Considerations of Environmentally Relevant Test Conditions for Improved Evaluation of Ecological Hazards of Engineered Nanomaterials

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Abstract

Engineered nanomaterials (ENMs) are increasingly entering the environment with uncertain consequences including potential ecological effects. Various research communities view differently whether ecotoxicological testing of ENMs should be conducted using environmentally relevant concentrations—where observing outcomes is difficult—versus higher ENM doses, where responses are observable. What exposure conditions are typically used in assessing ENM hazards to populations? What conditions are used to test ecosystem-scale hazards? What is known regarding actual ENMs in the environment, via measurements or modeling simulations? How should exposure conditions, ENM transformation, dose, and body burden be used in interpreting biological and computational findings for assessing risks? These questions were addressed in the context of this critical review. As a result, three main recommendations emerged. First, researchers should improve ecotoxicology of ENMs by choosing test endpoints, duration, and study conditions—including ENM test concentrations—that align with realistic exposure scenarios. Second, testing should proceed via tiers with iterative feedback that informs experiments at other levels of biological organization. Finally, environmental realism in ENM hazard assessments should involve greater coordination among ENM quantitative analysts, exposure modelers, and ecotoxicologists, across government, industry, and academia.

Introduction

As nanotechnology (the synthesis, manipulation, and measurement of matter at 10^{-9} meter scales)[1] rapidly evolves,[2] engineered nanomaterials (ENMs) are entering air, waters, soils,[3] and sediments where they could adversely affect organisms to ecosystems.[4-6] Actual environmental impacts of ENMs have not been documented, and there are uncertainties about the potential for, and how to evaluate, impacts.[7]

ENM ecotoxicology elucidates hazards and their mechanisms.[8] The scope overlaps with conventional ecotoxicology, although ENMs are particulate and diverse,[9] with varying cores, native or acquired surface chemistries,[10] conditional agglomeration or dissolution,[11] and size- plus composition-dependent electronic properties,[12] affecting their reactivity and biological interactions.[13]

Focusing ENM ecotoxicology invokes exposure scenarios[14, 15] relevant to ENM production,[16-19] use,[20-24] disposal,[25, 26] and product release (Fig. 1).[14, 27-31] Scenarios consider environmental fate and transport,[15, 32-39] bioavailability,[40, 41] and ENM uptake into ecological receptors.[42-46] Scenarios also specify processes[47, 48] and ecosystem services[49] such as food production.[50, 51]

In conventional chemical toxicology, observed and perceived exposures often diverge.[52] In ENM ecotoxicology, water is emphasized, while soil and sediment impacts have received less attention.[53] Where do exposures occur? What ENM forms[54] and quantities are involved? Which ecological receptors are affected?[55] Which local exposure conditions prevail? As with conventional chemical risk assessment, such questions unite hazard and exposure assessments.[15, 56]

Standardized test regimens do not derive from scenarios, since ENM test conditions are predefined (e.g., aqueous chemistry, temperature, pH, experiment duration, among others) for standardized endpoints.[57] Ideally, ENM hazards are studied at realistic exposures for ecologically relevant receptors. An example would be studying real soils under controllable yet realistic conditions, i.e. in greenhouses[58] or lysimeters.[59] However, requiring absolute realism in all ENM ecotoxicology would pose scientific challenges associated with: measuring ENMs analytically in environmental media; measuring toxicity across a representative range of environmental conditions; characterizing environmental ENM forms and their transformations so that toxicity is measured for representative materials; ENMs altering physical or chemical exposure media conditions; and few efficient approaches for estimating hazards and exposures necessary to evaluate risks before ENM products develop. Another challenge is internal to the scientific community: multiple dissimilar working definitions of environmental relevance intruding on scholarship, including peer review.[53] Environmental relevance remains undefined, leading to categorization of research around a few selected concepts.[60]

Previously, over 600 published studies were examined to compare modeled or measured environmental concentrations of ENMs versus concentrations administered in ENM ecotoxicity assessments.[53] The study found nominal concentration disparities, but also infrequent testing at low ENM concentrations. The study noted uncertainties in ENM exposure modeling, and that other toxicity testing conditions beyond ENM concentration—including aqueous chemistry, biological receptor, system complexity, and ENM form—relate to real-world conditions. However, the study did not establish what constitutes environmental relevance in the ecotoxicology of ENMs.[53]

Issues regarding environmental detection, uncertain concentrations, and unknown toxicity are not unique to ENMs; they are also raised for other emerging contaminants.[61] However, because the ENM industry is rapidly evolving and scientists seek to assist in advancing environmentally safe nanotechnology, environmental relevance in ENM hazard assessment should be prioritized. To accomplish this, representatives from academia, industry, and government regulation working in ecotoxicology, exposure modeling, and social science (Table S1) addressed four questions within this critical review: (i) What exposure conditions are used in assessing ENM ecotoxicity potential for model organisms? (ii) What exposure and design considerations drive mesocosm experiments for assessments of ENM environmental hazards? (iii) What is the state of knowledge regarding ENM environmental exposure conditions, via measurements or modeling simulations? (iv) How should concepts such as exposure conditions, ENM transformation, dose, and body burden be used in interpreting biological and computational findings for assessing risks? The main objective was to provide context and guidance to the meaning of environmental relevance (singular or multiple meanings) in ENM environmental hazard assessment. This critical review addresses the four motivating questions and expands on detailed topics that emerged during the project (Supporting Information). For each question, there are findings and recommendations. These serve to crystallize what is meant by environmental relevance in ENM ecotoxicology, and further coalesce ENM environmental exposure and hazard assessment endeavors.

What exposure conditions are used in assessing ENM ecological hazard potential for model organisms?

Findings

Many systems, approaches, and conditions (including standardized testing with model media, organisms, and endpoints)[62] have been used to assess ENM ecotoxicity.[63] The applicability and challenges of standardized testing protocols under aquatic conditions have been reviewed[57, 64] and vetted in workshops.[62] Also, the OECD reviewed and vetted its guidelines for testing ENMs[65] in an expert meeting.[66, 67] Readers are referred to those reports for deliberations of ENM standardized testing.

Research studies include laboratory-specific low-[68, 69] and high-throughput dose-response evaluations using select media with ENM compositional and receptor variants[70] for assessing uptake[71-73] and effect mechanisms.[69, 74, 75] Investigations include mechanistic gene transcriptional,[76-79] DNA damage,[80] metabolomics profiling,[81] and transgenerational[82, 83] experiments. Bottle-scale microcosms partially simulate limited levels of environmental complexity,[84] e.g. in using natural soils or sediments with[58] or without[85, 86] plants and associated rhizosphere influences,[87] or using seawater[88] or marine sediments[89, 90] and associated receptors based on expected ENM compartmentalization.[91] Non-standard microcosms assess ecological endpoints, e.g. microbial community composition[85, 92] and function[92] related to C and N cycling.[92, 93] Environmental factors are examined, including how ENMs interactively affect soil water availability[94] and soil bacterial communities.[95] Single-species experiments that assess ENM bio-association[96, 97] or bioaccumulation[71, 98] precede and motivate using microcosms for assessing dual species trophic transfer[98-101] and potential biomagnification.[102]

Pristine ENMs, including those with surface functionalization, capping agents, or adsorbed species or coatings,[103] are the most frequently assessed, although released and transformed versions are increasingly studied.[28, 30, 104] Results for textiles, paints, and nanocomposites[105] suggest that released particles significantly transform and age, and exhibit different environmental behavior and effects compared to pristine ENMs.[106, 107] Assessing changes in form and associated behavior or activity across the material's life cycle are uniquely challenging.

Nominal test exposure concentrations vary widely[53] (Fig. S1) and are sometimes related to scenarios such as repeated applications,[108] accidental spills,[109] or ranges over a spatial gradient.[110] High exposure concentrations may be used:[111] for assessing bioavailability in soil[58] in comparison to simpler media, or to accommodate analytical instrument detection limits. High concentrations are also used to establish no-effect limits, using limit tests; if an effect is not observed, the ENM is assumed to be non-toxic at lower concentrations,[62] although effects could occur at longer exposure times. However, this approach is problematic if agglomeration and sedimentation of ENMs are concentration dependent, or if effect mechanisms do not scale with concentration, which might occur if organisms adaptively respond to toxicants.

