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Viewpoint

Decreasing Uncertainties in Assessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials[†,‡]

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Determining the fate and interactions of nanomaterials in complex environmental contexts is required to assess exposure and possible harm as well as to inform regulation.

As the nanotechnology industry moves up into the rarified air of trillion dollar economics over the next several years (*1*), the number of simple and complex manufactured nanomaterials (NMs), and their uses, will grow tremendously. Large-scale production of engineered NMs presents the possibility that organisms and ecosystems may be exposed to new levels and qualities of substances with unknown consequences. Naturally occurring nanoscale materials are also ubiquitous in the biosphere, comprising the very building blocks of life and likely playing an important role in ecosystem

dynamics. Our evolutionary history includes millennia of adaptation and coexistence with these naturally occurring nanoscale materials. Managing risks associated with NM exposure will require the ability to quantify and differentiate the relative importance of manufactured, natural, and incidental sources of NMs at each step of the material's life cycle and understand the processes that govern NM transport, persistence, bioavailability, and toxicity.

In this Viewpoint, we highlight research issues being addressed in the Center for the Environmental Implications of NanoTechnology (CEINT) whose overarching objective is to develop the tools necessary to identify and mitigate the negative impacts of manufactured NMs in the environment. This research agenda is premised on the need to understand the hazards of NMs (e.g., toxicity, mutagenicity, impacts on ecosystem services) and underlying mechanisms as a basis for focusing study of the processes controlling exposure.

This premise has important implications for the determination of research priorities. While the goal of engineering out any *hazard* that NMs may present is not without merit, the properties of a material that produce the hazard may be closely related to those that make a given material useful in a specific application. A risk management strategy rooted in a fundamental understanding of the possible pathways of exposure to NMs leads to a broad array of options for managing risk: protective devices for workers in NM fabrication industries; standards for product disposal or recycling; the use of pollution reduction equipment; changes in human behavior; and, in extreme cases, an outright ban on the production of a given NM.

Research efforts in CEINT are structured to identify hazards and elucidate the general principles that determine NM behavior from the nanoscale to ecosystems. These efforts are bound together by a framework for risk assessment needed to inform policy and provide guidance in managing

existing and future concerns surrounding NMs. In the following sections, we discuss CEINT research organized around five areas of uncertainty regarding the environmental implications of NMs.

1. What Nanomaterial Properties and Environmental Conditions Control Their Spatial and Temporal Distribution in the Environment?

NMs may include individual nanoparticles (NPs), NP composites, macroscopic objects composed of NPs such as thin films, and many other objects composed of materials with the requisite characteristics of having at least one dimension of $1-100$ nm and displaying novel properties. Our focus here is on NMs that may serve as sources of NPs in the environment. Because of their size, NPs are practically "all" surface. Without a theoretical grounding in NP surface chemistry, it is impossible to predict their environmental transport and fate. Predicting exposure to manufactured NMs and the relevant exposure concentration begins with an assessment of the environmental availability of manufactured NMs throughout their life-cycle. This will largely be determined by the engineered matrices in which these materials are found, such as composites in intermediate or finished consumer goods (e.g., carbon nanotubes [CNTs] in tennis rackets, TiO_2 and nanosilver coatings, or ZnO in water/organic creams). Once released, modifications to NP surfaces and coatings—engineered *or* unintentional—will likely alter exposure vector(s) and hazard(s) through impacts on surface-chemistry-related factors such as mobility, persistence, reactivity, bioavailability, and biocompatibility $(2-4)$.

Nanomaterial Sources and Matrix Effects on Availability. Methods and protocols are needed to evaluate the potential release of NPs embedded in such intermediate and finished products which are likely very different from pristine NPs. Developing a rigorous methodology for characterizing NPs' release from NM-containing products is an important challenge, magnified by the diversity of products and uncertainty surrounding fabrication, use, and disposal.

