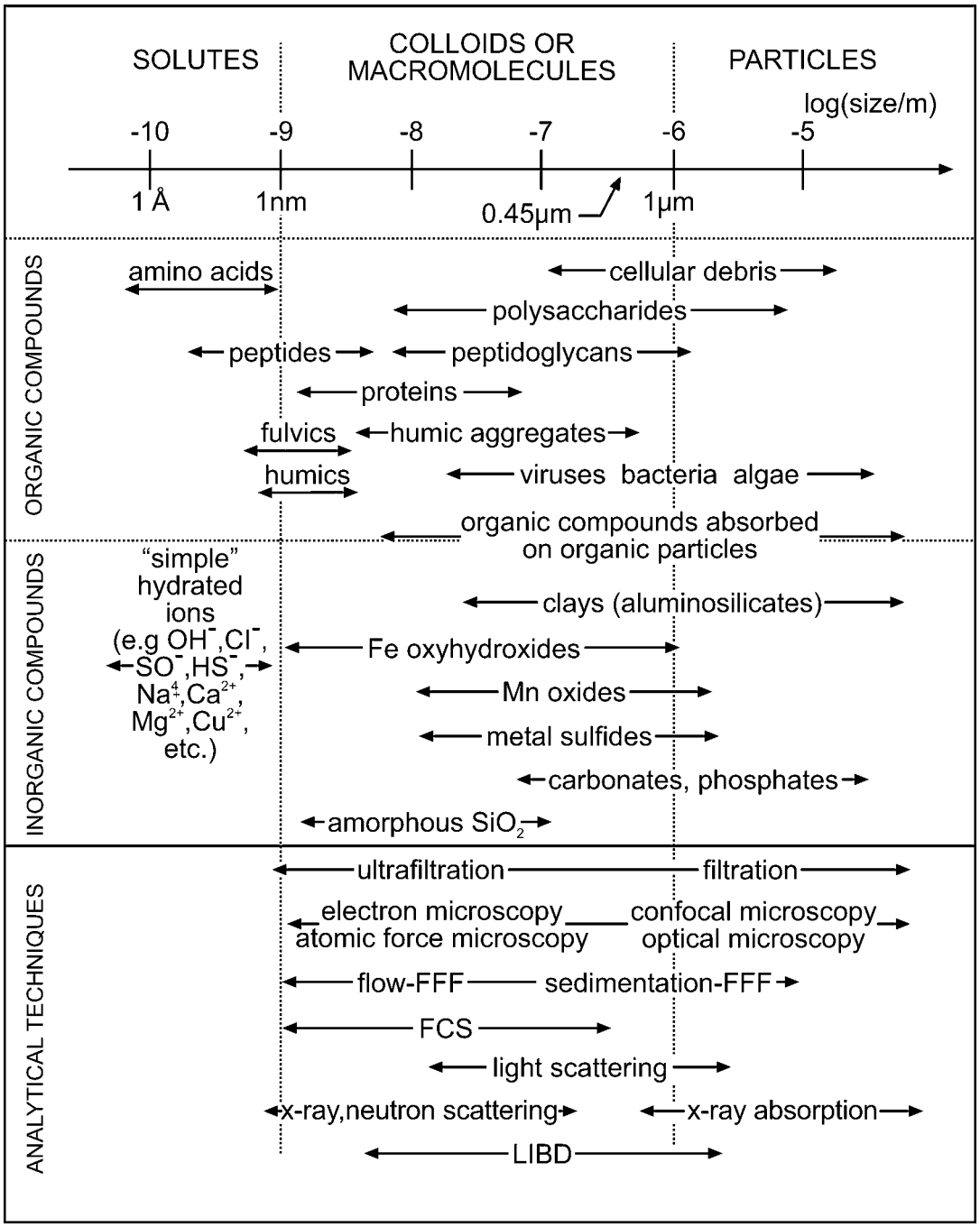

Fig. 1. Size distributions of various types of environmental colloids and particles and several of the analytical techniques used to characterize them. FFF = field-flow fractionation; FCS = fluorescence correlation spectroscopy; LIBD = laser induced breakdown detection. Taken from Redrawn and Wilkinson [5].

model particles (glass, anion exchange resin, bentonite, calcium carbonate, wax, and a polysaccharide), covering a wide range of positive and negative values in synthetic seawater that contained no organic matter, all converged to a narrow range of negative values when the samples were placed in natural seawater. These authors concluded that the adsorption of surface-active organic materials from seawater onto the surfaces of the particles masked their intrinsic properties and dominated their surface electrical properties.

Hunter and Liss [49] extended this concept to suspended particles in estuaries of fundamentally different inorganic chemical composition, further bolstering the hypothesis that the electrokinetic characteristics of particles in natural waters are dominated by adsorbed organic matter. Hence, HS are likely to form nanoscale coatings on solid phases [50] and reduce aggregation by charge stabilization [51] and fibrils are likely to increase aggregation via bridging mechanisms [52]. This interaction between NPs and HS has been demonstrated with CNTs and zero-valent iron and standard Suwannee River, USA, HS [53,54]. Recent results from one of the authors (J. Lead, University of Birmingham, UK, personal communication) suggest, not unexpectedly, that other factors, such as pH, calcium ion concentration, and the presence of other types of natural colloids, will be important and that sediments (via aggregation

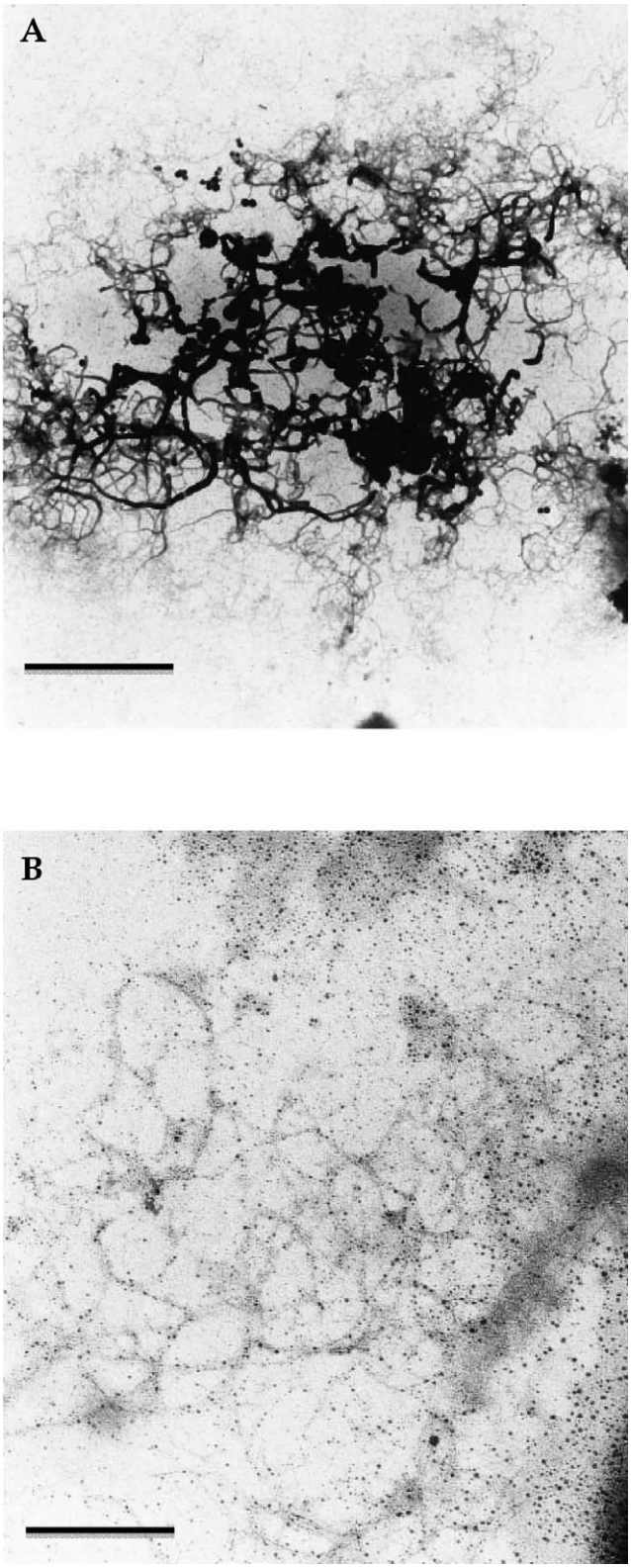

Fig. 2. (**A**) Natural hydrous iron oxide aggregates found between 6.5 and 7.5 m in the water column (scale bar = 1 μm). The micrographs display intimate mixtures of organic fibrils naturally stained by iron oxides. (**B**) Silver stain on organic fibrils found at 7 m in the water column (scale bar = 200 nm). These fibrils consist of polysaccharides that may be naturally stained by hydrous iron oxides.

and settling) are the likely final sink of NPs despite the stabilization of HS.

Determining the concentration and speciation of NPs in water

As yet, no peer-reviewed literature is available on concentrations (or speciation) of NPs in natural waters or sediments. Further, considerable problems require resolution before these measurements can be performed by the research community. Routine monitoring of concentration for regulatory purposes is even further away. Speciation (the physicochemical form or distribution of forms) analysis may require refinement of the methodologies employed for concentration analysis (inductively coupled plasma/mass spectrometry [ICP-MS] or atomic absorption spectrometry may be used for the determination of elemental composition) but should be attainable given time. Given the attention and resources currently devoted to this area, it is likely that considerable progress may be made rapidly.

As mentioned in the previous section, simple box models with many simplifying assumptions have indicated concentrations of the most common NPs (Ag and oxides of Ti, Ce, and Zn) could be expected to be present in natural waters in the range 1 to 10 μg/L, and total NP concentrations may approach 100 μg/L, while values in sediments may be higher [37]. Preliminary work using a technique called flow field–flow fractionation coupled to an inductively coupled plasma mass spectrometer (FlFFF-ICP-MS) [55] has the potential to provide concentration and perhaps speciation data on NPs. Although preliminary data are available showing NP concentrations in waters, these results are not yet published, and further work is required (M. Hassellov, University of Gothenberg, Sweden, personal communication). For more complex systems (sediments and soils and carbon-based NPs), no data are available. It is important that chemical and size characterization can be undertaken, and these are currently not straightforward and even not possible for certain materials and/or media.

Difficulties measuring NPs are related to measuring trace levels against a high background of natural colloids. For the specific (and likely least amenable to analysis) case of iron oxide NPs, there exists a large background of naturally occurring iron either in the dissolved phase (generally at low pH and reducing conditions) or in the solid phase, occurring in sizes from nm to μm and nM to μM concentration levels [56,57]. Into this complex milieu, iron oxide or zero-valent iron NPs may be discharged, and clearly distinguishing between the natural and manufactured materials may be extremely complex. For this system, isotopic labeling may be essential to perform any meaningful analyses. For other and, arguably, more important NPs, such as silver or cerium oxide, the background will be less significant, but unambiguously quantifying manufactured NPs will still be difficult.

In addition to concentration, speciation of the NP must also be identified and quantified to quantify behavior and transport processes as well as biological interactions. For all NPs, knowledge of the distribution between the primary particle and the aggregate and also the form of the aggregate is essential, as discussed previously. For inorganic NPs, solubility is likely to be important, as has been shown by Brunner et al [58]. The technique of FlFFF-ICP-MS is an excellent example of the necessary correct approach to quantification and speciation analysis in that it couples a size fractionation methodology (FlFFF) with a total element/isotope analysis (ICP-

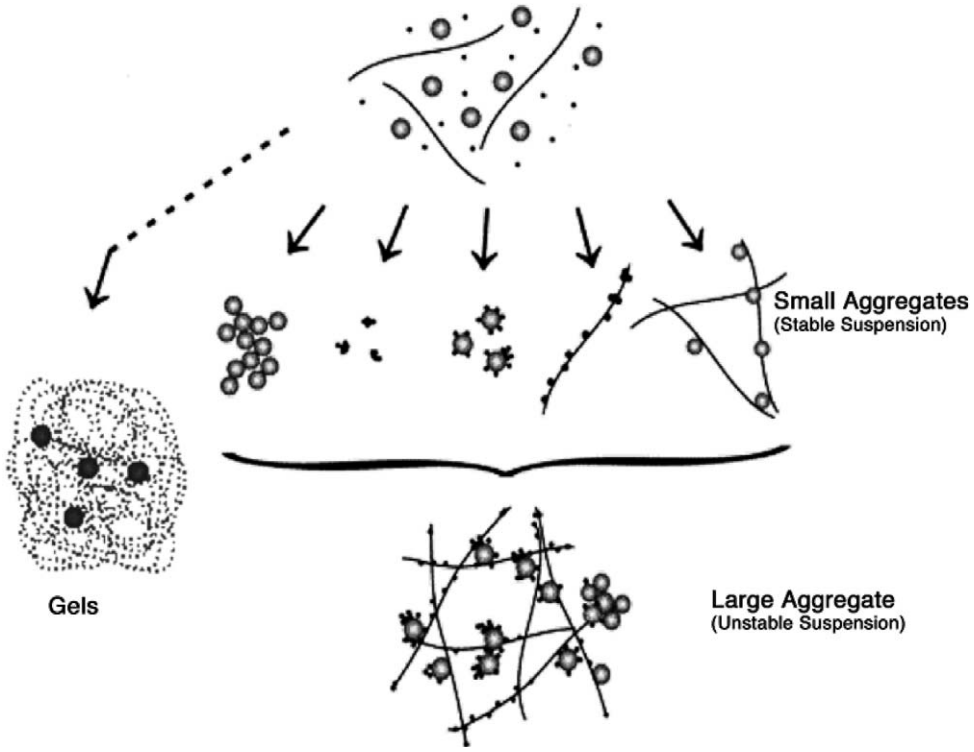

Fig. 3. Three-component model of aquatic colloids relevant to aggregation processes. Small points are fulvic-type material and other material, such as sugars and amino acids; circles represent inorganic colloids; and lines are biopolymers such as polysaccharide fibrils. Taken from Buffle et al. [52].

MS). Potentially, this can give all requisite information, such as concentrations within the dissolved phase and primary particle and aggregate fractions. Other systems for fractionation, including ultrafiltration, and analysis, including graphite furnace atomic absorption spectrometry, may be useful either separately or combined with FlFFF-ICP-MS. In addition, other higher-resolution, single-particle methods, such as electron microscopy and atomic force microscopy, are essential confirmatory tools. However, none of these tools are routine or easily incorporated into regulatory systems.

Fate and behavior in freshwater ecosystems

From approximately 2004 on, much discussion has centered on physicochemical properties of NPs in relation to behavior in natural waters, and it is almost universally agreed that the following properties are an essential requirement: Chemical composition, mass, particle number and concentration, surface area concentration, size distribution (including polydispersity of the primary particle and the nature of any aggregates), specific surface area, surface charge/zeta potential, surface contamination, and the nature of NP shell and any capping agents), stability, and solubility (using a 1-nm cutoff between dissolved and NP fractions). Other properties may also be important.

Measurement of some of these properties is routine, although accuracy is often difficult to ensure. Size distributions can be quantified by dynamic light scattering, electron microscopy, and other methods. Specific surface area can be measured by the technique developed by Brunaur, Emmett, and Teller [59], electron microscopy, or X-ray diffraction. Solubility (of inorganic NPs) can be measured by ultrafiltration or dialysis, followed by ICP-MS. In both the laboratory and the field, it is essential to ensure that these properties are measured in a realistic manner. For instance, the background of natural colloids present in environmental samples should be included in laboratory experiments, and the question then arises as to how this background affects these properties for relevant NPs. As discussed, HS may stabilize NPs by forming coatings, whereas fibrillar colloids may cause aggregation. In addition, experiments must ensure that other parameters, such as pH and ionic strength, cover a range of appropriate conditions. Similarly, as aggregation is a kinetic process, size distributions may easily change when the time period of measurements or experiment is long compared to the length of time for aggregation to occur. Therefore, conditions must be chosen so that changes in NP structure are minimal, and, in any case, measurements must be performed at the start and end of the requisite time scales to check this.

