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Abstract

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Nanomaterials are materials of which the discrete parts have one or more dimensions in the order of 100 nm or less. This characteristic provides nanomaterials with unique properties, resulting in an explosive increase in the economic and social importance of these materials in recent years. It is due to these properties why risks of nanomaterials may differ from those of conventional (i.e., non-nano) substances.

A proper approach for carrying out an adequate environmental risk assessment of nanomaterials and the relevant nano-specific properties to be tested in such an assessment, has not yet been determined. This is due to a scarcity of information on the production and use of these materials, their fate and their ultimate environmental effect(s) on ecosystems, as well as the causal relationship between the underlying fate and effect processes.

Environmental risk assessment of substances is based on the comparison of (predicted) environmental concentrations with effect levels. The National Institute for Public Health and the Environment (RIVM) recommends taking the unique properties of nanomaterials and their effects in the environment into account when developing and using models for predicting environmental concentrations of chemicals as well as for effect assessment. Quantifying the effective exposure concentrations of nanomaterials in ecosystems is considered a key issue, as for all relevant species of each nanomaterial, it is necessary to determine the properties that modify the effective exposure of the particles.

This is the conclusion drawn by the RIVM based on the results of a literature survey it carried out, as well as on accumulated general knowledge on the fate and effects of nanomaterials. Concerns expressed in international studies on the possible risks associated with the presence of nanomaterials in the environment motivated this study. There is also the question whether current risk assessment methodologies, which were developed for the conventional soluble chemical substances, are suitable for testing nano-specific properties.

Key words: nanomaterials, risk assessment, ECHA Guidance, exposure, toxicity

Rapport in het kort

Nanomaterialen in het aquatisch milieu: toxiciteit, blootstelling en risicobeoordeling

Het is nog onduidelijk hoe de milieurisicobeoordeling van nanomaterialen uitgevoerd moet worden en met welke nanospecieke stofeigenschappen daarbij rekening moet worden gehouden. Dit komt doordat nog weinig bekend is over de productie en het gebruik van deze materialen en de uiteindelijke effecten ervan op het ecosysteem.

Nanomaterialen zijn materialen waarvan de afzonderlijke delen één of meerdere dimensies in de orde van 100 nm of minder hebben. Deze karakteristiek geeft nanomaterialen specifieke eigenschappen, dusdanig dat hun economisch en maatschappelijk belang op dit moment zeer snel toeneemt. Het zijn juist deze eigenschappen waardoor de risico's van nanomaterialen voor ecosystemen anders kunnen zijn dan die van reguliere (niet-nano) stoffen.

Het Rijksinstituut voor Volksgezondheid en het Milieu (RIVM) stelt voor om bij het kwantificeren van effectieve blootstellingsconcentraties en effect niveau's van nanomaterialen rekening te houden met de specifieke eigenschappen van nanomaterialen en hun effecten, zoals vorm, grootte, en oppervlakte-karakteristieken, maar ook of nanodeeltjes samenklonteren tot grotere deeltjes afhankelijk van het type ontvangende water. Voor alle relevante vormen waarin het nanomateriaal voor kan komen in het milieu (het nanomateriaal zelf, samengeklonterde deeltjes, stoffen die vanuit het nanomateriaal in oplossing gaan, et cetera) moeten gegevens over blootstelling en effecten gegenereerd worden. Voor het identificeren en valideren van aanpassingen in modellen en testmethodieken is meer onderzoek nodig.

Dit blijkt uit een literatuurstudie van het RIVM, aangevuld met algemene kennis over het gedrag en de effecten van nanomaterialen. Aanleiding voor het onderzoek zijn zorgen die in internationale studies zijn geuit over de mogelijke risico's van nanomaterialen. Daarnaast is het de vraag of de bestaande risicobeoordelingmethodieken die voor reguliere, goed oplosbare, chemische stoffen zijn ontwikkeld voldoende rekening houden met de specifieke eigenschappen van nanomaterialen.

Trefwoorden: nanomaterialen, risicobeoordeling, ECHA Guidance, blootstelling, toxiciteit

Contents

Summary

Identification of nanomaterial-specific properties that impact the fate and toxicity of manufactured nanomaterials (NMs) in the environment is essential for a proper evaluation of the benefits and dangers of nanotechnology. The limited behavioural and toxicological information that is available for specific NMs is rarely put in context by comparison with the toxicity of the same material in either molecular or bulk form, as put forward (again) by the U.K. Royal Commission on Environmental Pollution in November 2008. In this study we executed this comparison for different physical-chemical properties of NMs in the aquatic environment to identify properties that may result in deviating fate and toxicity characteristics of NMs. Specific properties of NMs may result in unexpected fate characteristics and enhanced reactivity and toxicity of these materials, as compared to the corresponding bulk materials, including the dissolved species. In order to apply the environmental risk assessment paradigm of comparing exposure (predicted environmental concentration, PEC) with hazard (predicted no-effect concentration, PNEC) of manufactured NMs, it is essential to characterize both PEC and PNEC in terms of the most important NM species. The colloidal nature of aquatic suspensions of NMs is known to generally give rise to aggregation and agglomeration and, as a consequence, to settling to sediments. We conclude that the current method of characterizing environmental risk as the quotient of a PEC and a PNEC can be applied to NMs. However, current risk assessment guidelines should be updated in order to allow proper assessment of PEC and PNEC of NM species; assessment of the speciation of the NMs in dependence of water chemistry is a key feature in this respect.

1 Introduction

The recent large increase in production, species and utilization of manufactured nanomaterials (NMs) has raised concerns that the release of these materials into the environment may pose a serious threat (Moore, 2006; Nowack and Bucheli, 2007; Handy et al., 2008; Klaine et al., 2008). An increasing number of products contain some form of NMs, which may eventually end up in the aquatic environment (Moore, 2006). However, there are currently large gaps in our knowledge and understanding of the toxicity and exposure of NMs for aquatic organisms, which hampers risk assessment for NMs.

