Common Strategies and Technologies for the Ecosafety Assessment and Design of Nanomaterials Entering the Marine Environment

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ABSTRACT The widespread use of engineered nanomaterials (ENMs) in a variety of technologies and consumer products inevitably causes their release into aquatic environments and final deposition into the oceans. In addition, a growing number of ENM products are being developed specifically for marine applications, such as antifouling coatings and environmental remediation systems, thus increasing the need to address any potential risks for marine organisms and ecosystems. To safeguard the marine environment, major scientific gaps related to assessing and designing eco-safe ENMs need to be filled. In this Nano Focus, we examine key issues related to the state-of-the-art models and analytical tools being developed to understand ecological risks and to design safeguards for marine organisms.

Engineered nanomaterials (ENMs) are used extensively in a variety of emerging technologies and commercial products, including biomedicine, pharmaceuticals, and personal care, renewable energies, and electronic devices. As these materials are used, disposed of, and degraded, they can release ENMs into the environment. Fate and transport models indicate that ENMs entering soil and waterways will eventually reach the marine environment as nanowaste that can cause human injuries as well as ecological impact with significant socioeconomic consequences. In addition to accidental releases and exposures, a suite of new marine nanotechnologies, including antifouling paints and pollution remediation systems, are also being developed with great uncertainty about their eco-safety and sustainability for the marine environment. An increasing number of short-term, well-controlled laboratory studies have tested ENM’s toxicity on marine organisms and showed a wide variety of potential biological injuries. Whether ENMs cause similar injuries in the dynamic natural marine environment is uncertain because the fate, transport, and behavior of many ENMs in seawater, and thus their biological risks, remain poorly understood.

The chemistry of ENMs plays a crucial role as their bioavailability, bioaccumulation, and toxicity are difficult to predict in seawater because the materials undergo complex interactions/transformations when exposed to elevated ionic concentrations. For example, metallic ENMs undergo aggregation, sedimentation, corrosion, and reprecipitation in seawater, but the rates at which these processes occur depend heavily on the inherent dissolution rate of the dominant metal. Therefore, developing tools to predict, to estimate, and to compare the long-term effects and risks of ENMs presents many challenges. Meeting these challenges is a fundamental objective of many marine

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nanoecosafety focused research programs, as there is little doubt that organisms living in marine waters and sediments will be exposed to ENMs in effluents from factories and households or from urban runoff. In this Nano Focus, we examine the state-of-the-art approaches used to predict and to measure the exposure and toxicity of ENMs in marine ecosystems. We focus on the influence of key environmental conditions, such as salinity, chronic low-level exposures that probably will dominate natural coastal seascapes, the effects of ENMs in concert with other multiple environmental stressors, and the varying effects of ENMs as they age under natural environmental regimes. Our goal is to provide a synopsis of available methods, models, and insights necessary to highlight the gaps that exist and need to be filled for the design and production of ecosafe ENMs to be used for marine ecosystem sustainability.

In this Nano Focus, we examine the state-of-the-art approaches used to predict and to measure the exposure and toxicity of ENMs in marine ecosystems.

Characterization and Behavior in Seawater: From Models to Analytical Tools.
Predicting ENM exposure will lead to a better understanding of their fate and behavior in the marine ecosystem. Their dispersion stability is a key factor that determines their residence time in the water column, and thus their occurrence in the benthic or pelagic systems. Dispersion is influenced by a variety of parameters, including the intrinsic characteristics of the ENMs, the solution chemistry, and the interaction with surrounding components.7–12 Ranging from the basic theoretical prediction of the colloidal stability to the most relevant and holistic approach accounting for the system heterogeneity, a number of physicochemical mechanisms appear determinant in the fate of ENMs in the seawater column and sediments. In the seawater column, both hydrodynamic flow (river outlet, wave oscillations) and random diffusion (Brownian) favor the colloidal transport of particulate matter, competing with gravitational sedimentation (see Figure 1). The particle size and density thus determine the persistence in suspension. The size is that of the individual isolated nanoparticles (NP) or their homogeneous and heterogeneous aggregates (nano-objects and their aggregates and agglomerates). The aggregation dynamics consist of a two-step process where the suspended particles first collide, and then may attach to each other.13 The collision frequency and the attachment efficiency, respectively, drive these two steps, together determining the aggregation rate. The attachment efficiency results from the balance between repulsive and attractive interparticle forces, as predicted by DLVO theory and its numerous extensions.14–17 These interactions have been widely studied for synthetic systems with basic and homogeneous compositions, revealing how surface charge, which strongly depends on pH and salinity, plays a determining role in the electrostatic dispersion stability of particles, especially in those that have no steric protection against aggregation.18,19 Critical salt concentrations have been determined for the counterion nature and valence and solution pH by measuring the induced kinetics of aggregation.12,20–23 Bare NPs often display low dispersion stability in natural water, while functionalized NPs (e.g., using polyethylene glycol or a polyvinylpyrrolidone coating) may remain very stable at NaCl concentrations higher than that of seawater. Nevertheless, the marine environment is rarely homogeneous and of basic composition. Natural mineral (clay, carbonates, etc.) and organic matter (algae, exopolymers, etc.) reside suspended in the seawater column as geogenic and biogenic colloids,24 potentially interacting with the ENMs. The affinity between the NPs and these naturally occurring colloids, a consequence of the NPs’ high surface energy, drives the so-called heteroaggregation phenomenon. This has been studied under certain solution chemistries.10,21–23 Similar to metal and oxide ENMs aggregating at high salt concentrations are actually stabilized by interactions with proteins. Moreover, the kinetics of ENMs removal appeared also to correlate with their concentrations.