Effects of Nanoparticle Chemistry on Aggregation and Deposition. Conventional molecular descriptors used to predict the distribution of dissolved organic pollutants in the environment (e.g., the octanol−water partition coefficient, saturation vapor pressure, and aqueous solubility) have questionable meaning for NPs. This is especially true for aggregates of NPs which are on the order of tens to hundreds of nanometers and behave like distinct particles in aqueous systems $(5, 6)$. Colloid science has taught us that particle surface chemistry controls both aggregation and deposition, and that these processes are likely to have a profound effect on the transport and availability of NPs in the aqueous environment $(7-10)$. Further, NP aggregate structure is likely to vary based on physical and chemical processes that control aggregation kinetics which evidently play a critical role in determining NM reactivity (11). Aggregate effects represent a clear departure from the behavior of conventional chemical compounds implying a need for new risk assessment protocols.

Biological and Chemical Transformations of Nanomaterials. NMs may undergo a wide range of weathering or "aging" processes that will alter their surface chemistry and, therefore, transport and potential exposure routes. These transformations occur through processes such as redox reactions, interactions with organic macromolecules such as natural organic matter (NOM) or cellular material, dissolution, or adsorption of known pollutants (e.g., As, Hg, PCBs, PAHs). In air, manufactured and incidental NPs might be expected to condense low volatility compounds (organics and sulfate) (12). Photocatalytic NPs (e.g., TiO_2) might in turn photooxidize the condensed material. Interactions between NPs and organic macromolecules $(13-16)$ such as NOM, proteins, surfactants, and polyelectrolytes modify aggregation and deposition kinetics and therefore transport and potential exposure routes. Environmental and physiological conditions also modify reactivity of NMs as evidenced by changes in the toxicity of manufactured $(17, 18)$, natural (19), and incidental NPs (20), the generation of reactive oxygen species (ROS) $(21, 22)$, and reduced redox activity $(9, 23)$. Additionally, other chemical species that NMs interact with may themselves be altered in the process. For example, NPs may alter the conformation of proteins $(24, 25)$ interfering with cell signaling and possibly gene transcription.

Just as NPs may impact organisms, organisms may impact NPs. The accessibility of adsorbed or chemically bound macromolecules that stabilize NPs against aggregation may also prevent biological attack and increase NP persistence. Alternatively, bacteria may enhance the solubility of iron oxide NPs (26) or enhance the bioavailability of adsorbed heavy metals such as mercury (Hg) (27). Photolysis, oxidation, and subsequent dissolution (in the case of inorganic NPs) can release toxic metals into the environment (28), alter NP morphology (29) and other properties (30), and ultimately provide removal mechanisms for NPs in the environment.

2. Are There Fundamental Differences Between Natural, Incidental, and Manufactured Nanoparticles?

Naturally occurring NPs, in enormous varieties, are truly ubiquitous, distributed throughout the atmosphere, oceans, soil systems, terrestrial water systems (groundwater and surface water), and in and/or on most living organisms (31). They are even present in the deep Earth, and throughout the solar system and interplanetary space. Although the overall mass distribution of naturally occurring nanoscale particles is not known at this time, the oceans probably hold by far the largest reservoir. River, glacier, and windblown inputs to the world's oceans are constant and massive. As far as we know, naturally occurring nanoscale particles have been present throughout Earth history, and therefore life has evolved in their presence. However, aside from a few documented cases (e.g., magnetotactic bacteria (32)), the role that natural NMs have played in biological evolution is hardly understood.