Fate and behavior in marine ecosystems

The marine environment is generally more alkaline, has higher ionic strength, and has a wide variety of colloids and natural organic matter. Coastal runoff and atmospheric deposition could contribute to contamination of the marine environment (Fig. 4). In coastal environments, the types of organic matter in the water will vary with diffuse inputs and the type of discharges, but one might expect higher concentrations of organic matter in the coastal zone compared to pristine oceanic water sample [60–62]. In addition, the oceans exhibit changes in physicochemical characteristics with depth that may influence aggregation and colloid chemistry. For example, temperature is well known to change with depth, and there is stratification of current with depth that may present different salinity and types of organic matter [62]. As in freshwater, aggregates of NPs may sink very slowly to the ocean floor, but it is unclear if NPs will accumulate at the interface between cold and warm currents (Fig. 4) or be recycled by biota. Both

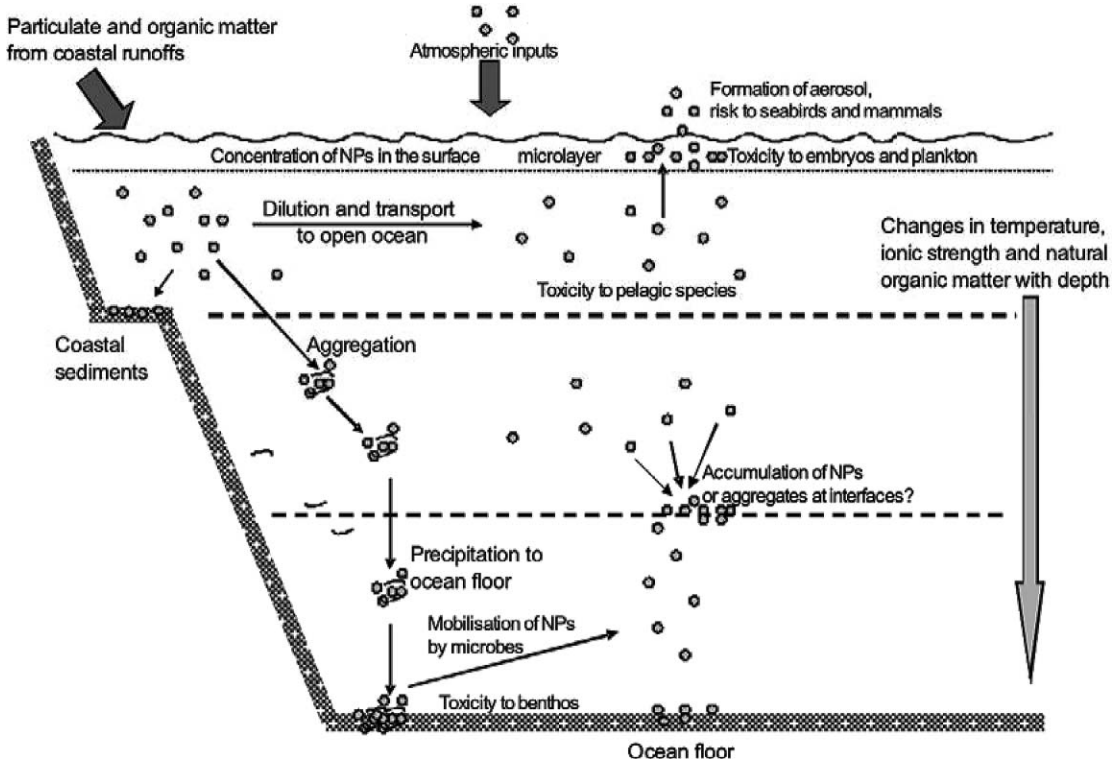

Fig. 4. Schematic diagram outlining the possible fate of nanoparticles (NPs) in the marine environment and the organisms at risk of exposure.

may be a particular risk to pelagic species that feed at these zones (such as vertical migrations in tuna), while deposition in the sediment may present a risk of exposure to benthic species. A risk may also exist of NPs accumulating in the surface microlayers of the oceans, where viscous and surface tension properties could trap NPs in the microlayer at the ocean surface [63,64]. Nanoparticles in this surface microlayer would presumably present a route of aerosol exposure risk to marine birds and mammals as well as the organisms living in the surface microlayer (Fig. 4). Photochemistry is especially pronounced in the surface microlayer, but the consequences are poorly understood and probably vary greatly among NPs.

To date, few systematic studies have investigated how changes in abiotic factors such as pH, ionic strength, or the presence of organic ligands in the water influence ecotoxicity. However, some chemical behaviors of NPs can be measured. The high ionic strength of seawater compared to freshwater will tend to cause aggregation. Experimental evidence from colloid chemistry in saline conditions suggests that even small increases in salinity above that of freshwater (~2.5‰) can dramatically decrease colloid concentrations by aggregation and precipitation processes [55]. For many estuarine organisms, such a small salinity change alone would have little biological effect, but this chemistry study predicts the rapid loss of colloids from the freshwater as soon as it enters the estuarine zone. Toxicity and behavior of NPs in even very dilute seawater is therefore likely to be very different from in freshwater. This further suggests that benthic estuarine organisms may be particularly at risk given the likelihood of these particles to agglomerate, aggregate, and settle.

The agglomeration, aggregation, and precipitation of manufactured NPs in seawater would result in the deposition of the NPs on sediment biofilms, perhaps with subsequent accumulation in the sediment and exposure to sediment-dwelling organisms. It could be argued that this behavior would limit the dispersion of manufactured NPs in the marine environment to the sediments immediately around the discharge. However, this may be a false logic because at the nanoscale, particles are subject to geochemical processes that could result in global dispersion of the material. For example, nanoscale (~5 nm) iron (oxyhydr)oxides that originate in freshwater run off and as part of diffuse coastal erosion contribute to the global cycling of iron through transport processes in the water column and cycling through diatoms and other organisms present in the marine environment [65]. Marine bacteria are also known to synthesize or accumulate NM (ferrihydrites at hydrothermal vents [66]; gold NP synthesis by marine bacteria [67]). Therefore, it should not be assumed that the processing of NPs in marine systems is the same as that of larger particles, and fate and behavior modeling of estuarine effluents may need some reconsideration for NPs.

POTENTIAL MECHANISMS OF BIOLOGICAL UPTAKE AND TOXICITY

Published quantitative research on uptake and accumulation of NMs by whole organisms is scarce. It is clear that organisms living in environments containing NPs will incorporate them within their bodies, mainly via the gut [68–70] with a possibility of translocation within the body. Most of the initial work in this area was undertaken on standard animal models (daphnids) used in ecotoxicology that are well studied and that can be observed whole under standard optical microscopes. Fernandes et al. [68] have demonstrated the uptake of fluorescent carboxylated nanoparticles by *Daphnia magna* and translocation from the gut to reserve fat droplets. The mechanism and significance of such uptake are still the focus of research. Nanoparticles can enter cells by diffusing through cell membranes [71] as well as through endocytosis [72] and adhesion

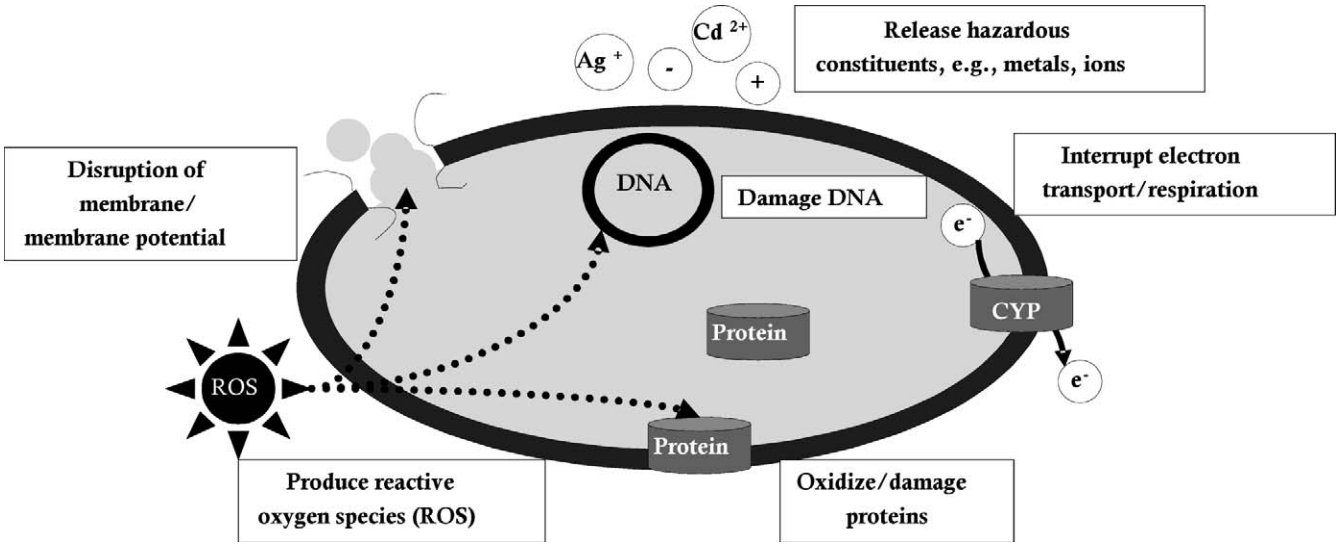

Fig. 5. Possible mechanisms of nanomaterial toxicity to bacteria. Different nanomaterials may cause toxicity via one or more of these mechanisms. CYP = cytochrome P.

[73]. Some NMs, such as quantum dots and CNTs, are intentionally designed to interact with proteins, nucleic acids, or cell membranes for labeling or drug delivery purposes [74,75]. Furthermore, bacteria can be used to deliver NPs [76]. However, the unintentional interactions are more relevant to environmental impacts because they are not controlled and they could adversely impact biota.

While toxicity mechanisms have not yet been completely elucidated for most NMs, possible mechanisms include disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species, and release of toxic constituents (Fig. 5). In certain applications, the toxicity mechanism is linked to the utility of the NM. In the case of magnetic NPs, a magnetic field is used to generate heat [77], and light-absorbing gold NPs attached to bacteria allow the use of lasers to target the bacteria to be killed [78]. However, unintentional toxicity mechanisms can be difficult to isolate and vary widely even within the same class of NM, such as fullerenes or nanosilver. For example, fullerol ($C_{60}[OH]_x$, the hydroxylated form of C_{60}) generates singlet oxygen and can behave as a potent oxidizing agent in biological systems but it is not noticeably cytotoxic [79]. Coating C_{60} with polyvinylpyrrolidone produces a NP that generates singlet oxygen that can cause lipid peroxidation and other cell damage [80]. Still other studies with fullerene water suspensions (nC_{60}) have shown antibacterial activity in the absence of light or oxygen, negating the exclusive influence of singlet oxygen [81]. Similarly, silver NPs may cause toxicity via multiple mechanisms. Morones et al. [82] reported several possible causes: Silver NPs adhered to the surface alter the membrane properties, therefore affecting the permeability and the respiration of the cell; they can penetrate inside bacteria and cause DNA damage, and they can release toxic Ag^+ ions. Degradation of lipopolysaccharide molecules, forming pits in the membrane, and changes in membrane permeability due to nanosilver have also been reported [83].

Damage to membrane integrity

The bacterial cell membrane is a semipermeable barrier that serves important cellular functions, such as regulation of material transport, energy transduction, and intercellular communication. Most imaging applications of NMs target the cell membrane since that is where a cell can be most easily labeled [84]. While quantum dots smaller than 5 nm or silver NPs smaller than 80 nm have been reported to enter bacterial cells [85,86], it is unlikely that bacteria would assimilate larger NMs. However, some NPs have been shown to attach to the cell surface and compromise the integrity and functions of the cell membrane. For example, silicon NPs and fullerene derivatives can embed themselves in the membranes [87]. Carboxyfullerene caused puncturing of bacterial cell membrane in a gram-positive bacterial strain that resulted in cell death [88]. Gold NPs have been reported to weaken membranes and cause heat shock responses in *Escherichia coli* [89].

Nanomaterials can also indirectly cause membrane damage through the generation of reactive oxygen species (ROS), which can oxidize double bonds on fatty acid tails of membrane phospholipids in a process known as lipid peroxidation. This increases membrane permeability and fluidity, making cells more susceptible to osmotic stress or hindering nutrient uptake [90]. Peroxidized fatty acids can trigger reactions that generate other free radicals, leading to more cell membrane and DNA damage.

Protein destabilization and oxidation

Nanomaterial–protein interactions have been optimized for a variety of biomedical applications. For example, quantum dots are used to target and fluorescently label proteins for imaging [91–93]. Multiwalled CNTs were used in biosensing applications to immobilize and optimize lactate dehydrogenase and alcohol dehydrogenase [94,95]. The toxicological interactions between NMs and proteins are related to either the NM physically interacting with proteins or the NM producing ROS or other damaging radicals. The structure and activity of glucose oxidase was altered using electrodes containing gold NPs or SWCNTs [96]. Nanomaterials that generate ROS can damage iron–sulfur clusters that behave as cofactors in many enzymes, leading to Fenton chemistry that catalyzes the production of more ROS generation [97]. Reactive oxygen species can also lead to the formation of disulfide bonds between

sulfur-containing amino acids, thus disturbing the structure and function of the protein.

Nucleic acid damage

Interactions of NMs with nucleic acids have applications in DNA labeling or DNA cleavage. Nucleotides can be tagged with NPs, such as quantum dots, which act as labeling agents for bioimaging applications [98–100]. As with NMs that are made to traverse the cell membrane, iron oxide NPs can be modified into nonviral NP gene transfection vectors to carry genetic information into the cell [101]. Photosensitive metallic and metal oxides that generate ROS as well as fullerenes are used for photodynamic therapy, targeting cells and DNA [102]. In contrast to the beneficial applications of NM–DNA conjugation, fullerenes have been reported to bind DNA and cause deformation of the strand, adversely impacting the stability and function of the molecule [103,104]. Quantum dots can also nick supercoiled DNA [105].

Some NMs indirectly damage DNA because of ROS production, which can lead to DNA strand breaks, cross-linking, and adducts of the bases or sugars [106]. Titanium dioxide NPs, such as those used in sunscreen, generate oxygen radicals that can nick supercoiled DNA [107]. Photosensitive fullerenes can cleave double stranded DNA on exposure to light, although this is highly dependent on the type of the fullerene derivative [108]. Despite these results, only a few studies on the genotoxicity of NMs using Ames tests or any other established protocol [109–111] have been published, and little is known about the potential mutagenic and teratogenic effect of NMs.

Studies on the carcinogenicity of NPs have focused on lung and overall respiratory systems in mammal models. Seriousness of effects seems to be a combination of both particle surface area and reactivity. From the results of these studies, it is difficult to extrapolate generalized effects in terms of dose responses, target species, and the appropriate metrics [112,113]. It is possible that NMs may effect tumor formation through DNA damage and increased cell proliferation associated with inflammation [4]. Given that this work has focused on the lung, it is unclear how much would apply to other tissues and organs.

Cell damage via reactive oxygen species

Many studies of NMs have attributed toxicity to ROS production. As mentioned in the previous sections, ROS are able to damage every cell component, and this interaction tends to trigger further radical formation. For example, Fenton chemistry occurs during the oxidation of proteins containing iron–sulfur groups, and harmful aldehydes are released during lipid peroxidation. While several assays are readily available to test the presence of ROS and the damage it does to cells, these assays must be evaluated carefully to avoid false readings due to interactions between the NPs and the assay reagents [114,115]. The particle aspect of NMs introduces new complications into the use of established assays, particularly those based on dyes.

Interruption of energy transduction

Electron transport phosphorylation and energy transduction processes may be disrupted if membrane integrity is compromised or if a redox-sensitive NM contacts membrane-bound electron carriers and withdraws electrons from the transport chain. Fullerene derivatives have been reported to inhibit *E. coli* respiration of glucose [116,117]. Cerium dioxide NPs may themselves be transformed after contact with living cells, oxidize membrane components involved in the electron transport chain, and cause cytotoxicity [118].

Release of toxic components

Certain NMs cause toxicity to bacterial cells by releasing harmful components, such as heavy metals or ions. Quantum dots are semiconductor nanocrystals that contain noble or transition metals, such as CdSe, CdTe, CdSeTe, ZnSe, InAs, or PbSe, in their core; CdS or ZnS in their shell; and an organic coating [119,120]. Uptake of QD by *E. coli* and *Bacillus subtilis* has been reported [85]. Although no acute cytotoxic effects were observed in that study, in the absence of an efficient efflux system, QD uptake will lead to accumulation of potentially toxic metals in the cells, where they have long residence times and cause toxic effects. In addition to the metals of the core/shell, some organic coatings may also be toxic [121]. Release of silver ions has been implicated in toxicity of silver NPs. It is believed that silver ions interact with thiol groups of proteins, resulting in inactivation of vital enzymes [122]. Silver ions have also been shown to prevent DNA replication [123] and affect the structure and permeability of the cell membrane [123].

Aluminum NPs have been found to inhibit the root growth of plants. Particles at 13-nm size suppressed root growth of five different plant species at 2-mg/ml concentration, while larger sizes, at 200 to 300 nm, had no effect [124]. Although the authors suggested that these effects were due to the presence of free hydroxyl groups on the particle surfaces, Murashov [125] suggested that some of these phytotoxicity effects may have resulted from increased solubility of nanoscale aluminum.

EFFECTS ON AQUATIC ORGANISMS

Preparation of nanoparticle suspensions

One of the key issues in the assessment of the ecotoxicology of NMs has been the protocol to use when preparing laboratory test exposures. By definition, these compounds are in general insoluble, susceptible to aggregation and, hence, likely to settle within a relatively short time. This will result in a dynamic exposure scenario, making it difficult to quantify the amount of NP suspension the test organism experiences in the bioassay. Although it could be argued that this would represent the real-world situation, it is important to recall that in the environment these materials will encounter turbulent waters in flowing rivers and a variety of chemicals, such as detergents and natural organic matter, that may act as dispersants. In addition, depending on their physical and chemical properties as well as the environment/media, many NMs tend to form large aggregates (effectively becoming microparticles), and it is not currently clear whether the materials in the aggregates possess the same toxic potential and bioavailability as single NPs. What is important to consider, however, is the form of NM that is found in the environment, and this is likely to depend on the formulation of the released NM along with the substances with which the NM interacts on entering the environment. For example, release of NMs via wastewater suggests that NMs will be mixed with significant quantities of household and industrial detergents, as described previously, or even coated with proteins as a result of interaction with microorganisms, and this could help to disaggregate these materials and increase the residence time in the water column. Further-

more, naturally occurring surfactants, such as humic and fulvic acids, may also help to disaggregate NPs [53]. It could be argued that this is similar to the development of test exposures and studies of substances that are not readily water soluble, such as hydrocarbons and some pesticides. As described later, however, it is important that results of such studies are widely accepted and are not marred by discussions focusing on protocols, as this would lead to uncertainty regarding the reliability of the results.