The current method of characterizing environmental risk of chemicals is based on the quotient of a predicted environmental concentration (PEC) and a predicted no-effect concentration (PNEC), and is elaborated into the Guidance on information requirements and chemical safety assessment (ECHA, 2008) and formalized in the European Union System for the Evaluation of Substances (EUSES; EU, 2008). There is no reason to assume that the general risk paradigm is not valid for NMs, i.e. PEC/PNEC is suitable). The question remaining, however, is how to account for NM-specific environmental behaviour in assessing PEC and PNEC, which is not addressed or worked out in the ECHA Guidance and EUSES (SCENIHR, 2007, 2009).

In this report we provide an overview on the latest developments on toxicity testing of NMs for aquatic organisms, on the exposure of organisms to NMs in the aquatic environment and on the risk assessment for NMs in aquatic ecosystems. In this report we will determine which aspects of the risk assessment of NMs have to be tackled and/or changed in the Guidance and EUSES. We will be drawing up a statement of affairs and using this to propose concrete adjustments to make the ECHA Guidance also suitable for nanomaterials.

The environment contains many natural NMs (Lead and Wilkinson, 2006; Hochella et al., 2008), but we will focus on manufactured NMs here. In this report we define NMs as manufactured materials with at least one dimension in the range between 1 and 100 nm, as used by many authors (Handy et al., 2008; Klaine et al., 2008). This can be nanofilms, nanosheets, platelets and surface coatings (1 dimension); nanowires, nanofibres and nanotubes (2 dimensions); or nanoparticles, including precipitates, colloids and quantum dots (3 dimensions). These manufactured NMs are often divided into different chemical classes, often based on different compound classes and their use, e.g., carbon nanotubes, metal containing NMs, and quantum dots (Klaine et al., 2008). However, regardless of the classification, even within a single chemical there will be immense diversity in crystal structures, sizes, morphologies, surface area and surface charges (Handy et al., 2008).

2   Recent literature (2007-2008)

A scan of the scientific literature on environmental aspects of NMs showed that it is remarkably rich in review articles and approximately half of the publications on environmental aspects of NMs since 1993 appeared in 2007 and 2008 (Table 1). However, the limited information on behaviour and toxicology that is available for specific NMs is rarely put in context by comparison with the toxicity of the same material in either dissolved or large particle form, as put forward by the U.K. Royal Commission on Environmental Pollution in November 2008 (Royal Commission on Environmental Pollution, 2008). In this recently published report an overview is given of the current knowledge and gaps in understanding NMs. Therefore, in this report we do not want to repeat again the available reviews, but encourage readers to have a look at the report of the U.K. Royal Commission on Environmental Pollution (2008). The main conclusions from this report dealing with the subjects of our report are incorporated. The added value of this report in comparison with previous reports and reviews is the inclusion of suggestions on how to adjust current risk assessment methods for chemicals to be able to assess the risk for NMs.

Table 1: Number of publications per year for different combinations of keywords using SCOPUS (until November 23rd 2008).

Keywords Year	Nanoparticles, Environment	Nanoparticles, Environment, Review	Nanoparticles, Aquatic	Nanoparticles, Aquatic, Review
2008	431	47	45	11
2007	403	33	13	1
> 1993	1866	158	83	16

Aquatic toxicity, exposure and risk assessment of chemicals are largely based on dissolved chemicals and their properties, particularly their octanol-water partition coefficient (K_{OW}). This approach seems inappropriate for NMs, since it is unknown whether the K_{OW} is important and/or usable for NMs (they form suspensions of particulate matter rather than solutions). The difference in behaviour of NMs from the bulk form or from the molecular dispersed or atomic state of the same material may give rise to different modes of mobility and toxicity in organisms and in the environment, and will therefore cause difficulties in assessing both PEC and PNEC, as described by SCENIHR (2007, 2009).

3 How do nanomaterials differ from conventional chemicals?

Suspensions of dispersed nanoparticles in water are colloidal solids-in-water systems, fundamentally different from the true solutions that conventional chemical substances form in water. The behaviour of nanomaterials in water is controlled by nano-specific physical interaction mechanisms. For conventional chemical substances, processes and mechanisms that control the concentrations to which organisms are exposed (both in ecotoxicity test systems and the environment) are well understood. Chemical substances dissolve in water; the dissolved molecules or ions undergo (equilibrium) partitioning between different chemical forms or species: ions form complexes, ions and molecules get associated with colloids and solids in water, are transported to other environmental media, such as air and sediment, and may be transformed or degraded by abiotic and microbial processes. The extent and rates of these processes are known in sufficient detail or can be tested by using standard protocols, so that exposure concentrations can be predicted from release rates by means of mathematical models. For nanomaterials, this situation is less straightforward. Are the same processes and mechanisms in control for these substances? Are the processes and mechanisms understood sufficiently? Are we capable of modelling them? Can we predict exposure concentrations from release rates? The answer must be repeatedly negative: at the moment our knowledge of the processes that control the concentrations of nanomaterials in water is insufficient to allow predictive modelling of exposure concentrations.

