ACCOUNTS
of chemical research

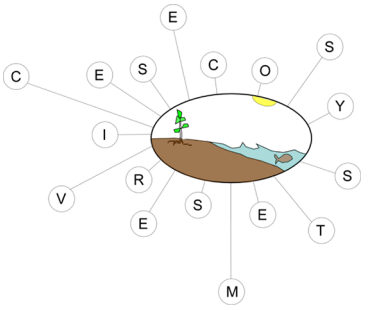
Ecological Nanotoxicology: Integrating Nanomaterial Hazard Considerations Across the Subcellular, Population, Community, and Ecosystems Levels

PATRICIA A. HOLDEN,[*,†,‡,§] ROGER M. NISBET,[†,§,∥] HUNTER S. LENIHAN,[†,‡,§] ROBERT J. MILLER,[†,‡,§] GARY N. CHERR,[†,⊥] JOSHUA P. SCHIMEL,[†,§,∥] AND JORGE L. GARDEA-TORRESDEY[†,#]

[†]*UC Center for the Environmental Implications of Nanotechnology (UC CEIN),* [‡]*Bren School of Environmental Science and Management,* [§]*Earth Research Institute, and* [∥]*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California, United States,* [⊥]*Departments of Environmental Toxicology and Nutrition, Bodega Marine Laboratory, University of California, Davis, Bodega Bay, California, United States, and* [#]*Department of Chemistry, The University of Texas at El Paso, El Paso, Texas, United States*

RECEIVED ON MARCH 5, 2012

CONSPECTUS



Research into the health and environmental safety of nanotechnology has seriously lagged behind its emergence in industry. While humans have often adopted synthetic chemicals without considering ancillary consequences, the lessons learned from worldwide pollution should motivate making nanotechnology compatible with environmental concerns. Researchers and policymakers need to understand exposure and harm of engineered nanomaterials (ENMs), currently nanotechnology's main products, to influence the ENM industry toward sustainable growth. Yet, how should research proceed? Standard toxicity testing anchored in single-organism, dose-response characterizations does not adequately represent real-world exposure and receptor scenarios and their complexities. Our approach is different: it derives from ecology, the study of organisms' interactions with each other and their environments. Our approach involves the characterization of ENMs and the mechanistic assessment of their property-based effects. Using high throughput/content screening (HTS/HCS) with cells or environmentally-relevant organisms, we measure the effects of ENMs on a subcellular or population level. We then relate those effects to mechanisms within dynamic energy budget (DEB) models of growth and reproduction. We reconcile DEB model predictions with experimental data on organism and population responses. Finally, we use microcosm studies to measure the potential for community- or ecosystem-level effects by ENMs that are likely to be produced in large quantities and for which either HTS/HCS or DEB modeling suggest their potential to harm populations and ecosystems.

Our approach accounts for ecological interactions across scales, from within organisms to whole ecosystems. Organismal ENM effects, if propagated through populations, can alter communities comprising multiple populations (e.g., plant, fish, bacteria) within food webs. Altered communities can change ecosystem services: processes that cycle carbon, nutrients, and energy, and regulate Earth's waters and atmosphere. We have shown ENM effects on populations, communities, and ecosystems, including transfer and concentration of ENMs through food chains, for a range of exposure scenarios; in many cases, we have identified subcellular ENM effects mechanisms.

To keep pace with ENM development, rapid assessment of the mechanisms of ENM effects and modeling are needed. DEB models provide a method for mathematically representing effects such as the generation of reactive oxygen species and their associated damage. These models account for organism-level effects on metabolism and reproduction and can predict outcomes of ENM-organism combinations on populations; those predictions can then suggest ENM characteristics to be avoided. HTS/HCS provides a rapid assessment tool of the ENM chemical characteristics that affect biological systems; such results guide and expand DEB model expressions of hazard. Our approach addresses ecological processes in both natural and managed ecosystems (agriculture) and has the potential to deliver timely and meaningful understanding towards environmentally sustainable nanotechnology.

Published on the Web 10/05/2012 www.pubs.acs.org/accounts
10.1021/ar300069t

Introduction

The use of engineered nanomaterials (ENMs) is rapidly increasing,[1] raising concerns about impacts to environmental organisms and processes.[2,3] Thus far, there is no direct evidence for in situ environmental harm by ENMs.[3,4] However, considering that ENMs are highly reactive[5] but mostly uncharacterized,[6] concern and uncertainty surround their fates and effects in the environment. What research can guide regulators and industry to avoid environmental harm? Given the pace of ENM development, research must be efficient to be influential. But efficiency is not just about speed; it is equally about *efficacy*, that is, delivering meaningful information regarding the ecological effects of ENMs. We are performing research to assess and predict effects of ENMs across ecological scales for a range of exposure scenarios. This is a departure from the convention of standardized toxicity testing for lethality of select organisms under artificial conditions.[7] This Account demonstrates why an emphasis on ecology in assessing nanoecotoxicology is important.

Drivers for Understanding Ecological Effects of ENMS

Ecology and Maintenance of Ecosystem Services. Ecosystem processes[3] and environmental services[2] are at risk as ENMs enter the environment. Human societies depend on collective *ecosystem services*,[8] for example, crop pollination, water and climate regulation, biodiversity conservation, and food production (Figure 1). Such services emerge from complex ecological interactions that cross biological levels of organization (population, communities, ecosystems) and physical scales (from the interfaces of individual particles to entire watersheds). A particularly high value[8] ecosystem service is nutrient cycling. Nutrient cycling occurs via reactions catalyzed by organisms with corresponding biochemical capacities; the myriad processes span many individuals within populations. Over short spatial scales, populations interact within communities, such as microbial communities in the oceans or in soils. Over larger spatial scales, multiple communities promote ecosystem functions. Maintaining ecosystem services (Figure 1) is broadly important, and because ecosystem services are delivered through complex ecological processes, ecologically focused research is required to understand ENM environmental impacts. This view defines *ecological nanotoxicology*.

Safer-by-Design Nanomaterials. Comprehensive nanotoxicology could help facilitate growth of the ENM industry.[1]

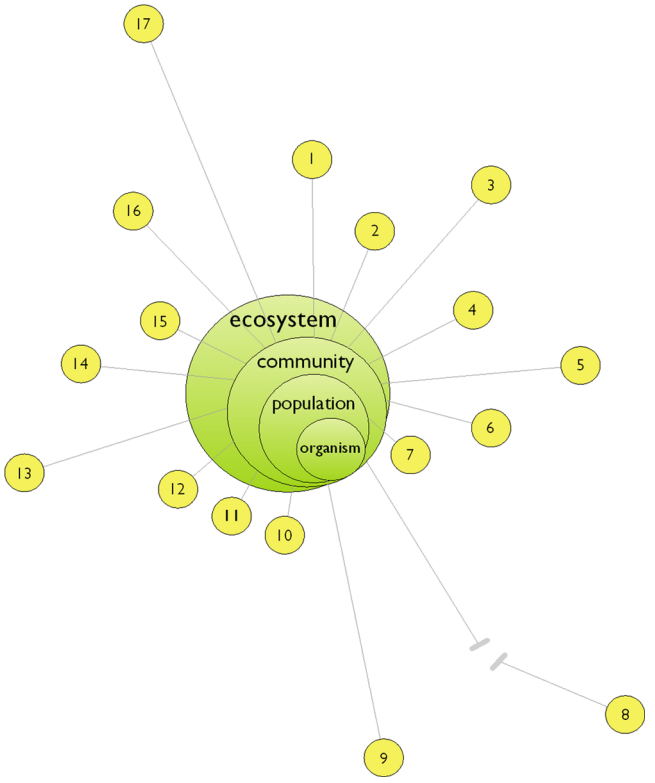


FIGURE 1. Ecosystem services[8] from natural organisms, populations, communities, and ecosystems. If ENMs interrupt biological systems (nested center circles), service provisions (intact spokes) are diminished. Numbers at spoke ends, where spoke length represents relative value,[8] signify gas (1), climate (2), disturbance (3), and water (4) regulation; water supply (5); erosion control (6); soil formation (7); nutrient cycling (8); waste treatment (9); pollination (10); biological control (11); habitat (12); food production (13); raw materials (14); genetic resources (15); recreation (16); cultural (17). The broken line for (8) reflects its off-scale magnitude.

