ERA-NET SIINN

Safe **I**mplementation of **I**nnovative

Nanoscience and **N**anotechnology

Deliverables 3.7

Guidelines for EHS Assessment

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Abstract

Concomitant with increased interest in and use of engineered nanomaterials (ENM), an awareness of safety issues has been developing. This has led to that various stakeholders (representatives of the industry, authorities, various interest groups, among others) are requesting appropriate risk assessment (RA) of these materials.

Risk assessment for ordinary chemicals and also for particles with sizes above the nanoscale, has been performed for a long time. The methods that have been employed in these instances are also the ones that in general have been considered appropriate for RA of ENM.

There is, however, only limited number of published cases of RA of ENM in the scientific literature database. Available studies typically deal with specific materials in a specific setting, and do not include more than a fraction of the expected life cycle or value chain for the material.

A number of challenges have been identified that are specific for RA of ENM. These challenges include the characterization of physical-chemical properties of ENM during various stages of the life cycle. Other challenges regard the actual exposure to man or the environment, the hazard potential for different forms of ENM, and their effects at relevant exposure levels. Still other challenges include the development of the most suitable dose metric, and the adoption of quantitative risk assessment approaches which would be of great benefit for risk managers.

The field of nanomaterials is changing with a very rapid pace, and new materials with significantly different properties are likely to be available in the near future. A very important consideration is to critically assess the appropriateness of current approaches for the different components of RA.

It is recognized that the pristine form of any nanomaterial is not necessarily the form which will be exposed to either humans (in occupational settings or as consumer) or the environment. Risk assessment has accordingly to take into account the entire value chain of the material, and focus the efforts on the stages where actual exposure is realistic.

Introduction

The development of technologies and materials that operate and/or function at the nanometer scale (engineered nanomaterials, ENM) has been forecasted to significantly influence several sectors of the economy and the society in general. In parallel, the consciousness about safety aspects for the successful implementation of nanotechnologies and ENM has manifested itself in several ways, including substantial public funding for scientific research regarding nanosafety.

The obvious driver behind the focus on risk and safety is the fact that these technologies and materials possibly behave differently than the same materials when they are either existing as chemicals in solution, or as particles of larger sizes (i.e. above several hundreds of nm). The term "nanotoxicology" has been used to characterize toxicological studies intended to find out potential environmental, health and safety (EHS) effects of ENM. Such studies have been underpinned by an assumption that materials engineered to utilize unique properties associated with the nanoscale must exhibit nanoscale specific toxicology. This assumption is, however, not uncontested. Performed research shows furthermore that toxicity of many nanomaterials is possible, and indeed predictable from non-nanoscale materials.

Despite a considerable amount of scientific studies during the last decade, question marks remain regarding the fate and behaviour of ENM in various settings, and whether or not these materials have any (detrimental) effect on human health or the environment.

The shortcomings in knowledge make proper risk assessment (RA) of ENM for human health and the environment difficult to perform. Among several key areas of knowledge that are needed for RA, the information about whether or not an exposure to ENM occurs is central, as well as knowledge about the physico-chemical properties of the material, and how these can influence the interaction with biological systems.

The task is made even more complex by two time-related scenarios, viz.:

- that ENMs exist along a value chain (see Fig 1 below), and thus have their own (external) life cycles, and
- that ENMs can possibly enter and subsequently undergo changes within an organism.

The possibility for interactions with biological processes may vary considerably along these two axes, which complicates the RA substantially.

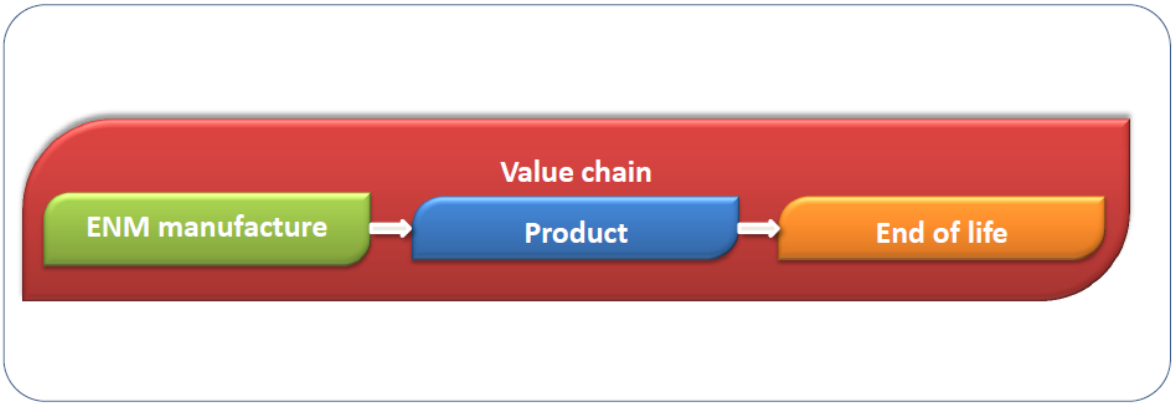

Figure 1. Schematic view of a value chain for a generic ENM which is incorporated into a product. At its final stage(s), the ENM encounter various end of life scenarios, which can include disposal, recycling, or possibly reuse. Exposure to both the environment and humans can take place along the entire value chain.

Why is then the RA of ENM a concern? The short answer is that risk management activities performed by stakeholders need a proper foundation, which is provided by RA.