One of many controversies regarding experiments with NP suspensions involves the development of semistable suspensions of carbon NPs. A number of the initial published ecotoxicology studies on carbon nanotubes and fullerenes (see the discussion later) used the organic solvent tetrahydrofuran (THF) in the preparation of suspensions. Tetrahydrofuran, however, is not representative of materials widely found naturally or via discharges in the environment. Typically, nC_{60} was prepared by adding water to C_{60} dissolved in THF and then removing the THF by evaporation, as described by Fortner et al. [126]. The use of THF as an intermediary solvent has raised concern as a confounding factor in the toxicity studies [127–130]. Brant et al. [128] demonstrated that, even after filtration and evaporation, low levels of THF remained trapped between the aggregated nC_{60} particles, suggesting that results of studies such as Oberdorster [131], Lovern and Klaper [132], Zhu et al [133], outlined later, were confounded in that they investigated the effects of C_{60} combined with THF rather than the effects of C_{60} alone. Tetrahydrofuran is classified by many regulatory bodies as a neurotoxin and so could in part explain some of the effects observed in the fish in the study by Oberdorster [131].

Attempts to clarify the controversy on the role of THF on the toxicity of nC_{60} have compared the response to THF–nC_{60} with nC_{60} prepared by long-term stirring of C_{60} powder in water or by sonication. In one study, Henry et al. [134], using larval zebrafish, compared the toxicity of THF-mediated fullerene suspensions to fullerene suspensions produced by stirring or by sonication. They also examined the toxicity THF alone in water. Survival of larval zebrafish was reduced in THF–C_{60} and THF–water but not in C_{60} water prepared by either stirring or sonication. Analysis of THF–C_{60} and THF–water by gas chromatography/mass spectrometry did not detect THF but found THF oxidation products γ-butyrolactone and tetrahydro-2-furanol. Further toxicity testing of γ-butyrolactone indicated that effects in THF treatments could result from γ-butyrolactone toxicity. Significant upregulation of genes involved in controlling oxidative damage was observed in fish from treatments containing THF, further reinforcing the conclusion that toxicity was a result of the formation γ-butyrolactone via the oxidation of THF [134].

In contrast, some studies have indicated toxic effects due to both nC_{60} prepared by THF and long-term stirring in water [111,130,133,135]. Furthermore, THF was not antibacterial at the maximum possible residual concentrations it could be found in nC_{60}. Although THF was not directly involved in toxicity at the concentrations it was present, it could have contributed to toxicity via peroxide formation. However, this was unlikely because water stabilizes THF and decreases its ability to form peroxides, which are short lived, while the nC_{60} suspensions retain their toxicity for over two years [136]. Therefore, these authors concluded that residual THF was not necessarily a determining factor in the toxicity of nC_{60}. However, the type of solvent used to make nC_{60} does affect the properties of the nC_{60} formed [137]. For example, sonicated nC_{60} was less toxic to bacteria than THF-prepared nC_{60} [135]. Whether sonication promotes hydroxylation of C_{60} has not been determined, although hydroxylated C_{60} (fullerols) had no noted toxicity [138].

Studies on bacteria

Microorganisms are of great environmental importance because they are the foundation of aquatic ecosystems and provide key environmental services ranging from primary productivity to nutrient cycling and waste decomposition. Consequently, an understanding of NM toxicity to microbes is important to evaluate the potential impacts of NMs in the environment. Furthermore, microorganisms are convenient (model) test organisms because they grow rapidly and are inexpensive to culture; have a high surface-to-volume ratio, making them sensitive to low concentrations of toxic substances; and facilitate studies at many levels ranging from a single biochemical reaction in bacteria to complex ecosystems containing a diversity of microorganisms [136]. Also, it is highly likely that bacteria will influence NM fate and behavior.

Microbial ecotoxicity tests can investigate survival, reproductive capacity, and mutation as well as nonlethal toxicity endpoints. Calculation of a minimal inhibitory concentration or minimal bactericidal concentration offers a standardized method to compare the lowest level of toxicant that prevents bacterial growth for the minimal inhibitory concentration or that actually reduces the number of viable cells for the minimal bactericidal concentration. In bioluminescent tests, diminished bioluminescence of certain bacteria, such as *Vibrio fischeri*, suggests that the test substance has antimicrobial activity [139,140]. Growth inhibition tests use plating methods or spectrophotometric methods to determine growth inhibition after exposure. Some environmental contaminants can be mutagenic and cause changes in the genetic code of receptor organisms. To examine a compound's potential mutagenicity, several assays determine the compound's ability to damage microbial DNA. Most of the tests, such as the Ames test and Mutatox test, look at the frequency of mutations caused by the toxicant that restore some previously damaged function to the microbe [141–144]. However, only a few of these assays have been applied to NMs [145–147]. The Scientific Committee on Emerging and Newly Identified Health Risks [4] has indicated that currently available protocols for the general assessment of mutagenicity or genotoxicity are likely to be applicable for nanomaterials, although practical work to date is currently limited. Biomarkers and other physiological processes that respond in a specific manner to toxic exposure are useful in evaluating chronic, nonlethal effects. Some biomarkers respond to specific contaminants or classes of contaminants, while others respond to contaminant stress in general [148]. Biomarkers include enzyme assays, which are used primarily with sediment microbial communities to examine the effect of a compound in the activity of common enzymes, such as dehydrogenases [149–151].

The literature describing toxicity of various NMs on microorganisms is very limited compared to that evaluating the toxicity on eukaryotic organisms; however, the toxic effects of NMs in prokaryotic systems are increasingly being characterized. Silver NPs and titanium dioxide are among the best-studied NMs with respect to microbial toxicity. Such materials are established as antimicrobial agents, and their nanocrystalline forms may act similarly [152–155]. The bactericidal

Table 1. Toxic effects of nanomaterials on bacteria

Nanomaterial	Toxic effects	References
Carbon-containing fullerenes		
C_{60} water suspension (nC_{60})	Antibacterial to a broad range of bacteria	[135,138,146]
C_{60} encapsulated in polyvinylpyrrolidone	Antibacterial to a broad range of bacteria	[80]
Hydroxylated fullerene	Bactericidal for Gram-positive bacteria	[223]
Carboxyfullerene (malonic acid derivatives)	Bactericidal for Gram-positive bacteria due to fullerene insertion into the cell wall; inhibitory or ineffective against Gram-negative bacteria	[224–227]
Fullerene derivatives with pyrrolidine groups	Inhibits growth of *Escherichia coli* by interfering with energy metabolism	[224,228,229]
Other derivatives of C_{60}	Inhibit the growth of *Mycobacteria*; antimutagenic in *Salmonella typhimurium*; antibacterial	[230–232]
Carbon nanotubes		
Single-walled	Antibacterial to *E. coli*, cell membrane damage	[233,234]
Multiwalled	Cytotoxic to microbes	[235]
Metallic		
Quantum dots	Penetrate cells by oxidative damage to membrane; uncoated quantum dots toxic to *E. coli* and *Bacillus subtilis*	[236–238]
Silver	Bactericidal; viricidal	[82,239]
Gold	Low toxicity to *E. coli* and *Staphylococcus aureus*	[240–242]
Metal oxides		
Magnetite	Low toxicity to *Shewanella oneidensis*	[243]
TiO_2	Accelerates solar disinfection of *E. coli* through photocatalytic activity and reactive oxygen species (ROS); surface coatings photocatalytically oxidize *E. coli, Micrococcus luteus, B. subtilis*, and *Aspergillus niger*	[244–246]
MgO	Antibacterial activity against *B. sutbilis* and *S. aureus*	[247]
CeO_2	Antimicrobial effect on *E. coli*	[118]
ZnO	Antibacterial activity against *E. coli* and *B. subtilis*	[248,249]
Others		
SiO_2	Mild toxicity due to ROS production	[166]

effect of silver compounds and silver ions is well known and has been applied in a wide range of disinfection applications from medical devices to water treatment [156,157]. While there is probably a particle effect, release of silver ions has been proposed as one of the toxic mechanisms of silver NPs [153]. Nano-TiO_2 in water treatment membranes inhibits fouling by *E. coli* when the system is placed under ultraviolet illumination [158]. Furthermore, antiviral properties of NMs have also been reported [159–161]. Nonlethal effects, such as inhibition of enzymatic activities, have been described in some cases [162–165].

One drawback to most of the bacterial studies is that they have evaluated toxicity on single bacterial isolates in optimal bacterial growth media. To date, there have been very few comprehensive studies of NM impacts on environmental microbial communities. Only a few representative materials have been tested under controlled conditions, and specific dose–response data are not always reported. The synergistic or antagonistic effects of mixtures have not yet been investigated. The toxic effects of various NM classes are summarized in Table 1. These effects range from no damage to moderate damage to cellular processes to cell mortality.

Studies on freshwater invertebrates and primary producers

Most of the work assessing the effects of NMs in freshwaters to date has focused on a narrow range of compounds and test species. Invertebrates have been the most commonly used organisms with little published work carried out as yet on vertebrates, primary producers, and unicellular freshwater organisms. Invertebrates have been used for a number of reasons: These organisms have well-defined and standardized test methods; some, such as daphnids, are transparent and may facilitate the observation of NPs within the organism, and many invertebrates filter the aqueous environment in search of food. This final reason makes these organisms one logical portion of the food web to examine since filtering of NP suspensions will likely result in a significant exposure to filter feeders.

The earlier published studies on freshwater invertebrates focused mainly on crustaceans, with *D. magna* being the most studied test species. Lovern and Klaper [132] exposed *D. magna* to C_{60} or TiO_2 (Degussa P25, 25-nm diameter). The particles were treated to break up the aggregates either by sonicating in medium for 30 min or by treatment with the organic solvent THF. Both the TiO_2 and the C_{60} particles were more potent at killing the organisms when prepared in THF than when prepared by sonication, and the C_{60} was more potent than the TiO_2. The question remains as to whether the particles prepared in THF were more toxic because they were better dispersed or because of THF-induced toxicity. Similar results were obtained by Zhu et al. [133]. These authors compared the effects of THF-treated with two-month water-stirred fullerenes on *D. magna* and on adult male fathead minnow (*Pimephales promelas*). Tetrahydrofuran-treated fullerenes proved to be more lethal than those prepared via water stirring to both *D. magna* and minnow. Zhu et al. [133] suggested that even sonication can enhance toxicity and therefore stressed the need to use environmentally relevant doses and preparation techniques.

Oberdörster et al. [130] tried to avoid the controversial use of THF in preparing aqueous fullerene suspensions by stirring the NPs in water for at least two months. They did not characterize the surface chemistry of the fullerenes and hence did

not determine degree of hydroxylation. They exposed the aquatic crustaceans *D. magna* and *Hyalella azteca* and marine harpacticoid copepods to a range of fullerene concentrations. These could not be prepared at high enough concentrations to cause 50% mortality of the invertebrate species exposed (LC50) for 48 or 96 h. The maximum concentrations tested were 35 mg/L for freshwater and 22.5 mg/L for full-strength (35-ppt) seawater since at higher concentrations the fullerene precipitated out of solution. Exposures for 21 d to 2.5 and 5 mg/L fullerenes, respectively, resulted in a significant delay in *D. magna* molting and significantly reduced offspring production, which could have negative impacts at the population level [130].

Adams et al. [166] compared the effects of a range of nanoscale materials (TiO_2, SiO_2, and ZnO) on a range of test species (*B. subtilis*, *E. coli*, and *D. magna*) under light and dark conditions. The study indicated that *D. magna* was the most sensitive test organism. Hund-Rinke and Simon [167] also tested the effects of photocalytic active NPs on *D. magna* and a green algae, *Desmodesmus subspicatus*. Suspensions were obtained by sonication, and endpoints assessed were 72-h EC50 growth (algae) and standard 48-h immobilization test (*D. magna*). Two products were studied (product 1 consisting of 25 nm TiO_2, of a crystalline form, mainly anatase, and product 2 consisting of 100 nm TiO_2, of pure crystalline anatase). On investigation, there was no evidence of product contamination, and product 1 resulted in a 72-h EC50 of 44 mg/L, whereas exposures to product 2 did not result in a clear concentration–response curve (no 72-h EC50 could be determined). No significant differences were obtained when the suspensions were irradiated prior to exposures [167]. As for the exposures conducted with *D. magna*, results seemed to indicate a stronger inhibition caused by the smaller particles, although no concentration–response curves could be obtained for both products. As for the algae, no differences were obtained on irradiation.

Further work using a freshwater alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), was undertaken by Franklin et al. [168], who compared effects on growth of exposures to nanoscale ZnO (30 nm) with bulk ZnO and $ZnCl_2$. Results from these investigators highlighted the importance of characterization of exposures, comparisons of effects between nanoscale and bulk, as well as an assessment of what fractions may lead to toxic effects, that is, dissolved or particulate. So far, very few studies have attempted this detail in assessment. Recent studies [68,169] have characterized the effects of exposing a range of aquatic crustaceans (*D. magna*, *Artemia salina*, and Gammarids) to several NPs (e.g., 20-nm fluorescent polystyrene particles, 25-nm TiO_2, 14-nm carbon black). Results indicate that particles were ingested, resulting in accumulation in the gastrointestinal tract. The particles also adhered to the exoskeleton surfaces of the exposed organisms, suggesting multiple routes of exposure and absorption. Fluorescent polystyrene NPs were rapidly taken up by neonate *D. magna* into their fat-storing droplets. In addition, LC50 (48 h) results ranged between 5 and 20 mg/L. Subtoxic doses of carbon black and TiO_2 NPs were also found to be associated with increased molting of the carapace by neonates [68,169]. In these studies, the methodology employed involved 30 min of sonication in standard water medium but not the use of any solvent.

A study by Roberts et al. [70] corroborates the previous observation that NMs can be readily ingested by daphnids. These authors studied the interactions between *D. magna* and a 1.2-nm water-soluble lysophophatidylcholine-coated SWCNT. These authors further demonstrated that daphnids were able to biomodify the SWCNT by stripping the lipid coating. Acute toxicity was observed only at the highest concentration (100% mortality in the 20-mg/L exposures). Suspensions were prepared by sonication [70].

Lovern et al. [170] revisited their studies on exposures of *D. magna* to C_{60} and TiO_2 but also to a fullerene derivative, $C_{60}HxC_{70}Hx$. They studied and quantified behavior and physiological parameters, including hopping frequency, feeding appendage beating, and heart rate. Whereas the latter was affected on exposures only to C_{60}, the previous two were altered on exposures to both fullerene types. Once again, suspensions were prepared by treating with THF [170].

Baun et al. [69] assessed the ecotoxicological effects of NMs in combination with other chemicals. Their study characterized the potential of C_{60} as a contaminant carrier, using atrazine, methyl parathion, pentachlorophenol, and phenanthrene as additional environmental contaminants and *D. magna* and *P. subcapitata* as test organisms. Suspensions were prepared by following a two-month stirring of C_{60} in medium, with subsequent mixing with the model contaminants. Results indicate different toxic effects can be obtained depending on contaminant type. Synergistic/additive and antagonistic effects were attained, stressing the importance of assessing further possible interactions of NMs with environmental contaminants [69].

Although most of the studies conducted have focused on crustaceans as test organisms (with *D. magna* the most prominent) and bulk measures, such as immobilization and movement, as endpoints, Gagné et al. [171] assessed the ecotoxicological effects of an as yet unstudied NM, quantum dots. The test species selected was the freshwater mussel *Elliptio complanata*, which belongs to an important guild, the filter feeders. These are species that may be particularly at risk given their ability to extract materials in suspension from the water column. Gagné et al. [171] studied the effects of cadmium telluride (Cd-Te) on a range of sublethal endpoints. such as oxidative stress, immunotoxicity, and genotoxicity. Exposures led to oxidative stress in gills and DNA damage in gills and digestive gland, with results indicating that toxic effects are associated to some extent with the dissolved phase [171].

Results thus far obtained from the acute tests conducted on *D. magna* indicate that the lethality of the NPs tested is relatively low but that there may still be cause for concern [130,132,133,169]. Preliminary results indicated increased oxidative stress in *D. magna* with increased carbon black concentrations [68,133]. Much work is still ongoing in this area, and the findings of NP accumulation within body compartments suggest that such research is essential. Sublethal effects, physiological effects, food web uptake, and interactions with other chemicals will need to be investigated further, as indicated previously. Finally, published studies on the effects of NMs on a wider range of invertebrates are still lacking.