As such, ways in which ENMs might be designed to be more compatible with human health and the environment have been reviewed.[4] Safe ENM design is not a monolith, however, as strategies that protect mammalian cells[9] do not necessarily protect whole populations in the natural environment.[10] Thus, a distinct objective of ecological nanotoxicology is discovering ENM properties that render them environmentally compatible *across* biological scales, not simply within a scale.

Evidence for Ecological Effects of ENMS

Terrestrial Environment. Soil bacteria, which are abundant and versatile catalysts, can sorb and disperse ENM agglomerates.[11] For some ENMs, such as CdSe quantum dots (QDs), bacterial membrane association generates damaging reactive oxygen species (ROS); QDs can then enter and accumulate, causing further stress and inhibiting growth.[12] QDs in bacteria can be transferred and concentrated (biomagnified)

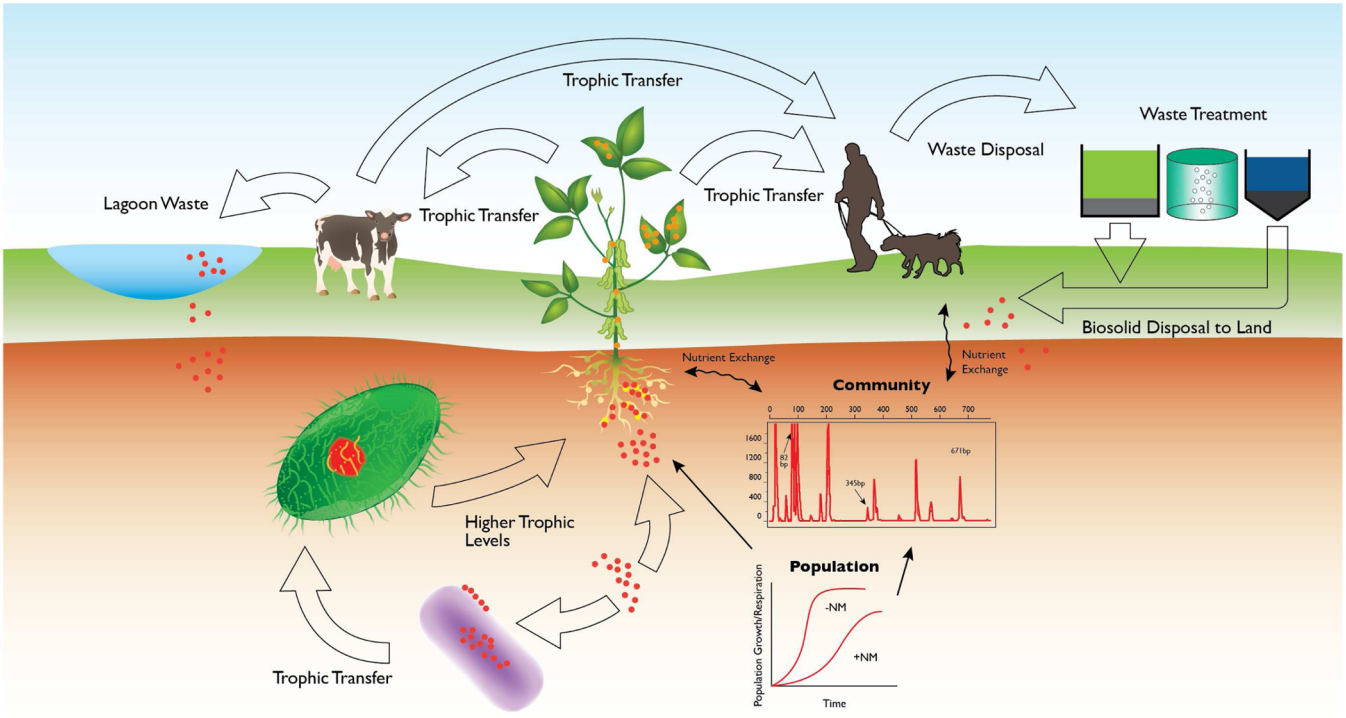


FIGURE 2. Conceptual terrestrial ecological nanotoxicology system where belowground ENMs (red dots) enter bacteria (purple) and protozoans (larger green) from soil or via trophic transfer, or can directly affect plant roots including root symbioses (yellow/green nodes on roots). ENMs translocated into aboveground plant tissues may change chemically (orange dots). ENM effects on bacterial population growth may be measured or modeled (lower right graph); population effects can alter bacterial communities (middle right graph), e.g., as per terminal restriction fragment length polymorphism (TRFLP) patterns of whole-soil DNA extracts. Below- and aboveground, ENM bioaccumulation, bioconcentration, trophic transfer, and translocation are possible (block arrows), including ENMs entering food supplies, affecting host health, and entering soil via waste streams. Nutrient exchange (squiggled lines) could change by effects on soil microbial communities and plants, or indirectly if ENMs change soil physiochemical characteristics controlling nutrient or water flow.

into protozoan predators,[13] affecting higher organisms. In soil microcosms, nano-TiO_2 (up to 2 mg g^{-1} soil) and -ZnO (up to 0.5 mg g^{-1} soil) ENMs altered bacterial community structure in a dose-dependent fashion,[14] and influenced taxa associated with ecosystem processes of N_2 fixation, methane oxidation, and complex C decomposition.[15]

ENMs could affect plant health and the food supply.[16] X-ray synchrotron techniques identified ZnO ENM derivatives, and chemical quantification showed Zn translocation throughout hydroponic soybean[17] and native desert plants;[18,19] bioaccumulated Zn reduced root growth.[17,18] For several agricultural plant types grown hydroponically, CeO_2 ENMs translocated into tissue and, without dissolving like ZnO ENMs, this led to genetic change, altered germination, and changed root and shoot growth.[17,20] Thus, high-volume metal oxide ENMs can affect a wide range of plants.

In a planted mesocosm study using soybean, Zn from nano-ZnO amended to soil was bioavailable and became distributed in beans and leaves; nano-CeO_2 invaded root nodules, interfered with N_2 fixation, and stunted plant growth.[21] Mesocosms as such can integrate population and community-level responses within the soil that coregulate nutrient cycling and plant yield (Figure 2).