The physical processes of attraction and repulsion between particles are well known and addressed extensively in text books on physical chemistry and colloid science (Israelachvili, 1991). For single molecules (atoms, ions) in water, repulsion at short distances and (Van der Waals) attraction at larger distances leads to an optimum inter particle distance of approximately 4 Å (0.4 nm), at which the so-called Lennard-Jones potential is minimal (Figure 1). This optimum inter particle distance is of the same order of magnitude as the size of small molecules (diameter of H_2O 2.75 Å or 0.275 nm).
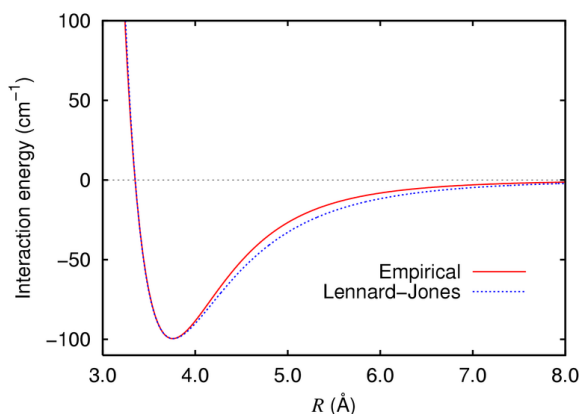

Figure 1. Empirical and theoretical interaction energies between two particles.

As a result, dissolved molecules in water remain truly dissolved and do not adhere to form multi-molecular clusters. Nanocolloids consist of small particles of solid matter, made up of many individual atoms or molecules. Such particles are roughly three orders of magnitude larger than single molecules. Attraction between solid (nano) particles is explained as the spatial sum of the Van der Waals attractions between the individual molecules (atoms, ions) that make up the solid phase. At the same time, electrostatic repellence or steric hindrance counteracts the Van der Waals attraction. This leads to inter (nano) particle potential curves as given in Figure 2. As shown here, generally attraction dominates at relatively short inter particle distances - note that the spatial scale of Figure 2 is orders of magnitude larger than the scale of Figure 1. If particles approach each other sufficiently close, Van der

Waals attraction will cause them to aggregate. If repellence or hindrance is strong enough, however, particles may not be able to approach each other close enough for Van der Waals attraction forces to become dominant, in which case aggregation is prevented and stable colloidal suspensions may exist.
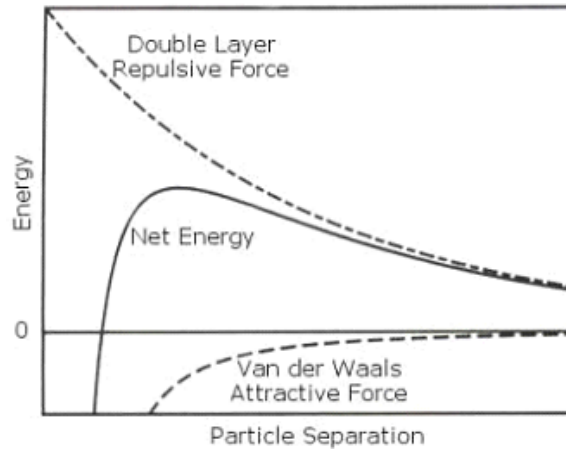

Figure 2. Spatial sum of interaction potentials of many (nano)particles.

Similar attractive forces explain how nanomaterials interact with other colloidal materials (e.g., natural organic matter) and suspended solids in water. While particles of nano size remain suspended as a result of Brownian motion, larger aggregates will undergo sedimentation (Figure 3).
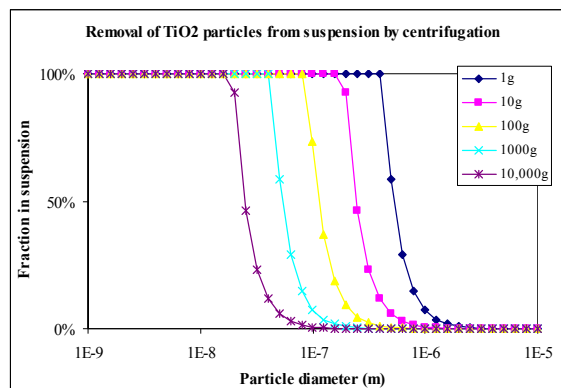

Figure 3. Influence of size of particles on overcoming gravity.

Processes of particle aggregation and the conditions under which colloidal suspensions remain stable or become unstable are well understood and described in the DLVO theory of colloid science (Lyklema, 2005). Colloidal interactions cause nanoparticles to behave differently from dissolved molecules. The processes and mechanisms that control concentrations in water are very different for dissolved molecules (atoms, ions) and nanomaterials of the same chemical substance. Environmental fate models for predicting exposure concentrations from release rates may work well for conventional chemical, but may fail totally for NMs if the nano-specific colloidal interactions are not accounted for in the model, which is the case in the current models used in EU environmental risk assessment. However, this problem of predicting exposure concentrations is already known for highly hydrophobic compounds.

Clearly, the colloidal nature of suspensions of nanomaterials is of great importance in understanding and predicting the environmental fate and the ultimate exposure concentrations of organisms. The particulate nature of the nanomaterials is of equally great importance for understanding and predicting the toxic effects to organisms. The strong tendency of nanomaterials to adhere to solid surfaces is expected to lead to nano-specific interactions at membrane passage, which determines the extent and rate of uptake into living cells. Once taken up, the same tendency should be expected to give rise to nano-specific interactions with subcellular structures. Studies on nanosize dependent toxicity for NMs in water are scarce, with only one study on TiO_2 nanomaterials. This study showed that toxicity was dependent on size, but the highest toxicity was found with particle sizes of around 20 nm, with a decline towards even smaller particles (Jiang et al., 2008). This shows that the maximum toxicity of NMs is not at their smallest possible size and indicates that toxicity induced by their crystalline structure, in the form of reactive oxygen species (ROS) formation, may decline when the particles become smaller than a certain size. More information should become available on the toxic mode of NMs and the effect of particle size on their toxicity.