Studies on freshwater vertebrates (fish)

A publication by Oberdörster et al. [130] using juvenile largemouth bass was the first nonhuman, nonrodent vertebrate study on NP toxicity to be published. As in the studies described previously, the nC_{60} was pretreated with THF as a transition solvent to aid suspension of the NP in water (i.e., create nC_{60}). The fish were exposed to 0.5 and 1 mg/L nC_{60} for 48 h and were found to exhibit signs of lipid peroxidation

Table 2. Toxic effects of nanomaterials on freshwater organisms

Nanomaterial	Toxic effects	References
Carbon-containing fullerenes		
Filtered C_{60} suspended in tetrahydrofuran (THF) (stirred overnight)	Largemouth bass (*Micropterus salmoides*) exposed to 0.5- and 1-mg/L treatments over 48 h. Significant lipid peroxidation was found in brains in fish exposed to 0.5 mg/L. Total glutathione levels were marginally depleted in gills.	[131]
Filtered C_{60} suspended in THF (stirred overnight)	The median lethal concentration (LC50) (48 h) for *Daphnia magna* on exposure to filtered C60 was 460 μg/L, the lowest-observed-effect concentration (LOEC) was 260 μg/L, and the no-observed-effect concentration (NOEC) was 180 μg/L. One hundred percent mortality was observed at 880 ppb and above. Survival of *D. magna* exposed only to THF was not significantly affected.	[132]
C_{60} water suspension (sonicated for 30 min)	The LC50 (48 h) for *D. magna* on exposure to filtered C60 was 7.9 mg/L, the LOEC was 0.5 mg/L, and the NOEC was 0.2 mg/L. Highest mortality (~60%) was observed at 9 mg/L (highest exposure concentration tested).	[132]
C_{60} water suspension (stirred for 2 months)	No LC50 (48 and 96h) could be determined for *D. magna* and *Hyalella azteca* at the maximum concentrations used (respectively, 35 and 22.5 mg/L); at higher concentrations, precipitation was observed. Exposures to 2.5 and 5 mg/L over 21 d delayed molting and reduced offspring production in *Daphnia.* No effects on movement, molting, or feeding were observed in *Hyalella* at concentrations up to 7 mg/L. In the fish exposures (fathead minnow, *Pimephales promelas*, and Japanese medaka, *Oryzias latipes*) carried out over 48 and 96 h, neither the mRNA nor the protein-expression levels of cytochrome P450 isozymes CYP1A, CYp2K1, and CYP2M1 were changed; the peroxisomal lipid transport protein PMP70 was significantly reduced in the minnow but not medaka, which the authors suggest indicated potential changes in acyl-CoA pathways.	[130]
C_{60} suspended in water (stirred for 2 weeks)	Exposures over 8 d resulted in a maximum of just under 50% mortality in *D. magna* at the concentrations studied (max. 35 mg/L). LC50 (48 h) was found to be over 35 mg/L (max. concentration studied). Fathead minnow (*P. promelas*) exposed to 0.5 mg/L over 48 h resulted in no mortality. Sublethal effects observed over the same period included elevated lipid peroxidation in brain and gill and increased expression of CYP2 family isozymes in liver as compared to control fish.	[133]
C_{60} prepared with THF (stirred overnight)	Exposures over 8 d resulted in 100% mortality in *D. magna* for solutions 1 and 2 ppm. LC50 (48 h) was found to be 0.8 mg/L. One hundred percent mortality was observed for fathead minnow (*P. promelas*) over 6 to 18 h.	[133]
C_{60} prepared with THF (stirred overnight)	Exposures of *D. magna* to 260 μg/L (LOEC) over 60 min when compared with the control resulted in increased hopping frequency, appendage movement and heart rate, and no difference in the rate of postabdominal claw curling.	[197]
$C_{60}HxC_{70}Hx$ prepared with THF (stirred overnight)	Exposures of *D. magna* to 260 μg/L over 60 min when compared with the control resulted in increased hopping frequency and appendage movement and no difference in both the rate of postabdominal claw curling and heart rate.	[197]
C_{60} (prepared with benzene, THF, and acetone)	Exposed zebrafish (*Danio rerio*) embryos suffered delayed embryo and larval development, decreased survival and hatching rates, and observed pericardial edema observed over 96 h at 1.5 mg/L. The addition of an antioxidant seemed to result in reduced toxic effects, indicating that oxidative stress may have resulted in developmental impairment.	[201]
Fullerol; a hydroxylated C_{60} derivative, $C_{60}(OH)_{16-18}$ (prepared with benzene, THF, and acetone)	No effects on zebrafish (*D. rerio*) embryos survival, hatching rate, heart beat, pericardial edema observed over 96 h at max. 50 mg/L.	[201]
C_{60} (stirred and sonicated for 7 d)	Exposures carried out on larval zebrafish (*D. rerio*) from 75 to 147 h postfertilization (hpf) at 25-mg/L concentration. No lethal effects observed. Gene expression changes relative to the control were considered to be minimal. However, further research to interpret these results is needed.	[134]
C_{60} (suspended in THF, stirred for 24 h)	Exposures carried out on larval zebrafish (*D. rerio*) from 75 to 147 hpf. Lethal effects observed for 5% THF–C_{60} (~40% mortality). Similarly, approximately 20% mortality observed for 5% THF–water treatment. Gene expression changes relative to the control were observed. THF–C_{60} had a stronger effect overall. Upregulation of genes with antioxidant activity in both THF treatments was observed. THF degradation products, namely, γ-butyrolactone, were suggested to be implicated in the observed enhanced toxicity.	[134]

Table 2. Continued

Nanomaterial	Toxic effects	References
C_{60} (stirred for 2 months)	Effects assessed on *Pseudokirchneriella subcapitata* and *D. magna* of a combination of C_{60} with phenanthrene, atrazine, methyl parathion, and pentachlorophenol. In algal tests, the presence of C_{60} increased the toxicity of phenanthrene and decreased the toxicity of pentachlorophenol. Similar results were obtained for *Daphnia*, although with different levels of magnitude. No significant differences were found for the other chemicals.	[69]
Carbon nanotubes		
Multiwalled (sonicated for 5 h)	Exposures over 5 d of the eukaryotic unicellular protozoan *Stylonychia mytilus* at concentrations of 0.1 to 200 mg/L. Dose-dependent growth inhibition was observed at concentrations above 1 mg/L. Colocalization of CNTs within the mitochondria was observed. Damage to micronucleus, macronucleus, and membrane was observed, and this was thought to be due to the mitochondria effects.	[133]
Lysophophatidylcholine coated single-walled (sonicated for 30 min)	*Daphnia magna* exposed over 96 h to concentrations up to 20 mg/L. Individuals observed to ingest carbon nanotubes rapidly. The lysophophatidylcholine coating was used as a food source, and the solubility of the NT was affected. One hundred percent mortality observed in 20-mg/L exposures. No LC50 determined but likely to be between 10 and 20 mg/L.	[70]
Single-walled (sodium dodecyl sulfate [SDS] used as dispersant; sonicated)	Exposures of rainbow trout (*Oncorhynchus mykiss*) over 10 d to 0.1 to 0.5 mg/L. Dose-dependent rise in ventilation rate, gill pathologies (edema, altered mucocytes, hyperplasia), and mucus secretion observed. No major hematological or blood disturbances observed. Significant increases of Na^+K^+-adenosine triphosphatase (ATPase) activity observed in the gills and intestine but not the brain. Oxidative stress–linked effects observed. Some pathologies observed in the brain and livers.	[202]
Raw single-walled (stirred for 30 min)	Zebrafish embryos exposed from 4 to 96 hpf to concentrations up to 360 mg/L. Hatching delay induced at concentrations over 120 mg/L. Mollecular and cellular analyses show that overall embryonic development was not affected. Delay in hatching was thought to be due to the Co and Mi catalysts used in the production of single-walled carbon nanotubes.	[203]
Double-walled	Zebrafish embryos exposed from 4 to 96 hpf to concentrations up to 240 mg/L. Hatching delay induced at concentrations from 240 mg/L.	[203]
Carbon black		
Carbon black	Zebrafish embryos exposed from 4 to 96 hpf to concentrations up to 240 mg/L. No hatching delay observed.	[203]
Carbon black	Uptake rapid in *D. magna* and *Lymnaea stagnalis*. Physical effects likely to impact on acute and sublethal endpoints.	[68]
Metallic		
Copper	The effects of copper nanoparticles (80 nm) on gill injury and lethality in zebrafish (*D. rerio*) were assessed and compared with the effects of soluble copper. Nanocopper was found to be acutely toxic to zebrafish with a 48-h LC50 of 1.5 mg/L. Nanocopper solutions at 100 μg/L resulted in enhanced morphological effects and global gene expression patterns in gills when compared to soluble copper, indicating the toxic effects are not mediated solely by solution.	[205]
Silver	Single Ag nanoparticles (5–46 nm) observed to be transported into and out of zebrafish (*D. rerio*) embryos through chorion pore canals and exhibit Brownian diffusion (not active transport). It is likely that deformities and abnormalities observed within the embryos are due to nanoparticle exposure at a critical concentration of 0.19 nM.	[206]
Metal oxides		
TiO_2 suspended with THF (filtered)	*Daphnia magna* concentration of 10 mg/L caused 100% mortality in *D. magna*. The LC50 for filtered TiO_2 was calculated at 5.5 mg/L, with the LOEC being 2.0 mg/L and the NOEC 1.0 mg/L.	[132]
TiO_2 sonicated	No experiments involving sonicated TiO_2 exhibited mortality greater than 9% in *D. magna* over the test exposures time. No LC50 could be determined, nor could the LOEC or NOEC.	[132]
TiO_2 sonicated	Exposures of rainbow trout (*O. mykiss*) over 14 d to 0.1 to 1 mg/L. Gill pathologies observed. No major hematological or blood disturbances observed. Significant decreases of Na^+K^+-ATPase activity observed in the gills and intestine. Oxidative stress–linked effects observed. The authors conclude that TiO_2 at the concentration studied are unlikely to affect ion regulation, but respiratory stress is of concern.	[204]

Table 2. Continued

Nanomaterial	Toxic effects	References
TiO_2 sonicated	Phytoplanktonic green algae (*Desmodesmus subspicatus*) exposed to TiO_2 (stirred throughout) irradiated with simulated sunlight. Two different products studied; the first was mainly anatase of 25-nm particle size, and the second was 100% anatase of 100-nm particle size. Exposures over 72 h to concentrations up to 50 mg/L. Median effective concentration (EC50) of 44 mg/L determined for product 1. No EC50 could be determined for product 2. Preillumination caused no additional effect. *Daphnia magna* exposed to concentrations up to 3 mg/L over 48 h. As for the algae, lower toxicity was observed for product 2 (with larger particle size). Immobilization results were highly variable, and a dose–response relationship could not be derived. Preillumination seemed to result in enhanced immobilization.	[194]
TiO_2 prepared with THF (stirred overnight)	Exposures of *D. magna* to 2 mg/L (LOEC) over 60 min when compared with the control resulted in no differences in hopping frequency, appendage movement, rate of postabdominal claw curling, and heart rate.	[197]
TiO_2 sonicated	Uptake rapid in *D. magna*. Physical effects likely to impact on acute and sublethal endpoints.	[68]
ZnO	Freshwater microalga (*P. subcapitata*) exposed to 25 to 600 μg/L over 72 h. Comparison of toxic effects between $ZnCl_2$, bulk ZnO, nano-ZnO (powdered form), and nano-ZnO (aqueous form, including 2% teric, a surfactant) was carried out on the basis of total zinc and dissolved zinc. No differences were found when assessed on the basis of dissolved zinc (0.1-μm filterable) with an EC50 determined as 60 μg/L.	[195]
Quantum dots		
Cadmium telluride (CdTe)	Freshwater mussels (*Elliptio complanata*) exposed to solutions up to 8 mg/L over 24 h. Observed reduction in phagocytic activity and hemocyte viability in the hemolymph. Lipid peroxidation was significantly increased at higher concentrations in gills and overall reduced in the digestive gland over all concentrations. A degree of DNA damage was observed. Comparisons with effects of Cd ions indicate that toxicity observed is due to a combination of ion and colloidal forms.	[198]
Others		
Nonionized fluorescent polystyrene nanoparticles	Particles were adsorbed to the chorion of Japanese medaka (*O. latipes*) eggs and accumulated in the oil droplets. Particles 39.4 nm in diameter shifted into the yolk and gallbladder during embryonic development. Adult medaka accumulated 39.4-nm nanoparticles mainly in the gills and intestine when exposed to a 10-mg/L solution. Nanoparticles were also detected in the brain, testis, liver, and blood. Concentrations of nanoparticles in the blood of male and female medaka were 16.5 and 10.5 ng/mg blood protein, respectively. The authors suggest that nanoparticles are capable of penetrating the blood–brain barrier and that they eventually reach the brain.	[200]
Negatively charged polystyrene fluorescent particles	Rapid uptake observed in *D. magna* with translocation into reserve lipid bodies recorded.	[68]

in the brain. Selective transport of NP to the brain of rodents has been observed (the authors suggest that this, along with the lack of neural antioxidant defense mechanisms, could explain the enhanced lipid peroxidation in the brain). In a subsequent study, Zhu et al. [133] demonstrated that THF-prepared C_{60} induced 100% mortality within 6 to 18 h of exposure in adult fathead minnow (*P. promelas*). Conversely, nC_{60} generated by water stirring had no impact on lethality over the same time period, although lipid peroxidation was observed in the gill, suggesting oxidative damage as well as a significantly increased expression of cytochrome P2 family isoenzymes in the liver as compared to control fish. Results of Zhu et al. [133] again suggest that the method of preparation can increase toxicity.

As described previously, Oberdörster et al. [130] stirred fullerenes in water for two months to develop a suspension free of THF. Two fish species, fathead minnow and Japanese medaka (*Oryzias latipes*), were exposed to at 0.5 mg/L of this fullerene suspension for 72 h. No changes were observed in mRNA or protein expression levels of cytochrome P450 isoenzymes CYP1A, CYP2K1, and CYP2M1. The peroxisomal lipid transport protein PMP70 was significantly reduced in fathead minnow but not in medaka; the authors attribute this to potential changes in acyl-CoA pathways [130].

Oberdörster et al. [172; http://www.wilsoncenter.org] explored the potential application of microarray technology to ecotoxicity screening of NPs. Specifically, their work focused on the development and application of a genomic-based, ecotoxicity screening method to nanoscale iron particles being used for environmental remediation. While these results are preliminary, they indicate the potential of using microarrays in the detection of toxic effects of nanoparticle exposure.

Similarly to some of the studies with invertebrates, Kashiwada [173] studied the uptake and fate of NMs in a vertebrate

test species. Eggs and adult fish were exposed to fluorescent polystyrene particles of a wide size range. Results suggest rapid uptake and translocation across the different organs, as suggested by previous work with invertebrates.

Zhu et al. [174] assessed the developmental effects of exposures to nC_{60} in zebrafish. Although the preparation method used was different from Oberdorster's [131], it also used dispersants, such as benzene and THF, which were then removed by slow boiling. Although exposures to C_{60} at 1.5 mg/L affected development, subsequent treatment with the antioxidgluthatione (an antioxidant) mitigated toxicity, supporting the notion that nC_{60} exerts oxidative stress [174].

Smith et al. [175] studied the toxicity of SWCNT to rainbow trout. Single-walled carbon nanotube exposure caused a dose-dependent rise in ventilation rate, gill pathologies (edema, altered mucocytes, hyperplasia), and mucus secretion with SWCNT precipitation on the gill mucus. Single-walled carbon nanotube exposure caused statistically significant increases in Na^+K^+-adenosine triphosphatase activity in the gills and intestine but not in the brain. A thiobarbituric acid–reactive substances test was carried out to assess relative amounts of lipid peroxidation products to detect any effects of the exposure on oxidative stress. Results demonstrated dose-dependent and statistically significant decreases especially in the gill, brain, and liver during SWCNT exposure compared to controls. Single-walled carbon nanotube exposure caused statistically significant increases in the total glutathione levels in the gills (28%) and livers (18%) compared to the solvent control. Pathologies in the brain included possible aneurisms or swellings on the ventral surface of the cerebellum. Liver cells exposed to SWCNTs showed condensed nuclear bodies (apoptotic bodies) and cells in abnormal nuclear division. Fish ingested water-containing SWCNTs during exposure (presumably stress-induced drinking) that resulted in precipitated SWCNTs in the gut lumen and intestinal pathology. Aggressive behavior and fin nipping caused some mortality at the end of the experiment, and this was attributed to gill irritation and brain injury, although the solvent could also have partly contributed to the aggression. Overall, the authors concluded that SWCNTs are a respiratory toxicant in trout and that observed cellular pathologies suggest cell cycle defects, neurotoxicity, and blood-borne factors that possibly mediate systemic pathologies [175].

Cheng et al. [176] also investigated the effects of CNTs but on another species of freshwater fish, *Danio rerio*. A range of endpoints was investigated with a delay in hatching observed at higher concentrations for both single- and multi-walled CNTs. Further investigation by the authors indicated that toxic effects could have been due to possible contamination of CNTs [176].

The effect of a range of metal NPs was assessed on rainbow trout [177] and zebrafish [178,179]. Results indicate that effects will depend on the type of material being studied with both copper [178] and silver [179] more likely to result in toxic effects at lower concentrations than titanium dioxide [177]. Table 2 presents a summary of the key published studies to date on the effects of NMs on freshwater taxa.

EFFECTS ON MARINE ORGANISMS

Despite the considerable literature dealing with the toxicity of NPs in freshwater systems, estuarine and marine species should not be neglected for several reasons. Major differences likely exist in the chemical behavior of NPs in seawater compared to freshwater that will impact on the fate and behavior of manufactured NMs and therefore the habitats or organisms being exposed (Fig. 4). It should be recognized that most industrial discharges are to estuarine or marine environments, and although concentrations of NPs have not been routinely measured in these effluents, many of the chemical manufacturers who may take on the role of large-scale production of NMs in the future are typically located with a marine or estuarine discharge. Coastal systems are likely to be the ultimate sink for any NMs, deliberately or purposely discharged into the environment, and they are also areas where high levels of suntan-related cosmetics may be found. Finally, there is a diverse array of algae and invertebrates in marine systems that do not necessarily have freshwater counterparts. Thus, there is a biodiversity argument that we should aim to protect all the major groups of organisms in the oceans as well as organisms with special conservation status, such as marine mammals.