Aquatic Environment. ENM physical properties likely affect toxicity across the spectrum of natural aquatic environmental media and biological interfaces of tissues and cells. Such variability is not part of typical model systems and requires nonstandard approaches. For example, iron doping of ZnO ENMs reduced dissolution and toxicity to mammalian cells and freshwater zebrafish embryos[9,22] but did not reduce toxicity (for up to 200 μg L^{-1} Zn exposure) to sea urchin embryos,[10] possibly due to a ZnO nanoparticle effect in the developing embryos. In vitro and in vivo responses of cells to ENMs may also differ: for example, chronic exposures of mussels to ZnO ENMs at concentrations for which other ENMs caused cellular damage,[23−25] that is, impairing hemocyte function in vitro, do not appear to affect hemocyte phagocytosis in vivo. This disconnect may be due to Zn(II) ion sequestration and elimination mechanisms that operate in whole organisms.

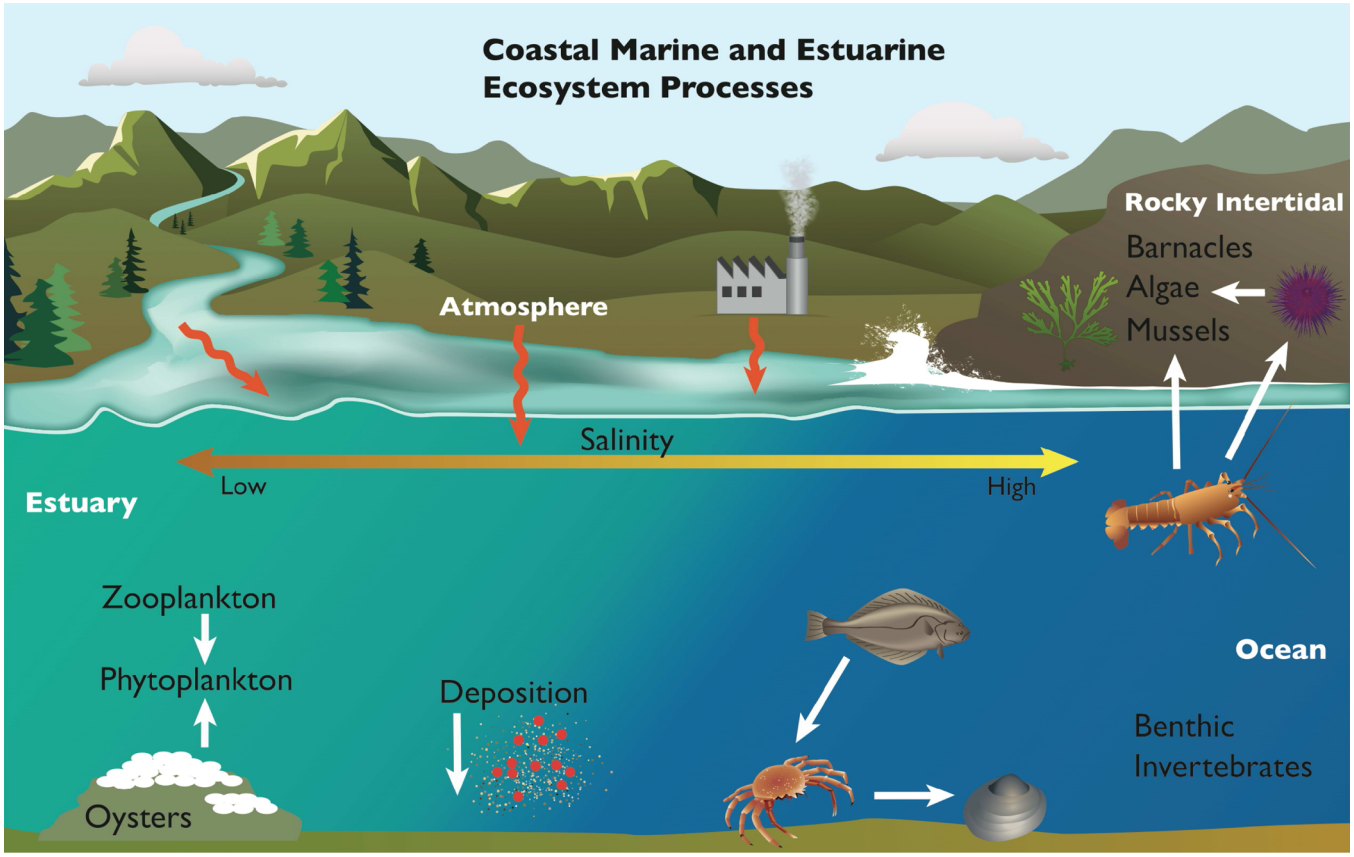


FIGURE 3. Conceptual aquatic ecosystem interactions with ENMs, focusing on coastal marine and estuarine conditions where there are extreme gradients in salinity, ranging from low (zones of freshwater discharge) to high (open ocean), and exposure to air and desiccation (the rocky intertidal zone). Along these gradients, ENM agglomeration, settling, and uptake exposes benthic populations and communities. Food webs involve organisms exchanging within the water column and across the benthic–water column interface, with phytoplankton and macroalgae bioaccumulating ENMs from the water, thereby enabling ENM bioconcentration in primary consumers, especially zooplankton and benthic herbivores, as well as benthic suspension and deposit feeders. Benthic invertebrates and fish predators (secondary consumers) are exposed to and bioaccumulate ENMs via uptake through consuming primary consumers. Similar processes occur in freshwater aquatic ecosystems. Orange arrows represent inputs of ENMs and white arrows represent mechanisms of trophic transfer.

Environmental conditions may also impact toxicity, as was demonstrated with marine phytoplankton, the primary producers that support ocean food webs and are integral to the global carbon cycle. Experiments with marine coastal phytoplankton showed no negative effects of TiO_2 in tests conducted under standard test conditions[26] with artificial lighting. However, experiments manipulating ultraviolet radiation (UVR) exposure showed that TiO_2 was toxic under UVR levels typical of marine surface waters.[27]

Benthic food webs (Figure 3) that support many fisheries rely on phytoplankton from the overlying water as food. Preliminary data suggest that marine mussels, a common seafood, suffer reduced growth and reproduction when they graze upon ZnO ENM-contaminated phytoplankton suspended in the water. Such data can be used to develop integrative models (see below), which can be used to deduce mechanisms of impact at population and ecosystem levels.

Exposure Considerations

ENMs enter aquatic and terrestrial environments through air emissions,[28] wastewater treatment plants (WWTPs),[29,30] and building façade runoff.[31,32] Amounts in the environment are unknown[33] and estimates vary,[34,35] but ENMs released with manufacturing, accidental spills, product wear, waste disposal, and recycling will reach destinations at concentrations depending on ENM dissolution, agglomeration, sedimentation, and other transport processes.[36] Depending on exposure route, organisms may not be impacted by ENMs.[37] Further, organisms may not be exposed if ENM bioavailability is low, for example, due to sorption into soil matrices.[38] Effects of bioavailable ENMs depend on dose,[3,14] and harm at organism or population levels that may not manifest in communities or across ecosystems, owing to homeostatic buffering effects.[39] However, it could be erroneous to assume that ENMs would have limited impacts

owing to environmental dilution: due to their small size and tendency to sorb organic material, ENMs may remain suspended in water indefinitely, dissolve, or aggregate and deposit in sediments, particularly in estuaries.[36,40] To date, much ecological nanotoxicology has involved medium to high ENM concentrations, in order to determine the potential for harm, for example, as might occur near concentrated "hot spots". In the absence of measured ENM environmental concentrations, mechanistic effects modeling (below) uses empirical results to make predictions at untested exposure concentrations.