In natural water, both homo- and heteroaggregation may occur simultaneously, their respective kinetics resulting directly from the corresponding collision frequencies and attachment efficiencies.25 In the seawater column, the natural colloids mainly consist of dissolved and particulate organic carbon, among which large and amorphous organic aggregates displaying low settling velocities are distinct from denser particles with higher settling velocities, such as diatoms.30,31 Their concentrations fall in the microgram per liter (μg/L) range. However, the release of ENMs in seawater is of terrestrial origin, implying their prior transport through surface water wherein suspended particulate matter is 3 orders of magnitude more abundant (mg/L), while the predicted ENMs concentrations range below microgram per liter (μg/L). The most widely used ENMs, based on TiO₂ and Ag NPs, are expected at 10⁻²–10⁻¹ and 10⁻⁵–1 mg/L, respectively.32 These concentrations are likely to favor the heteroaggregation and colloidal transport of persistent ENMs to the sea. Estuaries
and coastal marine environments are thus areas of high interest, where stable colloids brought by river encounter increasing salinity gradients that may cause their large agglomeration and sedimentation. This situation typically forms siltation areas, where ENMs and other pollutants bound to those colloids may bury and accumulate within sediments or persist in the water column.\textsuperscript{31,33–35} The NOM composition also plays a role in this balance. Seasonal variations influence phytoplankton and microbial activity that produces exudates made of fibrillar and acidic polysaccharide compounds. These biopolymers can remain dispersed or assemble together into gel networks.\textsuperscript{36–40} Their structure and abundance determine their potential roles in the fate of the suspended particulate matter, and thus of ENMs. Electrostatic or steric stabilization is often observed when the particle surface is saturated with adsorbed molecules.\textsuperscript{41,42} On the contrary, lower surface coverage added to large molecular weight may favor bridging flocculation\textsuperscript{43–45} and formation of large aggregates such as marine snow. Moreover, because of a given NP's size ratio, the resulting heteroaggregates may sometimes consist of biopolymers decorated with adsorbed NPs, as pearls on a necklace, or in other cases, of biogel incorporating the NP in their network.\textsuperscript{46,47} The unique term heteroaggregation accounts for a very wide panel of colloidal dynamics, ranging from flocculation to colloidal stabilization. Very contrasting final states may be reached, depending on the respective size and concentration of the natural colloids with regard to the ENM.\textsuperscript{45,46–50} It is the final size and density of the formed heteroaggregates that determine the persistence of ENMs in the seawater column. Those remaining in the colloidal size range, typically below 1 μm in size,\textsuperscript{24} promote further transport of ENMs and their exposure to pelagic organisms, while the formation and sedimentation of larger flocs instead concerns the benthic ecosystem.

\textbf{The State of the Art on Marine ENMs Ecotoxicology.} Many harmful effects of ENMs have been reported for marine organisms, but factors including physicochemical properties of ENMs, seawater parameters, interactions with both physical and chemical factors (i.e., ultraviolet and marine pollutants), and organisms’ physiology and ecology make ecotoxicological assessment difficult.\textsuperscript{4} Selected invertebrate organisms have been recognized as potential biological targets of ENMs exposure, including biofilm, phytoplankton, bivalves, and bottom grazers (reviewed in ref 6 for inorganic NPs). Little data are available on higher trophic levels such as fish and marine mammals.\textsuperscript{51–54} Biological responses and end points of toxicity have been investigated as (1) mechanisms of uptake and of translocation inside the body, (2) organelles/compartments/cells/tissues as targets of toxicity and/or retention, and (3) cellular pathways/mechanisms of toxicity.\textsuperscript{6} With few exceptions, there is a lack of epidemiological studies focusing on reproduction and development (e.g., early life stages, embryos, and larvae).\textsuperscript{55–58}