The purpose of the present report is to draw attention to unique features of ENM and how these are reflected in relevant RAs. More specific aims include to describe how RA of materials for human health and the environment is performed, and the challenges that are emerging regarding ENM; to overview the problems facing hazard identification and exposure assessment; and to bring attention to possible future complications to RA of nanomaterials and nanotechnologies.

Entry points and fate of ENM

It is currently accepted that there are several possible entry points for ENM to the human body, and in analogy to other mammals. Whether and to what extent ENM can enter non-vertebrate animal species is poorly investigated. Regarding plants, the knowledge is also sparse. Currently, there is no evidence that ENM can bypass the plant cell wall, but it is possible that ENM can be taken up by roots and root hair structures and thus translocated to other tissues of the plant (Ma et al 2010). If other possible uptake or entry mechanisms are realistic is unknown. Uptake into bacteria has been documented in some cases, and seems to primarily be a function of that the ENM damage the cell membrane and thus enter via passive routes. Otherwise, the present consensus is that ENM cannot cross the bacterial cell membrane (Maurer-Jones et al. 2013). Possible ENM-mediated effects on bacteria can also emanate from dissolution of the nanoparticles, where dissolved ions exert the effects, or due to reactivity of the ENM which generates reactive oxygen species (ROS) (Ma et al 2013).

Table 1 below outlines the major entry points and secondary translocation in humans.

Table 1. ENM administration routes and secondary translocation in the human body.

Process	**Involved structures**	**Comments**
Inhalation	Nasal cavity, lungs	Most likely pathway
Ingestion	Oral cavity, gastric and intestinal epithelia	Poorly investigated
Dermal uptake	The entire skin	Not relevant for intact skin
Systemic administration		Relevant for application of nano-pharmaceuticals and diagnostics
Secondary events		
Translocation to secondary organs via the circulation system following entry is documented. The possible importance of structural barriers (e.g. blood-brain-barrier, blood-placenta-barrier; blood-testes-barrier etc.) is poorly investigated.		
Translocation to the brain from the nasal cavity by means of retrograde axonal transport has been seen in animal experiments during high-dose exposures. Unclear if this scenario is realistic for humans under real-life conditions.		

Subsequent to entering the body and translocation to secondary organs, the ENM can interact with cells. Several entry pathways are described (see Fig. 2) as well as several intracellular responses. However, a key feature seems to be that ENM can induce oxidative stress which in turn leads to generation of free oxygen and nitrogen species, which can lead to a number of down-stream events.

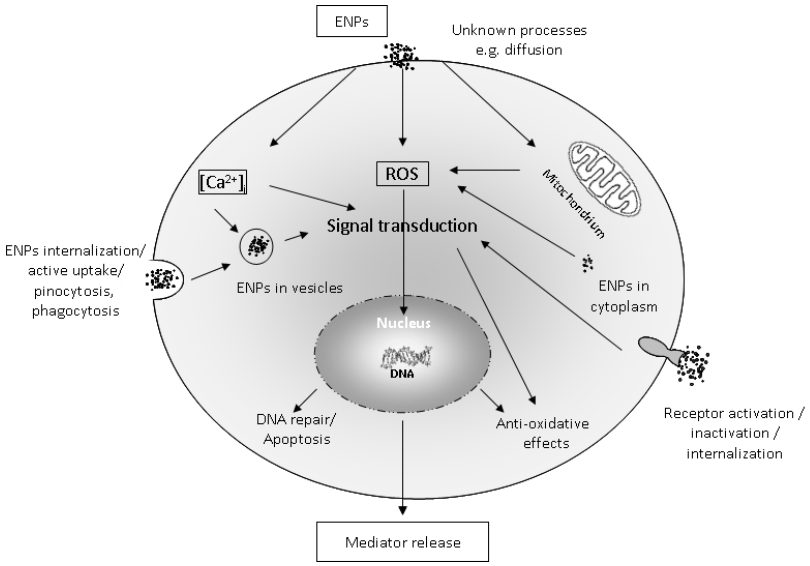

Figure. 2. Uptake pathways and cellular effects of ENM in mammalian cells (from Simkó and Mattsson 2010).

Risk and risk assessment

A definition of risk which is often used emanates from IUPAC (see Lewalle 1999): "the probability of adverse effects caused under specified circumstances by an agent in an organism, a population, or an ecological system". Such a definition is then the foundation for the common view that risk in the context of human and environmental toxicology is a function of hazard and exposure. Thus, there is no risk unless an agent (chemical, physical, or biological) is hazardous, at the levels where actual exposure does occur.

What is then risk assessment? A generally agreed upon definition is not available, but within the community where risk assessment for human health and the environment is performed and observed, it is seen as "a process by which scientists evaluate the potential for adverse health or environmental effects from exposure to naturally occurring or synthetic agents" (Society of Toxicology 1998). The goal of this activity is to provide risk managers (authorities, health and safety personnel etc.) with a science-based foundation for decision making in management of agents possibly affecting health and environment.

The principal stages of risk assessment are depicted in the flow chart in Fig. 3 and can be summarized as follows:

- Hazard identification

- Examination of dose-response relationships (hazard characterization)
- Exposure assessment
- To highlight uncertainties in the determination of hazards and dose-response relationships
- To evaluate possible modes (mechanisms) of actions for the hazard of concern.