Incidental NP inputs to the environment have risen dramatically since the beginning of the Industrial Revolution due to manufacturing emissions and the combustion of fossil fuels. While not widely appreciated, the distinctions between naturally occurring, incidental, and manufactured NPs are often blurred. For example, incidental CNTs and other fullerene-related nanocrystals have been reported to originate from propane stoves, wood fires, burning tires, and other sources $(33, 34)$, and fullerene C_{60} has been found in geologic deposits (35), candle soot, and meteorites (36). The bottom line is that releases of naturally occurring and incidental NPs into terrestrial and aquatic environments are large and, in the generic case, far more ubiquitous than releases of manufactured NMs. Do manufactured NMs with rare natural analogues have less predictable impacts than those with abundant natural analogues? Can the existence of natural or incidental analogues be useful in screening new manufactured NMs for potential environmental risk? How can this be quantified? (Figure 1). Can we distinguish among the properties of natural, incidental, and manufactured NPs, even if they are all the "same" material? An assessment of the risks posed by manufactured NMs should consider the relative exposures presented by all sources of NMs as well as the mechanisms that organisms have developed over time to "cohabitate" with them.
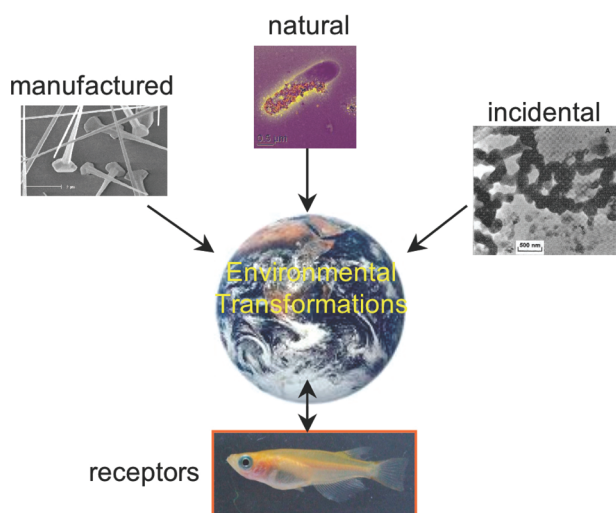
FIGURE 1. Sources of nanomaterials relevant to exposure assessment.

3. Are There True "Nano" Effects on Bioavailability, Toxicity, and Other Environmental End Points That Go Beyond the Effect of a Very High Specific Surface Area?

NPs are defined as having a characteristic length of $1-100$ nm for at least one dimension *and* having properties that differ from those of molecules or bulk materials of the same composition (*37-40*). Experiments performed with materials that are simply small versions of their bulk counterparts may not capture the truly "nano" dimensions of NP environmental behavior. Size-dependent behavior of NPs relating to physicochemical properties such as optical, electrical, and magnetic phenomena are well recognized, however the relationship between these physicochemical properties and specific biological end points is far from clear. For example, some studies with TiO_2 NPs have shown that once normalized by surface area, toxicity responses in vivo are similar regardless of size (*20*). This suggests that these nanoscale materials merely act as small versions of bulk TiO_2. In contrast, at least one study with Ag NPs has observed a "nano" effect with respect to surface-area normalized responses (*41*). Thus evaluations of NM impacts on organisms should be based on a consideration of the novel, size-dependent properties of these materials (perhaps defining *nano*toxicology).

Early life stage development generally comprises a period of elevated vulnerability to environmental stressors, including xenobiotics. Initial screens will serve as range-finding experiments where NMs are tested at many concentrations using toxicity, gross morphology, and for animals, gross behavior as end points. High sensitivity to low concentrations is an important attribute in testing the effects of materials such as NMs that may be present at trace levels. Developmental and toxicity screening methods developed by CEINT researchers use embryonic and larval stages that allow for the examination of specific tissue uptake and impacts on the reproductive, renal, and digestive systems, development of the cardiovascular system, and developmental changes in the nervous systems of medaka, killifish, and zebrafish, respectively (*42-44*). The medaka model is particularly powerful for assessing absorption, distribution, metabolism, and excretion (ADME) of NMs. A "see-through" strain of this species (*45*), in which the genes coding for skin pigmentation have been mutated, allows one to visually examine the internal organs in the adult fish noninvasively (*46*). This technique has been employed to elucidate tissue distribution of biotransformation enzymes and fluorescently tagged fullerenes (*47*).

The goal of studies such as these is ultimately to discover mechanisms that control NP interactions in living systems. While oxidative stress is often cited as one possible mechanism by which NMs may impact organisms at the cellular level (*48*), there are many other possible mechanisms that merit investigation including DNA damage, steroid hormone homeostasis, and protein folding.