Some challenges to ENM ecotoxicology are familiar to conventional chemical ecotoxicology while others are unique. With conventional chemicals, toxicity is related to the effective dose of a toxicant molecule crossing the cell membrane and disrupting essential processes. However, ENMs can exert effects as particulates (e.g., physical effects) and in a molecular or ionic form depending on the dissolution extent in the medium. The effective ENM dose—which impacts the assay results[112]—may be unknown or changing because it varies with media conditions or with dynamic physicochemical interactions of receptors and toxicants.[113] One effective dose metric may be insufficient to characterize an observed effect that could be related to both a physical interaction of the ENM particulate and the dissolved ionic form. Also, bioavailability and effective ENM doses[114] change because ENMs can transform abiotically[115] and biotically[116, 117] during assessment. While such influences on stability exist in conventional ecotoxicology, stability in a particulate exposure must also consider the ENM size distribution, and potential physical changes to the ENM such as dissolution and agglomeration. Therefore, effective and nominal doses may or may not be related.

Although multiple ENMs may co-occur in product formulations, different ENMs are infrequently studied together [118] or with co-contaminants.[119-121] ENMs can conditionally sorb and modulate the toxicity and uptake of other contaminants and vice versa.[122-134] ENMs can acquire coatings[135, 136] such as natural organic matter (NOM),[137, 138] and age with varying pH and sunlight.[139, 140] Assessment outcomes are affected by media chemistry, physical characteristics, and additives (e.g., soil pH,[141] temperature,[142] or organic matter content,[108, 143] and dispersing agents in aqueous media[144]). The exposure concentration at the receptor and consequent effects[145] depend on ENM dissolution[146] perhaps assisted by biotic ligands,[147] and speciation,[103] shed ions,[103] surface associations,[148] and heteroaggregation with colloids and particulate matter,[148-151] or agglomeration and settling.[152] All of these vary with ENM types and their varying properties, and ambient conditions.[148] Changes in ENM properties may change bioavailability[94, 153] and toxicity.[154]

Various test durations and endpoints have been studied, from acute responses measured by standard test protocols[141] to short term microbial biodiversity and community composition effects[108] or plant genotoxicity[111, 155] and nutrient composition changes.[156, 157] Multiple endpoints, toxicant characterization methods, and experimental controls increase assessment comprehensiveness[158] and reduce artifacts and misinterpretations.[152] Experimental controls based on coatings and dispersants enable determining if the apparent toxicity is attributable to the ENM itself or to ligand or surface groups. Yet whether and how experimental controls are used varies widely, for example metal salts that allow interpreting biological responses relative to ENM dissolution products versus intact ENMs.[159, 160]

The appropriate analytical method for quantifying, locating and characterizing organism-associated ENMs depends on ENM chemistry and amounts or concentrations. For ENMs exhibiting fluorescence or for ENMs containing heavy elements, high resolution microscopy can assess biological uptake and compartmentalization.[99, 102, 161-163] X-ray synchrotron methods can sensitively locate bioaccumulated metal oxide ENMs[164] and their transformation products in biota.[117, 165] No single analytical tool is suitable for all ENMs; however, the general lack of methodologies that can be routinely implemented to quantify ENM exposure in complex matrices continues to be a major challenge to the fundamental understanding of ecological effects.

Alternative testing strategies (e.g. miniaturized screening assessments using environmentally relevant bacteria)[166] can simultaneously assess many material types, controls, and concentrations,[70, 75] which is useful for ENMs not available in sufficient quantities for microcosms. However, the potential for interferences in screening assay performance, depending on the specific ENM properties and toxicity test conditions,[167] are increasingly recognized, and therefore should be controlled or accounted for in study designs.[152, 168]

To date, ENM ecological hazard assessments have not adequately explored numerous well-conceived and plausible exposure scenarios that are founded in theory, hypothesis, mechanism and occurrence probability; yet scenarios increase certainty and predictability when addressing nanotechnology-related material safety. For instance, hazard assessments have been mainly skewed towards as-produced ENMs without full consideration of potential aging, since aging cannot be fully standardized in a realistic context. However, such studies can and should further be conducted. Biological uptake, and the compartmentalization and speciation of ENMs, are still infrequently studied, limiting possibilities for attributing ENM exposure to effects at biological receptors.[9] Varying degrees of rigor have been applied in designing and incorporating controls that are relevant to experimental questions or hypotheses. Varying degrees of attention are paid to experimental artifacts. While great advancements have been made in ENM ecotoxicology, improvements are needed to increase the environmental relevance of future research.

Recommendations

To understand hazards for plausible exposure initiation scenarios, assessment conditions will need to depart from standardized testing protocols.[62, 169, 170] It may be helpful to link or compare data obtained for ENMs under plausible scenarios to those obtained with standard methods using standardized media (e.g., standard soil mixtures) to facilitate interpreting complex multivariate experiments or comparing results among multiple laboratories. Herein, several recommendations regard exposure conditions that should be used in assessing ENM ecotoxicity (Table 1).

Adequately characterize exposure conditions.

Soil ecotoxicity studies should specify the soil taxonomy[171] and characteristics such as pH, clay content, and organic matter content, using standard methods.[108, 172] Similarly, characterization of sediments should be provided. Natural soils and sediments are preferred, because artificial media do not harbor natural soil communities, and do not reflect chemical and physical characteristics (e.g. of specific surface area related to water holding, or of organic matter content) that influence ENM effects and bioavailability. Water characteristics determine ENM agglomeration, dissolution, and other behaviors affecting aquatic system compartmentalization (e.g., residing in the water column or depositing into sediments)[91] and hence need definition. When adequately documented, media characteristics can be used retrospectively to interpret conditional ENM bioavailability or effect mechanisms.

Determine and report the form and concentrations of ENMs.

ENMs in testing should represent the particulate material form relevant to the environmental scenario (i.e., as-produced materials near manufacturing sites, or as-released with associated product surface coatings or with relevant co-contaminants in waste streams, including industrial byproducts, Fig. 1). ENMs should be fully characterized and their history (including storage conditions and time, or other uses that may affect compositional or physical characteristics) should be adequately described to allow comparing between studies. For toxic coatings,[136] coating identity and degree of coverage should be related to observed effects. Impurities in ENMs introduced during product synthesis and handling should be characterized, since they can sometimes account for apparent ENM ecotoxicity.[152] Nominal concentrations should scale according to exposure scenarios, or to specific objectives such as mechanistic research or quantifying biotic uptake. Dose verification, including size distribution and ENM concentration, is also desirable, although heretofore challenging in soil and sediment exposures.

ENM physicochemical changes during release and in the environment should be studied to uncover properties of ENMs that reach biological receptors. All potential forms of ENMs, including transformation products and residual reagents used in synthesis, should be accounted for, such that all toxicants can be related to biological responses.[152] Study designs should anticipate dispersing agent effects[144] and the nature of transformed ENMs plus co-contaminants, by including controls to account for effects, fate, and kinetics of ENMs in the test medium.[154]

The ENM physicochemical states should be understood before conducting hazard assessments.[173, 174] This is important because some ENMs agglomerate or dissolve or otherwise change in laboratory media, resulting in non-uniform exposures and uncertain bioavailability. Such unevenness precludes relating measured effects to the applied dose. Spatial bio-association, bioaccumulation,[175] and intra-organism compartmentalization should be assessed (e.g., through imaging and mapping with sufficiently high resolution[117]) to locate ENMs and their components.

Relate biological receptors to the exposure scenario.

Important advances have been made toward characterizing the physicochemical factors influencing ENM behavior in environmental and test media, and towards utilizing that information to develop standardized methods for conducting ENM ecotoxicity testing.[62, 64, 67, 152] However, aquatic or terrestrial species[8, 159, 166] —and even different species belonging to the same order[176] —respond differently to ENMs using the same tests. Thus, test species and endpoints should be carefully chosen to enhance the relevance of ENM ecotoxicity testing. Some complex interrelationships and dependencies between species comprising ecosystems have been described.[8, 159, 177] However, focused research could rationally identify species for routine evaluations; likewise, the scientific rationale behind test species should be reported. Ecosystems are more complex than conditions of routine ENM ecotoxicity evaluations. Thus, research should define an optimal suite of test species and endpoints to determine the ecosystem response to a given ENM.

In general, biological receptors should be chosen for expected exposures stemming from realistic exposure scenarios. For example, relatively insoluble ENMs may, depending on their density, size and agglomeration state, rapidly settle out of suspension and associate with aquatic sediments. In that case, initial hazard assessment could focus on benthic, rather than pelagic, receptor organisms.[62] Conversely, for ENMs that rapidly dissolve under environmental exposure conditions, conventional ecotoxicological exposure scenarios may be applied and receptors chosen to assess dissolution product toxicity. However, ENM dissolution rates vary, and pelagic organisms can be more sensitive than benthic organisms.[100, 101] Thus, both ENM compartmentalization and form must be accounted for when choosing receptors.