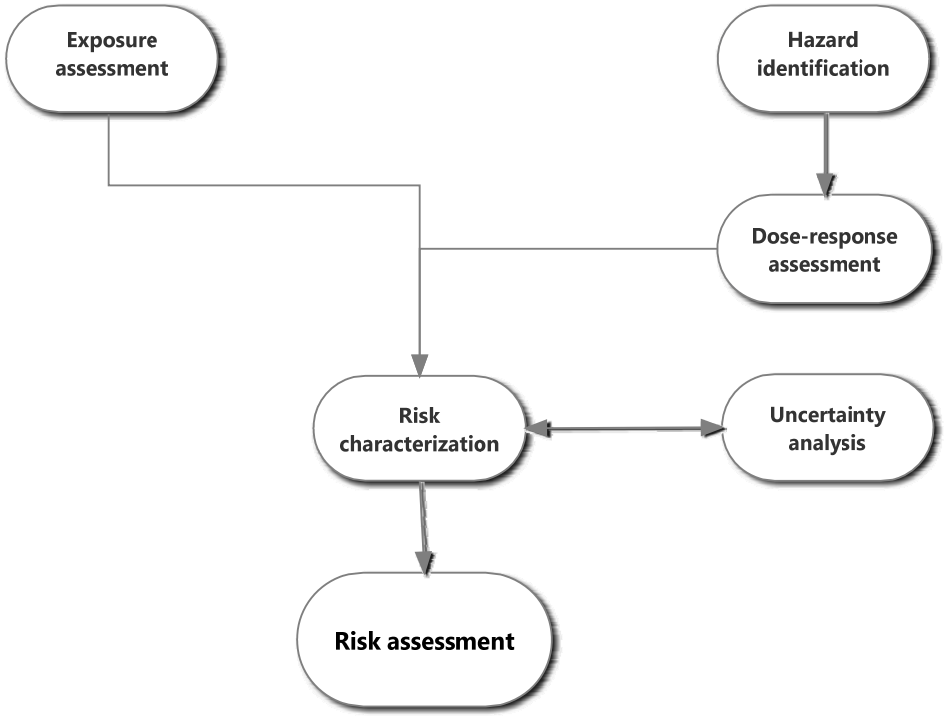

Figure 3. Flow chart depicting the different processes that are involved in risk assessment of agents for health and the environment. Importantly, there is no risk unless both exposure and hazard criteria are present.

A health risk assessment typically evaluates the evidence within several areas of studies (such as human studies, animal studies, cellular studies, modelling and *in silico* studies; "lines of evidence"). Obtaining data from different types of studies make it thus possible to integrate the various pieces of data, to perform an integrative risk assessment.

Due to advances in scientific knowledge as well as in modelling and measuring techniques, the procedures currently used for human and environmental risk assessment are required and expected to change substantially in the near future. Regarding risk assessment for human health, the approach currently used is hazard-driven with strong reliance on the use of laboratory inbred rat strains and to a lesser extent, inbred mouse strains as test species. Over time, these tests have been increasingly standardised by the introduction of good laboratory practice and ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) or OECD (Organisation for Economic Co-operation and Development) test guidelines. Some *in vitro* tests, in particular for genotoxicity and topical effects have been added. Many of the tests in current use are written into legislative requirements for the approval of various types of

products. To address uncertainties due to the need for extrapolations from animal experiment data to characterise effects that may occur in humans, conservative standard default values (also called assessment factors, uncertainty factors, or default factors) are common practice.

There are both political and scientific reasons behind a change in the way that human risk assessment is conducted. The primary changes that are proposed are:

- a paradigm shift from a hazard-driven process to one that is exposure-driven
- a progressive reduction of tests using laboratory animals.

The latter change is also reinforced by the REACH legislation of 2006 with the aim that animal testing is a last resort (Regulation (EC) No 1907/2006 concerning the registration, evaluation, authorization and restriction of chemicals). In turn, this ambition is founded on "The three Rs (3Rs)" guiding principle for animal use in testing (Replacement, Reduction, Refinement; Russel and Burch 1959).

Risk assessment can be performed according to different approaches, that are either qualitative (stating risk in terms like ´low´, ´intermediate´, ´high´ etc.); quantitative (giving e.g. a probability number for the risk in question); or a mixture of the two. Furthermore, the assessments can be founded in different analytical frameworks. Here we are outlining the principles and main steps for two different approaches, the weight of evidence approach and the integrated testing strategy (ITS) for safety assessment. The former approach has been employed for a longer time as a tool for evaluation of risks from chemicals to human health and the environment, whereas the ITS approach is more novel and largely driven by the needs to reduce animal testing. There are furthermore fewer examples of this approach available from the literature.

Weight-of-evidence approach for risk assessment

A weight of evidence approach for risk assessment is a framework for bringing together individual lines of evidence, either in a qualitative or a quantitative manner. This approach is common in many instances where risks to human health and/or the environment are assessed. When integration of the lines of evidence is taking place, it can either be done according to a standardized qualitative protocol, or based on structured decision or statistical models in its most advanced form. According to Linkov et al. (2009), most risk assessments for human health that employ a weight of evidence approach are actually qualitative and not using quantitatively rigorous methods. The authors analysed 54 published risk assessments concerned with human health and found that only four were employing formal statistical and thus quantitative methods. The most common approach was an assessment based on best professional judgment (expert judgment). In a "hierarchy" of weight of evidence methods, this approach is more appropriate for integrating data from several lines of evidence than just listing evidence. However, to accomplish transparency and reproducibility a weight of evidence method that is quantitative should be used (Linkov et al., 2007, 2009).

