Chapter 15
Environmental Risk Assessment of Emerging Contaminants—The Case of Nanomaterials

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Abstract Risk assessment is a powerful tool to help evaluate potential environmental and health risks of novel materials. However, traditional risk assessment frameworks and methods often face significant challenges when evaluating novel materials due to uncertainties and data gaps. Engineered nanomaterials is one prominent example of new, advanced materials whereby scientists, researchers and decision-makers are still discussing best practices to modify and update risk assessment frameworks after nearly two decades of research. This chapter focuses on how early warning signs within the environmental risk assessment development process for nanomaterials were addressed with a focus on characterizing uncertainty. We shed light on how environmental risk assessment of nanomaterials transitioned from a state of "known unknowns" to data-driven inputs to conducting risk assessments. We also discuss ecotoxicological testing considerations, and in particular how methodological and technical challenges were addressed. Finally, we provide recommendations on how best to transfer identified best practices and knowledge to other emerging technologies and advanced materials.

Introduction—Environmental Risk Assessment of Nanomaterials and the Role of Uncertainty

The development of new materials, their widespread use in society and eventually their end-of-life management raises potential concerns over their environmental risks and safety (Hansen et al. 2013a). Key questions often include "Is this material an emerging contaminant?" and "Will this material pose new, hitherto unknown, risks

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to the environment and society?” These questions have proven to, in fact, be very complex to answer since multiple factors influence the environmental distribution, fate, effects, and ultimately the risks posed by any material. When we are dealing with novel materials, however, the complexity increases compared to conventional or well-known materials, as the scientific uncertainty can often be difficult to quantify and decision-makers are subsequently left to make choices that are not necessarily supported by scientific evidence.

While this opens up a whole range of theoretical and practical questions and considerations on how best to deal with novel materials, the introduction of engineered nanomaterials in consumer products and industrial applications provides a recent example of the development and application of a group of novel materials that has proved challenging to assess and formulate risk-based decisions. This chapter will therefore focus on the case of nanomaterials, and illustrates how early warning signs were addressed to move the field of nano-environmental fate and effects from a state of “known unknowns” to data-driven input to risk assessments. While this chapter will relate to nanomaterial risk assessment, it does not aim to evaluate the frameworks or tools to evaluate risks of nanomaterials nor the underlying or associated regulations. We conclude the chapter with several reflections on the field of nanomaterial risk assessment and provide recommendations on how best to transfer the acquired knowledge to other emerging technologies and advanced materials.

First, it is important to highlight that (quantitative) risk assessments are performed in order to evaluate risks and to support decision-making, rather than primarily serving as an academic exercise. Ideally, risk assessment should fully rely on scientific evidence (e.g., causal relationships between exposure and effect, such as dose–response assessments). However, this seldom occurs for novel environmental contaminants that have greater degrees of uncertainty, and therefore, more research is often needed to complete risk assessments and make decisions regarding the risks. This means that decision-making based on and/or assisted by risk assessments will often take place in the face of uncertainty, and the evaluation of uncertainty plays (or should play) a major role in any risk appraisal. This is widely acknowledged in the current regulatory practice and uncertainty analysis is for example an integrated part of the chemical safety assessment procedures issued by the European Chemicals Agency (ECHA 2012). Uncertainty is, however, a very dynamic parameter (or set of parameters), and only through time can the environmental risks of novel materials be more fully understood. The use and development of engineered nanoparticles in a variety of consumer products and other applications is no exception to this; although scientific knowledge has advanced and expanded significantly since the first early warning signs of adverse effects of nanoparticles on environmental organisms (Oberdörster 2004), significant uncertainty persists in understanding their environmental risks even after nearly two decades of research (Grieger et al. 2019).

Many different risk assessment frameworks and tools for nanomaterials exist today (Grieger et al. 2012; Hristozov et al. 2012; Oomen et al. 2018; Franken et al. 2020; Sorensen et al. 2019), and though they are different in their scope, applicability and resulting outcomes, they generally follow the traditional “risk assessment paradigm”, i.e., that risk is a function of exposure and effects. Therefore, most procedures within these nano-risk assessment frameworks and tools begin with information gathering and material characterization steps and then advance to effects (or "dose–response") assessment and exposure assessment. The outcome of these last two steps, which is most often scenario-based, is then aggregated into a final risk characterization step, which essentially concludes with the identification of risk or that no risk is expected (and/or more information may be needed). As mentioned above, this outcome of the risk assessment procedure is accompanied (or should be accompanied) by an uncertainty analysis that informs decision-makers of identified data gaps, limitations, and/or uncertainties relevant for the conclusion reached.

In 2007, the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) highlighted the following as main areas of uncertainty for identification of environmental risks of nanomaterials: environmental fate, behavior, and mobility; degradation, persistency, and bioaccumulation; and adverse effects to a variety of organisms (SCENIHR 2007). In other words, all steps of the environmental risk assessment framework of nanomaterials were considered to have serious data gaps and high degrees of uncertainty. Further data gaps were identified that centered around testing methods, equipment for testing and analyses, and the most appropriate metrics for expressing test results.

In addition to the SCENIHR 2007 report, Grieger et al. (2009) analyzed and characterized the types, levels, and nature of different uncertainties within the field of environmental risks of nanomaterials in a systematic characterization of the "known unknowns" of nanomaterial safety. This analysis was conducted by applying the Walker and Harremoës framework (Walker et al. 2003) to 31 peer-reviewed scientific papers and reports published between 2004 and 2008 on potential environmental, health, and safety (EHS) risks of nanomaterials. Overall, this work provided valuable insight on the data gaps and uncertainties as identified by scientific experts, governmental agencies, regulatory bodies, and national/international organizations in the early phase of nanomaterial risk identification. In the analysis that mapped the main areas of uncertainty and data gaps according to the reviewed papers and reports, Grieger et al. (2009) found that testing considerations, characterization of nanomaterials, effects assessment, and exposure assessment all were associated with significant uncertainty (Fig. 15.1). These findings were further supported by other reviews of the EHS data of nanomaterials with regards to uncertainty and knowledge gaps of the environmental risks of nanomaterials (*e.g.*, Maynard 2006; USEPA 2007; DEFRA 2007; OECD 2007, Baun et al. 2008). The Grieger et al. (2009) analysis showed that within the general locations of nanomaterial characterization, effects assessment, exposure assessment, testing considerations, and other areas (Fig. 15.1), a number of sub-locations of uncertainty stood out in terms of their frequency of being mentioned in the reviewed materials. The most frequently cited sub-locations of uncertainty across all sub-locations were: (1) lack of reference materials and standardization, (2) characterizing the environmental fate and behavior of nanomaterials, and (3) determining environmental effects and/or ecotoxicity.

In addition to mapping the main areas of uncertainty related to nanomaterial environmental risk assessment, Grieger et al. (2009) also analyzed the level and