Select endpoints relevant to appropriate receptor and exposure scenarios.

Multiple effects measurements[152] should be applied to answer research questions. Rapid screening assessments should be prioritized within a testing strategy (Fig. 2).

Mechanistically understanding overt toxicity is needed, which may require measuring more omics[81] endpoints (i.e., gene transcripts, proteins, and pathway metabolites) and choosing variables for developing mathematical models to predict toxicity at untested concentrations or conditions.[8] Omics technologies can also identify potential modes of action (MOAs) that are conserved among different species. However, different scientific communities will have varying preferences in defining needs for omics-level investigations.

Perform adequate dosimetry to understand exposures.

Effects interpretation requires understanding the effective toxicant dose or other (perhaps indirect) basis of impacts.[9] For ENMs, the mass concentration basis of dosing may relate only partially to the effective applied dose, since biological effects often originate from surface interactions with receptors.[178] Furthermore, ENMs are more complex than conventional chemicals because ENM shape, aggregation state and surface area may influence toxicity.[67, 178-182] Thus, surface area applied has been suggested as a supplemental dosing metric.[178] However, ENM surface area in suspension/solid media is not a straightforward assessment given that ENMs may aggregate with a size distribution that is affected by the medium in which they are dispersed. In addition, coatings, either on pristine ENMs or acquired in the test media or environment, may alter toxicity.[104, 183]

ENM amounts and forms effecting biological impacts should be understood and related to the administered dose to inform environmental risk assessment.[9, 67, 184] This is the essence of dosimetry in ENM ecotoxicology. As with other exposure concerns related to hazard assessment, appropriate dose measurement depends on receptor and ENM characteristics, which are scenario-dependent. For example, mammalian cells are harmed by ENMs that become internalized, yet uptake pathways depend on ENM characteristics.[185] Then again, bacterial receptors that affect ecosystem-level processes may be impacted by externally-associated ENMs at the cell membrane, or even in the surrounding environment. In those cases, dosimetry relies on understanding ENM behavior in the complex media in which bacteria reside (e.g., soil, water, sediments, and extracellular polymers[186, 187]), which is scenario-driven. Endpoint observations of ENM damage will also depend on ENM processing in cells. During hazard assessments, understanding the history of biological interactions with internalized, or otherwise associated ENMs may not be feasible. Yet efforts should be made to measure and spatially associate ENM bioburden (quantity and form) within biological receptors, and to examine the relationships of applied ENMs to apparent effective dose and to effects.

Summary

Overall, it is not recommended to categorically exclude select conditions, environmental compartments, protocols, receptors, or endpoints, since any may be environmentally relevant. Rather, careful experimental designs around well-conceived, plausible exposure scenarios should be emphasized; also, ENM characteristics that influence biological responses under the dynamic conditions that occur in the environment and in biota should be characterized and quantified. One could imagine identifying key material-environment system determinants that could be systematically varied to provide test results across relevant determinant ranges. Such ideas are not specific to ENM ecotoxicology, but could establish defensible practices for making progress in hazard assessment while the ENM industry rapidly advances.

What exposure and design considerations drive mesocosm assessment of ENM environmental hazards?

Findings

Mesocosms are “enclosed experimental systems [that] are intended to serve as miniaturized worlds for studying ecological processes.”[84] While the distinctions between mesocosms and other experimental systems are not well delineated, mesocosms are generally larger experimental units and inherently more complex than bench-top microcosms or more simplified laboratory experiments.[84, 188-191] Mesocosms for ENM ecotoxicology are intended to increase the complexity of experimental systems, such that more realistic ENM physical compartmentalization, speciation,[117] and uptake into biota[58, 192] can be achieved alongside biotic effects.[89, 177] Also, the intent is to realistically characterize ENM fates and interactions with environmental system components, and to reveal fluxes among compartments of the ecosystems (e.g., the aqueous phase, sediments, and biota in aquatic systems) responsive to internal system influences that are unconstrained by investigator interventions.[25, 177, 193]

Mesocosms have been used for testing relative biotic effects of ENM variants (e.g., surface functionalization, capping agents, adsorbed surface species or coatings),[177] and discerning ENM effects separately from effects of dissolution products (ions or complexes).[194] Mesocosm testing may occur following individual organism and microcosm studies (Fig. 2). For example, to study how ENMs impact crops, one could first establish the potential for hydroponic plant population impacts,[111] use soil microcosms to understand ENM bioavailability via observing soil microbial community shifts,[85, 93] and then scale up to greenhouse mesocosms of soil-grown crops. This sequence could provide an understanding of plant-microbe interactions,[58, 87] ENM transformation and uptake in plants,[117] and effects on food nutritional quality.[157] Still, there are relatively few published studies using mesocosms to assess ENM ecological hazards,[53] and the design and operating variables (including size, media, biota, length of study, and ENMs tested) of existing mesocosm studies are wide ranging.[60]

By contrast, wastewater-associated ENMs,[195, 196] and their transformations,[197-199] effects, and fates[200] in wastewater treatment plants (WWTPs),[201, 202] along with the potential for ENMs to impact WWTP processes,[48, 203, 204] have been more extensively studied. Since sewage contains ENMs, WWTPs are inherent forms of mesocosms.[195, 205, 206] Studies at entire WWTP scales elucidate ENM fates during wastewater treatment, including significant association with biological treatment biomass[201, 207] that becomes biosolids.[76, 208] However, only 50% of biosolids produced in the U.S. are land-applied, and these biosolids are used on less than 1% of agricultural land in the U.S. (http://www.epa.gov/biosolids). Biosolids are land-applied even less in the European Union.[209] Thus, knowledge of ENM fates in WWTPs and how final residues are disposed regionally are needed to develop plausible exposure scenarios.

Concerns with mesocosms include factors that can be difficult to control (e.g., pH or oxygenation) and that mesocosms may respond to artifacts including “wall” or “bottle” effects.[84] Further, mesocosms can conflate direct and indirect toxicant effects, typically do not have a full complement of control conditions, and deliver inconclusive results (e.g., where bioaccumulation may be explicable by either direct toxicant uptake or by trophic transfer). Biological communities in mesocosms also lack realistic ecological interconnections, interactions, and energy flows. Nevertheless, outcomes can be improved by using carefully designed mesocosms and associated experiments.[84] For example, combined with analyzing mesocosm samples, performing practical "functional assays"[38] such as for heteroaggregation,[210] allows for anticipating phenomena and later interpreting ENM transformation and compartmentalization in mesocosms.[177] Similarly, batch physical association experiments—if conducted using realistic components, and over time frames that allow for quantifiable mass transfer—can assess ENM biomass association and readily suggest ENM fates in WWTPs.[207] Still, hydrodynamic conditions are different in simplified tests versus mesocosms, which are different from those in the natural environment. Hydrodynamic conditions will impact ENM fate and transport and thus exposure concentrations at receptor boundaries. The inability to capture real environmental hydrodynamic conditions in any experimental scale is a general shortcoming for both ecotoxicology and transport studies.

Recommendations

Although mesocosms do not fully simulate real environments,[84] mesocosms are useful and should be employed, albeit judiciously due to their resource intensity, within a strategy (Fig. 2). Recommendations regarding using mesocosms for assessing ENM environmental hazards are provided in Table 2.

Mesocosm studies must be designed and conducted around well-conceived questions related to plausible exposure scenarios; they should use select endpoints, potentially including sensitive omics measurements,[211] to answer questions or test hypotheses.[84] Internal process and constituent characterization should be thorough and equally responsive to well-conceived, realistic scenarios. Functional assays, i.e. “intermediary, semi-empirical measures of processes or functions within a specified system that bridge the gap between nanomaterial properties and potential outcomes in complex systems”,[38] should precede mesocosm designs and experiments, and aid interpreting mesocosm results (Fig. 2).

Mesocosm artifacts are avoidable by following best practices for design and operation, although possible interferences of particulate material testing with assays must be evaluated.[84] As for other hazard assessments, ENMs should be tested across the product life cycle, (i.e., ranging from as-produced to released from products) within a motivating exposure scenario. Similarly, suitable material controls should be used to test hypotheses regarding ENM-specific effects (Table 1).