The geochemistry of NMs, discussed in previous sections, suggests that determining the toxic effects of manufactured NPs on bacteria, diatoms, and sediment-dwelling organisms should be a priority issue. Published studies on the ecotoxicity of NMs to bacterial species are limited, even though the bactericidal properties of NMs have been reported (e.g., TiO_2 NPs and silver NPs [180]). Studies on microbes in marine sediments are particularly lacking.

To date, few ecotoxicological studies on marine invertebrates and fish have been reported. Information is limited to a few reports, and we are far from reaching any general consensus view on absorption, distribution, metabolism, excretion, toxic effects on body systems, or toxicity to different groups of marine organisms. Moore [181] argues that marine bivalves such as *Mytilus edulis* might take up NPs using endocytosis and demonstrated that polyester NPs were taken up into endosomes and lysosomes of mussels. Such modes of uptake may be especially relevant to marine organisms where aggregation of NPs on the surfaces of the organisms will occur. Moore [181] also raised concerns about NPs acting as delivery vehicles for other chemicals via the endocytosis pathway. Some evidence also exists that metals in the colloidal phase have a different bioavailability to aqueous metals for uptake by oysters [182]. It may also be possible for the surfaces of marine organisms to promote NP formation. Scarano and Morelli [183] noted that stable nanocrystals form on marine phytoplankton when exposed to cadmium. This raises the possibility that metal NP exposure and subsequent uptake could arise from the presence of appropriate conditions for crystal formation at the surface of the organism, even when manufactured NPs are not present in the original polluting material.

Salinity effects on body distribution and excretion of NPs are unknown for most marine organisms. A need also exists to establish target organs in marine fish and invertebrates. Crustaceans are well known for their ability to sequester toxic metals in granules in the hepatopancreas and other tissues [184]. It might therefore be possible for crustacea to do the same with metal NPs, and this would make these organisms potent bioaccumulators of NPs. Alternatively, processing of nanoscale granules may be different. At least one study on fish has included some salinity experiments to relate uptake with toxicity. Kashiwada [173] exposed the eggs of Japanese medaka to fluorescent NPs (30 mg/L) at a variety of different salinities. An increase in toxicity was seen with increasing salinity, along with a greater tendency for the particles to form aggregates. At a salinity of approximately 18.5 ppt, the greatest

fluorescence in the tissue (accumulation) was reached, and 100% of the eggs were dead within 24 h. At higher salinities approaching that of normal seawater, accumulation decreased, but the egg mortality rate remained high. Kashiwada's research demonstrated that the relationship between salinity (or rather ionic strength), aggregation chemistry, and the precipitation of NPs on the organism is a concern for toxicity.

A study by Templeton et al. [185] illustrates the need to adequately characterize nanoparticle test materials. They characterized the acute and chronic responses of the estuarine copepod *Amphiascus tenuiremis* to SWCNTs. While as-prepared SWCNTs were toxic, purified tubes were not toxic. These investigators used 3.3 N nitric acid to remove metallic and carbonaceous impurities. This process oxidized surface deformities in the tube and undoubtedly made the particle suspensions more stable. Once purified, they separated the NPs into size fractions using electrophoresis. The NPs were separated into three size fractions using electrophoresis: SWCNTs (53% of total), small CNTs (37% of total), and small noncarbon material (10% of total). The smallest size fraction caused significant reduction in life cycle molting success. These results suggest size-dependent toxicity of SWCNTs.

Studies assessing the effects of carbon black nanoparticles on reproduction and embryo development in the marine macroalgae *Fucus serratus* resulted in reduced fertilization success and higher incidence of incorrect polar body axis alignment [186]. During the study the algal zygotes were observed to be covered with a layer of carbon black nanoparticles, which the authors suggest may have interfered with the detection of incident light, which is crucial for alignment of the polar body axis.

Manufactured NPs could also be designed with surface properties that enable them to stay dispersed in saline solutions. It is therefore possible that some manufactured NPs will present exposure risks similar to those of other aqueous phase pollutants. The viscous properties of solutions will be a dominant force in the movement of dispersed NPs (i.e., a low Reynolds number) in marine surface waters: the surface microlayer [63]. This could result in the trapping of high concentrations of NPs in the particulates and exposure of marine invertebrates [64]. Also, as a result of viscous properties, the surface microlayer could have much higher NP concentrations than deeper layers. Organisms and forms in this ocean surface microlayer such as eggs and early life stages of organisms such as zooplankton may therefore be particularly vulnerable to manufactured NPs that show dispersion properties in seawater. Alternatively, some NPs in the surface microlayer might be beneficial in terms of degrading hydrocarbons that may also be present. For example, the presence of TiO_2 NPs in the surface microlayer might drive the photocatalytic degradation of oil pollution [187]. Of course, the free radicals produced by such degradation could also present a toxicological risk to organism in the surface microlayer.

TOXICITY TO SOIL ORGANISMS

Currently, very little information is available with which to assess the environmental risk of manufactured NPs to terrestrial ecosystems. One of the key hurdles in examining NPs in terrestrial systems is the detection of the manufactured NPs in the presence of natural NPs. Similarly, one of the key shortcomings in the few ecotoxicological studies to date is the proper determination of *dose* against which to judge effects and the separation of *particulate* dose from *dissolved* dose for some NPs. More research is needed in examining the fate, transport, and transformation of added NPs to the terrestrial environment so that particle dose characterization becomes quantitative. Soils differ from fresh and marine waters in that the solid phase provides a large and reactive sink for NPs, so that the applied dose may overestimate the actual dose to soil biota. A number of key processes are likely to affect the fate and bioavailability of NPs in the soil environment (Fig. 6).

Preparation of test media and dose characterization

One of the difficulties of measuring NP toxicity in soils is ensuring the homogeneous mixing of NPs with soil test media. Where NPs can be made into a stable suspension, dosing can take place by spraying the NPs evenly onto soil and mixing thoroughly. Problems may occur at high dose levels where stability of the NP suspensions may be compromised (aggregation), and repeated addition of lower doses, coupled with drying of the soil between dosing, may be needed. Where dry nanosized powders are mixed with soil, heterogeneity becomes a major problem if subsampling of the soil batch is performed later for ecotoxicological analysis. This may be minimized by mixing the NP with an inert carrier powder having a particle size closer to the NP size (compared to sieved soil), such as talc, although appropriate controls are needed to confirm that the inert carrier has no adverse toxicological effect on the endpoint in consideration.

The main techniques used to identify NMs in aqueous suspensions have been microscopy, usually atomic force, scanning, or transmission electron microscopy, and chromatographic techniques, such as field-flow fractionation [188–191] and ultrafiltration [192]. Most often these techniques are applied to NPs in standard solutions or in natural aquatic media. In soil science, natural NPs (soil colloids) have been studied for decades [193] with much work examining colloid generation [194,195], colloidal nutrients in soil [196], colloidal transport of contaminants [197–199], and colloid entrapment and deposition [199].

The accurate detection of NPs in soils requires their physical and quantitative separation from natural soil solids (both macro- and nanosize) and being able to distinguish them from natural nanosized NPs. Natural NPs in soil (traditionally termed soil colloids in soil science) are difficult enough to separate and characterize [200–202], and the further separation of NPs from these presents a significant analytical challenge.

Identification of NPs in soil requires the separation of the particles from the soil solid phase (desorption) and their dispersion into an aqueous suspension so that the techniques outlined previously can be used to identify the NPs extracted. To date, very little work has been conducted on the recovery and identification of NPs added to soil. It has recently been suggested that flow-field-flow fractionation may be a useful to identify NPs in soils and to distinguish them from natural NPs [202]. These workers used sodium dodecylsulfate to retain the ZnO NPs in suspension for soil spiking and to extract the NPs from soil. Distinction of NPs from natural colloids was by comparing spiked and unspiked samples. However, in this work, very high rates of ZnO NPs (12,000 mg/kg) were added, and this may be needed to overcome the contribution of background ZnO NPs in the soil suspensions. For metal MNPs, stable isotopic tracers may be useful to improve separation and identification from natural colloids in soil [203].

Nanoparticles have high surface reactivity, and, depending on surface charge and coatings, their adhesion to reactive soil

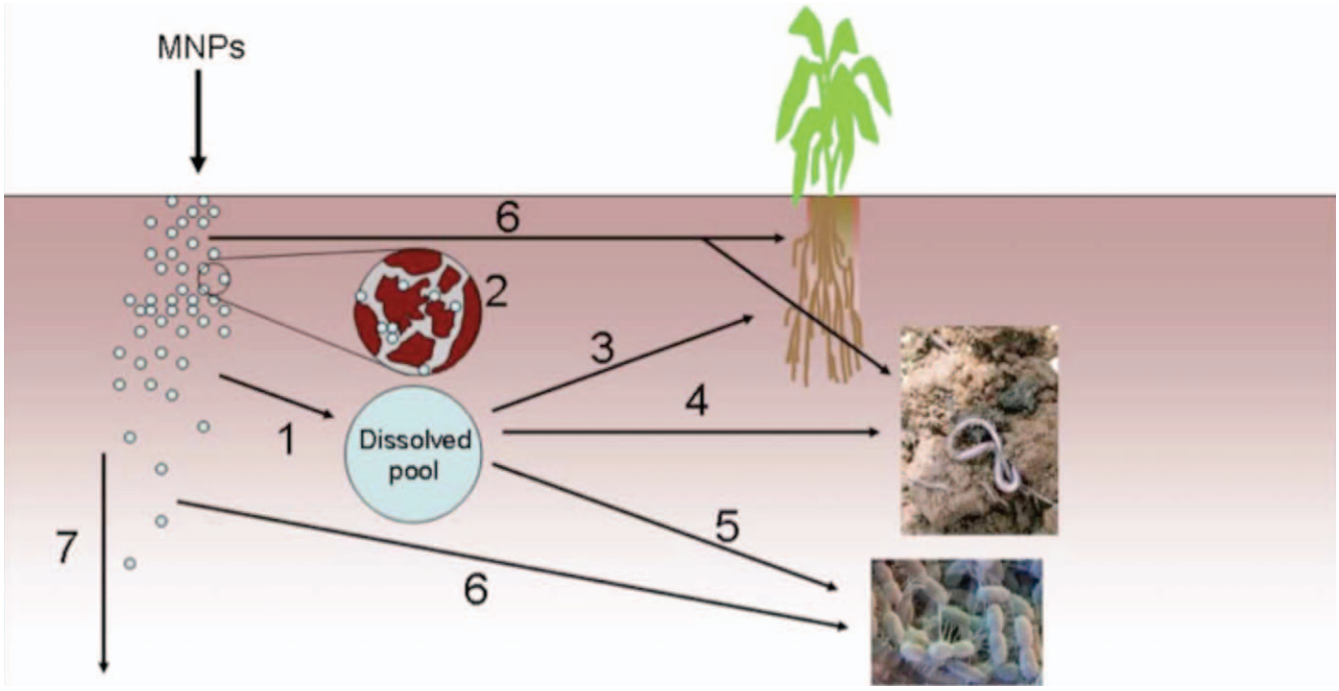

Fig. 6. Key processes in soil relating to transformation and potential risk from manufactured nanoparticulate particles (MNPs). (**1**) Dissolution; (**2**) sorption/aggregation; (**3**) plant bioccumulation; (**4**) invertebrate accumulation and toxicity; (**5**) microbial toxicity; (**6**) direct particle uptake/toxicity; (**7**) particle migration.

surfaces may be strong; partition coefficients for NP contaminants in soil have yet to be published. Data from transport studies of soil colloids, however, indicate that surface coatings on the NPs are important determinants of mobility and may enhance transport [204,205], and this has also been found for NPs used in groundwater remediation [206]. As yet, there are few data on transport of NPs through soils, and hence characterization of NP mobility and associated potential bioavailability remains to be elucidated. Recent studies examined the transport of eight NPs (fullerol [C_{60}-OH_m], SWCNTs, silica [57 nm], alumoxane, silica [135 nm], nC_{60}, anatase, and ferroxane) through spherical glass beads and found the attachment efficiencies to fall in the order as listed [207]. Similar studies in soils are now required.

One of the main drawbacks of current terrestrial ecotoxicological investigations of NPs is the lack of information on transformations of the materials after addition to the test medium. Nanoparticles are designed to have radically different properties than macroscopic or bulk material, and it is well known that chemical and physical properties of the contaminant added to soil will have a large influence on its fate and effects in the terrestrial environment [208–210]. It is also known that dissolution equilibria and kinetics for NPs are closely related to particle size, but relationships at the nanoscale are not fully defined and may depend on several factors—solute concentration, surface area, surface morphology, surface energy and charge, aggregation, adsorbing species, and so on [211]—with relationships at the micro- and bulk scale not always holding at the nanoscale. Some NP metals are regarded in bulk form as relatively insoluble (e.g., TiO_2), while others are sparingly soluble under some soil conditions and soluble in others (e.g., ZnO), but the dissolution and transformation of these materials at the nanoscale in soils is totally unknown. Dissolution is particularly difficult to study with NP metals, as most laboratory particle separation techniques (low-speed centrifugation or filtration through 0.45- or 0.22-μm filters) will not separate NPs from the true solution phase, and dialysis or ultracentrifugation is needed to define solution and particulate doses [212].

Bioaccumulation and toxicity of NMs in soils

Very few data exist by which to assess the potential environmental risk of NPs to the terrestrial environment, and this is seen as a key knowledge gap by regulators [213]. As yet, there are few reports in the peer-reviewed scientific literature of the assessment of ecotoxicity of NPs to soil biota *in soils.* Several reports have examined ecotoxicity to soil organisms, but the media used have been simple aqueous media [124,214–219], and persistence of the NPs in the test media was not assessed. These studies are summarized in Table 3.

Yang and Watts [218] reported the toxicity of alumina NPs (13 nm, coated with and without phenanthrene) to root growth of five plant species (cabbage, carrot, corn, cucumber, and soybean) exposed to aqueous suspensions of the NPs but only at high concentrations (2,000 mg/L). Loading of the alumina NPs with phenanthrene reduced the toxicity of the NPs. The NPs were not physically characterized prior to dosing, doses were not analytically confirmed, and in a letter to the editor of *Toxicology Letters*, Murashov [220] pointed out that the experimental protocol of Yang and Watts [218] did not distinguish toxicity caused by application of the aluminum in an NP form and toxicity of *solution* aluminum derived from the NP. Indeed, aluminum is a major component of soil minerals, known to be phytotoxic in acidic soils for almost a century [221], so the phytotoxicity observed by Yang and Watts [218] is not surprising and clearly indicates the need to accurately determine if the nanoparticulate form of a contaminant is toxic or if the soluble contaminant *derived from* the NP is toxic. Franklin et al. [168] reached similar conclusions for the toxicity of ZnO NPs to aquatic biota.

Table 3. Toxic effects of nanomaterials on soil organisms

Nanomaterial	Toxic effects	References
Carbon-containing fullerenes		
C_{60} granular and C_{60} water suspension (nC_{60})	None. Endpoints tested were respiration (basal and substrate induced), microbial biomass C, and enzyme activities. Small shift in bacterial and protozoan gene patterns by polymerase chain reaction denaturing gradient gel electrophoresis.	[243]
nC_{60}	No effect on respiration (basal), microbial biomass C (measured by substrate-induced respiration), and protozoan abundance. Reduction in numbers of bacteria. Small shift in bacterial and protozoan gene patterns by polymerase chain reaction denaturing gradient gel electrophoresis.	[244]
Carbon nanotubes		
Multiwalled	No effect on seed germination and root growth of corn, cucumber, lettuce, radish, and mustard rape. Reduced root growth of ryegrass.	[242]
Metallic		
Aluminum	No effect on seed germination of corn, cucumber, lettuce, radish, mustard rape, and ryegrass. Rhizotoxic to corn, lettuce, and ryegrass but stimulated radish and mustard rape root growth.	[242]
Zinc	Reduced seed germination of ryegrass and reduced root growth of corn, cucumber, lettuce, radish, mustard rape, and ryegrass.	[242]
Metal oxides		
Al_2O_3	Phytotoxic (germination and seedling growth), but see text.	[124]
	No effect on seed germination of corn, cucumber, lettuce, radish, rape, and ryegrass. No effect on root growth of cucumber, lettuce, radish, mustard rape, and ryegrass. Reduced root growth of corn.	[242]
TiO_2	Stimulatory to spinach seed germination and seedling growth at low dose, phytotoxic at high doses.	[246]
ZnO	Reduced seed germination of corn and reduced root growth of corn, cucumber, lettuce, radish, mustard rape, and ryegrass.	[242]

Zheng et al. [219] examined the effects of nano- and bulk TiO_2 on spinach seed germination and early plant growth in simple Perlite media containing a complete nutrient solution. Nano-TiO_2 significantly increased seed germination and plant growth at low concentrations but decreased these parameters at high concentrations. Bulk TiO_2 had little effect. The MNPs in Zheng et al. [219] were not physically characterized, and no details of size or surface reactivity of the materials were provided.