Increasing evidence supports the hypothesis that the immune system of marine bivalves represents a significant target of ENMs. The blue mussel Mytilus has been the species most utilized so far for marine ecotoxicological studies on the effects and mechanisms of action of ENMs on innate immunity.\textsuperscript{59,60} In vitro studies showed that different NPs...
Many harmful effects of ENMs have been reported for marine organisms, but factors including physicochemical properties of ENMs, seawater parameters, interactions with both physical and chemical factors, and organisms’ physiology and ecology make ecotoxicological assessment difficult.

are rapidly taken up by mussel hemocytes, affecting a large number of functional parameters, from lysosomal function to phagocytic activity and oxyradical production, and also inducing pro-apoptotic processes; the effects of NPs were mediated by stress-activated mitogen-activated protein kinase signaling, as in mammalian phagocytes.61–63 In vivo exposure to different NPs and, in particular, to nano-TiO2 chosen as a model NP type64,65 enabled formulation of a hypothesis on the possible pathways leading to nanoinduced immunomodulation60 (see Figure 2).

Due to the physiological mechanisms involved in the feeding process, nano-TiO2 agglomerates/aggregates formed in seawater are taken up by the gills and partly directed to the digestive gland, where intracellular uptake of NPs induces lysosomal perturbations and changes in the expression of antioxidant and immune-related genes. Nanoparticles can then potentially be translocated from the digestive system to the hemolymph and to circulating hemocytes, where nano-TiO2 induced changes both in functional parameters (lysosomal integrity, phagocytosis, reactive oxygen species, ROS, and NO production, induction of preapoptotic processes) and in transcription of antimicrobial peptides. Interestingly, the in vivo effects of nano-TiO2 on mussel immune parameters were observed at concentrations (1–10 μg/L) much lower than those usually utilized in ecotoxicity tests on aquatic species, and closer to predicted environmental concentrations.65 Recently, the rapidly expanding application of DNA microarrays and next generation sequencing (NGS) technologies offer new and broader research perspectives, from the whole transcriptome coverage to the Mytilus genome sequencing, leading to the identification of an increasing number of immune-related genes that could be targeted by different ENMs.66,67 Likely candidates are the members of the Toll receptor family recently identified in mussels;68 among these, transcription of the TRL-i isoform has been shown to be down-regulated in mussel hemocytes by in vivo exposure to nano-TiO2.69

Among invertebrates, the sea urchin is a successful marine model, globally distributed in almost all depths, latitudes, temperatures, and environments in the sea. The key to its successful survival is its potent immune system, which provides protection, robustness, and molecular plasticity.70 Thus, sea urchins represent an excellent model to uncover molecular and regulatory mechanisms promoting roles of the immune system on survival. Sea urchin immune cells have been demonstrated to activate their immune response machinery in response to different kinds of physical and chemical stressors, such as temperature shocks; pH decreases; exposure to UV–B radiation and heavy metals; and exposure to tin (SnO2), cerium (CeO2), iron (Fe3O4), and TiO2 NPs.71–76 The utility of the heat shock protein HSCP70/HSC70 as a general stress response marker to be used for monitoring both acute and chronic stresses has been demonstrated, with the only exception being NP exposure.76 In contrast, it is expected that specific pathways and biomarkers are selectively elicited in response to NPs, as already demonstrated in human immune cells.77,78 The availability of the sea urchin genome, which has been shown to be closely phylogenetically related to the human genome,79 offers the possibility to analyze its complex and sophisticated immune system and to compare the biological effects observed in sea urchins and human immune cells. Analysis of

Figure 2. Possible routes of engineered nanomaterial (ENM) uptake and disposal in the model marine bivalve Mytilus leading to immunomodulation.
the amazingly large repertoire of innate pathogen recognition proteins, such as the 20-fold expansion and diversification of the Toll-like receptors relative to human genes, would probably identify specific target genes for different NPs or ENMs. Complementary to the use of adults for studies on immune modulation, embryos have emerged as valid tools for studies on developmental and molecular perturbations induced by environmentally relevant classical and emerging contaminants, including metal NPs.

As an example, the in vivo exposure to nano-Ag causes dose-dependent developmental defects as well as alterations in swimming patterns in sea urchin embryos. However, the cause–effect relationship remains to be demonstrated. In conclusion, the value of the sea urchin model is twofold: first, it is proposed as a proxy to humans for the analysis of the effects of ENMs on the immune system, and second, it can be used as an alternative model for ecotoxicological studies (see Figure 3).

Engineered nanomaterials could give rise to genotoxic effects in exposed marine organisms; the loss of DNA integrity, if not properly repaired, may lead to mutations, birth defects, and long-term effects, such as cancer in vertebrates. The mechanisms of ENMs genotoxicity are still not well understood and it is often unclear if an effect on DNA is nanospecific. DNA strand breaks are one of the major types of oxidative damage to DNA via oxidative stress, which is generally assessed by the Comet assay, a technique widely used to evaluate the genotoxic effects of contaminants in bivalves' hemocytes, which are a potential target for genotoxicity.