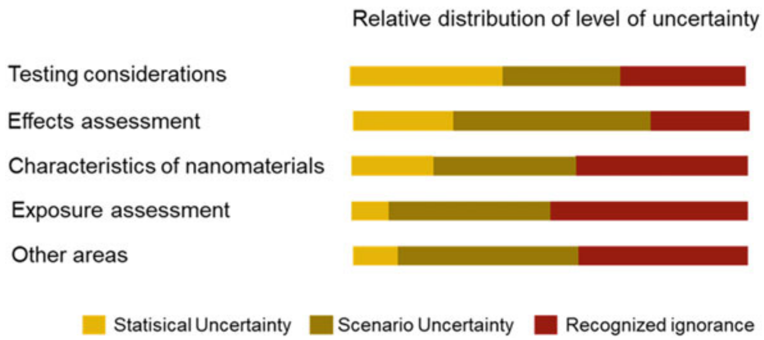

Fig. 15.1 Illustration of the relative distribution of the levels of uncertainty related to environmental, health and safety risks of nanomaterials among the five risk assessment areas where uncertainty was most predominant (redrawn from Grieger et al. 2009)

nature of these uncertainties. In accordance with the Walker and Harremoës framework, the level of uncertainty ranges from "ignorance" (i.e., unknown-unknowns) to "deterministic knowledge" (i.e., no uncertainty)—neither of which applies to nanomaterial risk assessment. Between these two extremes, other levels of uncertainty include "recognized ignorance" (i.e., known-unknowns), "scenario uncertainty" (i.e., known outcomes, unknown probabilities), and "statistical uncertainty" (i.e., known outcomes, known probabilities). While recognized ignorance is self-explanatory but impossible to quantify, the two other levels deserve a bit of explanation. In brief, scenario uncertainty relates to, e.g., scientific experiments where the outcomes are known (or can be expected) but dependent on a specific scenario, and therefore probabilities of the outcomes are unknown. In contrast to this, statistical uncertainty describes the uncertainty normally addressed in scientific studies, in that the possible outcomes, e.g., of an experiment, are known and the probabilities of these outcomes are also known or can be quantified using statistical models. While statistical uncertainty can be reduced by increased experimentation, scenario uncertainty can be reduced by more empirical research. Understanding and describing the level of uncertainty is therefore useful when evaluating whether more empirical research may help reduce uncertainties, while conducting more research can move the level of uncertainty from scenario uncertainty towards statistical uncertainty—a common approach for scientists modeling and quantifying uncertainty. In their analysis, Grieger et al. (2009) found that the level of uncertainty across all locations was between scenario uncertainty and recognized ignorance (Fig. 15.1). This showed that the general level of knowledge in 2006–2009 was at a relatively early stage of development. Finally, the nature of uncertainty was evaluated to be mainly epistemic, indicating that further research could be expected to reduce most of the uncertainties within the field.

Leveraging these key findings on the uncertainties of nano-EHS risks, the following sections take a closer look at how knowledge and data were acquired in the field of nanomaterial environmental risk assessment, including aspects of nano-effects and exposure assessments, since 2009. Using the Walker and Harremoës framework and the Grieger et al. (2009) application of their framework, we see that the scientific community made advancements that moved from "scenario uncertainty" towards "statistical uncertainty" in the reduction of nano-risk assessment uncertainties, particularly in ecotoxicity testing. Testing considerations will therefore be given a specific emphasis in the following sections, as this was the area of uncertainty that was most frequently cited in the systematic review of "known unknowns" of nano-EHS risks in Grieger et al. (2009). It should also be mentioned that since risk is a function of exposure and effects, it is equally important to reflect on the fate, effect, and exposure assessment when discussing risks of novel materials. These topics are, however, not included in this chapter, although a number of reviews have been published in these fields including Peijnenburg et al. (2015), Baun et al. (2017), and Nowack (2017).

We also note that in the following sections on ecotoxicity testing of nanomaterials and implications for nano-environmental risk assessment, the terms "relevance" and "reliability" are used often. These terms have specific meanings when used in a regulatory context (Box 15.1). Taken together, relevance and reliability form the cornerstones in defining test results as being deemed "adequate for regulatory purposes". Further, only data that are adequate for regulatory purposes can be used in risk assessment by regulatory bodies, and therefore new/updated methods for nanomaterials must be both reliable and relevant to have an impact on risk assessments (OECD 2005).

Box 15.1 The Organisation for Economic Co-operation and Development (OECD) definitions of regulatory reliability and relevance (OECD 2005)

"Reliability is defined as the extent of reproducibility of results from a test within and among laboratories over time, when performed using the same standardised protocol. The relevance of a test method describes the relationship between the test and the effect in the target species, and whether the test method is meaningful and useful for a defined purpose, with the limitations identified. In brief, it is the extent to which the test method correctly measures or predicts the (biological) effect of interest, as appropriate."

Ecotoxicity Testing of Nanomaterials—Developments and Implications for Risk Assessment

The number of studies regarding the ecotoxicological effects of nanomaterials has increased rapidly since the first paper was published in this field in 2004 (Oberdörster 2004). For example, in the project ENRHES (Engineered Nanoparticles—Review of Health and Environmental Safety), a comprehensive literature study revealed that 89 nano-ecotoxicity studies had been published from 2004 to 2009 (Stone et al. 2010a). Five years later, the NanoE-Tox database included 1,518 nano-ecotoxicity studies in

2015 (Juganson et al. 2015), and only six years after that in 2021, a search in literature databases resulted in more than 6,000 hits, e.g., 6,156 papers listed in Web of Science (search term "nano*" AND "ecotox"; May 2021). These findings correspond to a 69-fold increase in nano-ecotoxicity papers over an 11-year time span.

Although the number of scientific papers has increased quite dramatically over the past 10–15 years, the regulatory adequacy of the performed studies has been questioned by several authors (Hartmann et al. 2017; Hjorth et al. 2017). This is a critical aspect when evaluating the performed nano-ecotoxicity studies in the peer-reviewed literature, especially for decision-making purposes. Several authors have also expressed concerns about the regulatory adequacy of nano-ecotoxicity tests even if the tests were carried out in accordance with the existing OECD test guidelines (OECD TGs). In fact, the questions regarding the need for adapting the OECD TGs, or to provide test-specific guidance, have been raised multiple times since the early days of nanotoxicology. In response to the concerns raised, OECD launched a Working Party for Manufactured Nanomaterials in 2006. The work of this international cooperation culminated in 2020 with the publication of the OECD Guidance Document 317 on aquatic and sediment toxicological testing of nanomaterials (OECD 2020; Petersen et al. 2021). In the context of improving the regulatory adequacy of ecotoxicity tests for nanomaterials, this work is highly relevant and urgently needed (Hjorth et al. 2017; Hansen et al. 2017). The guidance is targeted at improving the reliability and relevance of test results obtained in experiments following the OECD TGs, e.g., for fulfilling information requirements in REACH (Nielsen et al. 2021). In addition, the OECD 317 guidance is expected to improve the general quality of scientific studies by providing urgently needed recommendations for "best principles" for ecotoxicity studies of nanomaterials (Hon et al. 2019).

While it is beyond the scope of this chapter to give a full account of all the considerations that have shaped the current guidance document for nanomaterial aquatic toxicity testing, we will provide a glimpse into the numerous testing considerations that have arisen during the past 15 years in the following sections. In particular, we elaborate on the need for nanomaterial characterization for exposure assessment, nanoparticle-specific testing considerations, the search for nano-specific effects, and finally the regulatory use of ecotoxicological data generated in standardized tests of nanomaterials.

Nanomaterial Characterization in Exposure Assessment

The physical and chemical (termed "physicochemical") characterization of nanomaterials before, during and after testing has been a major focus in improving the reliability of ecotoxicity test results. This is because the physicochemical parameters of nanomaterials (e.g., size, size distribution, shape, and charge) are considered to play critical roles in not only understanding potential exposures to, e.g., ecological organisms, but also how these exposures impact effects, and therefore risks. Since 2010, the field of nanomaterial characterization has significantly improved, but the number of relevant physicochemical characterization parameters and the importance of each one is still a topic debated in the scientific literature. It is nevertheless clear that in order to ensure proper, scientifically justified results from nano-ecotoxicity tests, exposure needs to be appropriately characterized, which relies on the characterization of nanomaterials across a range of physicochemical parameters. For regular, bulk-scale chemicals, the results of ecotoxicity tests are often evaluated using dose (concentration)-response curves, where the concentrations of the chemicals and therefore exposures are known. However, for nanomaterials, other physicochemical parameters than mass-based concentrations may be more relevant to describe exposures and risks, such as nanomaterial size, size distribution, shape, charge, zeta potential, or dissolution rate (Drasler et al. 2017).