The recommendations made regarding exposure conditions in assessing ENM hazard potentials for model organisms (including in microcosms) should be followed for mesocosm studies (Table 1). Additionally, mesocosm designs should incorporate exposure durations, which should be sufficiently long to address population growth, reproduction, bioaccumulation, trophic transfer, and possibly transgenerational effects. Sufficient measurements of ENM concentrations and time-dependent properties must be made for clear interpretations.

Key to successfully interpreting mesocosm studies is using validated methods for measuring ENMs in complex media. Measurements should include the size distribution, concentration and chemical composition of ENMs in the test system, including biological tissues,[161, 212-214] over time.[215] In some cases, transformation products are inventoried thoroughly during long term field-relevant exposures.[193] Detection schemes require sample preparation to assess *in situ* exposures (e.g., aqueous or solvent extractions) before quantitative analyses, or drying and embedding before visual confirmation by electron microscopy. The potential for artifact introduction should be recognized. Recovery methods continually develop, such as cloud point extraction for concentrating ENMs from aqueous matrices.[216, 217]

Depending on the exposure scenario, *in situ* aging may be a study objective. However, it is important to define what 'aging' really means and the specific application domain, since 'aging' is a wide-ranging term and can be used in different contexts, making comparisons impossible. At least, studies should be undertaken over sufficiently long time frames (e.g., acknowledging time scales of soil processes[141, 218]), which may include repeated ENM applications,[108] such that appropriate aging, i.e. time-dependent transformation under realistic conditions, could occur. Alternatively, pre-aged ENMs could be used. However pre-aging protocols (whether in the context of product use conditions or under environmental conditions) are not yet standardized and, while some convention could allow for comparing across studies, the appropriate aging protocol would depend on the envisioned exposure scenario.

Co-contaminants should be considered and potentially introduced into mesocosms, since some ENMs sorb, concentrate, and increase exposure to other contaminants.[130] Select endpoints should account for ENMs as chemosensitizers.[119, 120, 219, 220] Also, mesocosm study designs should anticipate and plan for measuring secondary effects (e.g., rhizosphere microbial shifts that affect plants[87] and soil nutrient turnover).

In summary, while few mesocosms have been used in assessing ENM ecotoxicity and are also rare for conventional chemical testing, such systems potentially offer greater realism. Still, mesocosm exposure and design considerations should derive from immediate environmental applicability. The value of mesocosms to ENM ecotoxicology can increase by following recommendations including: addressing context-dependent questions while using relevant endpoints; considering and minimizing artifacts; using realistic exposure durations; quantifying ENMs and their products; and considering ENM aging, co-contaminants, and secondary biological effects (Table 2). Further, it should be acknowledged that mesocosms do not fully recreate natural environmental complexity. For example, aquatic mesocosms do not recreate actual environmental hydrodynamic, or temperature cycling, conditions. Hydrodynamics can significantly impact ENM aggregation or heteroaggregation, and fate and transport (e.g., sedimentation, dissolution, etc.). Therefore, potential impacts on the resulting concentrations at the receptor boundaries should be considered.

What is the state of knowledge regarding ENM environmental exposure conditions, via measurements or modeling simulations?

Findings

ENM environmental exposure conditions herein refer to where, how much, and in what forms ENMs may occur in the environment. These are central issues for ecotoxicology of ENMs because they suggest test exposure scenarios[14, 15, 18] in which ENMs could impact biological receptors within environmental compartments (e.g., soil, sediments, air, and water) influenced by various factors (e.g., pH, redox potential, aeration, temperature, and ionic strength). These issues also influence outcomes of key regulatory concern: persistence, bioaccumulation, and toxicity.

In the parlance of exposure science,[221, 222] three knowledge domains inform ecotoxicology, including the ecotoxicology of ENMs[6]:

1) "far-field"[223] "environmental science"[222] regarding contaminant release, transport, fate, and transformation external to biological receptors;
2) "near-field"[223] exposures at the receptor that lead to biological outcomes, as affected by contaminant bioavailability, affinity, uptake, accumulation, and biological transformation; and
3) "at point" responses and outcomes that occur at the biological receptor and include toxicity (Fig. 3).

Discharges (locations, types, and amounts) underpin exposure scenarios,[14, 15, 37, 224] are initiated by situational contaminant releases (Fig. 1), and are referred to as source terms. Mass balance-based multimedia simulations[225] mathematically account for released contaminants as they are transported and exchanged between environmental media, where contaminants may be transformed and may ultimately concentrate, potentially with altered compositions and structures (Fig. 3).

Far-field exposure modeling approaches vary by question, the modeling purpose (e.g., regulatory compliance, priority setting, research, material design, or industrial use), the required spatial resolution (e.g., a site or region, and the need to resolve environmental heterogeneity), the temporal conditions (e.g., steady state, dynamic, or episodic), and the predictive accuracy required.[56, 226] Material Flow Analysis (MFA), which is a type of life-cycle inventory analysis, has been advanced to track ENM flows through various use patterns into volumes released into broad environmental compartments,[227] scaled to regional ENM concentrations that release via WWTPs to water, air, landfills, and soil.[227]

Such models estimate exposure concentrations in part via engineering assumptions and in part via heuristics (i.e. expert judgment regarding the potential amount released to various media).[228] Also, such material flow analysis models depend on the underlying data (e.g. production and release estimates) which are not readily available, making it difficult to validate model results and potentially leading to inaccurate estimates.[32, 228]

Multimedia models for ENMs[32, 37, 228-230] can predict environmental concentrations based on sources of continuous, time-dependent, or episodic releases[32, 228] and are similar to multimedia models that predict environmental concentrations of organic chemicals[231-233] and particle-associated organic chemicals.[233, 234] For ENMs, predicting particle size distribution—as affected by particle dissolution, agglomeration, and settling—is desired for various spatial and temporal endpoints. For one integrated MFA and multimedia model (MendNano and LearNano), user-defined inputs are flexible around product use and ENM release throughout material life cycles.[228] It is noted that although validation of multimedia models is a formidable task, various components of such models have been validated as well as model predictions with such models for particle-bound pollutants.

Most far-field[223] models of ENMs have major challenges. First, the quantities and types of ENMs being manufactured are unknown to the general public due to issues surrounding confidential business information, leading to a reliance on market research.[53] The resulting public uncertainty will persist while nanotechnology continues a course of rapid innovation, as is typical of new industries.[235] The rates of product use and ENM releases at all life-cycle stages (i.e., manufacturing, waste handling, product use, and product disposal) are also not defined.[225] There are challenges associated with modeling transport processes through specific media and across media (i.e., water, soil, air), highly divergent time scales of processes, lack of required input parameters, and the need for validation of results (e.g., through measurements of ENMs in the environment).[226] Several multimedia models developed for conventional chemicals could be adapted around ENMs, but few account for fate processes specific to nanoparticles (e.g., never in thermodynamic equilibrium).[37, 236] In addition, various transport models for a single medium and in the multimedia environment could be adapted for far-field analysis of ENMs, but few account for fate processes distinctive to ENMs (e.g., considering the complexity of ENMs aggregating and that their transport is governed by physical rather than thermodynamic driving forces).[37, 236] Moreover, their validation, which would require ENM monitoring data, is a major challenge.

The lack of understanding of many fundamental ENM behaviors under environmental conditions propagates into broad uncertainties, for example in predicting ENM removal to solids or aqueous fractions in WWTPs.[225, 227] ENM surface chemistries fundamentally affect ENM agglomeration or dispersion[237] and likely affect bioavailabilty.[94] Some species on ENM surfaces may degrade in the environment,[238] while other adsorbates can be acquired.[239, 240] Carbonaceous ENMs may be transformed or degraded by environmental processes such as photo-,[241, 242] enzymatic,[243, 244] chemical,[245] and bio-degradation.[246] Redox and other environmental conditions will affect nanomaterial surfaces, which for nano-Ag includes formation of sulfide that inhibits dissolution.[39, 247] Surface chemistry also affects transformation rates of primary particles and aggregates (e.g., in WWTP biological processes).[248] For many ENMs such as nano-ceria,[249] reactivity is highly size-dependent. To accurately model material fates thus requires understanding how material surface properties affect integrity, how both change under varying environmental conditions such as pH, clay content,[250] and organic matter content,[143, 251] and how surface properties and particle reactivity affect physicochemical processes that are parameterized in far-field models. This is especially true for ENMs used as pesticide delivery mechanisms, including carbon nanotube composites with specifically-reactive surface monomers. Yet only recently has modeling attempted to address differing properties of a material's structural variants (e.g., photocatalytic versus photostable nano-TiO_2).[230]