Oxidative stress after long-term exposure to nano-Ag was evidenced in mussel gills and digestive glands.

Figure 3. Sea urchin model to study immunity and development for the safe use of nanomaterials.
A loss of DNA integrity was found in marine mussel cells after in vitro exposure to nano-iron\textsuperscript{95} and in blue mussel after in vitro exposure to nano-Ag\textsubscript{2}S and CdS quantum dots.\textsuperscript{97} Concerning marine species, the effects of nano-TiO\textsubscript{2} have been investigated with respect to invertebrates\textsuperscript{98–102} and two articles have reported the susceptibility to micrometer rutile and nanometric anatase TiO\textsubscript{2} genotoxic potential of the top predator bottle-nose dolphin leukocytes\textsuperscript{51} and fibroblasts.\textsuperscript{34} Interactive genotoxic effects of C\textsubscript{60} fullerenes and fluoranthene were studied in marine mussels,\textsuperscript{103} highlighting that both fluoranthene and C\textsubscript{60} on their own caused concentration-dependent increases in DNA strand breaks, while combined exposure to C\textsubscript{60} and fluoranthene additionally enhanced the levels of DNA strand breaks, likely related to oxidative defense impairment. Recent evidence also showed that nano-TiO\textsubscript{2} and 2,3,7,8-tetrachlorodibenzop-p-dioxin (TCDD) can exert synergistic or antagonistic effects depending on the experimental condition, cell/tissue, and type of measured response, as evaluated by a battery of biomarkers including DNA primary damage (Comet assay), genome instability (RAPD assay), and chromosomal damage (Micronucleus test).\textsuperscript{103}

Biotransformation/detoxification may play a significant role in ENM toxicity, including genotoxicity, by affecting ENMs localization/disposition inside the cell. The accumulation and consequent toxicity of a compound is strongly influenced by the organism’s detoxifying/clearance capabilities. Biotransformation of ENMs might alter their life-cycle metabolism, but also might interfere with that of other compounds, as occurs in coexposure scenarios in the natural environment. Therefore, it is crucial to investigate the interactions of ENMs with the so-called “cell defense” (i.e., the cellular machinery activated in response to chemical stressors) in marine organisms that are naturally exposed to mixtures of compounds/toxicants present in the environment.\textsuperscript{6,104} Among actors on the first line of defense, P-glycoprotein (P-gp) is the most well-characterized membrane transport protein; it is also involved in the efflux of xenobiotics generally known as multidrug resistance (MDR, previously known as MXR), i.e., phase 0 and III of biotransformation.\textsuperscript{105,106} Several ENM-based approaches have recently been proposed to reverse/overcome the efflux-mediated resistance controlled by P-gp in human cell lines to favor cytotoxic drugs accumulation in tumor cells. Of course, such complex medical ENMs are transformed inside the body before expulsion.\textsuperscript{107–109} Little attention has been paid to marine organisms where MDR is actively involved in xenobiotic biotransformation and a similar interaction may occur. One example is the reported increase in the efflux of P-gp substrate Rhodamine B in the gills of the marine mussel Mytilus galloprovincialis caused by nano-TiO\textsubscript{2}.\textsuperscript{103} In parallel, an increase of TCDD bioconcentration in the whole body was observed in co-exposure conditions. Possible involvement of nano-TiO\textsubscript{2} on efflux functionality that, in turn, affects the disposition/cellular retention of other toxic compounds can be hypothesized.\textsuperscript{109} Concerning heavy metal biotransformation, metallothioneins (MTs) are ubiquitous, low-molecular-weight soluble proteins with high heavy-metal-ion-binding capacity. Their role in essential trace elements (Zn\textsuperscript{2+} and Cu\textsuperscript{2+}) homeostasis as well as detoxification from noxious ions such as Hg\textsuperscript{2+} and Cd\textsuperscript{2+} has been widely demonstrated in eukaryotic cells, including those of marine organisms.\textsuperscript{110} The increase of MT in response to a variety of oxide-based and metallic NPs has been reported in cells and/or tissues of several marine organisms. Nano-Ag increased MT gene expression in embryo and adult hepatopancreas cells of Crassostrea virginica\textsuperscript{95} in the nanomolar range. Conversely, no increase in MT-like proteins was reported in tissues of the ragworm Hediste diversicolor exposed to nano-Ag in sediments, whereas soluble Ag caused MT induction.\textsuperscript{111} Gold NPs (0.1 mg/L) were effective in tissues of the endobenthic clam S. plana.\textsuperscript{112} Among nano oxides, despite the number of studies on the effects of ZnO and nano-TiO\textsubscript{2}, only reports on nano-TiO\textsubscript{2} are available, demonstrating no effects on MT transcription in the tissues of in M. galloprovincialis.\textsuperscript{65,101} However, several studies have focused on the effects of nano-CuO, for which an increase of MT proteins has been documented in at least two molluscan species, M. galloprovincialis and S. plana.\textsuperscript{113–116} It is worth noting that in the latter studies, environmental Cu dissolution was very low or virtually absent, therefore MT induction had three possible explanations: (1) intracellular Cu dissolution to ionic forms; (2) oxyl-radical formation; or, most likely, (3) a combination of both, since metal and oxidative stress show overlapping features, cellular outcomes, and biochemical responses. It is widely accepted that MT genes are transcriptionally activated by different heavy metal ions penetrating into cells through a mechanism involving a zinc-sensitive transcription factor (MTF-1) and zinc displacement from a physiological pool of MTs by more electrophilic cations. In addition, oxyl radicals and, in general, oxidative stress are promoters of MT induction.\textsuperscript{117} Authors did not report MT modulation along with effects to the antioxidant enzymatic system and/or the occurrence of oxidative insults in the aforementioned works (except in ref 111, where no NP dissolution was observed). Assaying both MT heavy metal content and oxidation state and its concentration in marine organisms’ tissue thus represents a powerful tool to provide mechanistic information on the mode of action of metallic and metal-oxide NPs.