As an example, particle size determination has been of high priority in many studies due to the expectation that the biological effects of nanomaterials may be related to particle size and numbers of particles. In the media used for ecotoxicity testing, it is not trivial to determine the particle size distributions – a process most often performed by dynamic light scattering (DLS), which provides indirect measures of hydrodynamic diameters based on scattered light. The potential release of metal ions from some nanomaterials (e.g., Ag, CuO, and ZnO) is also a topic that has received a lot of attention over the past decade since the metal ions in many cases have been found to account for most of the toxicity observed (Notter et al. 2014). Therefore today, it is required that dissolution is accounted for in ecotoxicity tests involving metal-based nanomaterials (OECD 2020). Further, and similar to all transformation processes taking place during testing, it is the dissolution kinetics related to aquatic media that need to be documented rather than the dissolution constant. In fact, several studies have shown that the quantification of dissolution kinetics, as well as nanomaterial losses before, during and after incubation, was crucial for determining the actual exposure concentrations of nanomaterials in ecotoxicity tests (Sørensen and Baun 2015; Sekine et al. 2015; Cupi et al. 2015).

Throughout the development of the nano-ecotoxicology field, a number of physicochemical parameters have been suggested to be of importance for characterizing nanomaterials during testing (Stone et al. 2010b). Even today there is no full scientific understanding of which parameters govern the ecotoxicity of nanomaterials (Hjorth et al. 2017; Bondarenko et al. 2016; Hund-Rinke et al. 2015, 2016; Drasler et al. 2017). As mentioned before, the recent OECD Guidance Document No. 317 provides recommendations for regulatory-oriented testing, while from a scientific standpoint, the current recommendation is that as much characterization data as possible should be reported for ecotoxicity tests of nanomaterials. Hjorth et al. (2017) phrased this as: "a move from the traditional focus on controlling exposure, (as recommended) in TGs applied to dissolved chemicals, toward a focus on describing exposure through a range of different techniques (Wickson et al. 2014; Sørensen et al. 2016)".

Finally, it should be emphasized that most published studies on nano-ecotoxicology have focused on nanoparticle characterization in the stages before ecotoxicity testing. However, it has been increasingly recognized and deemed essential to quantify the actual exposure during testing to increase both the scientific value and the regulatory adequacy of nano-ecotoxicological studies (Hartmann et al.

2017). The lack of nanoparticle characterization during testing may be problematic for the interpretation of individual test results as well as for comparing studies, even if the same nanomaterials were tested using the same test method. In other words, it can be difficult to draw general conclusions even from test results using standard methods without full nanomaterial characterization. This, in turn, poses challenges for the development of validated in silico methods for predicting the ecotoxicological potential of nanomaterials based on physicochemical material properties.

Challenges in Nanomaterial Effect Assessments—Particles Are Not Dissolved Chemicals

Overall, standardized ecotoxicological tests have been challenged by the fact that nanomaterials do not behave like dissolved chemicals in aqueous suspensions. For example, it may be very problematic to maintain stable suspensions during the testing period, and even with extensive characterization, new and unexpected phenomena may be encountered as described in the previous section.

To illustrate these challenges, we will take a closer look at the algal growth rate inhibition test. This test is one of the three mandatory ecotoxicological tests to be carried out for classification, labeling, and risk assessment purposes for chemicals and nanomaterials in the European Union (EU). The procedure for the algal test is described in OECD TG201 (OECD 2011), as well as in the somewhat stricter standard of the International Organization for Standardization (ISO 2012). When nanomaterials are tested in algal growth rate inhibition tests, the nanomaterials may scatter light and therefore decrease the amount of light reaching the algal cells, thereby inhibiting or reducing the growth rate (i.e., "shading"), rather than contributing to or leading to any ecotoxicological effect on the algal cells. Shading has, in fact, been identified as a potential confounding factor of algal testing of nanomaterials since the very first publications in the area (Hund-Rinke and Simon 2006) and continues today (Hjorth et al. 2017; Nguyen et al. 2020). Although practical solutions exist to determine whether shading occurs (Fig. 15.2), it is still an open question as to what degree it influences the outcome of standard testing. Shading caused by nanomaterials in algal tests is a prominent example of a nano-specific influence that could be interpreted as an effect, but may also be a result of how tests are performed. Therefore, extrapolation of such effects from the algal growth rate inhibition tests to other organisms or the ecosystem will often not be valid due to this confounding effect of shading (Skjolding et al. 2016; Hjorth et al. 2017).

Further, the potential shading effects of nanomaterials have often been mentioned as a possible cause of the effects observed in algal toxicity tests (Handy et al. 2012; Petersen et al. 2015; Sørensen et al. 2016). A number of scientific studies on disclosing shading effects of nanomaterials have been conducted, predominantly by separating algal cells from the nanomaterial suspension (Fig. 15.2) to eliminate any direct toxicity, and illuminating the algae through the nanomaterial suspension

Fig. 15.2 Illustration of a testing setup for algal toxicity tests (**a**) and two setups to distinguish between physical and chemical effects of nanomaterials (**b** and **c**). In **b**, shading effects may be investigated using a double-vial setup, where algal cells are contained in the smaller inner-vial, surrounded by the nanoparticle suspension in the larger outer-vial. In **c**, the so-called "sandwich" setup reveals whether physical shading occurs in the algal test. Here one 6-well plate is filled with algal suspension without nanomaterials (c1) and another plate (c2) is filled with nanomaterial suspension without algae added. Plate c2 is placed on top of c1 and the combined "sandwich" setup is illuminated from above. For both modified setups (**b** and **c**), a decline in growth rate will be caused by physical shading caused by the tested nanomaterial (Modified from Sørensen et al. 2015). In addition to measuring growth rate (or biomass) in algal tests, changes in the algal pigment composition have also been a direct measure to quantify the effects of nanomaterial shading (Hjorth et al. 2016). This approach relies on the ability of algae to rapidly adapt their pigment composition in response to changing light conditions and therefore serves as an effective endpoint to quantify shading effects of nanomaterials (Hjorth et al. 2016)

(a so-called "sandwich" test) to determine if growth rate inhibition occurs as a result of nanomaterials obstructing the light available to the algae (Aruoja et al. 2009; Hartmann et al. 2010; Hund-Rinke and Simon 2006; van Hoecke et al. 2009). These studies have generally rejected this type of indirect shading as a cause of growth inhibition. In contrast, Sørensen et al. (2016) identified substantial growth rate inhibition when separating algae and platinum nanoparticles (PtNPs) in a double-vial setup.

Both the "sandwich" and separation setup shown in Fig. 15.2 aim to quantify whether shading from nanomaterials in suspension contributes to growth reduction. However, shading from nanomaterials attached directly to the algae (sometimes referred to as "cellular shading") is not considered by these approaches. Cellular shading may be highly important, as several studies have demonstrated nanomaterial attachment to algal cells (Aruoja et al. 2009; van Hoecke et al. 2009, Hartmann et al. 2013; Sørensen et al. 2016; Pang et al. 2020; Abdolahpur Monikh 2021). In general, it is believed that algal cells can overcome temporary shading and that this may not cause population effects due to growth rate reductions. Adhesion of nanomaterials to algal cells can however result in permanent shading but can also cause other physical effects like the limitation of nutrient availability.

The issues mentioned above for the algal test illustrate how each of the standardized ecotoxicity tests used for risk assessment purposes faces specific challenges when applied to particle suspensions rather than the dissolved chemicals that they were developed for (Skjolding et al. 2016). The standardized tests with fish and crustaceans are both challenged by keeping nanomaterial suspensions stable during incubation but also by a particle-specific change in exposure pathway by active or passive intake, either through ingestion or attachment on gills. This is fundamentally different from the exposure through molecular diffusion that is dominating in tests of dissolved chemicals.

As described above, it is not an easy task to document that the exposure to nanomaterials is controlled during an experiment. This is further complicated by the presence of organisms since their presence and interaction with nanomaterial suspension will interfere with the characterization techniques. For example, using DLS to determine the "in situ" size distribution of the suspension at the end of an algal test is hampered by the fact that samples extracted do not only contain algae but also that the algae have excreted exudates during incubation. Figure 15.3 shows two examples of the changes that mono-dispersed suspensions of gold and titanium dioxide nanoparticles undergo during 48 h of testing in a standardized algal toxicity test (from Hartmann et al. 2013). It is evident that the size distribution of particles in the medium is affected, but also that significant interaction with organisms also takes place.