Evolutionary/Multigenerational Responses. We speculate that exposure to new types or concentrations of NMs may have long-term, evolutionary influences on organisms. Multigenerational exposures to toxic NMs may alter reproductive strategies or exert fitness costs, as has been observed for other anthropogenic chemicals (*49*). Multispecies interactions such as competition and predator−prey interactions, or the ability of organisms to cope with other stressors, including natural stressors that previously shaped evolution might also be affected. Comparisons in reproductive strategies (*viz.* sexual/asexual) and quantification of changes in community structure is required. Genes that are altered in a heritable manner can be identified and tracked while changes in gene expression that may have population- or community-level consequences such as changes in fecundity require quantification over a range of NM exposures.

4. What Effects Might Nanomaterials Have On Critical Ecosystem Services?

Predicting the ecosystem impacts of NMs requires significant empirical progress in understanding (1) abiotic interactions between NMs and natural substrates and solutions; (2) interactions between NMs and an ecosystem's primary uptake compartments (plants, fungi, and bacteria) and the longevity and reversibility of ecological sources and sinks; and (3) the resulting consequences of NM exposure for productivity, organic matter decomposition, and trophic transfer. To date the existent and limited "lab-scale" research may be misleading—for example studies have found that C_{60} fullerenes are highly toxic to *E. coli* (*50*) or *B. subtilis* (*51*) in pure cultures, but a recent experiment that added C_{60} to natural soils found no effect of similar levels of C_{60} pollution on either microbial respiration or bacterial composition (*52*). These latter investigators hypothesized that the C_{60} fullerenes became bound to soil organic matter, thereby reducing their reactivity. While some classes of NMs may be toxic in the lab and inert in natural environments, it is also possible that other classes of NMs will become more lethal as a result of abiotic and biotic processing in complex ecosystems. It has been proposed that passage through the gut of organisms could strip stabilizing coatings off NMs, thus increasing their reactivity and toxicity (e.g., exposing the Cd-containing core of quantum dots). NMs may also have indirectly detrimental effects on ecosystems through their interactions with existing environmental contaminants. Facilitated transport has been observed with radionuclides on colloids (*53*) and "Trojan Horse" effects, wherein compounds that might not normally enter a cell are internalized when associated with a NP, are but two examples (*54, 55*) of indirect effects.

Ultimately we need a mechanistic understanding of the forms in which NPs are transported through and retained within ecosystem compartments. An ecosystem-scale conceptualization can then drive research choices to interrogate first-order impacts like determining simple environmental fate models and organismal effects of single NPs (see above section 3).

5. How Does One Do Risk Assessment On An Emerging Technology?

With all the potential complexity and nuances, what are the important issues for quantifying nanotechnology environmental risk? Risk assessment for NMs will depend critically

and sensitively on the issues and uncertainties identified in this paper. While risk assessment can serve as a powerful integrator of information gathered across a range of length scales and disciplines, the protean nature of NMs and their transformations, biological interactions, and environmental transport require us to rethink the existing risk assessment paradigms.

The first logical step of conventional risk assessment of a complex environment has been to identify the problem: the affected organism and the nature of the damage. For NMs, the nature of the hazard is one of the prominent unknowns. The second conventional step is to develop an accurate emissions inventory which brings additional uncertainty. Emissions estimates should include life cycle aspects of the particle: emissions from production (56) and waste generation (57), and emissions during storage, use, and disposal. Early efforts for NMs are estimated from production forecasts calculated using, for example, patent filings (58) and industry reports. Yet in the absence of measurements and no equivalent of a toxics release inventory, near-term emissions scenarios are going to remain highly speculative.

When faced with such uncertainties, simple models can still provide insight. Mass balance partitioning models have been used to predict NM concentrations in environmental compartments (59, 60). Such simple models are entirely appropriate for poorly characterized systems, but they offer limited guidance. Risk ranking methods have been proposed for situations in which urgent choice is needed in advance of NMs knowledge (61), but such insight has narrow application.