Approaches for Minimizing Ecological Effects of EMNS

Modeling, and Thereby Predicting, Ecological Toxicity. The pace of nanotechnology development threatens to exceed society's capacity to predict, and thus mitigate unwanted effects on ecosystem services. Biological receptors in the environment include a vast number of organisms, life stages, and biochemical pathways, yet their interactions with ENMs are inherently anchored in biochemical bases that should be amenable to hypothesis formulation and testing using mathematical modeling. Other Accounts in this special issue on Environmental Health and Safety Considerations for Nanotechnology highlight the power of *mechanistically based*, structure–activity relationships (SARs) in linking suborganismal processes to organismal performance, but SARs have limited transferability and utility for addressing concerns about nanomaterials' ecological effects – especially those involving populations and ecosystems. We require mechanistic approaches that are relevant at these higher levels of biological organization.

This need has been recently formalized in the adverse outcome pathways (AOP)[41] paradigm. A few AOPs make obvious contact with population dynamics, for example, those linking molecular mechanisms regulating egg yolk formation (vitellogenesis) to reduced reproduction,[42] but for many molecular responses the connection is less obvious. One common sublethal impact of toxicants is to change the rates at which individual organisms obtain energy and nutrients from their environment and utilize them for cellular function (Figure 4a). A powerful approach to modeling these changes, with a track record in ecotoxicology, is Dynamic Energy Budget (DEB) theory.[43–46] DEB theory focuses on the individual organism, with differential equations describing the rates at which an organism assimilates and utilizes energy and materials from food for maintenance, growth, reproduction, and development. These rates depend on the "state" of the organism (e.g., age, size, maturity) and its "environment" (e.g., food

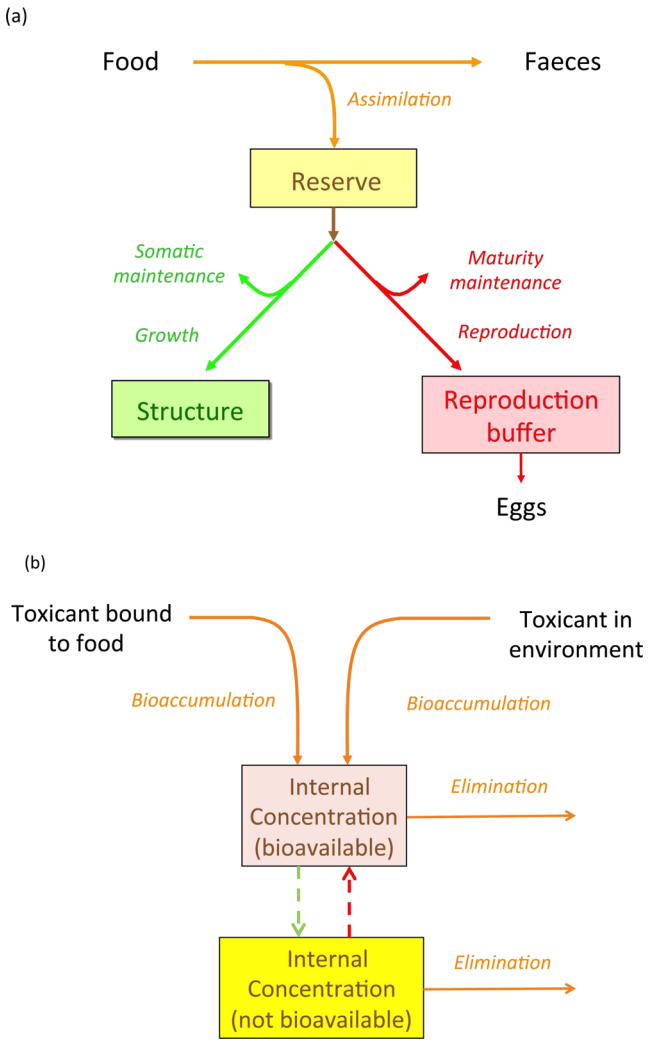


FIGURE 4. (a) Schematic representation of the transformations in the simplest *DEB model* capable of describing all life stages in an animal.[59] An embryo uses "reserve" to grow and develop. At "birth", a juvenile starts feeding. Assimilated food is stored as reserve and utilized by juveniles for maintenance, growth, and development (increase in maturity). Adults partition energy between maintenance, growth, and reproduction. (b) Mass transformations in a simple *toxicokinetic* model describing bioaccumulation. Toxicant enters the organism either through food or directly (e.g., transport across gills in a fish). Toxicant within the organism may or may not be bioavailable. A *toxic effect* model (not illustrated) specifies how bioavailable toxicant (red in panel (b)) impacts the transformations represented in panel (a).

density, temperature, exposure to contaminants). The connection to population dynamics is through "structured" or "individual-based" modeling techniques.[44,47,48] Furthermore, with some simplifying assumptions, the resulting population models share many properties with more traditional ecological models (which treat a population as a single aggregate entity),[49] thereby opening possible links to community and ecosystem dynamics. The use of DEB theory in ecotoxicology requires assumptions that describe

the uptake, release, and metabolism of toxic compounds, and their effects on the organism's physiology; specifically, *toxicokinetic* and *toxic effect* submodels are required.[50] The toxicokinetic submodels (Figure 4b) describe contaminant exchange with the environment and chemical transformations within an organism. The toxic effect submodel specifies how the basic DEB model parameters change in response to the toxicant.

There are at least five distinct ways in which DEB models may enhance our understanding of the ecological effects of nanomaterials:

1 *Existing DEB methodology* may characterize effects of nanoparticles on model parameters, as has been shown for marine phytoplankton exposed to metal oxide particles.[26] The approach yields metrics, including a "no-effects concentration" (NEC), that are independent of specific experimental protocols.[51−53]

2 *Individual to population projection*. By fitting a DEB model to a large body of data from short-term studies, it is possible to project effects on population growth rates. This approach can describe laboratory information on individuals from a broad range of contaminants and target organisms,[50] and help interpret field data.[54] We used this methodology to evaluate the effects of chronic exposure to metal oxide particles on lifetime reproduction rates of marine mussels.[55] DEB models give "added value" to measurements on individual organisms by using data from experiments lasting weeks or months to predict a population property that is expressed over years.

3 The DEB formalism allows modeling the *impact of multiple stressors*. DEB models can be used to evaluate the combined effects of ENM toxicity, reduced food availability, temperature, and other environmental factors that impact the energy available to the animal for growth and reproduction.[50]

4 With additional submodels, the DEB approach connects smoothly with *suborganismal information* and with *physicochemical processes in the environment*. The power of this approach was demonstrated in a recent study of bacterial responses to dissolved cadmium and CdSe QDs,[12,56] as outlined in Figure 5. There were supporting data on intracellular and extracellular dissolved Cd, and on ROS. Microbial studies exploit the individual-to-population connection in the opposite direction, with primary data coming from populations and model inferences relating to organismal and suborganismal processes.