The presence of exudates will not only influence the nanoparticle characterization (Fig. 15.3) but may also affect the toxicity of the nanomaterials. Exudates as well as naturally occurring organic matter (NOM) may form a coating on the nanomaterial surface. This is often referred to as an eco-corona. The influence of NOM on the ecotoxicity of nanomaterials has been a topic of many ecotoxicity studies (e.g., Arvidsson et al. 2020), while fewer studies have focused on eco-coronas composed of organism exudates (Docter et al. 2015; Nasser and Lynch 2016). Knowledge in this area is developing and shows that biomolecule coronas are established rapidly (Hjorth et al. 2017). This type of interaction is likely to occur for all nanomaterials but perhaps to different degrees depending on the composition and surface properties of nanoparticles. This aspect is of high importance for environmental risk assessment since the occurrence of pristine nanomaterials, once released into the environment, is unlikely.

In a recent meta-analysis of the influence of NOM on the aquatic ecotoxicity of nanomaterials, Arvidsson et al. (2020) examined 66 studies of metal and metal oxide nanomaterials. It was found that 84% of these studies showed a reduction in nanomaterial ecotoxicity in the presence of NOM. No strong correlation between the 50% effective, inhibitory or lethal concentrations (XC_{50} values) and concentrations of NOM occurred, but it was found that the toxicity decreased 1–10 times when NOM was present during testing (Arvidsson et al. 2020). This led the authors to suggest that XC_{50} values from experiments without NOM present may be used in environmental risk assessments of nanomaterials as reasonably conservative estimates of XC_{50} values with NOM present.

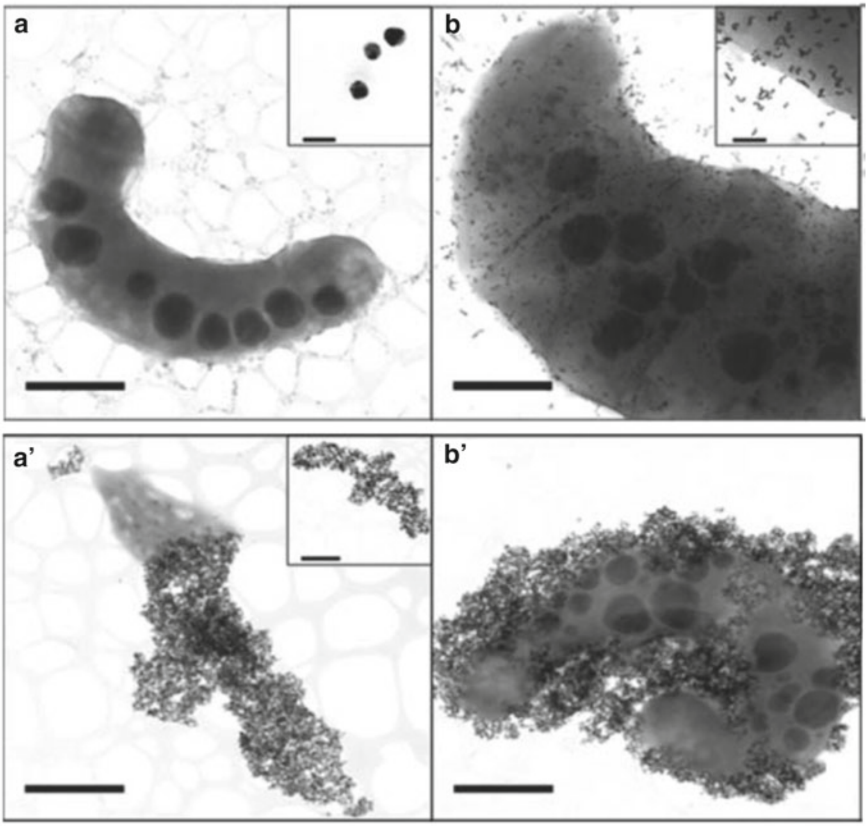

Fig. 15.3 Transmission electron microscopy (TEM) images of algal cells exposed to 1.9 mg/L gold nanoparticles (upper panel) and 35 mg/L titanium dioxide nanoparticles (lower panel). Scale bars are 2 μm. In the upper panel, TEM images were taken at the start of the test (**a**) and after 24 h of testing (**b**). Insert shows, **a**: individual gold nanoparticles (scale bar is 40 nm), **b**: attached gold nanoparticles at the edge of an algal cell (scale bar is 300 nm). In the lower panels, TEM images were taken after 24 h (**a**') and after 48 h (**b**') of testing. Modified from Hartmann et al. (2013)

Nanomaterial-Specific Effects and Modes of Action

While the discipline of nanoecotoxicology has developed significantly during the past decade, the exact toxic mechanisms or modes of action of nanomaterials remain unclear. This may be influenced by the technical challenges in testing, as discussed briefly above and as outlined by Skjolding et al. (2016) who highlighted that several factors must be accounted for to disclose whether a "nano-specific effect" occurs. As shown in Fig. 15.4, the different responses and potential confounding factors depend on the intrinsic and extrinsic properties of the nanomaterials. The response types relate to the dissolution in aqueous media, intake and discrete localization within the test organisms, as well as physical effects on test organisms (Fig. 15.4). As illustrated

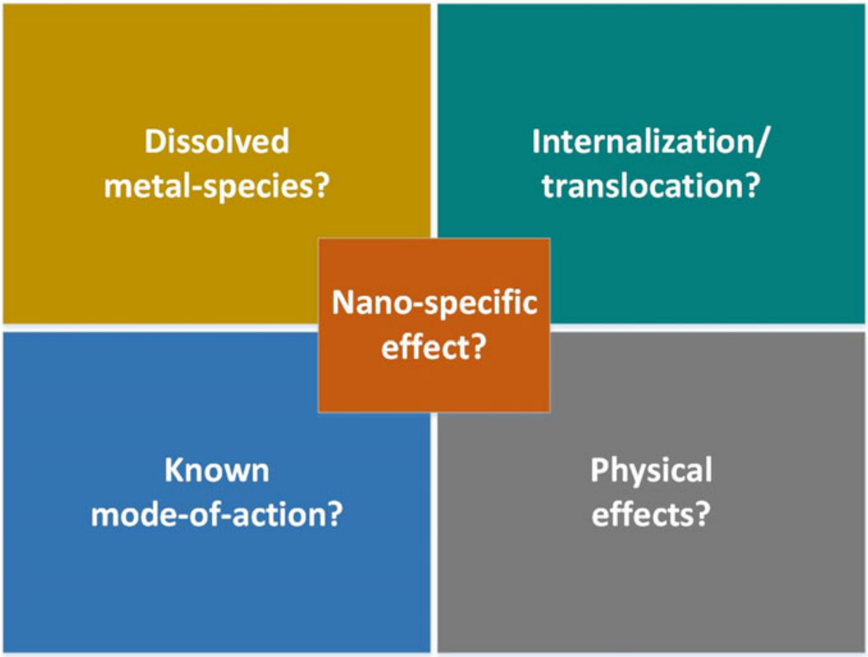

Fig. 15.4 Responses that occur concomitantly in ecotoxicity tests of nanomaterials which influence or dominate the outcome of the tests: Effects related to the dissolved fraction (top left), effects of internalization and translocation of nanomaterials due to their small size (top right), physical effects of the nanoparticles (bottom right), and the nanoparticle effects via a proposed mode of action related to, e.g., the generation of reactive oxygen species (bottom left). Based on Skjolding et al. (2016)

for the algal test above, the test setup may not allow for a differentiation of response type and since these occur simultaneously and (very) dynamically, it is often difficult to disclose an actual mode of action (Hjorth et al. 2017).