Recently, Lin and Xing [215] examined the toxicity of several NPs (multiwalled carbon nanotubes, Al, Al_2O_3, Zn, and ZnO) to germination and early root growth of six plant species in simple aqueous media at pH 6.5 to 7.5. The NPs were not physically characterized prior to exposure, and doses were not confirmed. The Zn-based NPs had the greatest effect on plant germination and root growth, with EC50 concentrations similar for both Zn- and ZnO-NPs of 20 to 50 mg/L, depending on plant species. The authors attempted to quantify the solution zinc dose in their experiments by centrifugation (3,000 *g* for 60 min) and filtration (0.7 μm). They reported that the centrifugation procedure did not fully separate the NPs from the solution phase (assessed using tapping mode/atomic force microscopy) but did not provide microscopic information on the solutions after filtration. Surprisingly, a 2,000-mg/L suspension of ZnO after centrifugation and filtration returned a solution Zn concentration of only 0.3 to 3.6 mg/L, significantly less than the concentration of Zn^{2+} in equilibrium with bulk ZnO at pH 6.5 to 7.5 [222]—approximately 10 to 900 mg/L.

To date, there are only two reports in the literature of the terrestrial effects of NPs performed in soil, both on fullerenes [216,218]. Tong et al. [216] examined the toxicity of nC_{60} in aqueous suspension and in granular form to soil microorganisms using soil respiration, microbial biomass, phospholipid fatty acid analysis, and enzyme activities as endpoints. The authors also examined the DNA profile of the microbial community. All tests were performed in the laboratory at optimal moisture conditions. In contrast to the observed microbial toxicity of nC_{60} in vitro [126], Tong et al. [216] found no effect of nC_{60} to any endpoint in the soil medium used (silty clay loam, 4% organic matter, pH 6.9). They suggested that this was due to the strong binding of nC_{60} to soil organic matter, although no evidence was provided that organic matter was the solid phase in soil reducing the effective dose. A similar set of experiments was performed by Johansen et al. [217], who examined the effect of nC_{60} added to a neutral soil (pH 6.7) with low organic C content (1.5%) on soil respiration, biomass C, bacterial and protozoan abundance, and the polymerase chain reaction denaturing gradient gel electrophoresis profiling of bacterial and protozoan DNA. No effects of exposure of nC_{60} were found on soil respiration, biomass C, and protozoan abundance, but reductions in bacterial abundance were observed through colony counts. The nC_{60} also caused only a small shift in bacterial and protozoan DNA, indicating a small change in community structure, similar to the results of Tong et al. [216].

These data highlight the need for more information on the interaction of NPs with soil components and more quantitative assessments of aggregation/dispersion, adsorption/desorption, precipitation/dissolution, decomposition, and mobility of manufactured NPs in the soil environment. This information will aid the interpretation of terrestrial ecotoxicity test data and will inform the correct protocols for the assessment of the ecotoxicity of NPs in soils.

CONCLUSIONS

The rapid advances in the understanding and manipulation of NMs undoubtedly will continue the explosive growth of

products incorporating NMs. Truly, their use may be limited only by one's imagination. As such, there will be a corresponding increase in NP release into the environment. Hence, it is essential that research to solve the problems and overcome the challenges that have been discussed in this review be accomplished. Without quantitative measures of both exposure and effects, ecological risk assessment cannot be conducted, and regulators will not have the tools to adequately manage NM in the environment. These problems and challenges are multidisciplinary and begin with accurately understanding the potential for environmental releases of NM and continue throughout characterizing environmental behavior, fate, and bioavailability. Environmental scientists, the majority of whom are used to dealing with truly soluble contaminants, can establish a foundation for conducting research on NPs by understanding the colloidal literature as well as the human health literature on airborne particulates. An excellent treatise is available on colloids and particles, and much of this information may be applicable to NPs [41]. The human health literature is replete with studies of the effects of inhalation and dermal exposure to fine and ultrafine particles.

In addition, for the study of NM behavior, fate, and effects in the ecosystem to progress, several issues must be addressed on a timely basis. Standard, characterized materials must be available to researchers. The variability between NM produced from different manufacturers can be large. In fact, many scientists have already experienced this and even note the batch-to-batch variation within one manufacturer (S. Klaine, Clemson University, Clemson, SC, USA, personal communication). Methods must be developed and standardized for creating test media (both soil/sediment and water) for conducting fate and effects testing. Particle and particle suspension characterization requirements should be standardized so that researchers know the minimum requirements necessary to adequately interpret results of their research. This undoubtedly will require developing and refining instrumentation. Importantly, these characterizations need to be relevant to field conditions.

It is obvious from the above discussion on NM effects that much research is needed in freshwater, marine, and soil ecosystems. Since the ultimate sink for NPs may be sediment and soils, more testing with organisms that inhabit these ecosystems is essential. However, research to date has dramatically illustrated the need for standardized testing protocols with NPs. This is particularly pressing in soil and sedimentary systems where progress in the development of standardized protocols and characterization are lagging far behind testing in aquatic systems.

In addition to single organism studies, food web research is needed to quantify the potential for bioaccumulation and, ultimately, human exposure. Methods for the detection and quantification of NPs in different organisms are necessary for this research. To facilitate this work, use of radioisotopes and stable isotopes should be considered.

Finally, since many NPs will enter aquatic environments and, as discussed here, since natural organic matter can stabilize these particles in the water column, more research is needed to better understand the interactions between NPs and natural organic matter. Further, the repercussions of these interactions on ecosystem fate, behavior, and bioavailability of NPs must be characterized.

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REFERENCES

1. British Standards Institution. 2007. Terminology for nanomaterials. PAS 136:2007. London, UK.
2. American Society for Testing and Materials. 2006. Standard terminology relating to nanotechnology. E 2456-06. West Conshohocken, PA.
3. Borm PA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdörster E. 2006. The potential risks of nanomaterials: A review carried out for ECETOC. *Particle and Fibre Toxicology* 3:11.
4. Scientific Committee on Emerging and Newly Identified Health Risks. 2007. The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials, 21–22 June 2007. European Commission, Brussels, Belgium.
5. Lead JR, Wilkinson KJ. 2006. Environmental colloids: Current knowledge and future developments. In Wilkinson KL, Lead JR, eds, *Environmental Colloids: Behaviour, Structure and Characterization.* John Wiley, Chichester, UK, pp 1–15.
6. Shi JP, Evans DE, Khan AA, Harrison RM. 2001. Sources and concentration of naoparticles (<10 nm diameter) in the urban atmosphere. *Atmos Environ* 35:1193–1202.
7. Oberdorster G, Oberdorster E, Oberdorster J. 2007. Concepts of nanoparticle dose metric and response metric. *Environ Health Perspect* 115:290.
8. Pulskamp K, Diabate S, Krug HF. 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol Lett* 168:58–74.
9. Muller K, Skepper JN, Posfai M, Trivedi R, Howarth S, Corot C, Lancelot E, Thompson PW, Brown AP, Gillard JH. 2007. Effect of ultrasmall supraparamagnetic iron oxide nanoparticles (Ferumoxtran-10) ob human monocyte-macrophages in vitro. *Biomaterials* 28:1629–1642.
10. Lead JR, Davison W, Hamilton-Taylor J, Buffle J. 1997. Characterizing colloidal material in natural waters. *Aquatic Geochemistry* 3:213–232.
11. Guo L, Santschi PH. 2006. Ultrafiltration and its applications to sampling and characterization of aquatic colloids. In Wilkinson K, Lead J, eds, *Environmental Colloids and Particles.* John Wiley, Hoboken, NJ, USA, pp 159–221.
12. Cameron FK. 1915. Soil colloids and the soil solution. *J Phys Chem* 19:1–13.
13. Yokoyama T, Huang CC. 2005. Nanoparticle technology in the production of functional materials. *Kona Journal* 23:7–17.
14. De Castro CL, Mitchell BS. 2002. Nanoparticles from mechanical action. In Baraton MI, ed, *Synthesis, Functionalization and Surface Treatment of Nanoparticles.* American Science, Valencia, CA, USA, pp 1–15.
15. Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE. 1985. C_{60}: Buckminsterfullerene. *Nature* 318:162.
16. Lekas D. 2005. Analysis of nanotechnology from an industrial ecology perspective. Part II: Substance flow analysis of carbon nanotubes. Project on Emerging Nanotechnologies Report. Woodrow Wilson International Centre for Scholars, Washington, DC.
17. Service RF. 1998. Superstrong nanotubes show they are smart, too. *Science* 281:893–894.
18. Holzinger M, Steinmetz J, Samaille D, Glerup M, Paillet M, Bernier P, Ley L, Graupner R. 2004. [2+1] cycloaddition for cross-linking SWCNTs. *Carbon* 42:941–947.
19. Qiao R, Ke PC. 2006. Lipid-carbon nanotube self assembly in aqueous solution. *J Am Chem Soc* 128:13656.
20. Qiao R, Roberts AP, Mount AS, Klaine SJ, Ke PC. 2007. Translocation of C_{60} and its derivatives across a lipid bilayer. *Nano Letters* 7:614–619.
21. Gu H, Soucek MD. 2007. Preparation and characterization of monodisperse cerium oxide nanoparticles in hydrocarbon solvents. *Chemistry of Materials* 19:1103–1110.

22. Sass J. 2007. Nanotechnology's invisible threat: Small science big consequences. NRDC Issue Paper. Natural Resources Defense Council, New York, NY, USA.
23. Pitkethly MJ. 2004. Nanomaterials—The driving force. *Nanotoday* 7:20–29.
24. Corma A, Chane-Ching JY, Airiau M, Martinez C. 2004. Synthesis and catalytic properties of thermally and hydothermally stable, high surface area SiO_2-CeO_2 mesostructured composite materials and their application for the removal of sulphur compounds from gasoline. *J Catalysis* 224:441–448.
25. Lin W, Huang Y, Zhou X, Ma Y. 2006. Toxicity of cerium oxide nanoparticles in human lung cancer cells. *Int J Toxicol* 25:451–457.
26. Dabbousi BO, RodriguezViejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R, Jensen KF, Bawendi MG. 1997. (CdSe)ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. *J Phys Chem B* 101:9463–9475.
27. Murray CB, Sun S, Gaschler W, Doyle H, Betley TA, Kagan CR. 2001. Colloidal synthesis of nanocrystals and nanocrystal superlattices. *IBM Journal of Research and Development* 45: 47–56.
28. Alivisatos AP, Gu WW, Larabell C. 2005. Quantum dots as cellular probes. *Annu Rev Biomed Eng* 7:55–76.
29. Li X-Q, Elliott DW, Zhang W-X. 2006. Zero-valent iron nanoparticles for abatement of environmental pollutants: Materials and engineering aspects. *Crit Rev Solid State Mater Sci* 31:111–122.
30. Zhang W. 2003. Nanoscale iron particles for environmental remediation: An overview. *Journal of Nanoparticle Research* 5: 323–332.
31. Royal Society/Royal Academy of Engineering. 2004. Nanoscience and nanotechnologies: Opportunities and uncertainties. Two year review of progress on government actions: Joint Academies' Response to the Council for Science and Technology's Call for Evidence. RS Policy Document 35/06. The Royal Society, London, UK.
32. Klopffer W, Curran MA, Frankl P, Heijungs R, Kohler A, Olsen SI. 2007. Nanotechnology and life cycle assessment. Woodrow Wilson International Centre for Scholars Project on Emerging Nanotechnologies, Washington DC.
33. Kramer JR, Benoit G, Bowles KC, Di Toro DM, Herrin RT, Luther GW, Manalopoulis H, Robilliard KA, Shafer MM, Shaw JR. 2002. Environmental chemistry of silver. In Andren AW, Bober TW eds, *Silver in the Environment: Transport, Fate, and Effects.* SETAC, Pensacola, FL, USA, pp 1–25.
34. Doty RC, Tshikhudo TR, Brust M. 2005. Extremely stable water-soluble Ag nanoparticles. *Chemistry of Materials* 17:4630–4635.
35. Zhang W-X, Elliott DW. 2006. Applications of iron nanoparticles for groundwater remediation. *Remediation* 16:7–21.
36. Biswas P, Wu P. 2005. Nanoparticles and the environment. *J Air Waste Manag Assoc* 55:708–746.
37. Boxall ABA, Chaudhry Q, Sinclair C, Jones A, Aitken R, Jefferson B, Watts C. 2007. Current and future predicted environmental exposure to engineered nanoparticles. Central Science Laboratory, Department of the Environment and Rural Affairs, London, UK.
38. Hoet PH, Nemmar A, Nemery B. 2004. Health impact of nanomaterials? *Nat Biotechnol* 22:19.
39. Klaine SJ, Cobb GP, Dickerson RL, Dixon KR, Kendall RJ, Smith EE, Solomon KE. 1996. An ecological risk assessment for the use of the biocide dibromonitrilopropionamide (DBNPA), in industrial cooling systems. *Environ Toxicol Chem* 15:21–31.
40. Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541–1583.
41. Wilkinson KJ, Lead JR, eds. 2006. *Environmental Colloids: Behaviour, Structure and Characterization.* John Wiley, Chichester, UK.
42. Madden AS, Hochella MF, Luxton TP. 2006. Insights for size-dependent reactivity of hematite nanomineral surfaces through Cu^{2+} sorption. *Geochim Cosmochim Acta* 70:4095–4104.
43. Gustafsson O, Gschwemd G. 1997. Aquatic colloids: Concepts, definitions and current challenges. *Limnol Oceanogr* 42:517–528.
44. Buffle J, Leppard GG. 1995. Characterisation of aquatic colloids and macromolecules. 1. Structure and behavior of colloidal material. *Environ Sci Technol* 29:2169–2175.
45. Honeyman BD, Santschi PH. 1992. The role of particles and colloids in the transport of radionuclides and trace metals in the ocean. In Buffle J, van Leeuwen HP, eds, *Environmental Particles*, Vol 1. Lewis, Boca Raton, FL, USA, pp 379–423.
46. Sigg L. 1994. The regulation of trace elements in lakes: The role of sedimentation. In Buffle J, de Vitre RR, eds, *The Chemical and Biological Regulation of Aquatic Systems.* Lewis, Boca Raton, FL, USA, pp 175–195.
47. Harrison RM, Harrad SJ, Lead JR. 2003. The global disposition of contaminants. In Hoffman DA, Rattner BA, Burton GA, Cairns J, eds, *Handbook of Ecotoxicology*, 2nd ed. Lewis, Boca Raton, FL, USA, pp 633–651.
48. Neihof RA, Loeb GI. 1972. The surface charge of particulates in seawater. *Limnol Oceanogr* 17:7–16.
49. Hunter KA, Liss PS. 1979. The surface charge of suspended particles in estuarine and coastal waters. *Nature* 282:823–825.
50. Gibson CT, Turner I, Roberts C, Lead JR. 2007. Quantifying the dimensions of nanoscale organic surface layers in natural waters. *Environ Sci Technol* 41:1339–1444.
51. Tipping E. 1981. The adsorption of aquatic humic substances by iron oxides. *Geochim Cosmochim Acta* 45:191–199.
52. Buffle J, Wilkinson KJ, Stoll S, Filella M, Zhang J. 1998. A generalized description of aquatic colloidal interactions: The three-colloidal component approach. *Environ Sci Technol* 32: 2887–2899.
53. Hyung H, Fortner JD, Hughes JB, Kim J-H. 2007. Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environ Sci Technol* 41:179–184.
54. Giasuddin ABM, Kanel SR, Choi H. 2007. Adsorption of humic acid onto nanoscale zerovalent iron and its effect on arsenic removal. *Environ Sci Technol* 41:2022–2027.
55. Stolpe B, Hassellöv M. 2007. Changes in size distribution of fresh water nanoscale colloidal matter and associated elements on mixing with seawater. *Geochim Cosmochim Acta* 71:3292–3301.
56. Davison W, de Vitre RR. 1992. Iron particles in freshwaters. In Buffle J, van Leeuwen HP, eds, *Environmental Particles*, Vol 1. Lewis, Boca Raton, FL, USA, pp 315–355.
57. Lyvén B, Hassellöv M, Turner DR, Haraldsson C, Andersson K. 2003. Competition between iron- and carbon-based colloidal carriers for trace metals in a freshwater assessed using flow field-flow fractionation coupled to ICPMS. *Geochim Cosmochim Acta* 67:3791–380.
58. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ. 2006. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol* 40:4374–4381.
59. Brunauer S, Emmett PH, Teller E. 1938. Adsorption of gases in multimolecular layers. *J Am Chem Soc* 60:309–319.
60. Middelburg JJ, Hennan PMJ. 2007. Organic matter processing in tidal estuaries. *Mar Chem* 106:127–147.
61. Hirose K. 2007. Metal-organic matter interaction: Ecological roles of ligands in oceanic DOM. *Appl Geochem* 22:1636–1645.
62. Yamashita Y, Tsukasaki A, Nishida T, Tanoue E. 2007. Vertical and horizontal distribution of fluorescent dissolved organic matter in the Southern Ocean. *Mar Chem* 106:498–509.
63. Wurl O, Obbard JP. 2004. A review of pollutants in the sea-surface microlayer (SML): A unique habitat for marine organisms. *Mar Pollut Bull* 48:1016–1030.
64. Simpkiss K. 1990. Surface effects in ecotoxicology. *Funct Ecol* 4:303–308.
65. Raiswell R, Tranter M, Benning LG, Siegert M, De'ath R, Huybrechts P, Payne T. 2006. Contributions from glacially derived sediment to the global iron (oxyhydr)oxide cycle: Implications for iron delivery to the oceans. *Geochim Cosmochim Acta* 70: 2765–2780.
66. Kennedy CB, Scott SD, Ferris FG. 2004. Hydrothermal phase stabilization of 2-line ferrihydrite by bacteria. *Chemical Geology* 212:269–277.
67. Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K. 2007. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids Surf B* 57:97–101.
68. Fernandes TF, Christofi N, Stone V. 2007. The environmental