5 DEB-based structured population models may describe the effects of ENMs on the *dynamics of interacting populations*[44,57] and thereby open the way to modeling community dynamics. Studies in progress use such models to elucidate effects of silver nanoparticles (NPs) on phytoplankton−zooplankton interactions.

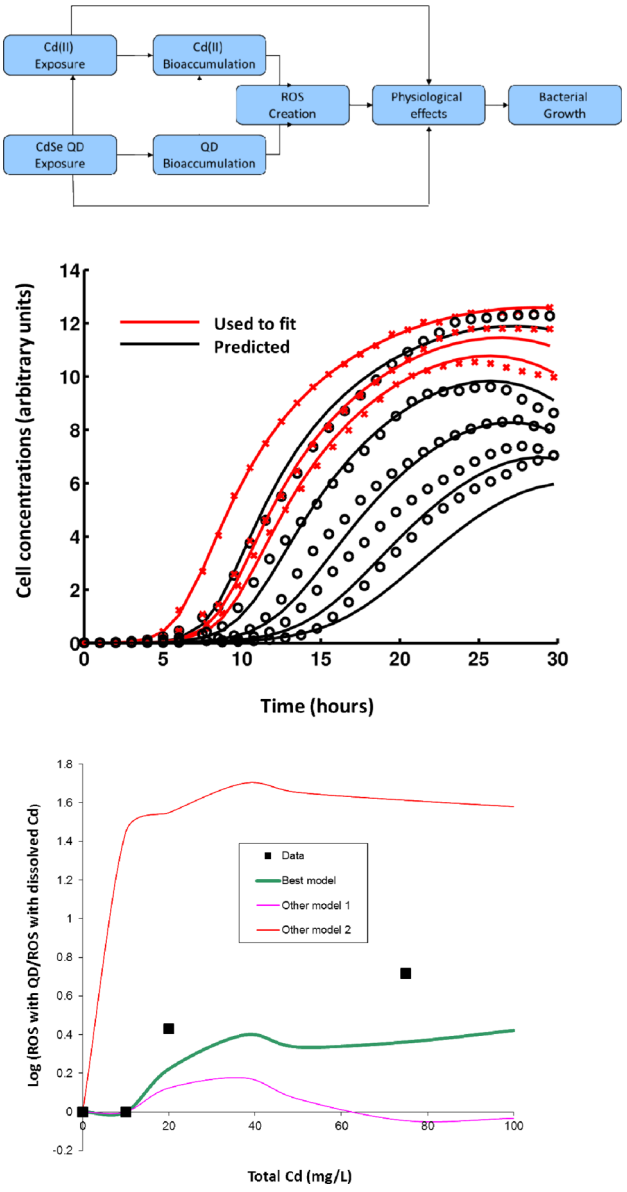


FIGURE 5. Identifying toxicity mechanisms for CdSe quantum dots (QDs) in populations of *Pseudomonas* bacteria. A variant of the standard DEB model was developed that included an explicit representation of ROS production and its effects within a cell[56,60] (panel a). The model was tested on data on toxicity of dissolved Cd salts[56] (panel b) and used to model toxic effects specific to QDs[60] (panel c). The DEB model of ionic Cd toxicity can predict effects at high concentrations from growth data at lower levels (panel b). By identifying ROS levels with one parameter in Kooijman's DEB model (the "aging acceleration"), it was possible to determine the plausibility of different potential mechanisms of QD toxicity. Panel c shows predicted values of a metric involving ROS levels after 15 h using three different toxic effect models. The "best model" assumed increase in maintenance processes such as cellular repair and maintenance of cross-membrane gradients as the dominant mechanism for QD toxicity.

In summary, DEB models offer a parameter-sparse representation of the organismal response to toxicants that can provide biologically interpretable metrics from toxicity tests. DEB models focus on individual organisms, but incorporate information from suborganismal studies and relate seamlessly to ecologically important studies on interacting populations. DEB modeling can support recent work on adverse outcome pathways (AOPs) by simplifying and extending the pathways to include ecological interactions without compromising the critical insights from molecular and cellular studies.

Making Appropriate and Accelerated Measurements of Effects Potentials and Mechanisms Using High Throughput Screening (HTS). HTS employing ENMs synthesized to systematically vary specific properties has allowed comprehensive testing of hypothesized effects-mechanisms in mammalian cells.[9] However, typical HTS models may not apply to ecosystems. Moving forward, HTS can apply to bacteria that were identified as sensitive in soil microcosm studies,[15] as was effective with laboratory bacteria.[58] Likewise, HTS assays were adapted to marine phytoplankton[26,27] and mussel hemocytes in High Content Screening (HCS). HTS and HCS enable screening large numbers of ENMs with varying properties for their effects on the physiology and growth of organisms responsible for important ecosystem services.

Letting Ecological Principles Drive Environmental Nanotoxicology. Ecological nanotoxicology is a system of hypothesis-testing endeavors, often beginning with individual- or population-level experiments to determine if growth or reproductive effects occur as a function of exposure to well-characterized ENMs with hypothesized stress-inducing properties (Figure 6). Experiments prioritize ENMs that are produced in large quantities and account for likely ENM environmental compartmentalization, i.e. ENM propensities to settle or modify in the environment.[36] Because of their cost, larger-scale experiments are undertaken if experiments, models, or the scale of ENM production suggest a potential for community- or ecosystem-level exposure and impacts. Such studies motivate additional hypothesis testing at subcellular to population levels. HTS/HCS and DEB modeling are used to extend the reach of low-throughput experiments and microcosm studies, such that ecological exposure and effects paradigms related to ENM properties can be developed efficiently, quickly, and accurately, thus allowing timely advice to ENM producers and regulators regarding industrially useful, but environmentally safe, ENM designs.

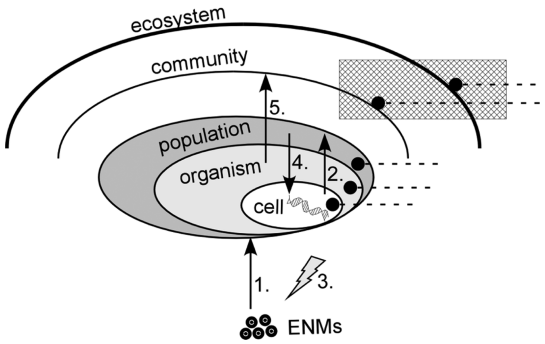


FIGURE 6. Conceptual, nested endeavors in ecological nanotoxicology. Horizontal (dashed) lines indicate biological levels (filled circle) for which individual experiments test effects hypotheses. For community and ecosystem levels, microcosms (hatched rectangle) are undertaken for ENMs with high production levels, when effects occur at lower biological levels, or when DEB models predict propagation of population-level effects to higher ecological levels. DEB modeling (arrows) explains and predicts effects at one level that are measured in another, or that derive from effects mechanisms attributable to ENM properties. The numbers alongside the arrows represent five DEB modeling scenarios (in the text).