For (environmental) risk management, we need to build models to accommodate the relevant complexities and behaviors attributed to NPs in the environment. One possible format appropriate for dealing with uncertainty is the Bayesian belief network. These models can use subjective judgments when data are unavailable, and they can be readily updated, a necessity in a rapidly evolving field (62). Bayesian models are powerful aids in localizing the areas of uncertainty in estimating risk. These calculations provide feedback to the experimental program in determining where research efforts will be best invested to further reduce uncertainty. While it is likely that modeling and wet laboratory research will need to go through multiple iterations, as the research effort evolves, updated information concerning possible risks posed by NMs and their environmental consequences is provided. Given the urgency and opportunity for anticipating environmental problems of an emerging technology before they occur, CEINT is working all aspects of the problem simultaneously, in a coordinated manner, to advance the understanding of nanomaterials in the environment and provide guidance to assess the possible related risks.

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Acknowledgments

Literature Cited

(1) Roco, M. C.; Bainbridge, W. S. *Societal Implications of Nanoscience and Nanotechnology*; National Science Foundation: Arlington, VA, March 2001.

(2) Lowry, G. V.; Wiesner, M. R. Environmental considerations: Occurrences and fate of nanomaterials in the environment. In *Nanotoxicology: Characterization, Dosing, and Health Effects*; Monteiro-Riviere, N., Tran, C. L. , Eds.; Informa Health Care USA: New York, 2007; pp 369–390.

(3) Wiesner, M. R.; Lowry, G. V.; Alvarez, P. J. J.; Dionysiou, D.; Biswas, P. Assessing the risks of manufactured nanomaterials. *Environ. Sci. Technol.* **2006**, *40* (14), 4337–4445.

(4) Biswas, P.; Wu, C. Y. Nanoparticles and the environment, critical review paper. *J. Air Waste Manage. Assoc.* **2005**, *55*, 708–746.

(5) Lecoanet, H. F.; Bottero, J.-Y.; Wiesner, M. R. Laboratory assessment of the mobility of nanomaterials in porous media. *Environ. Sci. Technol.* **2004**, *38*, 5164–5169.

(6) Brant, J. A.; Lecoanet, H.; Wiesner, M. R. Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. *J. Nanopart. Res.* **2005**, *7*, 545–553.

(7) Brant, J. A.; Labille, J.; Robichaud, C. O.; Wiesner, M. R. Fullerol cluster formation in aqueous solutions: Implications for environmental release. *J. Colloid Interface Sci.* 2007.

(8) Espinasse, B.; Hotze, E. M.; Wiesner, M. R. Transport and retention of colloidal aggregates of C_{60} in porous media: Effects of organic macromolecules, ionic composition and preparation method. *Environ. Sci. Technol.* **2007**, *41* (21), 7396–7402.

(9) Phenrat, T.; Saleh, N.; Sirk, K.; Kim, H.; Matyjaszewski, K.; Tilton, R.; Lowry, G. V. Stabilization of aqueous nanoscale zerovalent iron dispersions by anionic polyelectrolytes: Adsorbed anionic polyelectrolyte layer properties and their effect on aggregation and sedimentation. *J Nanopart. Res.* **2008**, *10*, 795–814.

(10) Mackay, M. E.; Tuteja, A.; Duxbury, P. M.; Hawker, C. J.; Van Horn, B.; Guan, Z. B.; Chen, G. H.; Krishnan, R. S. General strategies for nanoparticle dispersion. *Science* **2006**, *311* (5768), 1740–1743.

(11) Hotze, E. M.; Labille, J.; Alvarez, P. J.; Wiesner, M. R. Mechanisms of photochemistry and reactive oxygen production by fullerene suspensions in water. *Environ. Sci. Technol.* **2008**, *42* (11), 4175–4180.

(12) Robinson, A. L.; Donahue, N. M.; Shrivastava, M. K.; Sage, A. M.; Weitkamp, E. A.; Greishop, A. P.; Lane, T. E.; Pierce, J. R.; Pandis, S. N. Rethinking organic aerosols: Semivolatile emissions and photochemical aging. *Science* **2007**, *315*, 1259–1263.