A detailed description of the aspects of the weight of evidence approach for RA was recently presented by the European Commission´s Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR 2012). This memorandum describes in detail issues to consider when assessing the data for each of the following lines of evidence:

- stressor identification and characterization
- exposure (external exposure, internal exposure)
- human studies (epidemiological studies, human volunteer studies, biomarker studies in humans, clinical studies, other human data sources)
- hazard assessment (animal studies, *in vitro* studies)
- mathematical models, structure activity and other *in silico* data
- studies on modes/mechanisms of action
- omics-based studies (high throughput studies)
- developing methods and their assessments

Another important aspect of the weight of evidence approach is that the weight of individual studies is variable. Studies that fulfil the quality criteria will have a much larger weight than studies that are deficient in at least some criteria. Certain studies will have no weight whatsoever, due to poor quality or due to lack of relevance.

The assessments of the lines of evidences will very likely lead to identification of critical knowledge gaps, which in turn allows that specific research recommendations are formulated. The data gaps are also essential to note since they may prevent an overall conclusion on the risk.

Integrated testing strategy

There are a number of limitations of current safety testing paradigms in general, which also are valid for testing underpinning risk assessment of nanotechnologies and nanomaterials. Examples include that one single stand-alone animal test may not be especially appropriate and relevant for the human exposure situation. On the other hand, alternatives like *in vitro* and *in silico* approaches can only supplement an animal test, and provide only parts of the essential information which is needed. With these limitations in mind, the recent years have seen the advent of novel approaches of testing suitable for use in risk assessment. One such approach is ITS (integrated, or sometimes intelligent, testing strategies; cf. Kinsner-Ovaskainen et al., 2009, 2012; Hartung et al. 2013; Rovida et al. 2015). The aim of ITS is to integrate and combine different sets of data in a more efficient and informative way than in traditional testing strategies that are focused on one or a few approaches without the ambition to try to find an improvement. The information can originate from various sources, such as different test methods, batteries of test methods, tiered test schemes, modelling, high through-put screening, computational toxicology, exposure or epidemiological data. The idea is then to integrate this information into one decision-making process. The decision made can then lead to performing new tests that are more appropriate for the evaluation in question. ITS was initially supposed to replace *in vivo* test, but has also been developed into a strategy that is used to elucidate modes of action for different substances.

In vitro testing as a replacement of *in vivo* tests may be a long-term goal, but for all practical purposes, it is still not realistic to see that happen in the short time range. A major shortcoming of *in vitro* tests is that they investigate only one or a few of many steps in complex process. The *in vitro* tests are also focusing on one mode of action, whereas several pathways can cause the same toxicological effects in a living organism. Other limitations of *in vitro* studies concern that they may be useful for only a narrow range conditions, and that it is thus necessary to anyway employ several different methods. To what extent *in vitro* tests reflect the ADME (absorption, distribution, metabolism, excretion) processes are furthermore questionable.

Control banding

As is stated several times in this document, the knowledge necessary for a proper RA of a specific ENM, either for human health or for the environment, is mostly lacking. This is due to a lack of data regarding toxicology and/or exposure. In turn, this impinges on the risk management activities, which are underpinned by a rigorous RA. Especially in occupational situations, an alternative tool for controlling possibly harmful exposures has been introduced, viz. the so-called control banding (see Paik et al. 2008) for an overview of control banding and also of its relevance for exposures to ENM).

A number of different control banding tools have been developed and are in use in situations where knowledge about chemicals is insufficient for RA. In principle, this qualitative or semi-quantitative approach still requires a substantial amount of substance knowledge, and possible hazard and exposure levels, but in the absence of a real RA the approach sets specific actions (controls) that should be employed to prevent harm to people at work.

Nano-specific examples of control banding approaches are available in the literature (e.g. Brouwer 2012; van Duuren-Stuurman et al.. 2012). Even a very specific example for use in research laboratories has been developed (Groso et al. 2010).

ENM risk assessment

The scientific literature contains a significant number of studies that have relevance for risk assessment of ENM, particularly in the form of hazard identification studies. However, there are very few articles that actually perform a proper and complete RA for a given ENM, taking exposure, hazard identification and dose-response relationship studies, as well as uncertainty analysis into consideration. The following section highlights a few studies that have been identified as ENM risk assessments, and also indicates to what extent a “full” RA has been performed.

van Kesteren et al. (2014) made a risk assessment of a specific ENM, synthetic amorphous silica. This material is also known as food additive E551, which is present in a number of products including food, where it functions as an anticaking agent. The authors based the assessment on human kinetic studies (i.e. the exposure assessment) and on *in vivo* toxicity studies (both oral and i.v. administration studies; the hazard assessment). Importantly, this study considered sources of uncertainty and recommendations for improvement.

Another publication of ENM risk assessment, with environmental focus in this case, was recently published by Civardi and co-authors (Civardi et al. 2015). The study describes the case of particulate ("micronized") Cu, which contains nano-sized Cu particles. The material is intended for wood preservation, acting as an anti-fungal agent. However, certain strains of fungi develop Cu-resistance, and the authors hypothesize that these fungi can produce Cu-loaded spores that can be inhaled by humans, and possibly cause health effects. The study provides data on global annual use of the Cu-based particles, as well as data from relevant studies on hazard identification and characterisation. On the other hand, there is no data on human or environmental exposure to Cu-based NPs from treated wood. The conclusion is thus that the lack of exposure data precludes a risk assessment in this case.