Extending the Ecological Nanotoxicology Paradigm

Much current ecotoxicological research is directed at population-level impacts because this is a substantial focus of environmental impact assessment.[42] If the challenge is to understand how ENMs influence ecosystem services, research on mechanisms of injury at subcellular, cellular, individual, and population (i.e., subcommunity) levels should highlight keystone biological species that provide ecosystem services directly, or that indirectly facilitate them. An important next step is to identify community-level impacts of ENMs, such as altered predator–prey dynamics, loss of biodiversity[14] or community function, symbiosis interferences,[21] host community or disease pattern changes, and food web alterations, and to test whether safely designing materials reduces such impacts. Also, whether ENMs in nature are bioavailable, biotransformed, bioaccumlated, trophically transferred, and biomagnified[13] is critical to address in varying environmental media. An efficient and theoretically robust approach is to design experiments at subcommunity levels that parametrize models, the results of which can generate predictions about community- and ecosystem-level impacts, which are then tested in mesocosm experiments and/or by sampling patterns in nature.

Conclusions

Environmental nanotoxicology should emphasize ecological interactions at population, community, and ecosystem levels, while accounting for underlying organismal and cellular

effects. *Ecological nanotoxicology* powerfully links exposure and ENM chemical properties, biochemical mechanisms, and the ecological and physical processes that ultimately regulate ecosystem-level impacts and ecosystem services. Mechanistic models (e.g., DEB) are required to integrate information from multiple scales and to use experimentally measured effects, and underlying mechanisms, for predicting population level outcomes for realistic exposure regimes. Given the pace of nanotechnology development, such models in concert with HTS/HCS of multiple nanoparticle, organism, and mechanism combinations are needed for guiding safe nanotechnology.

This work was supported by the National Science Foundation and the Environmental Protection Agency under Cooperative Agreement DBI-0830117. Any opinions, findings, and conclusions are those of the authors and do not necessarily reflect the views of the National Science Foundation or the Environmental Protection Agency. This work has not been subjected to EPA review and no official endorsement should be inferred.

BIOGRAPHICAL INFORMATION

Dr. Patricia A. Holden received her B.S. and M.S. degrees in Civil & Environmental Engineering, and her Ph.D. from the University of California, Berkeley, in Soil Microbiology. She is a Professor in the Bren School of Environmental Science & Management at the University of California, Santa Barbara, where she also directs the UCSB Natural Reserve System. Her research regards urban water quality, pollutant effects and fates, and soil processes, drawing from environmental microbiology, microbial ecology, environmental physics and chemistry.

Dr. Roger M. Nisbet received his B.Sc. and Ph.D., both in Theoretical Physics, at the University of St. Andrews in Scotland. He was a Professor of Applied Physics at the University of Strathclyde until 1991, when he was appointed as Professor in the Department of Ecology, Evolution and Marine Biology at the University of California, Santa Barbara. His research interests cover many aspects of ecological theory and modeling, with particular emphasis on models that relate population dynamics to the physiology and behavior of individual organisms.

Dr. Hunter S. Lenihan received his Ph.D. degree in Marine Science, with an emphasis in population and community ecology, from the University of North Carolina at Chapel Hill. He is a Professor in the Bren School of Environmental Science & Management at the University of California, Santa Barbara. His ecotoxicological research includes quantifying the effects of anthropogenic contaminants in Antarctica. Other research involves disease ecology, fisheries management, deep-sea communities, aquaculture, and restoration ecology, most recently with a focus on coral reef ecosystems.

Dr. Robert J. Miller received his Ph.D. in Biology from the University of Massachusetts Boston and works on marine ecology. He is an Assistant Research Biologist in the Marine Science Institute at the University of California, Santa Barbara. His research focuses on primary production and food web support in coastal marine ecosystems, effects of contaminants on marine ecosystems, and benthic community ecology.

Dr. Gary Cherr received his Ph.D. from the University of California Davis, was an NIH postdoctoral fellow, and has worked in reproductive and developmental toxicology for over 25 years. His laboratory studies stressor impacts on marine invertebrates and vertebrates, and embryo defenses, including multidrug resistance transporters. His laboratory has investigated the impacts of oil spills on fish embryos as well as nanoparticle toxicology in marine invertebrate embryos and aquatic food webs. He is Professor of Environmental Toxicology and Nutrition at the University of California Davis, and is currently the Director of the University of California Davis' Bodega Marine Laboratory.

Dr. Joshua Schimel is Professor of Soil and Ecosystem Ecology at the University of California Santa Barbara, with a joint appointment between the Department of Ecology, Evolution & Marine Biology and the Environmental Studies Program, which he currently chairs. He received a B.A. in Chemistry from Middlebury College and a Ph.D. in Soil Science from the University of California, Berkeley. Before moving to UCSB, he was an Assistant Professor at the University of Alaska Fairbanks. His research targets how microorganisms in soil regulate ecosystem functioning, with emphases on Arctic ecology, microbial stress responses, and the connections between biotic, chemical, and physical drivers of soil processes.

Dr. Jorge Gardea-Torresdey is the *Dudley* Professor of Chemistry and Environmental Science and Engineering at The University of Texas-El Paso (UTEP). He received his Ph.D. from New Mexico State University in 1988. He has authored over 320 publications. Dr. Gardea has graduated 25 Ph.D. and 27 M.S. students. His research achievements are highlighted in the Lawrence Hall of Science of the University of California—Berkeley. He received the 2009 SACNAS Distinguished Scientist of the Year Award. Dr. Gardea's career has been highlighted by important journals, including the October 28, 2009 issue of *Environmental Science & Technology* (ES&T) and the December 3, 2009 issue of *Nature*. He was appointed in 2011 as Associate Editor of ES&T, ranked #1 in environmental sciences and engineering.

FOOTNOTES

All authors are investigators within the University of California Center for Environmental Implications of Nanotechnology (UC CEIN).
*To whom correspondence should be addressed.
The authors declare no competing financial interest.

REFERENCES

1 Roco, M. C. The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. *J. Nanopart. Res.* **2011**, *13*, 427–445.

2 Klaine, S. J.; Alvarez, P. J. J.; Batley, G. E.; Fernandes, T. F.; Handy, R. D.; Lyon, D. Y.; Mahendra, S.; McLaughlin, M. J.; Lead, J. R. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. *Environ. Toxicol. Chem.* **2008**, *27*, 1825–1851.

3 Bernhardt, E. S.; Colman, B. P.; Hochella, M. F.; Cardinale, B. J.; Nisbet, R. M.; Richardson, C. J.; Yin, L. Y. An ecological perspective on nanomaterial impacts in the environment. *J. Environ. Qual.* **2010**, *39*, 1954–1965.

4 Nel, A.; Grainger, D.; Alvarez, P.; Badesha, S.; Castranova, V.; Ferrari, M.; Godwin, H.; Grodzinski, P.; Morris, J.; Savage, N.; Scott, N.; Wiesner, M.: Nanotechnology

environmental, health, and safety issues. In *Nanotechnology Research Directions for Societal Needs in 2020: Retrospective and Outlook*; Roco, M. C., Mirkin, C. A., Hersam, M. C., Eds.; Springer: Berlin and Boston, 2010; pp 610.

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

6 Richman, E. K.; Hutchison, J. E. The nanomaterial characterization bottleneck. *ACS Nano* **2009**, *3*, 2441–2446.

7 Jager, T. Some Good Reasons to Ban ECx and Related Concepts in Ecotoxicology. *Environ. Sci. Technol.* **2011**, *45*, 8180–8181.