(13) Saleh, N.; Phenrat, T.; Sirk, K.; Dufour, B.; Ok, J.; Sarbu, T.; Matyjaszewski, K.; Tilton, R. D.; Lowry, G. V. Absorbed triblock copolymers deliver reactive iron nanoparticles to the oil/water interface. *Nano Lett.* **2005**, *5* (12), 2489–2494.

(14) Moreau, J. W.; Weber, P. K.; Martin, M. C.; Gilbert, B.; Hutcheon, I. D.; Banfield, J. F. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science* **2007**, *316*, 1600–1603.

(15) Singer, D. M.; Farges, F.; Brown, J. G. E. Biogenic uo2 - characterization and surface reactivity. *Am. Inst. Phys. Conf. Proc. 13th Int. XAFS Conf. 2007* **2007**, 277–279.

(16) Hardman, R. A toxicologic review of quantum dots: Toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.* **2006**, *114* (2), 165–72.

(17) Maynard, A.; Kuempel, E. D. Airborne nanostructured particles and occupational health. *J. Nanopart. Res.* **2005**, *7* (6), 587–614.

(18) Lovern, S. B.; Strickler, J. R.; Klaper, R. 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.* **2007**, *41* (12), 4465–4470.

(19) Jentzsch, T.; Penn, R. L. Influence of aluminum doping on ferrihydrite nanoparticle reactivity. *J. Phys. Chem.* **2006**, *110* (24), 11746–11750.

(20) Oberdorster, G.; Oberdorster, E.; Oberdorster, J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* **2005**, *113* (7), 823–39.

(21) Hotze, E. M.; Wiesner, M. R. Reactive oxygen species generated by aqueous suspensions of colloidal fullerene aggregates. *Abstr. Pap. Am. Chem. Soc.* **2005**, *230*, U1528–U1528.

(22) Lee, J.; Fortner, J. D.; Hughes, J. B.; Kim, J. H. Photochemical production of reactive oxygen species by C_{60} in the aqueous phase during UV irradiation. *Environ. Sci. Technol.* **2007**, *41* (7), 2529–2535.

(23) Phenrat, T.; Liu, Y.; Tilton, R. D.; Lowry, G. V. Adsorbed polyelectrolyte coatings decrease Fe^0 nanoparticle reactivity with TCE in water: Conceptual model and mechanisms. *Environ. Sci. Technol.* **2009**, *43* (5), 1507–1514.

(24) Chen, M.; von Mikecz, A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. *Exp. Cell Res.* **2005**, *305* (1), 51–62.

(25) Linse, S.; Cabaleiro-Lago, C.; Xue, W.; Lynch, I.; Lindman, S.; Thulin, E.; Radford, S. E.; Dawson, K. A. Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (21), 8691–8696.

(26) Ha, J.; Cordova, C. D.; Yoon, T. H.; Spormann, A. M.; Brown, G. E., Jr. Microbial reduction of hematite: Effects of particle size and exopolysaccharides. In *232nd ACS National Meeting, COLL-082, San Francisco, CA*; American Chemical Society: Washington, DC, 2006.

(27) Jew, A. D.; Rogers, S. B.; Rytuba, J. J.; Spormann, A. M.; Brown, J. G. E. Bacterially mediated breakdown of cinnabar and metacinnabar and environmental implications. In *American Geophysical Union Fall Meeting*, San Francisco, CA, 2006.

(28) Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett.* **2004**, *4* (1), 11–18.

(29) Jin, R. C.; Cao, Y. W.; Mirkin, C. A.; Kelly, K. L.; Schatz, G. C.; Zheng, J. G. Photoinduced conversion of silver nanospheres to nanoprisms. *Science* **2001**, *294* (5548), 1901–1903.

(30) Zhang, M.; Yudasaka, M.; Ajima, K.; Miyawaki, J.; Iijima, S. Light-assisted oxidation of single-wall carbon nanohorns for abundant creation of oxygenated groups that enable chemical modifications with proteins to enhance biocompatibility. *ACS Nano* **2007**, *1* (4), 265–272.