A multitude of studies has searched for modes of action or toxic mechanisms across a range of nanomaterials. For example, Lynch et al. (2014) summarized the possible mechanisms as follows:

- Dissolution, whereby the observed effects are caused by toxic ions;
- Nanomaterial surface effects, which lead to effects on the conformation of biomolecules;
- Nanomaterial structure effects, such as photochemical and redox properties resulting from bandgap or crystalline forms of nanomaterials;
- Nanomaterials as vectors, whereby nanomaterials may act as vectors to transport other toxic chemicals to sensitive targets (e.g., Trojan horse effects).

As mentioned above, metals and metal oxide nanomaterials (e.g., CuO, ZnO or Ag) are prone to dissolution. Further, many studies have focused on the role of the nanomaterial versus the released ions in explaining nano-ecotoxicity. For metals and

Table 15.1 Overview of use of standardized test methods in nanomaterial risk assessment

Use of testing data	Aim of testing	
	Classification	Environmental protection
Risk assessment step	Hazard identification Hazard ranking	Hazard assessment
Focus in relation to regulatory use	Reliability	Relevance
Experimental focus	Controlled experiments	Environmental realism
Results evaluation	Concentration–response curves	Assessment factors Species sensitivity distributions
Outcome - values	LC_{50}, EC_{50}, NOEC	PNEC
Nature of outcome	Relative	Absolute

metal oxide nanomaterials, the ability to generate reactive oxygen species (ROS, such as superoxide, hydroxyl radicals, and hydrogen peroxide) and induce oxidative stress is still the only distinct modes of action documented for nanomaterials in aquatic organisms (Ivask et al. 2014; Juganson et al. 2015; von Moos and Slaveykova 2014). However, even if this mode of action is suspected, it may be technically difficult to prove in tests, since the formation of extra- or intracellular ROS can trigger a cascade of cellular events (von Moos and Slaveykova 2014). Therefore, care has to be taken before conclusions can be drawn, even on this known mechanism of toxicity.

Finally, it is important to underline that while the ecotoxicity tests used for risk assessment do not allow an assessment of the mechanism causing the toxicity, this is in alignment with current regulatory approaches for the hazard assessment of conventional chemicals. The understanding of the potential mode of action of nanomaterials in environmental organisms is a question of high scientific relevance. This may in turn influence the design and endpoints of ecotoxicity testing for regulatory purposes, as shown by the recommendations of the 2020 OECD guidance document for aquatic toxicity testing of manufactured nanomaterials (OECD 2020). All this is tightly linked to the use and role of ecotoxicity data in risk assessment (Table 15.1) as will be further described in the following section.

Use of Ecotoxicity Data in Nanomaterial Risk Assessment

In risk assessment of "regular" (i.e., conventional) chemicals, ecotoxicity data are used in two distinct ways: 1) for "classification" purposes, i.e., to classify, label and determine the toxicity (T) criterion in a PBT assessment (PBT – persistence, bioaccumulation, toxicity), and 2) for "protection" purposes, i.e., the derivation of predicted no-effect concentrations (PNECs). Table 15.1 lists a number of differences in the purpose of ecotoxicity testing for these two uses of the data generated, also applicable to risk assessment of nanomaterials.

This approach is in agreement with the classical distinction in ecotoxicology between "anticipatory laboratory ecotoxicity testing" aimed at hazard identification and "assessment testing" aimed at environmental impact evaluation (Calow 1997; Hjorth 2016). As such, ecotoxicity testing using guidelines should support regulatory hazard ranking and labeling, whereas field testing should ideally inform decisions on environmental quality standards aimed at protecting the environment. For this reason, guideline tests focus on controlled experiments and inter-laboratory transferability (i.e., regulatory reliability), whereas environmental realism plays a much stronger role in the validity of field-scale tests.

In reality, for nanomaterials as well as for conventional chemicals, test results generated by following guideline recommendations for classification purposes as shown in Table 15.1 will also form the basis for evaluations of environmental protection. This has been termed as a "double use" of the data from guideline testing (Hjorth et al. 2017). The measures for regulatory adequacy, like relevance and reliability, will favor studies carried out according to internationally agreed-upon guidelines and standards (Hartmann et al. 2017). Therefore, there is a very strong focus on tests using the core set of organisms (i.e., fish, crustaceans, algae). The dataset generated with the original aim of ranking and classifying chemicals (i.e., a relative outcome) will then often be the only dataset available, and will therefore be used for defining environmental protection goals, i.e., an absolute outcome (Table 15.1).

For conventional chemicals, a precedent for this "double use" of ecotoxicity data has been established over the last 30 years. The use of the same test results at different stages in the risk assessment procedure relies on cut-off values and extrapolation methods that have been agreed upon by regulatory authorities and stakeholders, often following advice from expert groups (Syberg and Hansen 2015). For nanomaterials, the double use of guideline testing data for both purposes, as shown in Table 15.1, remains to be critically evaluated, though the appropriateness of extrapolation from guideline test data for PNEC determination has been questioned by several authors (Lützhøft et al. 2015; Baun et al. 2009). This critique has been made on the basis of the fundamental difference between dissolved chemicals and particles with regard to their behavior and effects in laboratory studies, compared to real-world behaviors and effects that may occur in complex environmental matrices.

The current procedures for establishing protective values have been transferred directly from dissolved chemicals to nanomaterials, relying mainly on so-called assessment factors, and in some cases, species sensitivity distributions (SSD). Both methods rely on the use of extrapolation approaches from laboratory studies to the protection of ecosystem functions. These approaches are founded on the basic toxicological notion that a higher concentration will lead to greater effects. Thus, monotonous concentration–response curves and stable suspensions during testing are inherent requirements for the ecotoxicity data to be valid for risk assessment purposes. As described above, this prerequisite is often severely challenged when nanomaterials are tested in standardized tests. In concentration–response experiments, changes in nanoparticle behavior may furthermore be nanoparticle concentration-dependent (e.g., stronger aggregation at high concentrations of nanoparticles) and this may alter the bioavailability of the particles and not necessarily result in monotonous concentration–response curves. For PNEC estimations by the assessment factor approach, this may be a problem for the validity of the extrapolation from standardized tests, since actual environmental effects may occur at lower concentrations than those used in the standardized tests, although this remains to be systematically investigated.

The other approach for PNEC determination, i.e., using SSDs, has only been used to a limited extent for nanomaterials (Sørensen et al. 2020). This field of application has significantly increased since the first nano-SSD was developed in 2013 by Gottschalk et al. (2013). For example, Chen et al. (2018) constructed SSDs considering nanomaterial structural characteristics, such as coating, size, shape, and experimental conditions for Ag, CeO_2, CuO, TiO_2 and ZnO nanoparticles. To account for the differences in the relevance and reliability of ecotoxicological data across studies, Semenzin et al. (2015) developed a nano-species sensitivity weighted distribution (n-SSWD). This approach was compared to a conventional SSD model as well as to a probabilistic SDD model for nanomaterials by Sørensen et al. (2020). In this model comparison, only studies regarding two reference materials, NM-300 K (silver) and NM-105 (titanium dioxide), were included and all data were evaluated by the nano-specific "nanoCRED" reliability criteria (Hartmann et al. 2016). While it was found that the conventional SSD generally yielded the most conservative but least precise output, the estimated hazardous concentrations for 5% of species (HC_5 values) of all models were within a narrow concentration range (Sørensen et al. 2020). Interestingly, the majority of studies were evaluated as being reliable from the regulatory perspective, although it was found that the degree of nano-specific characterization varied greatly (Sørensen et al. 2020). Generally, this study showed that for a large, well-curated data set, the output relevant for PNEC estimation was not very sensitive towards the choice of SSD model, but also that regulatory adequacy was improved by taking nano-specific considerations into account. This is important for the further development of nanomaterial risk assessment approaches, since PNEC values generated from SSD were not as dependent on extrapolation factors as PNEC values estimated by the assessment factor approach.