The classical question may arise of how to distinguish between solid (particulate) and liquid (dissolved) states. Can this be decided on the basis of particle size? Is there a borderline between dissolved and particulate? Fullerene and carbon nanotubes (CNT) take a peculiar position in this dilemma. From an organic chemical perspective, such materials are to be considered single molecules that cannot be seen as solids. Dispersed in water, they should be considered true solutions. Their environmental fate should be described rather well by means of the traditional processes and models used for conventional chemicals. How many individual molecules must come together in one cluster for the cluster to obtain a (nano-specific) solid character? This issue of size describes, in fact, the basic principle of nano-specificity of material properties. Molecules in the particle-water interface display both a dissolved and solid character. Nano-scale solids have such a great fraction of molecules in the interface that the material starts losing its solid character. This may coincide with appearance of size-specific (quantum) properties, e.g., light absorbance.

Another point of difference between nanomaterials and conventional chemicals is their solid nature itself. While conventional chemicals are (implicitly or explicitly) assumed to be and treated as true solutions, nanomaterials are solids that may dissolve to eventually form true solutions. When nanomaterials dissolve, the (nano-specific) solid and colloidal character including the possible toxic effects, ceases to exist, simply because the solid phase disappears. From this perspective, dissolution is to be regarded simply as a persistent reducing process that causes the active concentration of the nanosubstance to decrease. Conditions under which nanomaterials dissolve, the extent to which this occurs and the rate at which this takes place, are both substance-specific and not known for many nanomaterials. When nanomaterials dissolve, true solutions appear and toxic effects of the conventional form of the chemical substance start. The ultimate toxic effect of the nanomaterial results from the toxic effects of the nanoform on the one hand and the dissolved form on the other.

In assessment of the environmental fate and toxicity of chemical substances in nanoform, assessment of the entire suite of chemical dissolution, speciation and aggregation is to be considered. Single processes to be taken into account are:
1) Dissolution: suspended nanomaterial becomes smaller and may eventually completely dissolve. The toxicity, exposure and risk assessment of the individual chemical components released during dissolution of the NMs have to be considered. This can be done under the current Guidance-procedures. For example Griffitt et al. (2008) showed that nickel nanoparticles relatively quickly dissolve in water and the released Ni-ions were largely responsible for the measured toxicity. However, for slower dissolving NMs, the toxicity of the smaller-sized NMs during dissolution has to be considered as well.
2) Change in surface structure of NMs: molecules present in the aquatic environment can adsorb to NMs, or the outer layer of the NM can change due to chemical processes like oxidation. This can have two different effects. First: a change to the NM itself may change their suspension state by either

promoting or preventing aggregation, for instance, their adsorption to natural organic matter (NOM) often increases the stability of NM suspensions. Second: the adsorbed chemical to the NM surface or aggregation of NMs to NOM may result in increased toxicity of and increased exposure to either chemicals or NMs for organisms, respectively.

3)   Aggregation of NMs: individual NMs in water can attach to each other and form large aggregates. Aggregates can have a different relative surface area (= area per mass of material) compared to individual particles. This may change their toxic properties but little is known about this. The larger the aggregates, the smaller the Brownian movement to keep them in suspension and the larger the gravitational force to promote sedimentation of the aggregates, the fourth process influencing suspended NMs.

4)   Sedimentation: as soon as aggregates of NMs are too large to be kept in suspension by Brownian movement they will sink. Sedimentation will result in the removal of NMs from the water layer. Only through resuspension of the aggregates, for example, by enhanced water movement due to current or mixing of water, can the aggregated NMs re-suspend. However, individual atoms or molecules may dissolve and become available again in the water column (again, the current Guidance is sufficient for these dissolved chemicals). Also aggregation of NMs can result in sedimentation of the material, through which the concentration in the water will decline. This influences the exposure of organisms both in water (declined exposure) and in sediment (increased exposure).

NMs are thus in many ways not comparable to conventional chemical substances, for the latter a description of dissolved concentration and influence of (equilibrium) sorption processes can be sufficient to estimate toxicity and environmental exposure. In this context NMs are so-called 'difficult substances', which are known within the ECHA Guidance, e.g., metals. Special risk assessment methods are already available for this group of chemicals and may provide opportunities to assess the risks for NMs. We propose considering NMs as 'difficult chemicals' to be able to add special methods for their risk assessment. In this report we will suggest the type of nano-specific considerations needed to include NMs in the ECHA Guidance.

4 Aquatic toxicity testing of nanomaterials

The main problem with most toxicity tests performed with NMs on aquatic organisms is a lack of correct interpretation of the experimental data, which often comes down to inaccurate description of the properties of the NMs used and their fate in aquatic systems. In this chapter we will discuss the main issues causing this interpretation problem.

1)   Proper definition of the physical-chemical parameters of tested NMs
The first important information needed to gain proper understanding of the results for any study concerning NMs is a precise definition of the particles used. The following important aspects have at least to be defined:

  a.   size distribution
Many aspects concerning NMs are dealing with the size of the particles, e.g., suspension (Brownian movement) and reactive surface area (Grassian, 2008; Handy et al., 2008; Klaine et al., 2008). Therefore, the size distribution of the particles has to be determined precisely. For example, particles on the order of 4 nm diameter have about 50% of their atoms at the surface (Grassian, 2008), but this percentage of atoms at the surface is quickly declining with increasing particle size (see for example Fig. 4). Since it is difficult to commercially synthesize single-sized NMs, inhomogeneous samples are often produced (Grassian, 2008). Not only the size distribution of the NM powder has to be provided, but also the measured particle size of NMs suspended in water. This latter property is often determined using dynamic light scattering (DLS) and mostly results in larger particle sizes compared to the powder NM (Griffith et al., 2008; Wang et al., 2009; Zhang et al., 2008). Also, natural NMs in water may aggregate quickly to form particles in the size range 0.1 to 10 μm, which is the most stable form of this colloidal material in aquatic systems (Buffle and Leppard, 1995a). The actual impact of aggregation of NMs on their toxicity is unclear. However, they retain specific physicochemical properties that are characteristic for NMs (SCENIHR, 2009).