The Need To Develop Models. A major need in marine ecotoxicology for ENMs is the development of quantitative approaches for integrating more realistic ENM exposure scenarios
and predicting ecosystem impacts, thus enhancing the ability to design ecosafe ENMs. This can be accomplished through modeling exposure scenarios specific to estuaries and marine environments, testing those predictions with environmental fate and transport (F&T) experiments, and testing toxicity with organisms that could be exposed in both pelagic and benthic marine habitats (see Figure 4). This integration requires specifically: (1) generating predictive F&T models for specific ENMs and environments, such as multimedia environmental distribution (MendNano) models for coastal or estuarine ecosystems—similar models have been produced for terrestrial environments and allow flows of ENMs within and between environmental and biological media; (2) testing the predictions of the MendNano models through a combination of F&T experiments, high-content-screening (HCS) assays, microcosms, and computer-regulated mesocosm experiments at the level of individuals, populations, and/or communities; (3) analyzing the results of the multiple experiments for correlations using machine learning analysis and statistical modeling framework, and (4) using the correlation output to parametrize and update the MendNano F&T model. A full array of ENMs of different shapes and sizes, such as Cu, CuO, core–shell, and safe design materials as they are developed, can be studied when they are exposed to water, sediment, and biological matrices, and as they are transformed by physical (e.g., temperature, salinity, and dissolved and particulate organic material) and biological processes associated with estuarine environments and food webs composed of bacteria, plants, invertebrates, and fish. Both the benthic and pelagic communities, from sediment-dwelling invertebrates to fish and important estuarine fauna, represent potential targets of ENMs. The microbial community is crucial in determining ENMs’ F&T and effects; impacts to phytoplankton, for example, which are the primary producers in estuaries, probably have manifold ecological effects. The objectives of this approach are structured to assess the environmental implications of ENMs through integration models that generate theory, experiments that test those theories, and subsequent improvement of the models to generate better theory. Marine ecotoxicology efforts use this approach by linking ENMs’ chemical properties, exposure, and biochemical responses of injury with the ecological and physical processes that ultimately regulate marine ecosystem-level impacts and ecosystem services (see Figure 4). The general observations produced from this approach so far are the following:

1. Engineered nanomaterials can be toxic to bacteria in response to external aggression, algae and bacteria can release extracellular polymeric substances (EPS) that will potentially bind to metal ENMs, perhaps reducing their bioavailability and toxicity. The role of flocculation of EPS (“marine snow”) in the fate of ENMs remains vastly unappreciated.

2. Oysters are estuarine filter feeders that provide many important ecosystem services. As filter feeders, they process large volumes of water and are exposed to estuarine ENMs through ventilation for respiration, thus acting as potential ENM bioaccumulators from the
pelagic habitat. Benthic infaunal invertebrates, especially polychaete worms, crustaceans, echinoderms, and deposit-feeding clams, are probably exposed to ENMs deposited onto sediments, detritus, and biofilms.\textsuperscript{116,120} Together, these organisms, which are important in estuarine food webs, may store ENMs or their dissolution products in soft or hard tissue, and may also, through excretion, enhance ENM bioavailability. This may or may not represent an important pathway for human exposure, as well as for the trophic transfer of ENMs or their dissolution products. Fish are top predators in coastal ecosystems and can be exposed to metal ENMs directly through water column ingestion or gill tissue exposure. However, many fish species are also predators on numerous sediment-dwelling invertebrates that will have consumed ENMs, so this trophic transfer may represent an important route of exposure.