Overall, this section emphasizes that nanoecotoxicology tests serve different purposes, and different tests are needed to fulfill different regulatory needs in regard to risk assessment (Table 15.1). For hazard identification purposes, an ideal test would allow for controlled exposure conditions which, in combination with thorough nanomaterial characterization, would allow for reliable and reproducible benchmarking. For hazard assessment purposes, testing should ideally be carried out at environmentally realistic concentrations and under realistic conditions (Table 15.1). This type of testing will inherently be challenged in the current definition of regulatory reliability, but their relevance for the regulatory question at hand will be higher than that for the currently used approaches. For this, new tests/test designs and ecotoxicological endpoints are most likely needed, and extrapolation methods should be scrutinized in a systematic way (Lützhøft et al. 2015; Hjorth 2016; Aitken et al. 2011; Palmqvist et al. 2015; Syberg and Hansen 2015). Lastly, it is important to underline that the data with little regulatory relevance should not be confused with "bad or flawed data". This is because scientific studies without a regulatory focus or regulatory compliance have a very high value in themselves and are still needed to further develop the field of nanoecotoxicology (Hjorth et al. 2016; Wickson et al. 2014).

Reflections from Nanomaterial Toxicity Testing and Perspectives for Risk Assessment of Emerging Contaminants

In the preceding sections, some of the fundamental uncertainties related to performing risk assessment on novel materials have been described and exemplified by reviewing the development of regulatory-relevant ecotoxicity testing for nanomaterials over the past 15 years. Through this reflection, it becomes clear that the knowledge base has expanded significantly over this time period, and for several areas, the level of uncertainty has moved from "recognized ignorance" to "scenario uncertainty" to "statistical uncertainty". More than 15 years later, the fields of nano-EHS and risk assessment have expanded significantly, with thousands of peer-reviewed articles published on the topic of nano-ecotoxicity alone, and therefore a thorough review of papers and reports on nano-EHS knowledge would require a substantial undertaking, that may benefit from new advancements in data mining and automatic text analysis.

Also, through a reflection of the field of nano-ecotoxicology, it has become clear that it takes time and dedicated research efforts to reach a level where specific testing guidance can be given regarding relevant and reliable data generation for risk assessment of novel materials. In the case of ecotoxicity of nanomaterials, work in this field was initiated by the OECD in 2007 and ultimately finalized 13 years later through the publication of the previously-mentioned guidance document (OECD 2020). While this may seem like a long time period to develop this guidance, it is in fact faster than what has been observed previously for other emerging contaminants (Syberg and Hansen 2015). During this time, the production and application of nanomaterials have also increased significantly (Hansen et al. 2020), and the reliance on risk assessment for decision-making for nanomaterials has faced numerous challenges. This is partly due to the complexities and uncertainties described in this chapter but also due to underlying challenges for risk assessment frameworks for decision making (Grieger et al. 2009, 2019).

For novel materials and emerging environmental contaminants, this opens the question whether we must wait another 13 years to assess each new case, or whether we have learned from the case of nanomaterials, that a more proactive approach to risk assessment can be implemented. It is important to reflect on what we have learned from the nano-risk analysis that could be applicable to other fields that also are characterized by having sparse data and having a level of uncertainty that ranges between "recognized ignorance" and "scenario uncertainty."

Grieger et al. (2019) provided a perspective on this issue and concluded that the preceding 15 years of experience with nanomaterial risk analysis should be used to address potential risk issues of other emerging technologies or contaminants since the pace of innovation is surpassing the pace of risk identification and quantification. The authors suggested a number of best practices that may be applicable to other emerging and disruptive technologies (e.g., synthetic biology, 3-D printing, climate engineering technologies) (Box 15.2, Grieger et al. 2019).

Box 15.2 Five best practices for risk analysis of emerging technologies based on experiences from nanomaterial risk analysis (summary based on Grieger et al. 2019)

1. Promote Research Tailored for Regulatory Decision-Making Nano-risk research has largely been directed towards understanding the science rather than meeting decision-making and regulatory needs. While it is possible to link evolving nano-safety data to decision and policy-relevant needs using “bottom-up” strategies, the initiation of strategic, purposeful regulatory-relevant science programs (*i.e.*, using “top-down” strategies) at the start of major risk-based efforts for emerging technologies could help target research more effectively towards regulatory decision-making
2. Set Realistic Time, Cost, and Complexity Estimates to Develop Risk Analysis Similar to nano-risk analysis, the process of identifying risks, adapting or developing assessment protocols and procedures, and testing, validating, and harmonizing risk assessment methods for other emerging technologies are also likely be complex, time-consuming, and expensive. This may especially be the case if this process is based on the traditional approach of relying on experimental evidence and knowledge-based assessments for risk evaluations. It may help prepare and align stakeholder expectations early on to have realistic estimates of the time, costs, and degrees of complexities involved to derive concrete conclusions regarding risks. These estimates may help prepare industry, policymakers, and other decision-makers prioritize research efforts and funding programs directed at near-term methods, policy or decision-making while the underlying safety science is developed
3. Develop Proactive Strategies to Deal with Uncertainties in Risk Analysis and Decision-Making Scientific uncertainty has been one of the main obstacles in nanomaterial risk analysis. In general, standard approaches to handle uncertainties in risk assessment may not be well-suited for emerging technologies that are often characterized by having deep and extensive uncertainties in terms of potential risk pathways and consequences. Rather, risk assessment efforts for nanomaterials and other emerging technologies may benefit from including or being complemented by separate uncertainty assessments that identify and describe different scientific uncertainties and communicate how they may impact overall risk assessments and evaluations. Dynamic risk evaluation and management processes may be useful for dealing with emerging technological risks, as they allow for adaptive responses to quickly evolving scenarios or in light of new information. Adaptive and responsible risk governance frameworks that specifically account for uncertainty in risk evaluations, incorporate diverse stakeholder perspectives, and include procedural robustness may also be useful to proactively deal with uncertainty in risk analysis and decision-support

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4. Develop Mechanisms to Share Risk Data while Protecting Privacy, Confidentiality, and Proprietary Information
Concrete conclusions regarding the potential risks of nanomaterials have been hampered by challenges related to how nano-risk data have been managed and harmonized, along with issues of privacy, confidentiality, and intellectual property. Having more harmonized, multi-scale, and decision-directed approaches may help avoid some challenges related to data harmonization and integration. Future risk assessment and management efforts could rely on robust communication mechanisms between researchers and, with appropriate funding, integrate risk research efforts with respect to curation functionality, infrastructure, and communication processes at multiple levels of granularity from the onset

5. Critically Evaluate and Select Robust, Fit-For-Purpose Tools for Risk Analysis
While pursuing and deriving risk assessment for nanomaterials and other emerging technologies is clearly worthwhile in many cases (*e.g.*, following “traditional science” processes), it may also be lengthy and time-consuming, and there may be other risk evaluation approaches that could be more applicable for a given decision. A process that critically evaluates diverse evaluation frameworks and approaches followed by a transparent selection process for an emerging technology could be beneficial early-on to ensure that the most fit-for-purpose risk analysis framework is selected for utilization, further exploration, or to ultimately produce outcomes that meet decision-makers’ needs

While the perspectives and best practices put forward by Grieger et al. (2019) target a more general level for evaluating risks of other new or novel technologies, other articles have identified “early” risk indicators for nanomaterials (Subramanian et al. 2016; Arvidsson et al. 2018). In fact, as early as in 2008, Hansen et al. (2008) analyzed the introduction of nanomaterials and associated risks according to the “Late lessons for Early Warnings” presented by the European Environment Agency in 2001 (EEA 2001) and updated in 2013 (Hansen et al. 2013a). The same year, five early warning signs for harmful properties of nanomaterials were suggested by Hansen et al. (2013b), including novelty, persistency, bioaccumulation, the potential for being readily dispersed in the environment, and potential for causing irreversible action (e.g., toxicity). Hansen et al. (2013b) assessed these early warning signs using a set of five well-known nanomaterials, but more importantly for the field of emerging risk areas, they also discussed how these warning signs could be used by stakeholders in an effort to develop safe(r) nanomaterials and to communicate what is risk and uncertainties from a precautionary angle. The authors suggested that regulators could directly use the five early warning signs for precautionary action, ranging from a ban to the implementation of risk research. Also later in 2013, the early warning signs were incorporated into the hazard and exposure ranking tool, NanoRiskCat, which was aimed to support companies and regulators in their first-tier risk assessment and communication process regarding the known hazard and exposure potentials of consumer products containing nanomaterials (Hansen 2013c). The NanoRiskCat tool has been applied to all the 5,157 products claimed to contain nanomaterials in the NanoDataBase (nanodb.dk, visited 4 June 2021), and it also formed the basis for the suggestion for a new regulatory framework for nanomaterials called ReactNow (Hansen 2017).