  b.   composition
The composition of any material plays a central role in determining its properties, including reactivity, mobility and toxicity and therefore the exact chemical composition of the NMs must be defined. NMs can consist of a single element or molecule, or of a combination of different elements or molecules (composites). This last type of NMs consists of a core (which is itself usually referred to as the NM) and a shell around the core produced either deliberately (as with many quantum dots) or unintentionally (as in the oxidation of zero-valent iron NMs to form an iron oxide shell). Also a surface active agent or capping agent can be used in practical applications of NMs (e.g., organic molecules such as polymers or surfactants). However, it is difficult to quantify surface properties (surface coverage, number of functionalized groups) at the interface of NMs (Grassian, 2008). Finally, small amounts of material (e.g., heavy metals), known as dopants, can be added intentionally to alter the electrical and chemical properties of the NM (Royal Commission on Environmental Pollution, 2008).

  c.   possible contaminants
Contaminants at the surface and structural defects can also modify properties of NMs (Royal Commission on Environmental Pollution, 2008). During the manufacturing and release of NMs, contaminants may attach to the surface of the materials (e.g., heavy metals to carbon nanotubes; Plata et al., 2008). These contaminants may be released during toxicity tests or upon release into the environment and may cause additional risk for aquatic organisms. Furthermore, contaminants present in the medium or environment may be able to attach to NMs, which can act as carriers for these contaminants and may enhance the toxicity of these substances (Zhang et al., 2007; Baun et al., 2008). The choice of dispersants for NMs may be also problematic since some of the best dispersants from a chemical viewpoint (e.g., amides and furans) are toxic to organisms (Smith et al., 2007). For example,
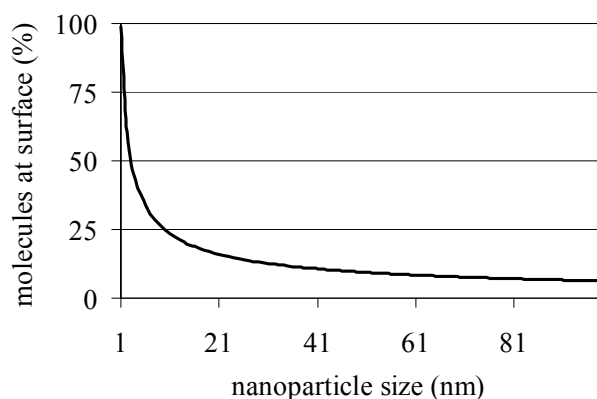
Figure 4. Example of nanoparticle size versus percentage molecules at the surface.

tetrahydrofuran (THF) is used to disperse fullerenes (e.g., Oberdörster, 2004) but THF has its own toxicity (Henry et al., 2007) and will thus interfere during toxicity testing. However, these problems are not exclusive to NMs, but can also cause problems in regular chemical testing. Recently, Spohn et al. (2009) showed that the measured toxicity of fullerene water-soluble side products which were formed in THF nC_{60} suspension were responsible for the observed acute toxic effects, whereas fullerenes themselves had no negative effect on either human lung epithelial cell line A549 in vitro or *Daphnia magna* in vivo.

d. Any other metric that might affect fate and effects
Other parameters which may affect toxicity of NMs are zeta potential and shape. The zeta potential gives an indication of the surface charge of the NMs. This surface charge can vary with the pH of the surrounding medium (Handy et al., 2008). Much attention is given to the shape of NMs in the atmosphere, since rod shapes nanoparticles are assumed to induce comparable effect to asbestos particles (Royal Commission of Environmental Pollution, 2008). In the aquatic environment, the effects of shape on ecotoxicology of NMs remains to be established (Handy et al., 2008).
Depending on the nature of the NMs it may also be appropriate to consider (SCENIHR, 2009):
- Photoactivation, some NMs may become activated by light, which may influence both their stability and activity
- Potential to generate active oxygen, since this is one accepted general mechanism for the adverse effects of NMs.

We can conclude that there is growing evidence to suggest that NM surface properties are distinctly different from both bulk solute and bulk solution, but often a quantitative understanding of surface composition, termination, charge and functionalization for NMs is lacking and difficult to acquire (Grassian, 2008).

2) Concentration of NMs in test medium
First, there is a discussion amongst scientists about the most accurate parameter to define the concentration of NMs in test media. Parameters often used are: concentration (g/L), reactive surface area (m^2/L) or number of particles (#/L). For a comparison between the toxicity of (dissolved) single molecules and NM of certain chemically comparable concentrations in g/L can be used; for inter-chemical comparison of NMs the surface area in m^2/L or number of particles may provide a better comparison (see Oberdörster et al., 2005 for discussions on nanoparticle concentration). However, not only the initial concentration of NMs in the test medium is important, but especially the actual concentration at different time points of the experiment.

Surface forces and Brownian motion exhibited at nanosize range may play a significant role in the self-assembly of nanostructures (Royal Commission on Environmental Pollution, 2008). Since NMs for the aquatic environment are characterized by forming a colloid dispersion in water, this means that the concentration of NMs in the test medium and environment is also influenced by agglomeration, aggregation, sedimentation and attachment to equipment and organisms (Lead and Wilkinson, 2006; Handy et al., 2008; Hochella et al., 2008; Klaine et al., 2008; see also this report chapter 4-3). This may change both the actual concentration of NMs and the size distribution of the NMs tested, as often large differences in size have been observed between the powder NM and the suspended NM (Griffith et al., 2008; Wang et al., 2009; Zhang et al., 2008). The surface of NMs can even be modified to prevent aggregation and improve stability in aqueous suspensions (e.g., for strongly hydrophobic NMs like CNT; Holzinger et al., 2004). Such modifications allow higher concentrations (in g/L) in the test medium and again stress the importance of knowing the structure of the studied NMs.