A recent study from Hristozov et al. (2014) used a quantitative weight of evidence RA approach for hazard identification and analysis. This is the first time that expert evaluation of data quality is applied for ENM RA according to the authors. However, the study focuses on hazard identification and does not include specific exposure assessment.

Risk assessment of occupational exposure to different ENM is sparingly published in the scientific literature. However, one example is a study by Koivisto et al. (2014) who assessed possible risks of inhalation exposure to nanodiamonds in a laboratory setting. The authors determined nanodiamond emission rates during handling, and performed cytoxicity studies in the human leukemia cell line THP-1. The conclusions of the study were that the exposure levels in this case were low (minute fractions of the total exposure to submicrometer urban air particles) and that the performed hazard assessment was insufficient for risk assessment.

Other examples of occupational RA were published by Liao et al. (2008) and Ling et al. (2011). The first of these studies focused on occupational TiO_2 exposures and inhalation, whereas the study from Ling and co-workers included both airborne TiO_2 as well as carbon black nanoparticles. The exposure assessment in the Liao study was complemented with experimental studies on the effects on the lungs. However, these studies have received substantial methodological criticism regarding exposure assessment (Morfeld et al. 2012) and are furthermore not covering more than limited aspects of the risk assessment procedures.

Hristozov and co-authors (Hristozov et al. 2012) pointed recently to that the available RA data is limited, and that especially studies on the exposure assessment part of RA are poorly characterised. Their conclusion was that most available studies serve as screening tools for hazard, and that the use for regulatory purposes is limited. Specific findings in the same directions were previously documented by Helland et al (2008). They studied 40 German and Swiss companies and reported that 65% of these did not consider RA at all, whereas the remaining fraction of companies at least sometimes performed some aspects of RA. A common theme among the companies was that they did not foresee any unintentional release during the life cycle of the ENM in question. The authors of the study also commented upon that the fate of ENM in the use and disposal stage received little attention among the investigated companies.

Challenges for ENM Risk Assessment

As noted in the previous section, there are not many outcomes of ENM RA that are available in the open scientific literature. This does not exclude that a substantial amount of such assessments have been performed, e.g. by competent authorities but also by the industry that manufactures, uses, or disposes of ENM and ENM containing products. The needs for proper RA are on the other hand stated in many documents (including *inter alia* Maynard et al. 2006; Borm et al. 2006; Savolainen et al. 2010; Simkó and Mattsson 2010; Klaine et al. 2012; Kuempel et al. 2012) , as well as the challenges for ENM RA, whether they are specific or not.

In general, it is considered that the currently available toxicology tests and assays are appropriate also for hazard evaluation of ENM (e.g. Oberdörster et al. 2005; OECD 2008, 2010; Kuempel et al. 2012). However, certain factors may influence the toxicity of ENM relative to larger particles, or to the dissolved form of the chemicals in question. A major question mark concerns exposure assessment of ENM. Due to the often complicated value chains and life cycles of ENM, especially since they can be expected to be integrated in ENM-containing materials and products, knowledge about their specific forms, fate and behaviour, and concentrations are difficult to obtain.

This section will outline in some detail views that are expressed in various documents on the subject of ENM RA, and also make a summary of the challenges as interpreted by the present authors.

SCENIHR Opinions

There are a number of organizations (national as well as transnational) that are concerned with safety aspects of ENM. Some of these also publish policy documents that identify the issues, propose specific actions, and suggest areas of research that are needed to close crucial knowledge gaps. A major player in this field is the European Commission, which has published several very important documents pertinent to ENM and nanotechnologies.

The European Commission´s independent Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has published several “Opinions” (overviews, assessments, guidelines, policy papers) on the subject of ENM including opinions on RA of ENM (SCENIHR 2006, 2007, 2009). The SCENIHR Opinion of 2006 concluded that the methods for evaluation of toxicity and ecotoxicity that are used in RA of chemicals may be insufficient to all of the issues arising with nanoparticles. This opinion especially pointed out that there is a need for a dose concept that included particle number and surface area, and not only mass. Other concerns were raised regarding the appropriate instruments for measurements of nanoparticles in different media, and about exposure assessment methods. Questions were also raised if existing methods for hazard characterization also include assessment of whether already existing medical conditions are affected by ENM; and also if current approaches are appropriate to analyse how ENM are distributed in the organism and in the environment.

A second opinion from SCENIHR was published in 2007. Two major considerations made in this opinion deal with determination of dose-response relationships (what metrics constitute dose?) and

a suggestion that RA of ENM needs to be performed on a case-by-case basis. Once more, the fate of ENM in different environments is stressed. The effects of physico-chemical properties on phenomena such as agglomeration, dissociation, and adsorption of environmental components need to be addressed in order for a RA to be performed. Another conclusion relates to ecotoxicological effects, viz. if key standard test taxa and recommended procedures are adequate. Concerns were also raised regarding in vitro mutagenicity (are current tests sufficiently sensitive?), environmental concentrations (how to predict them?), and bioavailability (are the commonly used species´ appropriate?).