The example given above illustrates that there are indeed many key findings and best practices we have learned from the field of nanomaterial risk assessment that may be transferred to other emerging risk issues. It should also be recognized that uncertainty and incomplete understanding will continue to be inherent parts of any risk analysis of novel chemicals, materials, or technologies. The nanomaterial risk analysis field has also shown that there are ways to deal with these issues, and decision-support can be provided even under high (and different) levels of uncertainty by drawing on past experiences, rather than to (continue to) expect and/or wait for full scientific evidence.

References

Abdolahpur-Monikh F, Chupani L, Arenas-Lago D et al (2021) Particle number-based trophic transfer of gold nanomaterials in an aquatic food chain. Nat Commun 12:899

Aitken RA, Bassan A, Friedrichs S et al (2011) Specific advice on exposure assessment and hazard/risk characterisation for nanomaterials under REACH (RIP-oN 3) – Final Project Report. European Commission, Brussels, Belgium

Aruoja V, Dubourguier H-C, Kasemets K et al (2009) Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata. Sci Total Environ 407:1461–1468

Arvidsson R, Furberg A, Baun A et al (2018) Proxy measures for simplified environmental assessment of manufactured nanomaterials. Environ Sci Technol 52:13670–13680

Arvidsson R, Hansen SF, Baun A (2020) Influence of natural organic matter on the aquatic ecotoxicity of engineered nanoparticles: recommendations for environmental risk assessment. NanoImpact 20:100263

Baun A, Hartmann NB, Grieger K et al (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17:387–395

Baun A, Hartmann NB, Grieger KD et al (2009) Setting the limits for engineered nanoparticles in European surface waters—Are current approaches appropriate. J Environ Monit 11:1774–1781

Baun A, Sayre P, Steinhäuser KG et al (2017) Regulatory relevant and reliable data for the environmental fate of manufactured nanomaterials. NanoImpact 8:1–10

Bondarenko OM, Heinlaan M, Sihtmae M et al (2016) Multilaboratory evaluation of 15 bioassays for (eco)toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID. Nanotoxicology 10:1229–1242

Calow P (1997) General principles and overview. In: Calow P (ed) Handbook of ecotoxicology. Blackwell Scientific Publications, Oxford, England

Chen G, Peijnenburg WJGM, Xiao Y et al (2018) Developing species sensitivity distributions for metallic nanomaterials considering the characteristics of nanomaterials, experimental conditions, and different types of endpoints. Food Chem Toxicol 112:563–570

Cupi D, Hartmann NB, Baun A (2015) The influence of natural organic matter and aging on suspension stability in guideline toxicity testing of ZnO, TiO_2, and Ag nanoparticles with Daphnia magna. Environ Toxicol Chem 34:497–506

DEFRA (2007) Characterising the potential risks posed by engineered nanoparticles; Second U.K. Government research report; Department for Environment, Food and Rural Affairs: London, UK

Docter D, Westmeier D, Markiewicz M et al (2015) The nanoparticle biomolecule corona: lessons learned—Challenge accepted? Chem Soc Rev 44:6094–6121
Drasler B, Sayre P, Steinhauser K et al (2017) In vitro approaches to assess the hazard of nanomaterials. NanoImpact 8:99–116
ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.19: Uncertainty analysis. European Chemicals Agency, Helsinki, Finland
EEA (2001) Late lessons from early warnings: the precautionary principle 1896–2000, European Environmental Agency, Copenhagen, Denmark
Franken R, Heringa MB, Oosterwijk T et al (2020) Ranking of human risk assessment models for manufactured nanomaterials along the Cooper stage-gate innovation funnel using stakeholder criteria. NanoImpact 17:100191
Gottschalk F, Kost E, Nowack B (2013) Engineered nanomaterials in water and soils: a risk quantification based on probabilistic exposure and effect modeling. Environ Toxicol Chem 32:1278–1287
Grieger K, Hansen SF, Baun A (2009) The known unknowns of nanomaterials: describing and characterizing uncertainty within environmental, health and safety risks. Nanotoxicology 3:1–12
Grieger KD, Linkov I, Hansen SF et al (2012) Environmental risk analysis for nanomaterials: review and evaluation of frameworks. Nanotoxicology 6:196–212
Grieger K, Jones JL, Hansen SF et al (2019) What are the key best practices from nanomaterial risk analysis that may be relevant for other emerging technologies? Nat Nanotechnol 14:998–1001
Handy RD, Cornelis G, Fernandes TF et al (2012) Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ Toxicol Chem 31:15–31
Hansen SF, Maynard A, Baun A et al (2008) Late lessons from early warnings for nanotechnology Commentary. Nat Nanotechnol 3:444–447
Hansen SF, Maynard A, Baun A et al (2013a) Nanotechnology—Early lessons from early warnings. Chapter 22 in “Late Lessons from Early Warnings vol. II”. European Environment Agency, European Commission, Copenhagen, Denmark
Hansen SF, Nielsen K, Knudsen N et al (2013b) Operationalization and application of “early warning signs” to screen nanomaterials for harmful properties. Environ Sci Process Impacts 15:190–203
Hansen SF, Jensen KA, Baun A (2013c) NanoRiskCat—A conceptual tool for categorization and communication of exposure potentials and hazards of nanomaterials in consumer products. J Nanoparticle Res 16: 2195
Hansen SF (2017) React now regarding nanomaterial regulation. Nat Nanotechnol 12:714–716
Hansen SF, Hjorth R, Skjolding LM et al (2017) A critical analysis of the environmental Dossiers in the OECD sponsorship programme for manufactured nanomaterials. Environ Sci Nano 4:282–291
Hansen SF, Hansen OFH, Nielsen MB (2020) Advances and challenges towards consumerization of nanomaterials. Nat Nanotechnol 15:964–965
Hartmann NB, Von der Kammer F, Hofmann T et al (2010) Algal testing of titanium dioxide nanoparticles-testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269:190–197
Hartmann NB, Engelbreckt C, Zhang J et al (2013) The challenges of testing insoluble metal and metal oxide nanoparticles in algal bioassays: titanium dioxide and gold nanoparticles as case studies. Nanotoxicology 7:1082–1094
Hartmann NB, Ågerstrand M, Lützhøft HCH et al (2017) NanoCred: a transparent framework to assess the regulatory adequacy of ecotoxicity data for nanomaterials—Relevance and reliability revisited. NanoImpact 6:81–88
Hjorth R (2016) Environmental risk assessment and management of engineered nanomaterials—The role of ecotoxicity testing (PhD Thesis) Technical University of Denmark, Kgs. Lyngby, Denmark
Hjorth R, Sørensen SN, Olsson ME et al (2016) A certain shade of green: can algal pigments reveal shading effects of nanoparticles? Integr Environ Assess Manag 12:200–202