(31) Hochella, M. F. J.; Lower, S. K.; Maurice, P. A.; Penn, R. L.; Sahai, N.; Sparks, D. L.; Twining, B. S. Nanominerals, mineral nanoparticles, and earth systems. *Science* **2008**, *319*, 1631–1635.

(32) Blakemore, R. Magnetotactic bacteria. *Science* **1975**, *190* (4212), 377–379.

(33) Murr, L. E.; Bang, J. J.; Esquivel, E. V.; Guerrero, P. A.; Lopez, D. A. Carbon nanotubes, nanocrystal forms, and complex nanoparticle aggregates in common fuel-gas combustion sources and the ambient air. *J. Nano. Res.* **2004**, *6*, 241–251.

(34) Bang, J. J.; Guerrero, P. A.; Lopez, D. A.; Murr, L. E.; Esquivel, E. V. Carbon nanotubes and other fullerene nanocrystals in domestic propane and natural gas combustion streams. *J. Nanosci. Nanotechnol.* **2004**, *4* (7), 716–718.

(35) Heymann, D.; Chibante, L. P. F.; Brooks, R. R.; Wolbach, W. S.; Smalley, R. E. Fullerenes in the cretaceous-tertiary boundary-layer. *Science* **1994**, *265* (5172), 645–647.

(36) Becker, L.; Poreda, R. J.; Nuth, J. A.; Ferguson, F. T.; Liang, F.; Billups, W. E. Fullerenes in meteorites and the nature of planetary atmospheres. In *Natural Fullerenes and Related Structures of Elemental Carbon*; Rietmeijer, F. J. M., Ed.; Springer: Netherlands, 2006; Vol. 6, pp 95−121.

(37) Academies des Sciences et Technologies de Paris, Nanoscience, nanotechnologies; www.academie-sciences.fr; 2004, 18.

(38) Royal Society of London. Nanoscience and Nanotechnology: Opportunities and Uncertainties; www.nanotec.org.uk; 2004, 19 (04).

(39) Hansen, S. F.; Larsen, B. H.; Olsen, S. I.; Baun, A. Categorization framework to aid hazard identification of nanomaterials. *Nanotoxicology* **2007**, *1* (3), 243–250.

(40) Nanoscale Science Engineering and Technology Subcommittee, The Committee on Technology, National Science and Technology Committee. *The National Nanotechnology Initiative Strategy Plan*; National Nano-technology Coordination Office: Arlington, VA, 2004.

(41) Carlson, C.; Hussain, S. M.; Schrand, A. M.; Braydich-Stolle, K. L.; Hess, K. L.; Jones, R. L.; Schlager, J. J. Unique cellular interaction of silver nanoparticles: Size-dependent generation of reactive oxygen species. *J. Phys. Chem. B* **2008**, *112* (43), 13608–13619.

(42) Wassenberg, D. M.; Di Giulio, R. T. Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome p4501a inhibitors in *Fundulus heteroclitus*. *Environ. Health Perspect.* **2004**, *112*, 1658–1664.

(43) Oxendine, S. L.; Cowden, J.; D.E., H.; Padilla, S. Adapting the medaka embryo assay to a high-throughput approach for developmental toxicity testing. *Neurotoxicology* **2006**, *27*, 840–845.

(44) Roy, N. M.; Linney, E. Zebrafish as a model for studying adult effects of challenges to the embryonic nervous system. In *Source Book of Models for Biomedical Research*; Conn, P. M., Ed.; Humana Press, Inc.: Totowa, NJ, 2007.

(45) Wakamatsu, Y.; Pristyaznhyuk, S.; Kinoshita, M.; Tanaka, M.; Ozato, K. The see-through medaka: A fish model that is transparent throughout life. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10046–10050.

(46) Hardman, R. C.; Volz, D. C.; Kullman, S. W.; Hinton, D. E. An *in vivo* look at vertebrate liver architecture: Three-dimensional reconstructions from medaka (*Oryzias latipes*). *Anat. Rec.* **2007**, *290* (7), 770–782.