(3) Dynamic energy budget (DEB) theory\textsuperscript{124} 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. Engineered nanomaterials may disrupt energy utilization, as do many other anthropogenic contaminants. Dynamic energy budget models use theory to provide estimates of dose-specific responses of ENM concentrations on individual growth, survival, and reproductive capacity.\textsuperscript{125,126}

(4) The outputs of DEB models can be used in population dynamic model simulations to predict the potential impact of ENM injuries on populations. For small organisms such as phytoplankton, for which population dynamics can be measured in microcosms or mesocosms, the population dynamic models can be used as predictions and then tested empirically with follow-up experiments. For larger organisms, for which population level manipulations are impractical, the models can provide theoretical impacts.

A major need in marine ecotoxicology for engineered nanomaterials (ENMs) is the development of quantitative approaches for integrating more realistic ENM exposure scenarios and predicting ecosystem impacts, thus enhancing the ability to design ecosafe ENMs.

Risk Assessment, Including Nanotechnology Applications in Marine Waters. The increasing exposure of aquatic organisms to ENMs may lead to extended ecological risks. Considerable research efforts in Europe have focused on evaluating the risks from ENMs in the aquatic environment (see Table 1). In the United States, the Nanotechnology Environmental and Health Implications Working Group of the National Nanotechnology Initiative coordinates the research that aims to develop protocols, standards, instruments, models, and validated data for ecological risk assessment (RA) of ENMs.\textsuperscript{127}

Despite the significant research efforts, a key issue in both the European Union and the United States that most research has not sufficiently addressed is that pristine ENMs undergo aging and transformation reactions during incorporation into products, and weathering and aging when released into the environment.\textsuperscript{121} Thus, at each step of the supply chain (lifecycle), there is a potential risk of exposure to ENMs with different physicochemical properties, which would affect their toxicity. Some of the few results for textiles, paints, and nanocomposites\textsuperscript{32} suggest that the released particles undergo significant aging\textsuperscript{128} and exhibit different environmental behavior and effects compared to the pristine ENMs.\textsuperscript{45} Aged and pristine Cu ENMs showed different fates and toxicity in an aquatic compartment under the same environmental conditions.\textsuperscript{129} In addition, aged nano-TiO\textsubscript{2} from sunscreen caused lower mortality in \textit{Daphnia magna} compared to bare nano-TiO\textsubscript{2}.\textsuperscript{130} These studies have confirmed that aged ENMs can exhibit differences in solubility, aggregation, and reactivity that can affect their aquatic mobility and toxicity. Moreover, it is hypothesized that many ENMs are (designed to be) persistent, which might lead to long-term exposure of aquatic and sediment species. However, longer-term ecological effects have not yet been comprehensively researched, especially for ENMs used in real products. Experiments with single species have provided evidence of changes being transferred across generations, causing epigenetic, mutational, or reproductive effects.\textsuperscript{131} Therefore, testing of longer-term genotoxicity on multiple species should be developed. Quantitative ecological risk analyses of ENMs would typically involve deterministic modeling of exposure–dose–response relationships. However, this would be affected by severe uncertainty and data variability. Therefore, it is recommended that the ecological risk modeling of ENMs is addressed in a probabilistic manner using stochastic approaches such as the Monte Carlo and the Latin hypercube simulations.\textsuperscript{132} In this case, distributions of hazard estimates would be derived instead of single values, which could be plotted against distributions of exposure estimates in order to identify central tendencies of expected risk and associated high-end probability of exposure. With this approach, predicted no effect concentrations (PNEC) can be estimated based on species sensitivity distribution (SSD) methodologies,\textsuperscript{32} which are well suited to deal with high uncertainty.
and variability. Such SSD modeling procedures produce the biological responses of each species of a target environmental system and combine them into a cumulative SSD for the aquatic compartment. Probabilistic estimation of ecological risks can then be conducted by linking this SSD to the probability of critical predicted environmental concentrations (PEC). Such modeling approaches for ecological risk estimation and the resulting risk mitigation measures can provide a strong basis for guidance in industrial and regulatory contexts.

**ENMs for Environmental Applications: Smart Nanoparticles for Sea Water Remediation.** Most of the remediation technologies available today are based on the adsorption, ion exchange, amalgamation, chemical degradation or precipitation, and bioremediation. While effective in many cases, these methods are often costly and time-consuming, particularly pump-and-treat methods that require expensive investments and procedures. In this context, the high adsorption capacity of ENMs for certain pollutants has been demonstrated in many cases. There are a variety of examples in the literature that exploit the use of NPs for the removal of heavy metals from aqueous solutions, such as using $\beta$-FeO(OH) nanocrystals to remove As(V),$^{133}$ SiO$_2$ NPs for removal of Cr(III),$^{134}$ as well as Al$_2$O$_3$ NPs$^{135}$

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**TABLE 1. Overview of European Union Research Projects Providing Data and Methods for Ecological Risk Assessment of Engineered Nanomaterials (ENMs) in the Aquatic Environment**