Evaluating computational model predictions is a challenge for ENMs, which presently are estimated to occur in the environment at low (ng/L, to mg/L) concentrations.[32, 230, 252-254] Also, detection methods for ENMs in environmental media and distinguishing ENMs from natural chemical analogs are still under development,[161, 255-257] with more evaluation strategies needed including a framework for validating new ENM analytical detection methods.[215] Fullerenes from incidental sources (combustion products) were quantified in river sediments collected from locations across the globe[258] and quantified in the atmosphere over the Mediterranean Sea.[259] Perhaps related to a viable exposure scenario, fullerenes were quantified at relatively high concentrations in treated wastewater effluent[260] and at ng/L to μg/L[261] concentrations in river waters receiving effluent discharge. While not necessarily nanoscale, similarly high concentrations of TiO_2 were reported for sediments sampled near a WWTP outfall.[262]

The greatest uncertainty in ENM exposures is near-field (Fig. 3), at the receptor where toxicant dose manifests as internal dose. Heteroaggregation is a dominant fate process for ENMs when they interact with natural colloids.[37, 263-265] Given sufficient residence time for ENMs in environmental matrices, heteroaggregation (i.e., kineticallycontrolled association with colloids) and to a lesser degree homoaggregation will affect localized compartmentalization, including stability in the water column and therefore, sedimentation.[177] However, these processes do not preclude biological impacts under simulated environmental conditions, as has been shown for nano-ceria in a complex aquatic mesocosm.[177] Exposure can be confirmed by quantifying receptor body burdens, thereby allowing for quantitatively relating near-field exposure to biological effects.[177] Thus, in the absence of detailed, biologically complex, near-field models for local exposures to environmental receptors, the ability to trace ENMs to biological receptors sampled directly from the environment becomes the best available approach to relate far-field exposures to biological impacts.[177]

Overall, material flow models and multimedia modeling of ENMs have advanced to inform ENM ecotoxicology. Available far-field modeling frameworks are adaptable to changing inputs despite uncertainties in production volumes. Major uncertainties remain at the nexus of ENM surface and core chemistries as related to nanomaterial transport, aggregation, and degradation characteristics. However, fundamental research (e.g., of conditional hetero- and homoaggregation, and of dissolution) is needed to discover and parameterize complex fate processes. New approaches, such as assays that can be used to rapidly probe surface associations,[38] demonstrate how to populate far-field models and how to determine near-field exposures associated with effects. Although existing models can simulate particle movement, deposition, and some transformations, the knowledge state regarding ENM environmental exposure conditions via measurements or modeling simulations cannot be assumed to accurately represent actual conditions at biological receptors.

Recommendations

Despite significant progress in modeling potential ENM environmental distributions, several key constraints exist. Models predict the average concentration within broad compartments such as soils, sediments, water, or wastewater, even when constrained to regional releases. Actual concentrations at biological exposure sites are typically unknown, but can be constrained within upper and lower bounds. Due to the potential for bio-association, bioaccumulation, and hetero- or homoaggregation, predicted environmental ENM concentrations may inaccurately estimate bioactive concentrations at receptors. Following are recommendations for using measurements or modeling simulations to understand conditions of ENM environmental exposures.

- The limitations of modeling approaches should be recognized more broadly, such that generic model predictions are not used to rigidly define or judge ecotoxicity testing conditions.
- Modeling should be simplified where possible and coupled with evaluation and iteration while varying assumptions to develop an understanding of the sensitivity of modeling parameters.
- Meteorological conditions play an important role in ENM transport, and thus should be carefully documented when assessing model predictions relative to monitoring.
- ENM surface characteristics relevant to the receptor and the exposure context should be considered by modelers and experimentalists. Considerations should include: which ENM characteristics prevail near receptors, how this changes in the environment, and how particle stability, migration and bioavailability are affected.

- Batch experimental approaches to rapidly assess relationships between environmental factors (e.g., pH, redox potential or aeration, ionic strength, and organic matter content) and ENM physicochemical characteristics that affect fate processes (e.g., homo- and heteroaggregation, sedimentation, and dissolution) could be more routinely incorporated into assessments (Fig. 2), to justify further testing and for interpreting other testing results.
- Improvements in quantifying ENMs in complex media, including biological tissue, are needed to validate multimedia models and to determine near-field exposures to biological receptors.
- Improved fundamental understanding of how ENM variants (e.g., in transformable coatings, shapes, sizes, or primary chemistry) comparatively migrate under varying environmental conditions can be advanced through research that intentionally develops and uses relatively stable ENMs as tracers.[188, 189, 266]

In summary, understanding exposure conditions should continue to improve from fundamental experimental research of ENM environmental behaviors. Modeling should continue to improve with such understanding, and avenues for model evaluation should continue to grow. Environmental relevance in ENM ecotoxicology need not be defined or constrained by model outputs, especially given recognized uncertainties and the need for continued model evolution. Lastly, echoing prior recommendations,[15] the efforts of exposure modelers, analytical methods developers, and ecotoxicologists should continue to influence each other for maximum individual and mutual benefit.

How should concepts such as exposure conditions, ENM transformation, dose, and body burden be utilized in interpreting biological and computational findings for assessing risks?

Findings

Many of the outstanding research issues and recommendations for evolving ENM ecotoxicology (Tables 1 and 2) are echoed in the discourse for other chemicals of emerging concern (CECs).[267] These include the need for systematically understanding ENM and decomposition product toxicity across various receptors within linked levels of biological organization,[8, 268, 269] quantifying actual exposures[270] and uptake[271] into environmental receptors,[57, 161] gaining mechanistic insights into and biological markers for acute and chronic low level exposures,[272, 273] and understanding how environmental factors including co-contaminants affect ENM transformation[274] and biological impacts. Still, how can the potential for exposure and impacts of ENMs be anticipated, prevented, managed, or mitigated? Further, what data and tools do decision makers need to inform their work? Innovation in nanotechnology hinges on having the science to evaluate ENM safety.

While no formalized process for incorporating all exposure conditions and concepts of ENM transformation, dose, and body burden into risk assessments currently exists, a proposed framework approach to risk characterization over the life cycle of ENMs has been published and is available.[63] This framework advocates an initial decision cut-off in regards to exposure; in the absence of exposure, the need for further assessment is diminished or negated.[26, 63] In this available framework, ENMs that are certain to rapidly dissolve into ionic components in a destined environmental compartment would be assessed for risk based on the released components rather than the original nanoparticles.[63] Persistent

ENMs are expected to accumulate in matrices such as sediments.[63] The consequences of ENMs to successive generations, biodiversity, and ecosystem services[49] are not addressed by model organism-specific assays of discrete growth and mortality.[8, 63] Nonetheless, in this available framework, toxicity endpoints associated with standardized testing protocols for sediment, aquatic, and terrestrial standard population-level endpoints over short and long time frames are advocated for assessing hazards of simulated ENM concentrations in the environment.[63] In this framework, sunlight is an environmental variable, bioaccumulation is measured, and ENM modifications during product and material life cycles that may change bioavailability are considered.[63] While such a framework has broad organizational appeal, priority setting within the framework is required and thus could focus on tests that are relatively well aligned with likely exposure scenarios.

Even with a risk assessment framework that considers ENMs across product life cycles and considers sediments, water, and soil in testing endpoints,[63] major hurdles hinder regulatory agencies, and research scientists, in using concepts such as exposure conditions, ENM transformation, dose, and body burden in interpreting biological and computational findings for assessing risks. Toxicity tests developed for dissolved chemicals typically require significant modification for use with ENMs.[57] Tests may not apply to ENMs if they are not appropriate for solids.[275] Additional scientifically-based hazard information from the peer-reviewed literature may or may not be available for consideration. ENMs used in ecotoxicity tests, which are sometimes laboratory-synthesized to overcome uncertainty regarding proprietary coating or other commercial formulations, may be insufficiently analogous to allow for extrapolating information or risk comparisons.

Issues include the need to know test material characteristics and how they relate to testing results and the ENM life cycle. Even if an initial risk assessment considers ENM solubility,[63] ENM dissolution is not instantaneous; therefore, at what stage of dissolution does the contaminant no longer pose a hazard as an ENM? Also, where biological impacts stem from ENM surface characteristics, how can mass concentration be used to judge hazards? Environmental ENM effects in benchtop experiments can be indirect, stemming from physical nutrient depletion,[152] or amplifying organism uptake of co-contaminants.[276] Other indirect physical effects derive from ENMs adhering to the organism surface,[277] light shading,[278] or internal food displacement.[99] Near-field exposures (Fig. 3) can result in biological hazards from specific ENMs based on their properties (e.g., electronic).[75, 279]

By definition, ecological risk assessment (ERA) is "the process for evaluating how likely the environment will be impacted as a result of exposure to one or more environmental stressors."[280] ERA involves predicting effects for individuals, populations, communities and ecosystems, and concerns itself with valuable[49] ecosystem services such as nutrient cycling.[280] Thus, conducting ERAs for ENMs could benefit from an ecological outlook. All levels of biological organization, and interactions between them, would be considered when assessing responses to ENM exposure (Fig. 3).