3) Dissolution

Dissolution of NMs may result in the release of the dissolved or ionic form of the material, which may induce additional toxicity. A concern for many metal-based NMs is the dissolution of soluble metal ions from the surface of the NMs, so that NMs will act as delivery vehicles for free metal ions (Handy et al., 2008). Due to dissolution of metals from metallic NMs, part of the observed toxicity may be caused not by the metallic NMs but their dissolved metal ions. In fact, it has been claimed that the toxicity of certain metallic particles (ZnO nanoparticles) is mostly or entirely due to the toxicity of the dissolved metal species (Franklin et al., 2007). Griffitt et al. (2008) showed that the lethal concentration (LC_{50}) of metal ions was indeed often lower compared to their metallic nanomaterials at comparable mass concentrations, indicating that metal ions contributed more to toxic effects than metal nanomaterials, but the particulate form did contribute to toxicity. For juvenile zebra fish (*Danio rerio*) silver and copper nanomaterials showed higher toxicity than their respective ionic forms. Not only the test solution, but also the presence of organisms can affect particle dissolution, e.g., substantially larger amounts of copper dissolve in incubations with test organisms compared with the absence of test organisms, which likely results from dissolution by the organisms following ingestion (Griffitt et al., 2007, 2008).
Solubility of NMs is an important property that needs to be addressed (SCENIHR, 2009). A different aspect of dissolution is the disappearance of NMs from the aquatic environment, together with their possible effects. Dissolution is a persistency reducing process for NMs and the dissolution rate of NMs determines how long they will remain as particles in water. The concentration of NMs in the aquatic environment is thus largely dependent on their solubility. Secondly, solubility determines the concentration of dissolved species originating from NMs. The effects on toxicity for aquatic organisms depend on the relative toxicity of the NM compared to the toxicity of the solution or released ions.

4) Dose-response unknown for NMs

Since the actual concentration and size distribution of NMs in test media are difficult to determine due to aggregation and dissolution, it is often not possible to calculate the dose-response relationship for NM toxicity on aquatic organisms. For NMs in general, it appears that their toxicity is potentially quite complex and not straightforwardly related to size only (Grassian, 2008). From TiO_2 nanoparticle (size 20 and 250 nm) toxicity data, Oberdörster et al. (2005) concluded that particle surface area for particles of different sizes but of the same chemistry is a better dose-metric than particle mass or particle number. Later studies by Grassian et al. (2007), however, did not show an increase in TiO_2 toxicity of 5 nm compared to 21 nm particles. A possible explanation may be that the formation of reactive oxygen species (ROS) is at a maximum for particle size near 20 nm and decreases for smaller particles (Jiang et al., 2008).

5   Exposure to nanomaterials

The actual concentration of (manufactured) NMs in the aquatic environment is largely unknown. No peer-reviewed literature is available on concentrations or specification of NMs in natural waters, although NMs are already applied on a large scale (Handy et al., 2008; Klaine et al., 2008). In this chapter we discuss the most important caveats concerning our knowledge on the exposure of organisms to NMs in the aquatic environment.

1)   Release of NMs into environment not yet quantified (process, waste, wear and tear)
Increase in production of NMs is large, which is expected to enhance the release of NMs to the environment. Different pathways for NMs release into the environment have to be considered, both intended and unintended:
- direct release of NMs, e.g., NMs used for remediation of soil pollution (Zhang, 2003)
- indirect release of NM, e.g., TiO_2 from cosmetics and sun lotion
- wear, e.g., carbon black from car tires (Royal Commission on Environmental Pollution, 2008, section 3.14)
- waste, e.g., NMs expelled from fuel combustion engines
- production of NMs for direct use (Royal Commission on Environmental Pollution 2008, section 3.18)
Although the exact amount of NMs released into the environment is unknown, we may assume that this will increase in the forthcoming years. Good estimations of these NM fluxes are needed to be able to identify possible hazards for humans and the environment.

2)   Measuring NMs/colloids in natural waters is difficult due to technical limitations
The detection of NMs in the environment is complicated by the likely low concentrations of NMs (ng L^{-1} to low $\mu g\ L^{-1}$; Boxall et al., 2007) and the relatively high concentrations of natural NMs and organic carbon (mg L^{-1}) or background levels of trace metals (Buffle and Leppard, 1995b; Handy et al., 2008; Klaine et al., 2008). Determining size distribution of particles or the total concentration is not enough to determine the presence, concentration and size of manufactured NMs in the environment. Techniques for determining particle size of colloids, e.g., Field-Flow Fractionation (FFF) and Dynamic Light Scattering (DLS) and chemical composition, e.g., Inductively Coupled Plasma Mass Spectrometry (ICP-MS), have to be coupled (e.g., FFF-ICP-MS) to measure NMs in natural waters (for more details on methods to measure NMs see Grassian (2008), Hassellöv et al. (2008). Furthermore, studying NMs in aquatic environments is increasingly complicated because of their polydispersity, complexity and spatial and temporal variability (Klaine et al., 2008).