implications of nanomaterials. In Monteiro-Riviere N, Lang Tran C, eds. *Nanotoxicology: Characterization, Dosing and Health Effects.* CRC, Boca Raton, FL, USA, pp 405–420.
69. Baun A, Sorensen SN, Rasmussen RF, Hartmann NB, Koch CB. 2008. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C_{60}. *Aquat Toxicol* 86:379–387.
70. Roberts AP, Mount AS, Seda B, Souther J, Qiao R, Lin S, Ke PC, Rao AM, Klaine SJ. 2007. In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. *Environ Sci Technol* 41:3025–3029.
71. Lin S, Keskar D, Wu Y, Wang X, Mount AS, Klaine SJ, More JM, Rao AM, Ke PC. 2007. Detection of phospholipid-carbon nanotube translocation using fluorescence energy transfer. *Applied Physics Letters* 89:143118.
72. Kim JS, Yoon T-J, Yu KN, Kim BG, Park SJ, Kim HW, Lee KH, Park SB, Lee J-K, Cho MH. 2006. Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol Sci* 89: 338–347.
73. Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Shulz H, Semmler M, Imhof V, Heyder J, Gehr P. 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect* 113:1555–1560.
74. Gao XH, Chan WCW, Nie SM. 2002. Quantum-dot nanocrystals for ultrasensitive biological labeling and multicolor optical encoding. *Journal of Biomedical Optics* 7:532–537.
75. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. 2005. Quantum dot bioconjugates for imaging, labeling and sensing. *Nature Materials* 4:435–446.
76. Diao JJ, Hua D, Lin J, Teng HH, Chen D. 2005. Nanoparticle delivery by controlled bacteria. *Journal of Nanoscience and Nanotechnology* 5:1749–1751.
77. Hilger I, Hiergeist R, Hergt R, Winnefeld K, Schubert H, Kaiser WA. 2002. Thermal ablation of tumors using magnetic nanoparticles—An in vivo feasibility study. *Investig Radiol* 37:580–586.
78. Zharov VP, Mercer KE, Galitovskaya EN, Smeltzer MS. 2006. Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. *Biophys J* 90:619–627.
79. Pickering KD, Wiesner MR. 2005. Fullerol-sensitized production of reactive oxygen species in aqueous solution. *Environ Sci Technol* 39:1359–1365.
80. Kai Y, Komazawa Y, Miyajima A, Miyata N, Yamakoshi Y. 2003. Fullerene as a novel photoinduced antibiotic. *Fullerines, Nanotubes and Carbon Nanostructures* 11:79–87.
81. Lyon DY, Fortner JD, Sayes CM, Colvin VL, Hughes JB. 2005. Bacterial cell association and antimicrobial activity of a C-60 water suspension. *Environ Toxicol Chem* 24:2757–2762.
82. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* 16:2346–2353.
83. Sondi I, Salopek-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 275:177–182.
84. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. 2002. In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science* 298:1759–1762.
85. Kloepfer JA, Mielke RE, Nadeau JL. 2005. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. *Appl Environ Microbiol* 71:2548–2557.
86. Xu XH, Brownlow WJ, Kyriacou SV, Wan Q, Viola JJ. 2004. Real-time probing of membrane transport in living microbial cells using single nanoparticle optics and living cell imaging. *Biochemistry* 43:10400–10413.
87. Jang H, Pell LE, Korgel BA, English DS. 2003. Photoluminescence quenching of silicon nanoparticles in phospholipid vesicle bilayers. *J Photochem Photobiol A Chem* 158:111–117.
88. Tsao N, Kanakamma PP, Luh TY, Chou CK, Lei HY. 1999. Inhibition of *Escherichia coli*-induced meningitis by carboxyfullerence. *Antimicrob Agents Chemother* 43:2273–2277.
89. Hwang ET, Lee JH, Chae YJ, Kim BC, Sang B-I, Gu MB. 2007. Analysis of nanoparticles' toxic modes of actions by using recombinant bioluminescent bacteria. *Abstracts*, American Institute of Chemical Engineers Meeting, Salt Lake City, UT, USA, November 4–9, 60e.
90. Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microb* 3: 3–8.
91. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. 2003. Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat Biotechnol* 21:47–51.
92. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. 2005. Quantum dot bioconjugates for imaging, labeling and sensing. *Nature Materials* 4:435–446.
93. Jaiswal JK, Goldman ER, Mattoussi H, Simon SM. 2004. Use of quantum dots for live cell imaging. *Nature Methods* 1:73–78.
94. Antiochia R, Lavagnini I. 2006. Alcohol biosensor based on the immobilization of meldola blue and alcohol dehydrogenase into a carbon nanotube paste electrode. *Anal Lett* 39:1643–1655.
95. Tsai YC, Chen SY, Liaw HW. 2007. Immobilization of lactate dehydrogenase within multiwalled carbon nanotube-chitosan nanocomposite for application to lactate biosensors. *Sensors and Actuators B: Chemical* 125:474–481.
96. Liu JQ, Paddon-Row MN, Gooding JJ. 2004. Heterogeneous electron-transfer kinetics for flavin adenine dinucleotide and ferrocene through alkanethiol mixed monolayers on gold electrodes. *J Phys Chem B* 108:8460–8466.
97. Imlay JA. 2003. Pathways of oxidative damage. *Annu Rev Microbiol* 57:395–418.
98. Dyadyusha L, Yin H, Jaiswal S, Brown T, Baumberg JJ, Booy FP, Melvin T. 2005. Quenching of CdSe quantum dot emission, a new approach for biosensing. *Chem Commun* 25:3201–3203.
99. Sapsford KE, Pons T, Medintz IL, Mattoussi H. 2006. Biosensing with luminescent semiconductor quantum dots. *Sensors* 6:925–953.
100. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. 2002. In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science* 298:1759–1762.
101. Xiang JJ, Tang JQ, Zhu SG, Nie XM, Lu HB, Shen SR, Li XL, Tang K, Zhou M, Li GY. 2003. IONP-PLL: A novel non-viral vector for efficient gene delivery. *Journal of General Medicine* 5:803–817.
102. Wang SZ, Gao RM, Zhou FM, Selke M. 2004. Nanomaterials and singlet oxygen photosensitizers: Potential applications in photodynamic therapy. *Journal of Material Chemistry* 14:487–493.
103. Takenaka S, Yamashita K, Takagi M, Hatta T, Tanaka A, Tsuge O. 1999. Study of the DNA interaction with water-soluble cationic fullerene derivatives. *Chemistry Letters* 28:319–320.
104. Zhao X, Striolo A, Cummings PT. 2005. C_{60} binds to and deforms nucleotides. *Biophys J* 89:3856–3862.
105. Green M, Howman E. 2005. Semiconductor quantum dots and free radical induced DNA nicking. *Chem Commun* 1:121–123.
106. Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 3:3–8.
107. Wakefield G, Green M, Lipscomb S, Flutter B. 2004. Modified titania nanomaterials for sunscreen applications—Reducing free radical generation and DNA damage. *Mater Sci Technol* 20: 985–988.
108. Takenaka S, Yamashita K, Takagi M, Hatta T, Tsuge O. 1999. Photo-induced DNA cleavage by water-soluble cationic fullerene derivatives. *Chemistry Letters* 4:321–322.
109. Sera N, Tokiwa H, Miyata N. 1996. Mutagenicity of the fullerene C_{60}-generated singlet oxygen dependent formation of lipid peroxides. *Carcinogenesis* 17:2163–2169.
110. Babynin EV, Nuretdinov IA, Gubskaia VP, Barabanshchikov BI. 2002. Study of mutagenic activity of fullerene and some of its derivatives using His+ reversions of *Salmonella typhimurium* as an example. *Russian Journal of Genetics* 38:453–457.
111. Dhawan A, Taurozzi JS, Pandey AK, Shan WQ, Miller SM, Hashsham SA, Tarabara VV. 2006. Stable colloidal dispersions of C-60 fullerenes in water: Evidence for genotoxicity. *Environ Sci Technol* 40:7394–7401.
112. Borm PJA, Schins RPF, Albrecht C. 2004. Inhaled particles and lung cancer, Part B, Paradigms and risk assessment. *Int J Cancer* 110:3–14.
113. Morfeld P, Albrecht C, Drommer W, Borm P. 2006. Dose-response and threshold analysis of tumour prevalence after intratracheal instillation of six types of low and high surface area particles in a chronic rat experiment. *Inhalation Toxicology* 118: 215–225.

114. Lyon DY, Brunet L, Hinkal GW, Wiesner MR, Alvarez PJJ. 2008. Antibacterial activity of fullerene water suspensions (nC_{60}) is not due to ROS-mediated damage. *Nano Letters* 8:1539–1543.
115. Worle-Knirsch JM, Pulskamp K, Krug HF. 2006. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Letters* 6:1261–1268.
116. Mashino T, Okuda K, Hirota T, Hirobe M, Nagano T, Mochizuki M. 1999. Inhibition of *E. coli* growth by fullerene derivatives and inhibition mechanism. *Bioorg Med Chem Lett* 9:2959–2962.
117. Mashino T, Nishikawa D, Takahashi K, Usui N, Yamori T, Seki M, Endo T, Mochizuki M. 2003. Antibacterial and antiproliferative activity of cationic fullerene derivatives. *Bioorg Med Chem Lett* 13:4395–4397.
118. Thill A, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM. 2006. Cytotoxicity of CeO_2 nanoparticles for *Escherichia coli*: A physico-chemical insight of the cytotoxicity mechanism. *Environ Sci Technol* 40:6151–6156.
119. Dabbousi BO, RodriguezViejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R, Jensen KF, Bawendi MG. 1997. (CdSe) ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. *J Phys Chem B* 101:9463–9475.
120. Kim S, Fisher B, Eisler HJ, Bawendi M. 2003. Type-II quantum dots: CdTe/CdSe (core/shell) and CdSe/ZnTe (core/shell) heterostructures. *J Am Chem Soc* 125:11466–11467.
121. Hoshino A, Fujioka K, Oku T, Suga M, Sasaki YF, Ohta T, Yasuhara M, Suzuki K, Yamamoto K. 2004. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano Letters* 4:2163–2169.
122. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. 2003. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol* 69:4278–4281.
123. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 52:662–668.
124. Yang L, Watts DJ. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol Lett* 158:122–132.
125. Murashov V. 2006. Comments on ''Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles.'' *Toxicol Lett* 164:185–187.
126. Fortner JD, Lyon DY, Sayes CM, Boyd AM, Falkner JC, Hotze EM, Alemany LB, Tao YJ, Guo W, Ausman KD, Colvin VL, Hughes JB. 2005. C_{60} in water: Nanocrystal formation and microbial response. *Environ Sci Technol* 39:4307–4316.
127. Brant JA, Labille J, Bottero J-Y, Wiesner MR. 2006. Characterizing the impact of preparation method on fullerene cluster structure and chemistry. *Langmuir* 22:3878–3885.
128. Brant J, Lecoanet H, Hotze M, Wiesner M. 2005. Comparison of electrokinetic properties of colloidal fullerenes (n-C_{60}) formed using two procedures. *Environ Sci Technol* 39:6343–6351.
129. Andrievsky GV, Kosevich MV, Vovk OM, Shelkovsky VS, Vashenko LA. 1995. On the production of an aqueous colloidal solution of fullerenes. *J Chem Soc Chem Commun* 12:1281–1282.
130. Oberdörster E, Zhu S, Blickley TM, McClellan-Green P, Haasch ML. 2006. Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerene (C_{60}) on aquatic organisms. *Carbon* 44:1112–1120.
131. Oberdörster E. 2004. Manufactured nanomaterials (Fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth Bass. *Environ Health Perspect* 112:1058–1062.
132. Lovern SB, Klaper RD. 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C_{60}) nanoparticles. *Environ Toxicol Chem* 25:1132–1137.
133. Zhu Y, Zhao Q, Li Y, Cal X, Li W. 2006. The interaction and toxicity of multi-walled carbon nanotubes with *Stylonychia mytilus*. *Journal of Nanoscience and Nanotechnology* 6:1357–1364.
134. Henry TB, Menn F-M, Fleming JT, Wilgus J, Compton RN, Sayler G. 2007. Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. *Environ Health Perspect* 115:1059–1065.
135. Lyon DY, Adams LK, Falkner JC, Alvarez PJJ. 2006. Antibacterial activity of fullerene water suspensions: Effects of preparation method and particle size. *Environ Sci Technol* 40:4360–4366.
136. Lyon DY, Thill A, Rose J, Alvarez PJJ. 2007. Ecotoxicological impacts of nanomaterials. In Wiesner MR, Bottero J-Y, eds, *Environmental Nanotechnology: Applications and Impacts of Nanomaterials.* McGraw-Hill, New York, NY, USA, pp 445–480.
137. Markovic Z, Todorovic-Markovic B, Kleut D, Nikolic N, Vranjes-Djuric S, Misirkic M, Vucicevic L, Janjetovic K, Isakovic A, Harhaji L, Babic-Stojic B, Dramicanin M, Trajkovic V. 2007. The mechanism of cell-damaging reactive oxygen generation by colloidal fullerenes. *Biomaterials* 28:5437–5448.
138. Sayes CM, Fortner JD, Guo W, Lyon D, Boyd AM, Ausman KD, Tao YJ, Sitharaman B, Wilson LJ, Hughes JB. 2004. The differential cytotoxicity of water-soluble fullerenes. *Nano Letters* 4:1881–1887.
139. Jennings VLK, Rayner-Brandes MH, Bird DJ. 2001. Assessing chemical toxicity with the bioluminescent photobacterium (*Vibrio fischeri*): A comparison of three commercial systems. *Water Res* 35:3448–3456.
140. Steinberg SM, Poziomek EJ, Engelmann WH, Rogers KR. 1995. A review of environmental applications of bioluminescence measurements. *Chemosphere* 30:2155–2197.
141. Ames B, Maron D. 1983. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* 113:173–215.
142. Ames B, McCann J, Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat Res* 31:347–364.
143. Madill REA, Brownlee BG, Josephy PD, Bunce NJ. 1999. Comparison of the Ames Salmonella assay and Mutatox genotoxicity assay for assessing the mutagenicity of polycyclic aromatic compounds in porewater from Athabasca oil sands mature fine tailings. *Environ Sci Technol* 33:2510–2516.
144. Sun TSC, Stahr HM. 1993. Evaluation and application of a bioluminescent bacterial genotoxicity test. *J AOAC Int* 76:893–898.
145. Eggink A, Fiser J, Terekhov A, Turco R, Appelgate B. 2005. The effect of carbon nanoparticles on the infectivity of bacteriophage using a T4 phage-based/bioluminescent *Escherichia coli* assay. *Abstracts*, American Society for Microbiology, Atlanta, GA, USA, June 5–9, p 516.
146. Lyon DY, Fortner JD, Sayes CM, Colvin VL, Hughes JB. 2005. Bacterial cell association and antimicrobial activity of a C-60 water suspension. *Environ Toxicol Chem* 24:2757–2762.
147. Mori T, Takada H, Ito S, Matsubayashi K, Miwa N, Sawaguchi T. 2006. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. *Toxicology* 225:48–54.
148. Jamil K. 2001. *Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment.* Science, Enfield, NH, USA.
149. Bitton G, Koopman B, Agami O. 1992. MetPAD: A bioassay for rapid assessment of heavy metal toxicity in wastewater. *Water Environ Res* 64:834–836.
150. Juvonen R, Martikainen E, Schultz E, Joutti A, Ahtiainen J, Lehtokari M. 2000. A battery of toxicity tests as indicators of decontamination in composting oily wastes. *Ecotoxicol Environ Saf* 47:156–166.
151. Knobeloch LM, Blondin GA, Lyford SB, Harkin JM. 1990. A rapid bioassay for chemicals that induce pro-oxidant states. *J Appl Toxicol* 10:1–5.
152. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. 2003. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol* 69:4278–4281.
153. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* 16:2346–2353.
154. Sondi I, Salopek-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interf Sci* 275:177–182.
155. Wolfrum EJ, Huang J, Blake DM, Maness PC, Huang Z, Fiest J, Jacoby WA. 2002. Photocatalytic oxidation of bacteria, bacterial and fungal spores, and model biofilm components to carbon dioxide on titanium dioxide-coated surfaces. *Environ Sci Technol* 36:3412–3419.
156. Bosetti M, Masse A, Tobin E, Cannas M. 2002. Silver coated materials for external fixation devices: In vitro biocompatibility and genotoxicity. *Biomaterials* 23:887–892.