Release and exposure scenarios (Fig. 1), use of functional assays for assessing environmental compartmentalization (Fig. 2),[38] and combined life-cycle and multi-media modeling[228] have important roles in focusing ENM ecotoxicology. Less recognized is that mechanistically based models of dynamic biological effects are informed by hazard assessment research. Different types of process-based, dynamic models (e.g., Adverse Outcome Pathway or AOP,[281, 282] and Dynamic Energy Budget or DEB[283]) allow for predicting effects from exposures stepwise, starting at subcellular levels, into individuals, through populations, and conceivably to communities and ecosystems. Developing process-based models requires researching key effects processes[71, 284, 285] and ecological feedbacks.[153] Models are formalized to describe interactive processes culminating in toxicity such as reactive oxygen species (ROS) generation and cellular damage. Process-based mathematical expressions evolve with empirically-based discoveries or through model reconciliation with experimental data. Parameters are independent of toxicity testing protocols, although models could be informed by standard test results. Thus, ENM ecotoxicity research could support predictive toxicology by informing and populating process-based, dynamic ecological effects models.

A comprehensive fate and effects research agenda is needed for addressing ENM quantification in complex media.[286, 287] Such an agenda has allowed for assessing experimental compartmentalization,[288] and sensitively assessing environmental persistence,[289] toxicity, bioaccumulation,[287, 290] trophic transfer,[291] and indirect effects from the uptake of ENMs coated in other hazardous materials.[292, 293] Such research could substantially inform ENM risk assessment for a relevant environmental exposure scenario. However, most ENMs have not been studied comprehensively along the entire exposure and effects continuum (Fig. 3). Further, the approach is not sustainable. Rather, the need is to develop efficient approaches applicable within an overall approach to rapidly evaluate the large number of ENMs under commercialization (Fig. 2). A research agenda that focuses on distilling key determinants of exposure and hazard for ENM-environment systems that can be measured experimentally would be most compelling.

Thus, while the science of ENM ecotoxicology and exposure characterization has advanced, there are disconnects between how regulators review ENM-based products for environmental safety and the research that is conducted to evaluate hazards. Except for results published in open source outlets or directly reported, research may be unknown to government bodies. Ongoing synthesis of published research results is challenging due to high variability across study conditions and ENMs tested, and due to effort needed to regularly update such comparisons. Moreover, there is a systematic resistance to publishing "no effect" studies in the peer-reviewed literature.[294] As a result, relying only on published research to inform regulatory decisions can present challenges. A life cycle-based framework facilitates exposure modeling and hazard testing to support risk assessment. However, extrapolation of effects to untested concentrations, study, or environmental conditions, and across biological levels of organization, requires understanding dynamic biological process-based effects, which current standard tests neither deliver nor sufficiently inform. Ultimately, exposure scenarios are useful for framing and focusing ENM ecotoxicology, and some version of a tiered intelligent testing and risk assessment (Fig. 2) strategy is needed.

Such a conceptual tiered strategy considering the impact of the ENMs' varying properties on ecological risks at different life cycle stages was proposed in the EU FP7 MARINA (Managing Risks of Nanoparticles) project[295] and is being further developed in the EU NANoREG programme. This strategy considers several domains (exposure, fate, kinetics, and hazard) represented by specific tools ranging from relatively simple in the lower tiers to more complex and specific in the higher tiers. The framework aim is to structure information collection and generation for cost-efficient risk assessment, compliant with 3R animal-use testing principles (i.e., replacement, reduction, and refinement), which should also be pursued by means of grouping ENMs.

A strategy for grouping ENMs based on releases, uses, physicochemical properties, bioaccumulation, bioavailability, and effects for both human and ecological risk assessment is currently in development across a number of EU research projects such as MARINA, NANoREG, SUN, and GUIDEnano. These efforts have been challenged by the complexity of ENM identity and interactions, but this approach is necessary, as the costs for safety assessment on a case-by-case basis would be exorbitant.[295] Therefore, a vision on ENM grouping is needed, which should apply in a regulatory context.[296, 297] Applying grouping in regulatory risk assessments should enable read-across, i.e., filling a data gap by using information on one ENM, or a non-ENM, for another substance in the same group.

Recommendations

The abovementioned tools should be fitted into a risk assessment strategy for ENMs. This strategy should be flexible enough to address different assessment goals depending on the user's needs, considering all data already available as a starting point, contingent upon data quality evaluation[298] and selecting the most appropriate tools to fill existing data gaps. Such a strategy should ideally be exposure-driven, starting with identifying the most relevant exposure scenarios in the ENM life cycle, and evaluating completeness and quality of the available data from a risk assessment perspective. This facilitates careful prioritization of ENMs to optimize testing efforts and can inform more realistic ecotoxicological investigations. Doing so can allow one to screen-out irrelevant exposure routes, eliminate unnecessary testing, and support prioritization of exposure scenarios.

Exposure assessment should begin with an analysis of plausible exposure scenarios; where none is expected, further testing may be precluded for the applicable use patterns and volumes.[26, 63] Researchers and regulators need to understand actual exposures at biological receptors. This exposure-driven approach can also provide important information on realistic environmental conditions to affect test designs for improved interpretation of laboratory toxicology studies. Such practices can ensue in the interim, while research continues to discover best hazard assessment practices.

Experimental ENM toxicity assessments, using ecologically relevant receptors and across linked biological levels of organization, should inform developing and parameterizing dynamic process-based models. Such models should respond to future scenarios and predict impacts. ENM characteristics, exposure conditions, and ENM transformation, dose, and body burden should be used in interpreting biological and computational findings for assessing ENM risks. ENM test results should be benchmarked to results for appropriate controls to establish relative hazard (e.g., comparing dissolving ENMs to their dissolution products or comparing persistent ENMs to non-nano analogs). This applies to pinnacle concerns in ecological fate assessment of bioaccumulation, biomagnification, and biopersistence.[299]

How to develop, interpret, and use pertinent information in ENM environmental risk assessment is a larger issue that should become part of an extended dialog among regulators, industry, civil society organizations, researchers, and other societal members so that the fundamental research will inform decision making. Collaborative decisions are recommended for focusing ENM ecotoxicology towards relevant scenarios, including testing the most relevant materials throughout ENM life cycles and employing appropriate hazard assessment approaches, towards meaningful ecological risk assessment.

Summary and Outcomes: Towards Defining “Environmental Relevance”

The overarching question motivating this critical review was: how can we ensure that hazard assessment in ENM ecotoxicology is as environmentally relevant as possible? The answer requires considering how ecotoxicity tests are performed, what constitutes pertinent concentration and test conditions for ENMs (Table 1), the main biotic and abiotic attributes of the environment, how ecologically oriented hazard assessment is undertaken (Table 2), and how the resulting information should be interpreted. Answering this question yielded three primary insights. First, environmental relevance is informed by a logical consideration of what exposures might occur, to which receptors, and to what outcomes. The consideration should begin with a plausible release and exposure scenario (Fig. 1), and use best available knowledge and technologies to develop the full assessment approach. Concerns regarding ENM concentrations used in hazard assessments are paramount, but are not the only concerns. ENM concentrations should be selected to assess potential effects, but overly high concentrations that fundamentally change media conditions should be avoided. Still, concentrations ranging above and below predicted ENM average concentrations must be assessed for understanding potential organismal effects, underlying mechanisms and their concentration dependencies, and for informing process-based dynamic biological effects models. In addition to the nanomaterial, the conventional material (e.g., dissolved metal) should be tested. ENM distributions and fates in broad environmental compartments do not equate to concentrations and forms near, or effective at, actual biological receptors.[114] Therefore, research results on ENM effects should not be disregarded on the limited basis of environmentally relevant exposure concentrations when the study conditions were predicated on a broader hypothesis.