8 Costanza, R.; dArge, R.; deGroot, R.; Farber, S.; Grasso, M.; Hannon, B.; Limburg, K.; Naeem, S.; Oneill, R. V.; Paruelo, J.; Raskin, R. G.; Sutton, P.; vandenBelt, M. The value of the world's ecosystem services and natural capital. *Nature* **1997**, *387*, 253–260.

9 George, S.; Pokhrel, S.; Xia, T.; Gilbert, B.; Ji, Z. X.; Schowalter, M.; Rosenauer, A.; Damoiseaux, R.; Bradley, K. A.; Madler, L.; Nel, A. E. Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. *ACS Nano* **2010**, *4*, 15–29.

10 Fairbairn, E. A.; Keller, A. A.; Madler, L.; Zhou, D. X.; Pokhrel, S.; Cherr, G. N. Metal oxide nanomaterials in seawater: Linking physicochemical characteristics with biological response in sea urchin development. *J. Hazard. Mater.* **2011**, *192*, 1565–1571.

11 Horst, A. M.; Neal, A. C.; Mielke, R. E.; Sislian, P. R.; Suh, W. H.; Madler, L.; Stucky, G. D.; Holden, P. A. Dispersion of TiO_2 nanoparticle agglomerates by Pseudomonas aeruginosa. *Appl. Environ. Microbiol.* **2010**, *76*, 7292–7298.

12 Priester, J. H.; Stoimenov, P. K.; Mielke, R. E.; Webb, S. M.; Ehrhardt, C.; Zhang, J. P.; Stucky, G. D.; Holden, P. A. Effects of soluble cadmium salts versus CdSe quantum dots on the growth of planktonic Pseudomonas aeruginosa. *Environ. Sci. Technol.* **2009**, *43*, 2589–2594.

13 Werlin, R.; Priester, J. H.; Mielke, R. E.; Kramer, S.; Jackson, S.; Stoimenov, P. K.; Stucky, G. D.; Cherr, G. N.; Orias, E.; Holden, P. A. Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. *Nat. Nanotechnol.* **2011**, *6*, 65–71.

14 Ge, Y.; Schimel, J. P.; Holden, P. A. Evidence for negative effects of TiO_2 and ZnO nanoparticles on soil bacterial communities. *Environ. Sci. Technol.* **2011**, *45*, 1659–1664.

15 Ge, Y.; Schimel, J. P.; Holden, P. A. Identification of soil bacteria susceptible to TiO_2 and ZnO nanoparticles. *Appl. Environ. Microbiol.* **2012**, *78*, 6749–6758.

16 Rico, C. M.; Majumdar, S.; Duarte-Gardea, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food Chem.* **2011**, *59*, 3485–3498.

17 Lopez-Moreno, M. L.; de la Rosa, G.; Hernandez-Viezcas, J. A.; Castillo-Michel, H.; Botez, C. E.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (Glycine max) plants. *Environ. Sci. Technol.* **2010**, *44*, 7315–7320.

18 de la Rosa, G.; Lopez-Moreno, M. L.; Hernandez-Viezcas, J.; Montes, M. O.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Toxicity and biotransformation of ZnO nanoparticles in the desert plants Prosopis juliflora-velutina, Salsola tragus and Parkinsonia florida. *I. ;J. Nanotechnol.* **2011**, *8*, 492–506.

19 Hernandez-Viezcas, J. A.; Castillo-Michel, H.; Servin, A. D.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Spectroscopic verification of zinc absorption and distribution in the desert plant Prosopis juliflora-velutina (velvet mesquite) treated with ZnO nanoparticles. *Chem. Eng. J.* **2011**, *170*, 346–352.

20 Lopez-Moreno, M. L.; de la Rosa, G.; Hernandez-Viezcas, J. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO_2 nanoparticles and assessment of their differential toxicity in four edible plant species. *J. Agric. Food Chem.* **2010**, *58*, 3689–3693.

21 Priester, J. H.; Ge, Y.; Mielke, R. E.; Horst, A. M.; Cole-Moritz, S.; Espinosa, K.; Gelb, J.; Walker, S. L.; Nisbet, R. M.; An, Y.-J.; Schimel, J. P.; Palmer, R. G.; Hernandez-Viezcas, J. A.; Zhao, L.; Gardea-Torresdey, J. L.; Holden, P. A.: Soybean susceptibility to manufactured nanomaterials: evidence for food quality and soil fertility interruption. *Proc. Natl. Acad. Sci.* **2012**, *109*, E2451−E2456.

22 Xia, T. A.; Zhao, Y.; Sager, T.; George, S.; Pokhrel, S.; Li, N.; Schoenfeld, D.; Meng, H. A.; Lin, S. J.; Wang, X.; Wang, M. Y.; Ji, Z. X.; Zink, J. I.; Madler, L.; Castranova, V.; Lin, S.; Nel, A. E. Decreased dissolution of ZnO by iron doping yields nanoparticles with reduced toxicity in the rodent lung and zebrafish embryos. *ACS Nano* **2011**, *5*, 1223–1235.

23 Moore, M. N.; Readman, J. A. J.; Readman, J. W.; Lowe, D. M.; Frickers, P. E.; Beesley, A. Lysosomal cytotoxicity of carbon nanoparticles in cells of the molluscan immune system: An in vitro study. *Nanotoxicology* **2009**, *3*, 40–45.

24 Canesi, L.; Ciacci, C.; Betti, M.; Fabbri, R.; Canonico, B.; Fantinati, A.; Marcornini, A.; Pojana, G. Immunotoxicity of carbon black nanoparticles to blue mussel hemocytes. *Environ. Int.* **2008**, *34*, 1114–1119.

25 Gagne, F.; Auclair, J.; Turcotte, P.; Fournier, M.; Gagnon, C.; Sauve, S.; Blaise, C. Ecotoxicity of CdTe quantum dots to freshwater mussels: Impacts on immune system, oxidative stress and genotoxicity. *Aquat. Toxicol.* **2008**, *86*, 333–340.

26 Miller, R. J.; Lenihan, H. S.; Muller, E. B.; Tseng, N.; Hanna, S. K.; Keller, A. A. Impacts of metal oxide nanoparticles on marine phytoplankton. *Environ. Sci. Technol.* **2010**, *44*, 7329–7334.

27 Miller, R. J.; Bennett, S.; Keller, A. A.; Pease, S.; Lenihan, H. S. TiO_2 nanoparticles are phototoxic to marine phytoplankton. *PLoS One* **2012**, *7*, e30321.

28 Cassee, F. R.; van Balen, E. C.; Singh, C.; Green, D.; Muijser, H.; Weinstein, J.; Dreher, K. Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Crit. Rev. Toxicol.* **2011**, *41*, 213–229.

29 Kiser, M. A.; Westerhoff, P.; Benn, T.; Wang, Y.; Perez-Rivera, J.; Hristovski, K. Titanium nanomaterial removal and release from wastewater treatment plants. *Environ. Sci. Technol.* **2009**, *43*, 6757–6763.

30 Kaegi, R.; Voegelin, A.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Burkhardt, M.; Siegrist, H. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ. Sci. Technol.* **2011**, *45*, 3902–3908.