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*Nano FOCUS* 

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and supported Au NPs on alumina\textsuperscript{136} and as colloids,\textsuperscript{137} for removal of Hg(II). Although the ability of inorganic NPs to trap pollutants is best known for cations of elements such as the previous and Ni(II) and Cd(II), they can also be functionalized to trap organic molecules specifically and/or to act as photocatalysts to promote the complete degradation of toxic organic matter. A comprehensive overview of the different manufactured nanomaterials along with the pollutants they could potentially remediate has been reviewed elsewhere.\textsuperscript{2,138} The toxic species once absorbed onto the NPs can be removed by applying mild (and affordable) gravitational (centrifugation) or magnetic (in the case of magnetic NPs) field gradients. Other strategy when dealing with organic compounds is to promote their reduction (e.g., to transform perchlorates to Cl\textsuperscript{−}) with sacrificial electrodes, as iron NPs that will reduce halogenated compounds during their oxidation. Moreover, nanoremediation can present complementary properties to bioremediation, not just by decreasing pollutants, but also by dispersing the pollutants, and by synergetic interactions with biota. The majority of these works have been performed in the lab with synthetic water due to complexity and restrictions to disperse NPs in the natural environment.

All this research interest is because nanomaterials have a number of physicochemical properties that make them particularly attractive for water purification and environmental remediation. Their capacity to be dispersed in water allows them to travel farther than larger, macrosized particles, thus achieving wider distribution and permitting the whole volume to be quickly scanned with a relatively small amount of material.\textsuperscript{2,139,140} These unique properties can be employed to degrade and to scavenge pollutants (see Table 2).

Nanoremediation may address different levels of pollution with specific targeted technologies, which may be used in a stepwise strategy (see Figure 6), e.g., (i) cleaning very polluted sites toward less polluted soil, after which biota may be able to remediate pollutants further; (ii) cleaning less polluted sites with existing microbiota by developing synergies between the NPs and the involved biota to increase the potential for natural attenuation; and finally, (iii) cleaning low but hazardous concentrations of pollutants (microcontaminants). Very polluted areas, where all signs of life have disappeared, and therefore precluding the option of bioremediation, are a big challenge for remediation and a wonderful opportunity to test the reactivity, catalytic behavior, and large surface area per unit mass of NPs. In such cases, NPs are needed so they can induce/catalyze the initial chemical breakdown or adsorb and then slowly release the contaminants in order to achieve a level of contamination where microbial processes can take place, enhancing the sequential synergy of nanotechnology and bioremediation. Ideally, the ENM would degrade into harmless substances once its job is finalized. In addition, while cleaning very polluted water into less polluted water is feasible, cleaning low pollution levels in water to safe water (especially in the case of microcontaminants) is also a challenge that could be overcome by nanoremediation. However, the intrinsic nature of the inorganic NPs may also cause damage to the environment if dispersed uncontrollably.

Marine pollution by petroleum products (i.e., oil spills) often produces disastrous effects with negative impacts, affecting both the environment and socioeconomic development. In this context, the use of ENMs for in situ remediation represents a promising and cutting-edge solution, by ensuring a quick and efficient removal of pollutants.\textsuperscript{2} However, the use of such materials should not pose any additional risk to the marine ecosystem in which they are released.\textsuperscript{2,141}

Developing safe and effective remediation technologies for petroleum products based on the design and synthesis of new ecofriendly ENMs might be a challenging solution for promoting in situ nanoremediation for the marine environment. Such innovative ecofriendly ENMs should meet the highest standards of environmental safety and effectiveness, supporting economic development in terms of industrial competitiveness and innovation. Their application will contribute to the management, in more efficient and sustainable ways, of several systematic sources of petroleum pollution in various productive sectors, such as maritime transport, refining, mining, and liquefied natural gas terminals in marine waters. Finally, the use of ecofriendly ENMs, which provide rapid and effective removal of petroleum products without compromising the ecosystem, is a priority for the safeguard of the oceans.