3)   NM behaviour in the aquatic environment
The behaviour of NMs in water will depend both on properties of the NMs (again stressing the importance of NM-structure knowledge) and on environmental parameters (pH, ionic strength, natural organic matter). This can influence important processes which alter the dispersion of NMs in water: aggregation/disaggregation, adsorption/desorption, sedimentation/resuspension and dissolution. The state of NMs in aquatic environments will thus vary from dissolved ions and isolated nanoparticles to nanoparticle aggregates.

a.   NM properties
The properties of the NM itself will primarily determine how the material behaves in aquatic systems. When present, the shell of the NM is again the main part to look at. Strong hydrophobic NMs, like underivatized fullerenes and CNTs are extremely insoluble in water (Andrievsky et al., 1999) and will likely exist on the water's surface or at the water–sediment interface until physical mixing occurs within the aquatic environment (Farré et al., 2009). However, changes to the structure of these carbon NMs, e.g., addition of hydrophilic groups, will enhance their presence in the bulk water phase itself (Chiang et al., 1996). Shell modification can also be made to other hydrophobic NMs to improve their

dispersion in water. Also, dissolution of NMs depends firstly on the chemical constituent of the material, as described in chapter 4-3.

b. pH

The pH of the water partly determines the surface charge of NMs (Handy et al. 2008). The surface charge of NMs can strongly influence the aggregation kinetics and thus sedimentation rate of NMs (Buffle and Leppard, 1995a). Changes in pH, which may occur in natural waters, can therefore result in changes of both mobility and aggregation state of manufactured NMs. Particle aggregation occurs at all pH values and organic acids can destabilize TiO_2 nanoparticle suspensions (Pettibone et al., 2008). These authors also observed for TiO_2 that 5 nm particles can form larger aggregates compared to 32 nm particles under the same conditions of pH and solid concentrations. Metal nanoparticles may undergo dissolution in acidic aqueous environments to yield ions in solutions and result in the formation of smaller nanoparticles during dissolution (Borm et al., 2006). Thus both the amount of free metal ions and the size of metal nanoparticles may increase at decreasing pH values, which may occur both in aquatic environments and inside organisms. Measuring the zeta potential as a function of pH can provide predictions of colloidal NP stability (Handy et al., 2008).

c. ionic strength (salinity and water hardness)

The DLVO theory (see chapter 3 for more details) describes the attractive and repulsive forces acting on closely adjacent particles. These forces include Borne repulsion, diffuse double layer potential, and Van der Waals attraction (Handy et al. 2008). Van der Waals forces between surfaces separated by a medium can be regarded as material constants and are always attractive, the electrical double layer (EDL) consists of the layer of charge at the surface of a particle and can be both attractive and repulsive, depending on charge. Identical NMs have the same charge and thus result in a repulsive force. EDL thus forms a barrier which prevents identical particles to get close to each other. Increase in salinity will introduce opposite charged salt ions in the EDL, which results in a compression of the EDL. The NMs can then approach each other more closely and start to be affected by attractive forces acting on shorter length scales (Van der Waals forces) and aggregation will increase (see Handy et al., 2008 for an extended description of these processes). Elevation of water hardness is expected also to increase aggregation due to compression of the EDL or due to specific sorption of Ca^{2+}-ions (Handy et al., 2008).

d. natural organic matter (NOM): quantity and quality

Manufactured NMs can bind to NOM, forming larger colloid structures, which may enhance the stability of NM suspensions (Lead and Wilkinson, 2006). Natural NMs in the size range 0.1 to 10 µm are considered to be the most stable colloids in aquatic systems (Buffle and Leppard, 1995a). Humic substances are likely to form nanoscale coatings on NM surfaces, giving them an overall negative charge that results in reduced aggregation through both electrostatic and steric stabilization (Buffle and Leppard, 1995a; Lead and Wilkinson, 2006; Gibson et al., 2007). On the other hand, large fibrils may increase aggregation via bridging mechanisms (Buffle and Leppard, 1995a; Buffle et al., 1998). Thus, NMs dispersion stability will depend on the type and amount of NOM in water, but these aggregates can remain in suspension over long time periods in river water containing NOM (Giasuddin et al., 2007; Hyung et al., 2007). Whether or not a humic substance enhances colloidal stability is unknown, since no grouping of even the relatively simple humic substances according to structural models can be made (Lead and Wilkinson, 2006). Given the relatively high concentration of NOM in rivers, it is assumed that NMs aggregation and sedimentation will be dominated by interactions with NOM (Klaine et al., 2008). The current lack of understanding of this interaction causes serious problems in predicting the fate and transport of NMs in aquatic ecosystems.

Interaction of NMs with environmental parameters thus influences the speciation between dissolved, colloidal and particulate phases due to dissolution, aggregation and sedimentation (Grassian, 2008; Klaine et al., 2008). For natural small colloids or aggregates it is known that they can also accumulate

at the air-water interface due to flotation (Buffle and Leppard, 1995b). Especially in oceans this layer forms an important habitat for organisms, but already it has been shown that pollutants may have increased concentrations in this layer (Wurl and Obbard, 2004). This indicates that manufactured NMs may also end up in this layer. Thus, knowledge of the different phases of NMs is needed to determine the resulting exposure of organisms to the released NMs in the environment. This stresses the importance of studying the fate and behaviour of manufactured NMs in the aquatic environment (Klaine et al., 2008).

4) Unknown hazards of NMs in aggregates for aquatic organisms
The smaller surface area of aggregates may result in a decreased potential toxicity, but interactions may also result in enhanced potential toxicity. Here, we describe the possible interactions of NMs with other chemicals and substances in water, which may result in changing exposure and toxicity for organisms to these aggregates.

a. NMs can act as carriers for other toxic substances
There is a risk that NMs will be delivery vehicles for other toxic substances, or that there may be synergistic toxic effects when NPs are present in mixtures of other chemicals (Zhang et al., 2007; Handy et al., 2008). Some NMs have a net negative surface charge and will therefore bind cationic pollutants such as metals, indicating that NMs can influence the toxicity or bioaccumulation of other contaminants (Handy et al., 2008). For example, carp exposed to Cd in the presence of TiO_2 nanoparticles showed greater accumulation than for Cd alone (Zhang et al., 2007). However, much is unknown about the toxicity of pollutants adsorbed to different NMs.

b. NMs uptake after attachment to NOM
The role of colloidal material, like NOM, as carriers of toxic compounds has been identified already for 'normal' dissolved chemicals in water (Buffle and Leppard, 1995b), which is also implemented in models for bioavailability of chemicals in surface water (reviewed by Handy and Eddy, 2004). NOM may also aggregate with NMs and this complex can enter filter-feeding organisms in search of food in the aqueous environment (Klaine et al., 2008). Although direct uptake of NMs by filter feeding organisms has been shown (Gagné et al., 2008), the indirect uptake pathway of NOM-NM aggregates has not yet been shown.