In the SCENIHR opinion from 2009, the Committee stresses that methodology for both ENM exposure estimations and for hazard identification needs to be further developed, validated, and standardised. Issues for characterization of ENM include that the material should be characterized in its manufactured form but also as it is used in biological systems for safety evaluation. Furthermore, characterization needs to take agglomeration/aggregation of particles into consideration. Of high relevance for RA is also the characterisation of ENM as they are used in products, as this likely would be the form that consumers are exposed to.

Regarding specific metrics, the specific surface area as determined by the BET method (Brunauer, Emmet and Teller method for ENM surface areas determination; ISO 2010) is highlighted. For RA, knowledge about background vs incidental exposure is important. The issue of how coating of the particles with bio-molecules (e.g. the “protein corona”) affects behaviour is highly important. Other issues dealing with hazard identification include possible effects on liver and spleen, issues of direct or indirect genotoxicity. Environmental RA also has to deal with how ENM behave in water, and in soil and sediments, under the influence of factors such as pH, ionic strength, presence of natural organic material. This opinion from 2009 once more argues for a case-by-case approach to RA.

Life Cycle Analysis of ENM

Life Cycle Analysis (LCA) is a comprehensive framework that quantifies ecological and human health impact of a product or system over its complete life cycle (Hischier and Walser 2012). This concept is generally accepted and has been the subject for a substantial number of studies. There are even internationally accepted Application guidelines for how to perform such analyses (ISO 2006; 14040 and 14044). Current developments in LCA were recently overviewed by Finnveden et al. (2009).

A generic LCA has normally the following components:

- Goal and scope definitions

- Inventory analysis

- Life cycle impact assessment
  - which environmental compartment and organisms are actually affected
  - to what magnitude
  - by which characteristics of NPs
  - emissions adequately measured

- Interpretation
  - including uncertainty analysis

Only a few studies have applied LCA to nanotechnologies and ENM. Lazarevic and Finnveden identified 25 such studies (Lazarevic and Finnveden 2013). These only looked at part of the life cycle, with no quantitative studies addressing the impact of NMs to health and the environment.

For ENM RA purposes, it is of vital importance that the material´s life cycle is comprehensively mapped. As can be seen from Figure 4, the life cycle contains several stages, which each very likely keep the ENM in a stage specific form, or appearance. Measurements of the released amounts, as well as description of the forms are necessary for each of these steps. The release of ENM, irrespective of its form, can possibly occur during production of the ENM containing product. Such a release is likely of primary concern for the worker, although environmental exposure cannot be excluded *per se*. The consumer can possibly experience exposure during use and maintenance of the product. A major part of the release to the environment is expected to occur at the end of life, where recycling, reuse of material, and various disposal activities are taking place. Occupational exposure can possibly also take place in connection with these processes.