(47) Kashiwada, S. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). *Environ. Health Perspect.* **2006**, *114*, 1697–1702.

(48) Nel, A.; Xia, T.; Madler, L.; Li, N. Toxic potential of materials at the nanolevel. *Science* **2006**, *311* (5761), 622–627.

(49) Meyer, J. N.; Di Giulio, R. T. Genetic adaptation and associated fitness tradeoffs in killifish (*Fundulus heteroclitus*) inhabiting a contaminated estuary. *Ecol. Appl.* **2003**, *13*, 490–503.

(50) Chae, S. R.; Wang, S.; Hendren, Z.; Wiesner, M. R.; Gunsch, C. K. Effects of fullerene nanoparticles on *Escherichia coli* k12 respiratory activity in aqueous suspension and potential use for membrane biofouling control. *J. Membr. Sci.* **2009**, in press.

(51) Lyon, D. Y.; Adams, L. K.; Falkner, J. C.; Alvarez, P. J. J. Antibacterial activity of fullerene water suspensions: Effect of preparation method and particle size. *Environ. Sci. Technol.* **2006**, *40* (14), 4360–4366.

(52) Tong, Z.; Bischoff, M.; Nies, L.; Applegate, B.; Turco, R. F. Impact of fullerene (C_{60}) on a soil microbial community. *Environ. Sci. Technol.* **2007**, *41*, 2985–2991.

(53) Kersting, A. B.; Efurd, D. W.; Finnegan, D. L.; Rokop, D. J.; Smith, D. K.; Thompson, J. L. Migration of plutonium in ground water at the Nevada test site. *Nature* **1999**, *397* (6714), 56–59.

(54) Bastian, S.; Busch, W.; Kuhnel, D.; Springer, A.; Meibner, T.; Holke, R.; Scholz, S.; Iwe, M.; Pompe, W.; Gelinsky, M.; Potthoff, A.; Richter, V.; H., I.; Shirmer, K. Toxicity of tungsten carbide and cobalt-doped tungsten carbide nanoparticles in mammalian cells in vitro. *Environ. Health Perspect.* **2009**, *117* (4), 530–536.

(55) Cai, D.; Mataraza, J.; Qin, Z.; Huang, Z.; Huang, J.; Chiles, T.; Carnahan, D.; Kempa, K.; Ren, Z. Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing. *Nat. Methods* **2005**, *2* (6), 449–454.

(56) Robichaud, C. O.; Tanzil, D.; Weilenmann, U.; Wiesner, M. R. Relative risk analysis of several manufactured nanomaterials: An insurance industry context. *Environ. Sci. Technol.* **2005**, *39* (22), 8985–8994.

(57) Plata, D. L.; Gschwend, P. M.; Reddy, C. M. Industrially synthesized single-walled carbon nanotubes: Compositional data for users, environmental risk assessments, and source apportionment. *Nanotechnology* **2008**, *19* (18), 14.

(58) Ogilvie-Robichaud, C.; Uyar, A. E.; Darby, M. R.; Zucker, L. G.; Wiesner, M. R. Estimates of upper bounds and trends in nano-TiO_2 production as a basis for exposure assessment. *Environ. Sci. Technol.* **2009**, *43* (12), 4227–4233.

(59) Blaser, S.; et al. Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. *Sci. Total Environ.* **2008**, *390*, 396–409.

(60) Mueller, N. C.; Nowack, B. Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.* **2008**, *42*, 4447–4453.

(61) Linkov, I.; Satterstrom, F. K.; Steevens, J.; Ferguson, E.; Pleus, R. C. Multi-criteria decision analysis and environmental risk assessment for nanomaterials. *J. Nanopart. Res.* **2007**, *9*, 543–554.

(62) Borsuk, M.; Stow, C.; Reckhow, K. A Bayesian network of eutrophication models for synthesis, prediction, and uncertainty analysis. *Ecol. Modell.* **2004**, *173*, 219–239.

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