157. Chou WL, Yu DG, Yang MC. 2005. The preparation and characterization of silver-loading cellulose acetate hollow fiber membrane for water treatment. *Polymers for Advanced Technology* 16:600–607.
158. Kwak SY, Kim SH, Kim SS. 2001. Hybrid organic/inorganic reverse osmosis (RO) membrane for bactericidal anti-fouling. 1. Preparation and characterization of TiO_2 nanoparticle self-assembled aromatic polyamide thin-film-composite (TFC) membrane. *Environ Sci Technol* 35:2388–2394.
159. Marchesan S, Da Ros T, Spalluto G, Balzarini J, Prato M. 2005. Anti-HIV properties of cationic fullerene derivatives. *Bioorg Med Chem Lett* 15:3615–3618.
160. Piotrovsky L, Dumpis M, Poznyakova L, Kiselev O, Kozeletskaya K, Eropkin M, Monaenkov A. 2000. Study of the biological activity of the adducts of fullerenes with poly(N-vinylpyrrolidone). *Molecular Materials* 13:41–50.
161. Schinazi R, Sijbesma R, Srdanov G, Hill C, Wudl F. 1993. Synthesis and virucidal activity of a water-soluble, configurationally stable, derivatized C_{60} fullerene. *Antimicrob Agents Chemother* 37:1707–1710.
162. Liu JQ, Paddon-Row MN, Gooding JJ. 2004. Heterogeneous electron-transfer kinetics for flavin adenine dinucleotide and ferrocene through alkanethiol mixed monolayers on gold electrodes. *J Phys Chem B* 108:8460–8466.
163. Patolsky F, Tao G, Katz E, Willner I. 1998. C_{60}-mediated bioelectrocatalyzed oxidation of glucose with glucose oxidase. *Journal of Electroanalytical Chemistry* 454:9–13.
164. Antiochia R, Lavagnini I. 2006. Alcohol biosensor based on the immobilization of meldola blue and alcohol dehydrogenase into a carbon nanotube paste electrode. *Anal Lett* 39:1643–1655.
165. Tsai YC, Chen SY, Liaw HW. 2007. Immobilization of lactate dehydrogenase within multiwalled carbon nanotube-chitosan nanocomposite for application to lactate biosensors. *Sensors and Actuators B: Chemical* 125:474–481.
166. Adams LK, Lyon DY, Alvarez PJJ. 2006. Comparative ecotoxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. *Water Res* 40:3527–3532.
167. Hund-Rinke K, Simon M. 2006. Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and Daphnids. *Environ Sci Pollut Res Int* 13:225–232.
168. Franklin NM, Rogers NJ, Apte SC, Batley G, Gadd GE, Casey PS. 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. *Environ Sci Technol* 41:8484–8490.
169. Stone V, Kinloch I, Clift M, Fernandes TF, Ford A, Christofi N, Griffiths A, Donaldson K. 2007. Nanoparticle toxicology and ecotoxicology: The role of oxidative stress. In Zhao Y, Nalwa HS, eds, *Nanotoxicology*. American Scientific, Los Angeles, CA, USA, pp 281–296.
170. Lovern SB, Strickler JR, Klaper RD. 2007. Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C_{60} and $C_{60}HxC_{70}Hx$). *Environ Sci Technol* 41:4465–4470.
171. Gagné F, Auclair J, Turcotte P, Fournier M, Gagnona C, Sauve S, Blaise C. 2008. Ecotoxicity of CdTe quantum dots to freshwater mussels: Impacts on immune system, oxidative stress and genotoxicity. *Aquat Toxicol* 86:333–340.
172. Oberdörster E, Larkin P, Rogers J. 2006. Rapid environmental impact screening for engineered nanomaterials: A case study using microarray technology. Project on Emerging Technologies. Woodrow Wilson International Centre for Scholars and the Pew Charitable Trusts, Washington DC.
173. Kashiwada S. 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). *Environ Health Perspect* 114: 1697–1702.
174. Zhu X, Zhu L, Li Y, Duan Z, Chen W, Alvarez P. 2007. Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials: Buckminsterfullerene aggregates (nC_{60}) and fullerol. *Environ Toxicol Chem* 26:976–979.
175. Smith C, Shaw BJ, Handy RH. 2007. Toxicity of single walled carbon nanotubes on rainbow trout (*Oncorhyncos mykiss*): Respiratory toxicity, organ pathologies, and other physiological effects. *Aquat Toxicol* 84:415–430.
176. Cheng J, Flahaut E, Cheng SH. 2007. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. *Environ Toxicol Chem* 26:708–716.
177. Federici G, Shaw BJ, Handy RH. 2007. Toxicity of titanium dioxide to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. *Aquat Toxicol* 84:415–430.
178. Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, Barber DS. 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ Sci Technol* 41:8178–8186.
179. Lee KL, Nallathamby PD, Browning LM, Osgood CJ, Xu X-HN. 2007. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano* 1:133–143.
180. Fu J, Cong Y, Gao J, Yu X, Pan C, Li B, Han Y. 2007. Photoluminescent nanoparticles of organic-inorganic hybrids prepared by phase transfer complexation at the organic-aqueous solution interface. *Nanotechnology* 18:025704–025712.
181. Moore MN. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ Int* 32: 967–976.
182. Guo LD, Santschi PH, Ray SM. 2002. Metal partitioning between colloidal and dissolved phases and its relation with bioavailability to American oysters. *Mar Environ Res* 54:49–64.
183. Scarano G, Morelli E. 2003. Properties of phytochelatin-coated CdS nanocrystallites formed in a marine phytoplanktonic alga (*Phaeodactylum tricornutum*, Bohlin) in response to Cd. *Plant Sci* 165:803–810.
184. Barka S. 2007. Insoluble detoxification of trace metals in a marine copepod *Tigriopus brevicornis* (Muller) exposed to copper, zinc, nickel, cadmium, silver, and mercury. *Ecotoxicology* 16: 491–502.
185. Templeton RC, Ferguson PL, Washburn KM, Scrivens WA, Chandler GT. 2006. Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. *Environ Sci Technol* 40:7387–7393.
186. Nielsen HD, Berry LS, Stone V, Burridge TR, Fernandes TF. 2008. Interactions between carbon black nanoparticles and the brown algae *Fucus serratus*: Inhibition of fertilization and zygotic development. *Nanotechnology* 2:88–97.
187. Ziolli RL, Jardim WF. 2002. Photocatalytic decomposition of seawater-soluble crude-oil fractions using high surface area colloid nanoparticles of TiO_2. *J Photochem Photobiol A Chem* 147: 205–212.
188. Von Der Kammer F, Forstner U. 1998. Natural colloid characterization using flow-field-flow-fractionation followed by multidetector analysis. *Water Sci Technol* 37:173–180.
189. Hassellöv M, Lyvén BR. 1999. Sedimentation field-flow fractionation coupled online to inductively coupled plasma mass spectrometry—New possibilities for studies of trace metal adsorption onto natural colloids. *Environ Sci Technol* 33:4528–4531.
190. Burleson DJ, Driessen MD, Penn RL. 2004. On the characterisation of environmental nanoparticles. *J Environ Sci Health Part A Toxic Hazard Subst Environ Eng* 39:2707–2753.
191. Gimbert LJ, Haygarth PM, Beckett R, Worsfold PJ. 2005. Comparison of centrifugation and filitration techniques for the size fractionation of colloidal material in soil suspensions using sedimentation field-flow fractionation. *Environ Sci Technol* 39: 1731–1735.
192. Lahoussineturcaud V, Wiesner MR, Bottero JY. 1990. Fouling in tangential-flow ultrafiltration—The effect of colloid size and coagulation pretreatment. *J Membr Sci* 52:173–190.
193. Cameron FK. 1915. Soil colloids and the soil solution. *J Phys Chem* 19:1–13.
194. Quirk JP, Schofield RK. 1955. The effect of electrolyte concentration on soil permeability. *J Soil Sci* 6:163–178.
195. Zhang H, Selim HM. 2007. Colloid mobilization and arsenite transport in soil columns: Effect of ionic strength. *J Environ Qual* 36:1273–1280.
196. Gerke J, Jungk A. 1991. Separation of phosphorus bound to organic matrices from inorganic phosphorus in alkaline soil extracts by ultrafiltration. *Commun Soil Sci Plant Anal* 22:1621–1630.
197. Noack AG, Grant CD, Chittleborough DJ. 2000. Colloid movement through stable soils of low cation-exchange capacity. *Environ Sci Technol* 34:2490–2497.

198. Karathanasis AD. 1999. Subsurface migration of copper and zinc mediated by soil colloids. *Soil Sci Soc Am J* 63:830–838.
199. Kretzschmar R, Borkovec M, Grolimund D, Elimelech M. 1999. Mobile subsurface colloids and their role in contaminant transport. *Adv Agron* 66:121–193.
200. Noack AG, Grant CD, Chittleborough DJ. 2000. Colloid movement through stable soils of low cation-exchange capacity. *Environ Sci Technol* 34:2490–2497.
201. Gimbert LJ, Haygarth PM, Beckett R, Worsfold PJ. 2006. The influence of sample preparation on observed particle size distributions for contrasting soil suspensions using flow field-flow fractionation. *Environ Chem* 3:184–191.
202. Gimbert LJ, Hamon RE, Casey PS, Worsfold PJ. 2007. Partitioning and stability of engineered ZnO nanoparticles in soil suspensions using field-flow fractionation. *Environ Chem* 4:8–10.
203. Gulson B, Wong H. 2006. Stable isotope tracing—A way forward in nanotechnology. *Environ Health Perspect* 114:1486–1488.
204. Kretzschmar R, Robarge WP, Amoozegar A. 1995. Influence of natural organic-matter on colloid transport through saprolite. *Water Resour Res* 31:435–445.
205. Seaman JC, Bertsch PM. 2000. Selective colloid mobilization through surface-charge manipulation. *Environ Sci Technol* 34: 3749–3755.
206. Hydutsky BW, Mack EJ, Beckerman BB, Skluzacek JM, Mallouk TE. 2007. Optimization of nano- and microiron transport through sand columns using polyelectrolyte mixtures. *Environ Sci Technol* 41:6418–6424.
207. Lecoanet HF, Bottero J, Wiesner MR. 2004. Laboratory assessment of the mobility of nanomaterials in porous media. *Environ Sci Technol* 38:5164–5169.
208. Korcak RF, Fanning DS. 1985. Availability of applied heavy metals as a function of type of soil material and metal source. *Soil Sci* 140:23–34.
209. Smolders E, Degryse F. 2002. Fate and effect of zinc from tire debris in soil. *Environ Sci Technol* 36:3706–3710.
210. Voegelin A, Pfister S, Scheinost AC, Marcus MA, Kretzschmar R. 2005. Changes in zinc speciation in field soil after contamination with zinc oxide. *Environ Sci Technol* 39:6616–6623.
211. Borm P, Klaessig FC, Landry TD, Moudgil B, Pauluhn J, Thomas K, Trottier R, Wood S. 2006. Research strategies for safety evaluation of nanomaterials. Part V: Role of dissolution in biological fate and effects of nanoscale particles. *Toxicol Sci* 90: 23–32.
212. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS. 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. *Environ Sci Technol* 41:8484–8490.
213. U.S. Environmental Protection Agency 2008. Draft nanomaterial research strategy. EPA/600/S-08/002. Washington, DC.
214. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti M, Fiévet F. 2006. Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters* 6:866–870.
215. Lin D, Xing B. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environ Pollut* 150:243–250.
216. Tong ZH, Bischoff M, Nies L, Applegate B, Turco RF. 2007. Impact of fullerene (C-60) on a soil microbial community. *Environ Sci Technol* 41:2985–2991.
217. Johansen A, Pedersen AL, Karlson U, Hansen BJ, Scott-Fordsmand JJ, Winding A. 2008. Effects of C_{60} fullerene nanoparticles on soil bacteria and protozoans. *Environ Toxicol Chem* 27:1895–1903.
218. Yang L, Watts DJ. 2005. Particle surface characteristics may play an important role in phytotoxicity of aluminia nanoparticles. *Toxicol Lett* 158:122–132.
219. Zheng L, Hong F, Lu S, Liu C. 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. *Biol Trace Elem Res* 104:83–91.
220. Murashov V. 2006. Letter to the editor: Comments on ''Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles'' by Yang L, Watts, DJ, *Toxicol Lett 2005:158, 122–132. Toxicol Lett* 164:185–187.
221. Magistadm OC. 1925. The aluminium content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci* 20: 181–211.
222. Lindsay WL. 1979. *Chemical Equilibria in Soils.* John Wiley, New York, NY, USA.
223. Rozhkov SP, Goryunov AS, Sukhanova GA, Borisova AG, Rozhkova NN, Andrievsky GV. 2003. Protein interaction with hydrated C(60) fullerene in aqueous solutions. *Biochem Biophys Res Commun* 303:562–566.
224. Mashino T, Okuda K, Hirota T, Hirobe M, Nagano T, Mochizuki M. 1999. Inhibition of *E. coli* growth by fullerene derivatives and inhibition mechanism. *Bioorg Med Chem Lett* 9:2959–2962.
225. Tsao N, Kanakamma PP, Luh TY, Chou CK, Lei HY. 1999. Inhibition of *Escherichia coli*-induced meningitis by carboxyfullerence. *Antimicrob Agents Chemother* 43:2273–2277.
226. Tsao N, Luh T, Chou C, Wu J, Lin Y, Lei K. 2001. Inhibition of group A streptococcus infection by carboxyfullerene. *Antimicrob Agents Chemother* 45:1788–1793.
227. Tsao N, Luh TY, Chou CK, Chang TY, Wu JJ, Liu CC, Lei HY. 2002. In vitro action of carboxyfullerene. *J Antimicrob Chemother* 49:641–649.
228. Mashino T, Nishikawa D, Takahashi K, Usui N, Yamori T, Seki M, Endo T, Mochizuki M. 2003. Antibacterial and antiproliferative activity of cationic fullerene derivatives. *Bioorg Med Chem Lett* 13:4395–4397.
229. Mashino T, Usui N, Okuda K, Hirota T, Mochizuki M. 2003. Respiratory chain inhibition by fullerene derivatives: Hydrogen peroxide production caused by fullerene derivatives and a respiratory chain system. *Bioorg Med Chem Lett* 11:1433–1438.
230. Babynin EV, Nuretdinov IA, Gubskaia VP, Barabanshchikov BI. 2002. Study of mutagenic activity of fullerene and some of its derivatives using His+ reversions of *Salmonella typhimurium* as an example. *Russian Journal of Genetics* 38:359–363.
231. Bagrii EI, Karaulove EN. 2001. New in fullerene chemistry (a review). *Petroleum Chemistry* 41:295–313.
232. Bosi S, Da Ros T, Castellano S, Banfi E, Prato M. 2000. Antimycobacterial activity of ionic fullerene derivatives. *Bioorg Med Chem Lett* 10:1043–1045.
233. Kang S, Pinault M, Pfefferle LD, Elimelech M. 2007. Single-walled carbon nanotubes exhibit strong antimicrobial activity. *Langmuir* 23:8670–8673.
234. Wei W, Sethuraman A, Jin C, Monteiro-Riviere NA, Narayan RJ. 2007. Biological properties of carbon nanotubes. *Journal of Nanoscience and Nanotechnology* 7:1284–1297.
235. Biswas P, Wu CY. 2005. Critical review: Nanoparticles and the environment. *J Air Waste Manag* 55:708–746.
236. Kloepfer JA, Mielke RE, Nadeau JL. 2005. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. *Appl Environ Microbiol* 71:2548–2557.
237. Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, Gaub HE, Stolzle S, Fertig N, Parak WJ. 2005. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Letters* 5:331–338.
238. Hardman R. 2006. A toxicologic review of quantum dots: Toxicity depends on physicochemical and environmental factors. *Environ Health Perspect* 114:165–172.
239. Sondi I, Salopek-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interf Sci* 275:177–182.
240. Nyberg L, Turco RF, Nies L. 2008. Assessing the impact of nanomaterials on anaerobic microbial communities. *Environ Sci Technol* 42:1938–1943.
241. Zharov VP, Mercer KE, Galitovskaya EN, Smeltzer MS. 2006. Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. *Biophys J* 90:619–627.
242. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. 2004. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjug Chem* 15:897–900.
243. De Windt W, Boon N, Van den Bulcke J, Rubberecht L, Prata F, Mast J, Hennebel T, Verstraete W. 2006. Biological control of the size and reactivity of catalytic Pd(0) produced by Shewanella oneidensis. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology* 90:377–389.
244. Rincon A, Pulgarin C. 2004. Effect of pH, inorganic ions, organic matter and H_2O_2 on *E. coli* K12 photocatalytic inactivation by TiO_2 Implications in solar water disinfection. *Appl Catal B: Environ* 51:283–302.
245. Rincon A, Pulgarin C. 2004. Bactericidal action of illuminated

TiO_2 on pure *Escherichia coli* and natural bacterial consortia: Post-irradiation events in the dark and assessment of the effective disinfection time. *Appl Catal B: Environ* 49:99–112.
246. Wolfrum EJ, Huang J, Blake DM, Maness PC, Huang Z, Fiest J, Jacoby WA. 2002. Photocatalytic oxidation of bacteria, bacterial and fungal spores, and model biofilm components to carbon dioxide on titanium dioxide-coated surfaces. *Environ Sci Technol* 36:3412–3419.
247. Huang L, Li DQ, Lin YJ, Wei M, Evans DG, Duan X. 2005. Controllable preparation of Nano-MgO and investigation of its bactericidal properties. *J Inorg Biochem* 99:986–993.
248. Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M. 1995. Effect of ceramic powder slurry on spores of *Bacillus subtilis*. *Journal of Chemical Engineering, Japan* 28:556–561.
249. Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M. 1996. Effect of particle size and heating temperature of ceramic powders on antibacterial activity of their slurries. *Journal of Chemical Engineering, Japan* 29:251–256.