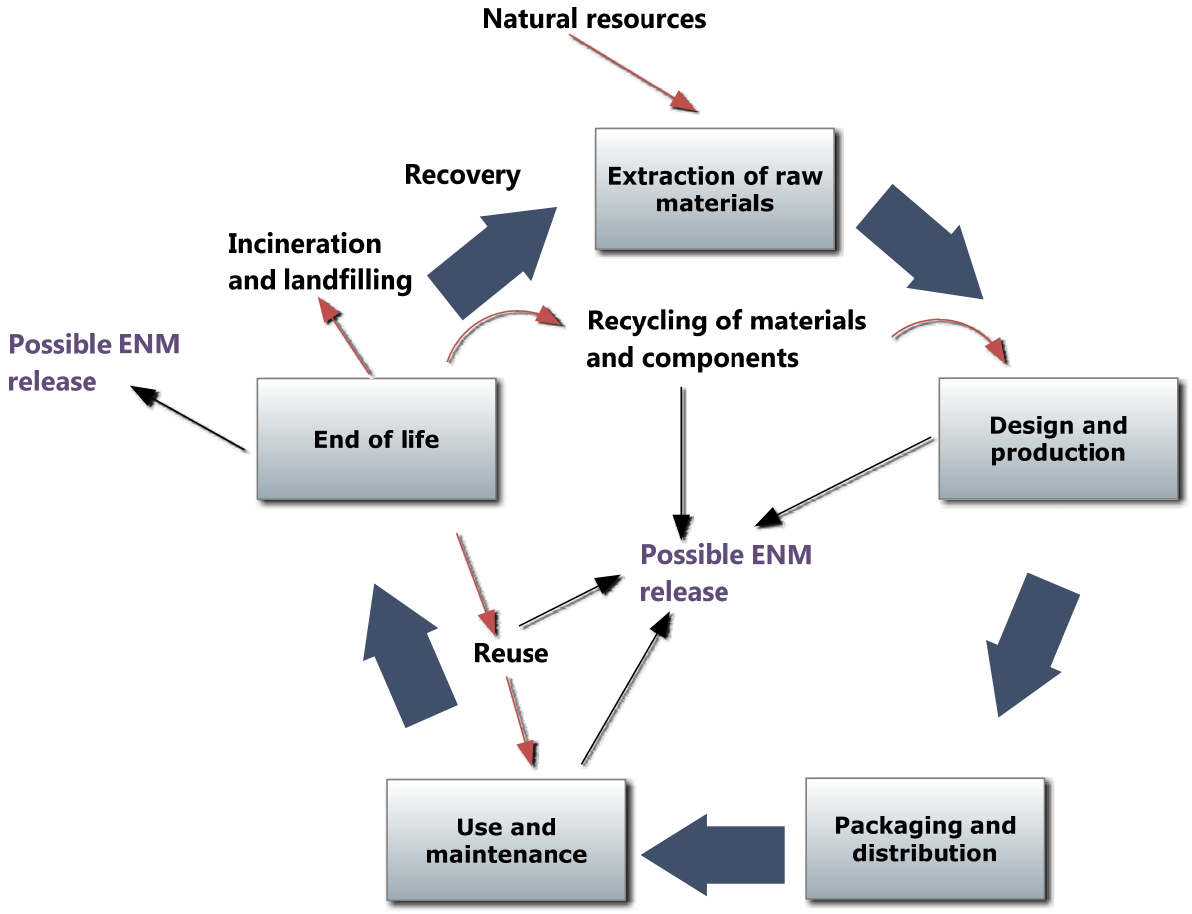

Figure 4. Generic life cycle of an ENM which is integrated into a product.

The question of dose in ENM research

When performing hazard identification and hazard characterisation studies, a central question is which dose(s) is/are investigated. In conventional chemical toxicology, the dose is described as the administered mass of the material, which also is having a specific chemical composition. For “nano toxicology” this approach has been challenged, since nanomaterial properties are largely influenced by the particle size, and also by other physical-chemical characteristics of the ENM.

Several studies have addressed the question which is the most appropriate metric when describing dose (see e.g. Peijnenburg et al. 2015; Simkó et al 2014). The latter paper proposes an ENM dose model which is analogous to the radiation protection dose model, using “deposited and equivalent dose”. The authors put forward that a suitable dose metric is the deposited nanoparticle surface area per tissue mass, and takes into account both primary and also agglomerated nanoparticles. Furthermore, this work also introduces weighting factors that are based on other physical-chemical properties of the ENM. These weighting factors consider the specific surface area, the surface textures, the zeta-potential as a measure for surface charge, the particle morphology such as the shape and the length-to-diameter ratio (aspect ratio), the band gap energy levels of metal and metal oxide nanoparticles, and the particle dissolution rate.

The ENM dose question is not trivial and is a major challenge for hazard related studies of nanomaterials and nanoparticles. Despite the awareness of this problem, and that some studies addressing this problem have been published, systematic experimental work which is aimed at solving this problem is lacking.

Grouping and computational approaches

The diversity of materials that have dimensions at the nanoscale is simply forbiddingly large, and is expected to grow to even larger numbers. If current approaches to assess the risks of these materials take place on a case-by-case-basis as is recommended by present policy papers (see e.g. SCENIHR 2009), the assessment procedures will simply be too cumbersome to allow that even a small fraction of the materials are appropriately assessed. Thus, approaches that streamline the work are highly sought after, and will likely be the cornerstones of future assessments. However, until now, these ambitions have not taken off. A recent paper from Arts et al. (2014) summarizes current “grouping” protocols that are developed for use on ENM. Their conclusion is that the suggested protocols are promising and go beyond mere structure activity relationships (SARs), and also include that the life cycle of the material is considered.

In general, a number of alternative and computer-based approaches for toxicity testing are getting more frequently used where appropriate. These methods include biokinetic modelling, SARs and QSARs (quantitative structure activity relationships).

QSARs are mathematical models that are used for prediction of toxicological behaviour of chemicals based on knowledge from a library of chemical structures. The assumption is that similarity in chemical structure also predicts the effects in a biological system. A number of QSAR models have been developed and tested both for eco-toxicology (Altenburger et al. 2003) and human toxicology purposes (Bernauer et al. 2005; Natarajan and Basak 2011). Of special interest for nanotoxicology is the

potential to use QSARs for grouping of materials (Burello and Worth 2011), which is needed in a setting where novel nanomaterials are constantly developed.

The potential usefulness of QSARs for toxicological testing has also been recognized by OECD and its Working Party on QSARs. This WP has released a tool box for the validation of QSARs (OECD 2004), which was followed by further guidance at the EU-level (ECHA 2008). Lately, it has been recommended by OECD (OECD, 2012) to include methods like QSAR in the future planning of risk assessment.

Summary of challenges

There are a number of challenges that make RA of ENM unique and possibly also to some extent more complicated than RA of traditional chemicals in solution and of particles of larger size than nanosized particles. Without prioritization, some of the major challenges include:

- Engineered nanomaterials are sophisticated materials that currently appear in many forms, made up of many different chemical elements, and sometimes in combination with other materials. It is likely that the development of new materials will proceed even faster, leading to that the repertoire of materials needing assessment becomes forbiddingly large. Thus, there is a need to find ways to perform predictive risk assessment, based on e.g. grouping principles that can include a number of materials.

- A given ENM exists along a value chain with its specific life cycle, from the initial research stages to the final disposal. To what extent is the material existing at the nanoscale along this chain, and to what extent are humans and/or the environment actually exposed to the material?

- A number of physical-chemical properties of ENM characterize these materials. Although some knowledge has accumulated during the last years, the respective properties´ influence on the toxicity of ENM needs further investigation.

- An ENM in an environmental setting (water, soil and sediment, or air) or in an organism such as the human will be exposed to different kind of matrices (which can be both organic and inorganic). A significant challenge deals with understanding the effects of these matrices on the properties of the ENM.

The table below expands on these considerations and give the most important challenges and corresponding needs and knowledge gaps.

Table 2. Overview of major RA challenges.