In addition to tethering ENM ecotoxicology to exposure initiation scenarios (Fig. 1), the concept of employing tiered approaches in hazard and risk assessment resonated (Fig. 2). Multi-stage approaches to ENM hazard assessment are advocated.[114] A highly developed tiered approach for health and safety testing of nanotechnologies has been published[26] and strategies for tiered risk assessment and grouping are underway.[295, 297, 298] Staging ENM ecotoxicology efforts, such that potential interactive impacts at all levels of biological organization (Fig. 2 and 3) are evaluated, could simultaneously inform risk assessment and predictive process-based effects model development. As some ENMs can cause biological impacts from ENM properties or characteristics,[300] ENM ecotoxicology should be oriented to logical exposure initiation scenarios (Fig. 1) based on ENM life cycles, via testing tiers (Fig. 2). Finally, coordination is recommended among multiple disciplines in ENM environmental analysis, fate and transport modeling, and hazard assessment, towards rapidly advancing research using tiered approaches around realistic exposure scenarios.

Acknowledgements

Funding was from the National Science Foundation and the U.S. Environmental Protection Agency under Cooperative Agreement DBI-1266377. Any opinions, findings, and conclusions are those of the authors and do not necessarily reflect the views of the National Science Foundation, the U.S. Environmental Protection Agency, or the National Institute of Standards and Technology. Although this document has been reviewed in accordance with

U.S. Environmental Protection Agency policy and approved for publication, the views are those of the authors and may not necessarily reflect official Agency policy. Mention of trade names or commercial products should not be interpreted as endorsement or recommendation for use. The authors acknowledge the helpful comments of three anonymous reviewers.

Supporting Information Available

Supporting Information includes a description of the process and methods used to develop this critical review, one supporting table, and one supporting figure.

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Table 1: Summary recommendations regarding exposure conditions used in assessing ENM hazard potentials for model organisms
Develop logically conceived, plausible exposure and receptor scenarios in designing experiments, addressing background chemical, physical, and biotic conditions, biological endpoints, and exposure periods.
Thoroughly characterize exposure media, including natural soils, waters, and sediments, and provide metadata to allow cross-comparisons and for mining influential factors across studies.
Select ENM forms—including ENM mixtures or other co-contaminants—and scale exposure concentrations to logically well-defined exposure scenarios, or to scenario-independent mechanistic and process-based modeling goals, or to best available analytical instrumentation limitations.
To the fullest extent possible, characterize ENMs under the exposure conditions and over the time frame of hazard assessments to allow homogeneous exposure or understanding bioavailability and relating endpoint measures to effective forms.
Examine and choose multiple endpoints for ENM physicochemical characterization, toxicity quantification, and toxicant characterization, subject to the exposure scenario or similar context.
Quantify body burden and determine compartmentalization of ENMs and transformation products in receptors, to allow comparing effective doses to measured biological responses. Carefully consider body burden assay approaches to avoid artifacts.
Adopt appropriate experimental control treatments for media additives such as dispersants, and for ENM transformation products that are expected during hazard assessment.
Incorporate appropriate rapid screening approaches, as prioritized tiers in an ecological hazard assessment hierarchy (Fig. 1).
Adapt these approaches in response to relevant knowledge generation.

Table 2: Summary recommendations regarding exposure and design considerations in mesocosm assessment of ENM environmental hazards
Establish mesocosms only to address clearly defined questions and hypotheses, including those motivated by testing results from lower complexity microcosms, and as related to expected exposure scenarios.
Design and operate mesocosms using established authoritative principles, and aim to minimize artifacts.
Follow the recommendations for assessing hazard potentials in using individualized or microcosm experiments (Table 1)
Make experiment and exposure durations sufficiently long to best represent the exposure scenario underpinning the mesocosm design.
Anticipate and address challenges in detecting and quantifying ENMs in complex media and myriad receptors in mesocosms, such that mass balances can be performed, and bioaccumulation and trophic transfer quantified.
Attend to issues of ENMs aging, or otherwise significantly transforming, over the duration of mesocosm operation, and control for, or otherwise assess, specific outcomes related to aged materials.
Consider assessing effects and ENM interactions with co-contaminants, since they are realistic constituents of most compartments into which ENMs are released.
Anticipate, recognize, and plan to assess secondary effects on non-target receptors that contribute to ecological processes in complex systems represented by mesocosms.
Increasingly use sensitive, e.g., omics type, endpoints as they are responsive to questions and hypotheses motivating mesocosm studies.

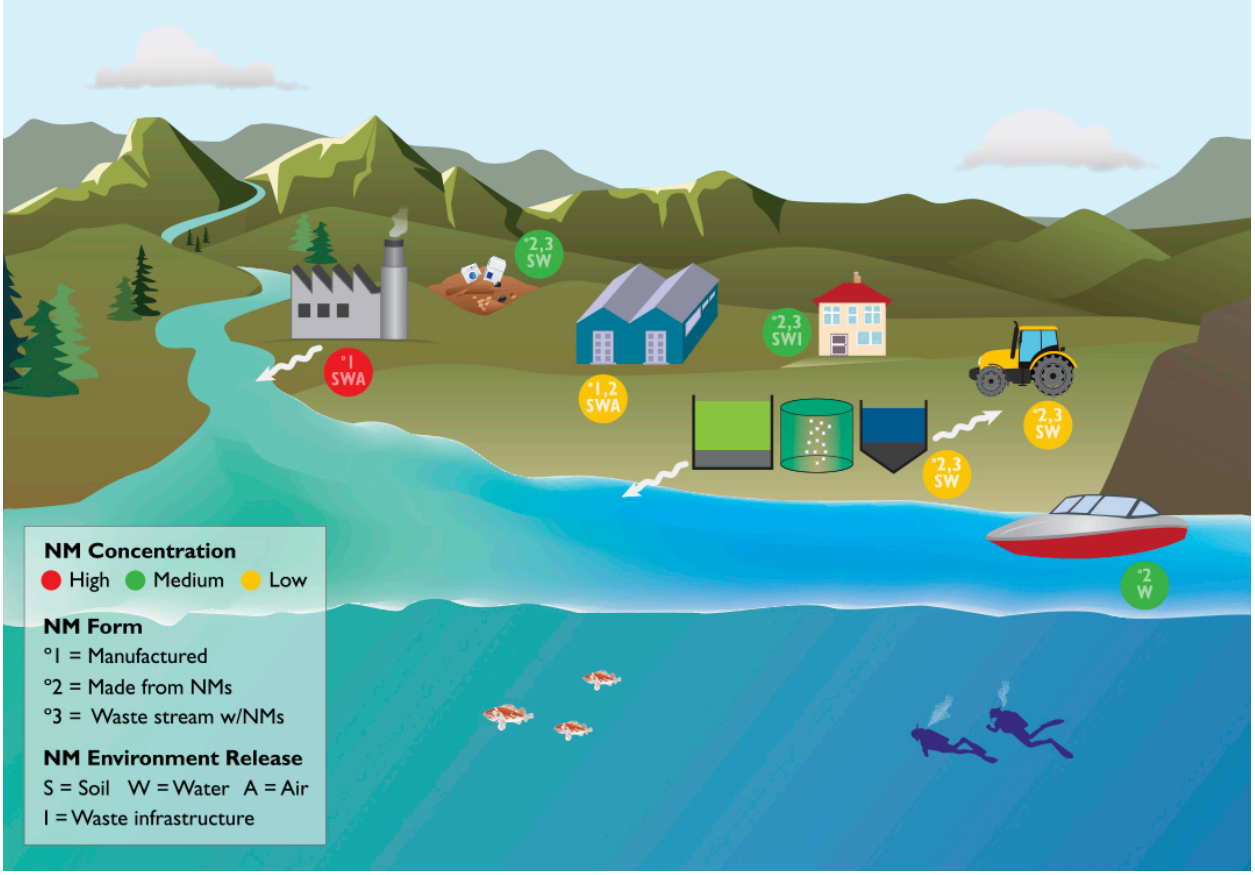

Figure 1: Conceptual environmental release scenarios for engineered nanomaterials (ENMs) across their life cycles. Potential release sites, clockwise from upper left, include: Primary ENM manufacturing; Landfill with solid waste including nano-enabled electronics, consumer goods, and permitted industrial waste; Secondary processing or goods manufacturing sites using ENMs; Consumer (household) use of ENM-enabled products; Agricultural ENM-enabled product use; Marine or freshwater ENM-enabled product, including coatings, use; Waste treatment with aqueous effluent and solids residuals that may contain ENMs or transformation products thereof. According to the legend, colored circles adjacent to each location indicate the highest expected relative nanomaterial (NM) concentration; the NM forms are as-produced (°1) or in products (°2) or in mixed waste streams (°3); release destinations include waste infrastructure and major environmental compartments (soil, water, air).