Hjorth R, Skjolding LM, Sørensen SN et al (2017) Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials. NanoImpact 8:28–37
Leong HS, Butler KS, Brinker CJ et al (2019) On the issue of transparency and reproducibility in nanomedicine. Nat Nanotechnol 14:626–635
Hristozov DR, Gottardo S, Critto A et al (2012) Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 6:880–898
Hund-Rinke K, Baun A, Cupi D et al (2016) Regulatory ecotoxicity testing of nanomaterials—Proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology 10:1442–14447
Hund-Rinke K, Herrchen M, Schlich K et al (2015) Test strategy for assessing the risks of nanomaterials in the environment considering general regulatory procedures. Environ Sci Eur 27:1–12
Hund-Rinke K, Simon M (2006) Ecotoxic effect of photocatalytic active nanoparticles TiO_2 on algae and daphnids. Environ Sci Pollut Res 13:225–232
ISO (2012) Water quality - Fresh water algal growth inhibition test with unicellular green algae. ISO 8692:2012. International Organization for Standardization, Geneva, Switzerland
Ivask A, Juganson K, Bondarenko O et al (2014) Mechanisms of toxic action of Ag, ZnO and CuO nanoparticles to selected ecotoxicological test organisms and mammalian cells in vitro: a comparative review. Nanotoxicology 8:7–71
Juganson K, Ivask A, Blinova I et al (2015) NanoE-Tox: new and in-depth database concerning ecotoxicity of nanomaterials. Beilstein J Nanotechnol 6:1788–1804
Lützhøft HCH, Hartmann NB, Brinch A et al (2015) Environmental effects of engineered nanomaterials: estimations of predicted no-effect concentrations (PNECs). Danish Environmental Protection Agency, Copenhagen, Denmark
Lynch I, Weiss C, Valsami-Jones E (2014) A strategy for grouping of nanomaterials based on key physico-chemical descriptors as a basis for safer-by-design NMs. Nano Today 9:266–270
Nasser F, Lynch I (2016) Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on Daphnia magna. J Proteomics 137:45–51
Maynard A (2006) Nanotechnology: a research strategy for addressing risk, project on emerging nanotechnologies, Report No. PEN 3; Woodrow Wilson International Center for Scholars: Washington, DC
Nielsen MB, Baun A, Mackevica A et al (2021) European nanomaterial regulation—Methodological challenges for registration and safety assessment. Environ Sci Nano 8:731–747
Nguyen MK, Moon J-Y, Lee Y-C (2020) Microalgal ecotoxicity of nanoparticles: an updated review. Ecotoxicol Environ Saf 201:110781
Notter DA, Mitrano DM, Nowack B (2014) Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. Environ Toxicol Chem 33:2733–2739
Nowack B (2017) Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context. Nanoimpact 8:38–47
Oberdörster E (2004) Manufactured nanomaterials (fullerenes, C(60)) induce oxidative stress in the brain of Juvenile Largemouth Bass. Environ Health Perspect 112:1058–1062
OECD (2005) Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment [ENV/JM/MONO(2005)14], Organisation for Economic Co-operation and Development, Paris, France
OECD (2007) Current Developments/Activities on the Safety of Manufactured Nanomaterials/Nanotechnologies, Tour de table at the 2nd meeting of the working party on manufactured nanomaterials, Berlin, Germany, April 25–27, 2007; Report No. ENV/JM/MONO(2007)16. Organisation for Economic Co-operation and Development, Paris, France
OECD (2011) Test No. 201: freshwater alga and cyanobacteria, growth inhibition test. Organisation for Economic Co-operation and Development, Paris, France
OECD (2020) Guidance document on aquatic and sediment toxicological testing of nanomaterials. Series on Testing and Assessment No. 317. ENV/JM/MONO(2020)8. Organisation for Economic Co-operation and Development, Paris, France

Oomen AG, Steinhäuser KG, Bleeker EAJ et al (2018) Risk assessment frameworks for nanomaterials: scope, link to regulations, applicability, and outline for future directions in view of needed increase in efficiency. NanoImpact 9:1–13

Pang C, Skjolding LM, Selck H et al (2020) A "point-of-entry" bioaccumulation study of nanoscale copper phthalocyanine in aquatic organisms. Environ Sci Nano 8:554–564

Palmqvist A, Baker L, Forbes VE et al (2015) Nanomaterial environmental risk assessment. Integr Environ Assess Manag 11:519

Peijnenburg WJGM, Baalousha M, Chen J et al (2015) A review of the properties and processes determining the fate of engineered nanomaterials in the aquatic environment. Crit Rev Environ Sci Technol 45:2084–2134

Petersen EJ, Goss GG, von der Kammer F et al (2021) New guidance brings clarity to environmental hazard and behaviour testing of nanomaterials. Nat Nanotechnol 16:482–483

Petersen EJ, Diamond SA, Kennedy AJ et al (2015) Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. Environ Sci Technol 49:9532–9547

SCENIHR (2007) The appropriateness of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials; European Commission Health and Consumer Protection Directorate-General, Directorate C—Public Health and Risk Assessment, C7—Risk Assessment: Scientific Committee on Emerging and Newly Identified Health Risks Brussels, Belgium

SCENIHR (2009) Risk assessment of products of nanotechnologies; European Commission Health and Consumer Protection Directorate-General, Directorate C—Public Health and Risk Assessment, C7—Risk Assessment: Scientific Committee on Emerging and Newly Identified Health Risks Brussels, Belgium

Semenzin E, Lanzellotto E, Hristozov D et al (2015) Species sensitivity weighted distribution for ecological risk assessment of engineered nanomaterials: the n-TiO_2 case study. Environ Toxicol Chem 34:2644–2659

Sekine R, Khurana K, Vasilev K et al (2015) Quantifying the adsorption of ionic silver and functionalized nanoparticles during ecotoxicity testing: test container effects and recommendations. Nanotoxicology 9:1005–1012

Skjolding LM, Sørensen SN, Hartmann NB et al (2016) A critical review of aquatic ecotoxicity testing of nanoparticles—The quest for disclosing nanoparticle effects. Angew Chem Int Ed 55:15224–15239

Stone V, Hankin S, Aitken R et al (2010a) Engineered nanoparticles: Review of health and environmental safety (ENRHES) (RPRT), Engineered nanoparticles. European Commission, Brussels, Belgium

Stone V, Nowack B, Baun A et al (2010b) Nanomaterials for environmental studies: classification, reference material issues, and strategies for physico-chemical characterisation. Sci Total Environ 408:1745–1754

Subramanian V, Semenzin E, Hristozov D et al (2016) Sustainable nanotechnology decision support system: bridging risk management, sustainable innovation and risk governance. J Nanopart Res 18:1–13

Syberg K, Hansen SF (2015) Environmental risk assessment of chemicals and nanomaterials—The best foundation for regulatory decision-making? Sci Total Environ 541:784–794

Sørensen SN, Wigger H, Zabeo A et al (2020) Comparison of species sensitivity distribution modeling approaches for environmental risk assessment of nanomaterials—A case study for silver and titanium dioxide representative materials. Aquatic Toxicol 225:105543

Sørensen SN, Baun A (2015) Controlling silver nanoparticle exposure in algal toxicity testing—A matter of timing. Nanotoxicology 9:201–209

Sørensen SN, Hjorth R, Giron Delgado C et al (2015) Nanoparticle ecotoxicity—Physical and/or chemical effects? Integr Environ Assess Manag 11:722–724

Sørensen SN, Engelbrekt C, Lützhøft HCH et al (2016) A multi-method approach for disclosing algal toxicity of platinum nanoparticles. Environ Sci Technol 50:10635–10643

Sørensen SN, Baun A, Burkard M et al (2019) Evaluating environmental risk assessment models for nanomaterials according to requirements along the product innovation stage-gate process. Environmental Sci Nano 6:505–518

US EPA (2007) Nanotechnology White Paper, Report No. EPA 100/B-07/001; Science Policy Council, United States Environmental Protection Agency: Washington, DC

Van Hoecke K, Quik JT, Mankiewicz-Boczek J et al (2009) Fate and effects of CeO_2 nanoparticles in aquatic ecotoxicity tests. Environ Sci Technol 43:4537–4546

Von Moos N, Slaveykova VI (2014) Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae–state of the art and knowledge gaps. Nanotoxicology 8:605–630

Walker W, Harremoes P, Rotmans J et al (2003) Defining uncertainty: a conceptual basis for uncertainty management in model-based decision support. J Integr Asses 4:5–17

Wickson F, Hartmann NB, Hjorth R et al (2014) Balancing scientific tensions. Nat Nanotechnol 9:870