c. impact of different routes of uptake for different types of organisms
Species can be directly exposed via the water phase to NM through contact of membranes, skin and gills (Handy et al., 2008; Klaine et al., 2008). NMs can attach to organisms and may interact with cell membranes (Lyon et al., 2005). The second route of uptake for biotic species is via the gastrointestinal tract (Smith et al., 2007; Federici et al., 2007). The latter may result in a change of NMs aggregation during transport in the gastrointestinal tract (due to change in pH and change in organic matter content of the contents of the gastrointestinal tract), with possible alterations of the toxicity of the NMs compared to the surrounding water. When NOM-NM aggregates break down through biotic interactions, this may again release free NMs and induce enhance toxicity.

6   Risk assessment for nanomaterials

Currently, it is extremely difficult to evaluate how safe or how dangerous NMs are because of the almost complete lack of our knowledge about so many aspects of their fate and toxicology, which are largely determined by the lack of understanding on how NMs act in the aquatic environment (Royal Commission on Environmental Pollution, 2008). Toxicity or the absence of toxicity as measured under standardized laboratory conditions may not be representative for natural conditions, due to exposure related differences and artefacts. Due to the limitations discussed in the previous two chapters it is not possible at the moment to prepare a risk assessment for NMs in aquatic ecosystems. In this chapter we will discuss the points for which the Guidance for NMs has to be modified compared to the 'normal' chemicals, i.e., dissolved chemicals. The current Guidance for chemical compounds in the aquatic environment compares the PEC with the PNEC and uses the hydrophobicity of the chemical of interest (expressed by means of the octanol water partitioning coefficient) as the basis for quantifying exposure. The underlying paradigm of risk being proportional to the extend by which PEC exceeds PNEC is also suitable as the basis for the risk assessment of NMs. However, there are still a number of issues which have to be tackled, in analogy to what was done with metals in the last few decades. Similar to NMs, the exposure of metals cannot be quantified on the basis of octanol-water partitioning. In the last twenty years major steps forward have been made in our knowledge on the effects and exposure of this group of chemicals in the aquatic environment. They are now considered special cases and have their own models for estimating effects and exposure. This may be an example for the risk assessment of NMs, although the latter consist of far more different types of compounds, thus even stronger efforts may be needed to adjust the Guidance for NMs, as was the case for metals.

The main issue of considerable uncertainty about the potential health and environmental impacts of NMs is that the knowledge of these materials lags significantly behind the pace of innovations. The areas of concern could change as new scientific information arises (Royal Commission on Environmental Pollution, 2008). In the longer term, more sophisticated third and fourth generation nanoproducts may represent a further change in functionalities and properties, which would be even more difficult to capture in a regulatory system that is primarily focused on the bulk chemical properties of the material (Royal Commission on Environmental Pollution, 2008). One of the properties from these newly developed NMs can be a carrier function. NMs can be intentionally built to have a carrier function for substances in organisms, like certain drugs in the human body. The exposure route of NM carriers for other chemicals is unknown in the aquatic environment at this moment. This carrier function may enhance the observed unintentional capacity of NMs to act in some way as carrier for other chemical substances, e.g., enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide NMs (Zhang et al., 2007).

The UK Royal Commission on Environmental Pollution (2008) concluded that new governance arrangements are necessary to deal with ignorance and uncertainty in the rapidly developing area of NMs, with three priorities:
  i.   functionality: focus on the properties and functionalities of specific NMs as the key driver rather than treat NMs as one single class
  ii. information: research programmes on the properties and functionalities of NMs are needed to inform risk assessment
  iii. adaptive management: recognition of the degree of ignorance and uncertainty and time it will take to address these

We agree with their conclusion and present some of the main issues that should be tackled for a proper risk assessment of NMs in aquatic systems.

We conclude that the following actions are necessary to improve risk assessment for NMs and have to be tackled and implemented before any guidance for NMs can be agreed upon:

- Exposure of organisms to NMs:
  Develop equipment to measure NMs in environmental samples to define the predicted environmental concentration (PEC) of NMs in aquatic ecosystems. Develop and test hypotheses on the fate of NMs in environmental and laboratory samples.
- Toxicity tests of NMs:
  Carefully execute toxicity tests under standardized conditions with well characterized NMs (including concentration and surface area), clean NMs, and aggregation/dissolution status of NMs, above all taking effective exposure into account to assess the predicted no-effect concentrations (PNEC) of NMs.
- Risk assessment of NMs:
  Define a priority assessment for NMs testing with respect to those types of NMs that are expected to express a higher reactivity or toxicity than expected from their 'bulk form'. Prioritize research which is based both on this priority assessment and on the actual production of NMs.

Based on this study we have the following suggestions how to deal with PEC/PNEC for NMs in the aquatic environment:
- For toxicity measurements from which the PNEC is derived, exposure concentrations *in the toxicity test system* have to be known for all relevant species of the studied NM: primary NM, clustered/aggregated NM and dissolved chemical specimens.
- Information has to be gathered on the species that may contribute most to the observed toxicity of NMs (a good example is given by Griffitt et al., 2008).
- Data have to be collected for all relevant species in order to evaluate PEC. We do not believe that only physical properties (size, shape) and physicochemical properties (surface characteristics) of NMs will provide sufficient data for a relevant estimation of the PEC of the different species that could develop from the NM in question. Also the processes in the real world that determine path and fate of the different species should be known, at least for the most toxic species. There is a need for environmental fate models that are adapted to account for environmental fate processes of most relevant species that may originate from NMs to predict how the ecosystem and humans are exposed.

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