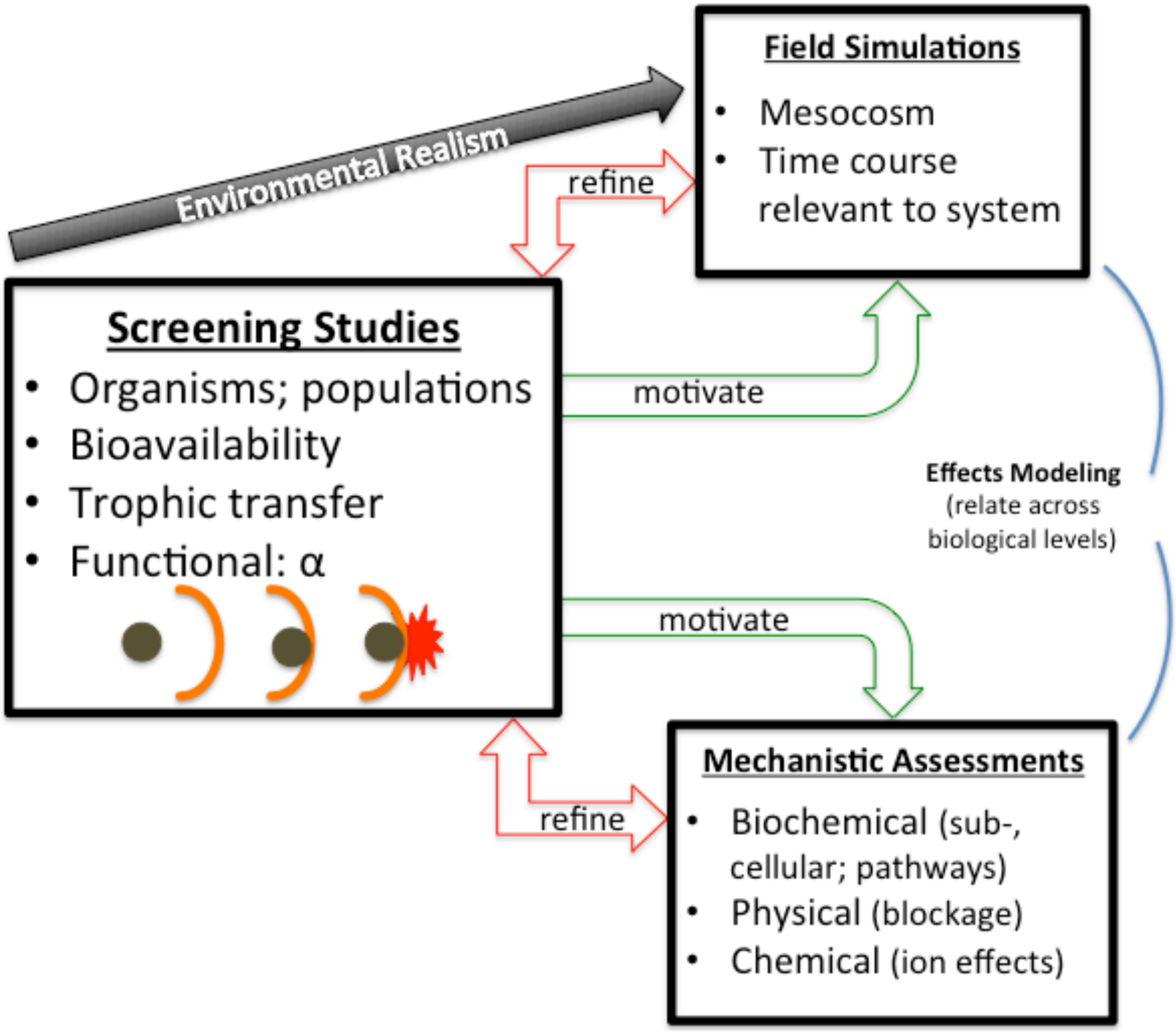

Figure 2: Interactive "tiers" of ENM ecological hazard assessment. Screening studies (left) are recommended starting points for ENM assessments, and are typically conducted under laboratory conditions, using microcosms, batch reactors, or microplates, depending on the experiment. Examples include assessing organismal to population growth effects, bioavailability (including to communities), ENM physical behavior, and trophic transfer. If screening assay results warrant, mesocosms (upper right box) may be initiated to simulate actual environmental conditions in longer-term experiments, to determine the potential for ENMs to impinge on ecosystems. Screening assay results may also motivate determining mechanisms (lower right box) of observed effects on cells or macromolecules and to characterize biochemical, physical, and chemical interactions of ENMs with biological receptors. Knowledge gained within each tier is used to refine the approaches in the other tiers, thereby improving the relevance of each activity. Results inform development of dynamic process-based mathematical models (curved lines linking across tiers) of biological effects.

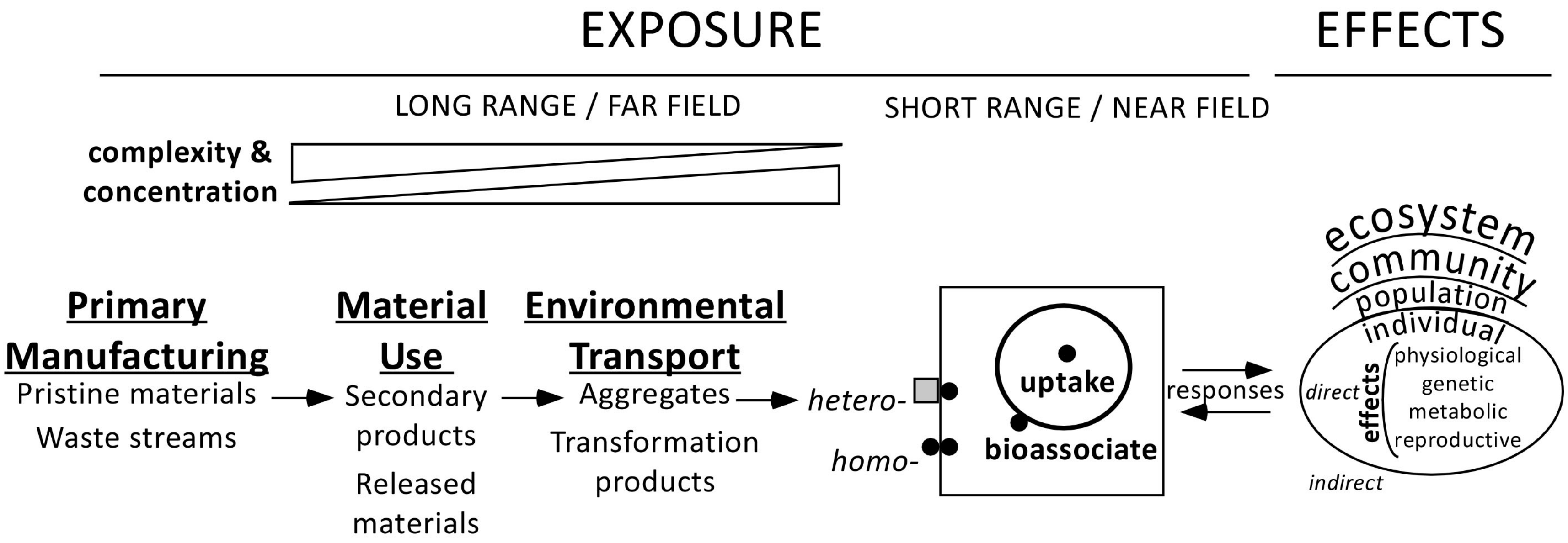

Figure 3: Conceptual exposure and effects assessments in ENM environmental risk assessment. Exposure derives from far-field emissions, transport, and transformation processes leading to environmental accumulation (bottom "wedge" of ENM complexity and concentration) of either more complex mixtures, or of specific ENMs or transformation products. Top "wedges" depict that either ENM complexity or ENM concentration can increase or decrease along the path of far-field ENM transport. At biological receptors, adverse effects are predicated on near-field exposures. Homo- and heteroaggregation (of multiple particles, here depicted as two) are particle-specific phenomena that may prevent near-field exposure. Biotic responses can influence bioavailability, and thus near-field exposures. Direct effects to biota may manifest across all levels of biological organization (subcellular, to individual, population, community, and ecosystem); effects can also be indirect, e.g., from physical effects of ENMs on nutrient availability.

TOC Art (for Table of Contents Only)