**FUTURE PERSPECTIVES AND FORESEEN CHALLENGES**

As mentioned, the deliberate or accidental release of ENMs into the oceans will impact marine organisms, especially if solutions for their safe manufacturing are not properly considered. To this purpose, two main organizations, the University of California Center for Environmental Implications of Nanotechnology (UC CEIN) and the Marine Focus Group in the framework of Hazard Working Group of the NanoSafety Cluster of the European Commission, focused their mission on studying the impacts of ENMs on the marine environment. The UC CEIN was established in 2008 with funding from the U.S. National Science Foundation and the U.S. Environmental Protection Agency, with the aim of studying the impact of nanotechnology on the environment, including the identification of hazard and exposure scenarios that take into consideration the novel physicochemical properties of ENMs. The UC CEIN has made great progress in...
assembling a multidisciplinary team to develop the scientific underpinnings, research, knowledge, education, and outreach that is required for assessing the safety of nanotechnology in the environment. The approach includes high-throughput/high content screening (HTS and HCS) studies to develop structure–activity relationships (QSARs) that can be used to predict the impact of ENMs on organisms in freshwater, marine, and terrestrial environments.142,143 A major goal of UC CEIN is to integrate research goals and themes to better estimate realistic nanomaterial exposure scenarios (F&T), predict ecosystem impacts, and design safer materials. UC CEIN’s strategies for accomplishing this goal include iterative learning between environmental modeling, chemistry, toxicology, ecosystem impacts, and sociological research efforts. Marine studies have included species of phytoplankton as primary producers, and copepods and mussels as primary consumers linking photosynthesis as well as ENM transfer to higher trophic levels. The effect of ENMs on embryo development is also a focus of the Center. The UC CEIN has been renewed (2013–2018) and new directions are planned to address the integrated marine nanotoxicology effort. A central role is to identify which ENMs have important environmental impacts versus those that are safe. UC CEIN’s marine ecotoxicology effort uses this approach by linking ENM chemical properties, exposure, and biochemical responses of injury with the ecological and physical processes that ultimately regulate ecosystem-level impacts and ecosystem services.

Within the NanoSafety Cluster of the European Commission, the Marine Ecotox Focus Group in the framework of the Hazard Working Group (http://www.nanosafetycluster.eu/working-groups/2-hazard-wg/marine-ecotox.html) was established in 2011. Its mission is based on the following assumptions: (1) the marine environment is likely to be a sink of ENMs as it is for most manmade pollutants, and it is therefore possible that certain ENMs may bioaccumulate and biomagnify along the

TABLE 2. Remediation Mechanisms of Actions of Different Engineered Nanomaterials

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<th>CATALYST</th>
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<th>HYBRIDES II</th>
<th>Core—shell</th>
<th>Tautomeric species (&quot;oxygen/electron sponges&quot;)</th>
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Figure 5. Integration of engineered nanomaterials (ENM) chemistry, exposure modeling, high-throughput screening (HTS) and high-content screening (HCS) assays, and injury responses across different levels of biological organization. Dynamic energy budget (DEB) models enable the linkage of individual responses to population and community levels.
marine trophic chain, thus potentially affecting marine biological resources (wild and farmed); (2) ENMs may be transferred to humans through diet by consumption of contaminated seafood products; (3) ENMs may lead to a deterioration of marine environmental quality (coastal areas including natural and recreational interests) with social and economic repercussions; (4) some ENMs can, however, be used to reduce marine pollution, through selected applications, such as nanoremediation by binding and removing specific contaminants. The progress in the development of nanotechnologies and nanoenabled products is vast and continuously progressing. The aim of the Focus group is to offer a platform for linking nanotechnologists with ecotoxicologists, environmental scientists, analytical chemists, biochemists, molecular biologists, industries, and end users (public) to provide the proper scenario suitable for an overall risk assessment of ENMs in the marine environment. By doing this, the focus group will provide appropriate support for decision and policy makers, including an understanding of the risks that may occur for ENMs that fall outside the definition, guidance, further development of measurement techniques, and help dealing with changes during the life cycle in agreement with recent work.\textsuperscript{144}

Again, at the EU level, the Quality-Nano pan-European infrastructure for quality in nanomaterials safety testing is providing support for a full assessment of potential impacts of past and next generation of ENMs at all stages of their lifecycle including interactions with environmental matrices and biota (http://www.qualitynano.eu/).

At the national level, the Italian Marine Nano Ecotoxicology working group was formally announced in November 2012 and further enlarged at the International level during the first Marine NanoEcoToxicology Workshop (MANET) held in Palermo in 2012 and fully dedicated to addressing the ecotoxicology of ENMs in the marine environment. Currently, it groups more than 50 scientists from 23 countries including France, Germany, Sweden, the United Kingdom, and the United States, involving both universities and research centers. Its mission is to encourage applied and basic research as a support for a safe and sustainable use of ENMs, fostering interdisciplinary approaches for research and training in the field of nanocotoxicology and promoting informed discussion on ethical and relevant social issues as well. In this context, the Italian Marine Nano Ecotoxicology working group acts as a reference group for the evaluation of the health and environmental impacts of ENMs with regard to scientific research, industrial innovation, and regulatory issues. It has the potential to act as a growing network integrating multidisciplinary skills and acquire the necessary expertise to fill the existing prioritized gaps.

Conflict of Interest: The authors declare no competing financial interest.

REFERENCES AND NOTES


Figure 6. Combining nano- and bioremediation.


133. Deliyanni, E. A.; Bakoyannakis, D. N.; Zouboulis, A. I.; Matis, K. A. Sorption of As(V) Ions by Akaganeite-Type Nanocrystals. Chemosphere 2003, 50, 155–163.