31 Kaegi, R.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Mueller, E.; Vonbank, R.; Boller, M.; Burkhardt, M. Release of silver nanoparticles from outdoor facades. *Environ. Pollut.* **2010**, *158*, 2900–2905.

32 Kaegi, R.; Ulrich, A.; Sinnet, B.; Vonbank, R.; Wichser, A.; Zuleeg, S.; Simmler, H.; Brunner, S.; Vonmont, H.; Burkhardt, M.; Boller, M. Synthetic TiO_2 nanoparticle emission from exterior facades into the aquatic environment. *Environ. Pollut.* **2008**, *156*, 233–239.

33 Von Der Kammer, F.; Ferguson, P. L.; Holden, P. A.; Masion, A.; Rogers, K. R.; Klaine, S. J.; Koelmans, A. A.; Horne, N.; Unrine, J. M. Analysis of engineered nanomaterials in complex matrices (environment and biota): general considerations and conceptual case studies. *Environ. Toxicol. Chem.* **2012**, *31*, 32–49.

34 Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. *Environ. Sci. Technol.* **2009**, .

35 Gottschalk, F.; Ort, C.; Scholz, R. W.; Nowack, B. Engineered nanomaterials in rivers - Exposure scenarios for Switzerland at high spatial and temporal resolution. *Environ. Pollut.* **2011**, *159*, 3439–3445.

36 Keller, A. A.; Wang, H. T.; Zhou, D. X.; Lenihan, H. S.; Cherr, G.; Cardinale, B. J.; Miller, R.; Ji, Z. X. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environ. Sci. Technol.* **2010**, *44*, 1962–1967.

37 Birbaum, K.; Brogioli, R.; Schellenberg, M.; Martinoia, E.; Stark, W. J.; Gunther, D.; Limbach, L. K. No evidence for cerium dioxide nanoparticle translocation in maize plants. *Environ. Sci. Technol.* **2010**, *44*, 8718–8723.

38 Tong, Z.; Bischoff, M.; Nies, L.; Applegate, B.; Turco, R. F. Impact of fullerene (C60) on a soil microbial community. *Environ. Sci. Technol.* **2007**, *41*, 2985–2991.

39 Levin, S. A. Ecosystems and the biosphere as complex adaptive systems. *Ecosystems* **1998**, *1*, 431–436.

40 Thio, B. J. R.; Zhou, D. X.; Keller, A. A. Influence of natural organic matter on the aggregation and deposition of titanium dioxide nanoparticles. *J. Hazard. Mater.* **2011**, *189*, 556–563.

41 Ankley, G. T.; Bennett, R. S.; Erickson, R. J.; Hoff, D. J.; Hornung, M. W.; Johnson, R. D.; Mount, D. R.; Nichols, J. W.; Russom, C. L.; Schmieder, P. K.; Serrrano, J. A.; Tietge, J. E.; Villeneuve, D. L. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* **2010**, *29*, 730–741.

42 Kramer, V. J.; Etterson, M. A.; Hecker, M.; Murphy, C. A.; Roesijadi, G.; Spade, D. J.; Spromberg, J. A.; Wang, M.; Ankley, G. T. Adverse outcome pathways and ecological risk assessment bridging to population-level effects. *Environ. Toxicol. Chem.* **2011**, *30*, 64–76.

43 Kooijman, S. A. L. M. Quantitative aspects of metabolic organization: a discussion of concepts. *Philos. Trans. R. Soc. London, Ser. B* **2001**, *356*, 331–349.

44 Nisbet, R. M.; Muller, E. B.; Lika, K.; Kooijman, S. A. L. M. From molecules to ecosystems through dynamic energy budget models. *J. Anim. Ecol.* **2000**, *69*, 913–926.

45 Kooijman, S. A. L. M. *Dynamic Energy and Mass Budgets in Biological Systems*, 2nd ed.; Cambridge University Press: New York, 2000.

46 Kooijman, S. A. L. M. *Dynamic Energy Budget Theory for Metabolic Organization*, 3rd ed.; Cambridge University Press: New York, 2010.

47 Grimm, V.; Railsback, S. F. *Individual-based Modeling and Ecology*; Princeton University Press: Princeton, 2005.

48 *Structured-Population Models in Marine, Terrestrial, and Freshwater Systems*; Tuljapurkar, S., Caswell, H., Eds.; Chapman & Hall: 1997; Vol. 18, pp 656.

49 Gurney, W. S. C.; Nisbet, R. M. *Ecological Dynamics*; Oxford University Press: New York, 1998.

50 Muller, E. B.; Nisbet, R. M.; Berkley, H. A. Sublethal toxicant effects with dynamic energy budget theory: model formulation. *Ecotoxicology* **2010**, *19*, 48–60.

51 Baas, J.; Jager, T.; Kooijman, S. Estimation of no effect concentrations from exposure experiments when values scatter among individuals. *Ecol. Modell.* **2009**, *220*, 411–418.

52 Jager, T.; Kooijman, S. A biology-based approach for quantitative structure-activity relationships (QSARs) in ecotoxicity. *Ecotoxicology* **2009**, *18*, 187–196.

53 Jager, T.; Vandenbrouck, T.; Baas, J.; De Coen, W. M.; Kooijman, S. A biology-based approach for mixture toxicity of multiple endpoints over the life cycle. *Ecotoxicology* **2010**, *19*, 351–361.

54 Muller, E. B.; Osenberg, C. W.; Schmitt, R. J.; Holbrook, S. J.; Nisbet, R. M. Sublethal toxicant effects with dynamic energy budget theory: application to mussel outplants. *Ecotoxicology* **2010**, *19*, 38–47.

55 Muller, E. B.; Hanna, S. K.; Lenihan, H. S.; Miller, R. J.; Nisbet, R. M.: Impact of engineered zinc oxide nanoparticles on the energy budgets of Mytilus galloprovincialis. *Ecotoxicology*, submitted for publication.

56 Klanjscek, T.; Nisbet, R. M.; Priester, J. H.; Holden, P. A. Modeling physiological processes that relate toxicant exposure and bacterial population dynamics. *PLoS One* **2012**, 7.

57 McCauley, E.; Nelson, W. A.; Nisbet, R. M. Small-amplitude cycles emerge from stage-structured interactions in Daphnia-algal systems. *Nature* **2008**, *455*, 1240–1243.

58 Ivask, A.; Suarez, E.; Patel, T.; Boren, D.; Ji, Z. X.; Holden, P.; Telesca, D.; Damoiseaux, R.; Bradley, K. A.; Godwin, H. Genome-Wide Bacterial Toxicity Screening Uncovers the Mechanisms of Toxicity of a Cationic Polystyrene Nanomaterial. *Environ. Sci. Technol.* **2012**, *46*, 2398–2405.

59 Sousa, T.; Domingos, T.; Poggiale, J. C.; Kooijman, S. Dynamic energy budget theory restores coherence in biology. *Philos. Trans. R. Soc., B* **2010**, *365*, 3413–3428.

60 Klanjscek, T.; Nisbet, R. M.; Priester, J. H.; Holden, P. A.: Dynamic energy budget approach to modeling mechanisms of CdSe quantum dot toxicity. *Ecotoxicology*, in revision.