Challenge	Corresponding needs and knowledge gaps
Exposure assessment data for products entire life cycle	- Value chain characterization - MNM behaviour during product manufacture, use, aging, disposal
Relevant detection and characterization	- Behaviour in complex media - Methods for determinations of realistic concentrations of ENM - Noise (background) levels - Behaviour in different organismal environments
Realistic hazard assessment	- Toxikokinetic modelling in organisms - Effects due to matrix interactions - Long term, low dose and persistency effects - High throughput and high content data for endpoint identification and mode of action - Relevant experimental controls - Relevant dose metrics
Risk assessment approach development	- Improved exposure assessment - Case-by-case vs grouping approaches - Quantified RA methods - Uncertainty analyses

Future materials

The RA efforts that have been performed so far are very likely concerned primarily with ENM that belong to the so-called “first generation” nanomaterials. This category includes passive nanostructures, such as metals and metal oxides, and also carbon nanotubes. There are also to some extent products available that are containing active nanostructures (second generation nanomaterials), such as biological and non-biological sensors, compounds for medical use, and

certain compounds for microelectronics. To what extent these kind of materials and products have been assessed for their possible risk to human health and the environment is unknown.

The anticipated integration of third (self-assembly and networking properties) and fourth generation (molecular devices with “intelligent” properties) nanomaterials is belonging to the future. Such materials are not constituents of any present consumer products.

The risk assessment overviews that are presented in this document are primarily relevant for the passive nanostructures, but are likely not appropriate for any future materials, including most of the second generation ENM. It is thus prudent to be prepared for coming scenarios, especially those that include the new sophisticated materials that are likely to be developed, with/without nano-components.

In general it is important to understand how the physical form and chemical composition of new materials interact synergistically to determine toxicology:

- physical-chemical properties are important for how dose is understood
- how materials move within, interact with, and are transformed by biological systems are also determined by their physical-chemical properties.

New materials will be very challenging for RA since they are likely complex multicomponent materials. It is also expected that hybrid materials will be developed that blur the boundaries between biological and non-biological components. Active materials that are designed to change behaviour according to their environment or a received set of signals are furthermore expected. All these properties, as well as many that we are unaware of presently, are likely complicating future RA efforts. To what extent present day RA approaches are appropriate for future safety considerations cannot be answered today, the question marks regarding materials, use, and their potential for release are simply too overwhelming at this stage. Needless to say, RA of ENM will be even more challenging in the future.

Conclusions

The possible effects of ENM on human health and the environment are sometimes ascribed to the „novelty“ of these materials. This could be interpreted as that “novel” types of responses occur, and that novel approaches for RA are needed. However, there are no data available that support such assumptions. The responses in biological systems (which can be molecules, cells, tissues, or organisms) to ENM are not unique, they are also appearing when chemicals in solution or larger particles are interacting with biological components. Furthermore, RA of ENM requires knowledge about the exposure as well as of hazard potential and dose-response relationships. This is once again in line with what is required for conventional RA of chemicals.

The specific challenges for ENM risk assessment are thus of another character. Central to all RA-related activities is the need for profile life cycles, as depicted in Fig. 4 below. For each stage along the value chain, the properties of the ENM need to be known, as well as to what extent there is a real exposure at that stage, and if exposure at these levels, and with an ENM in that specific form actually has hazard potential. Corresponding knowledge about the effects of the pristine form of the ENM is thus having very limited, if any, value for risk assessment.

For each product containing ENM, the life cycle aspect is needed in order to perform RA. This framework strongly encourages the user/assessor to consider how the ENMs physical-chemical properties, the hazards, and/or exposures may be altered during the materials life cycle.

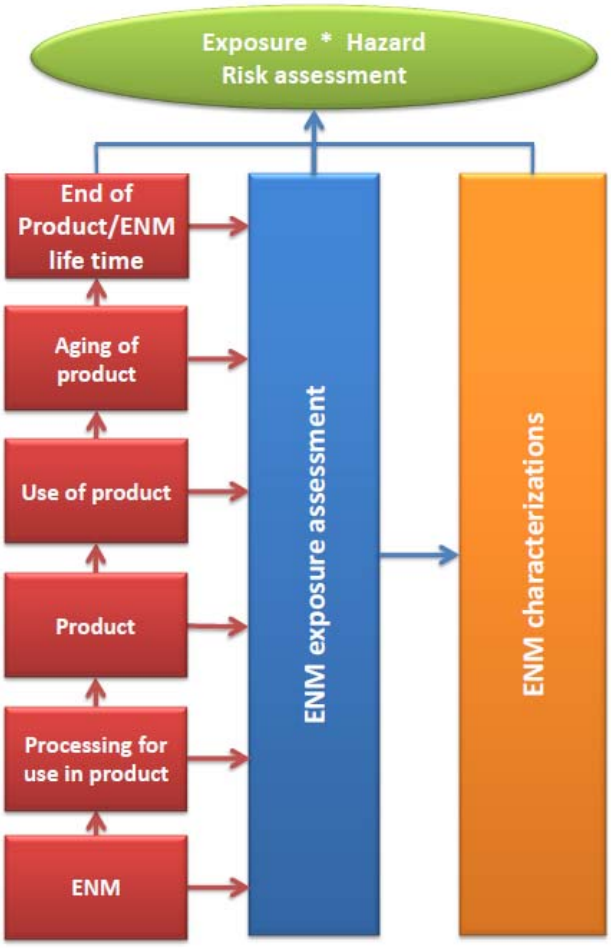

Figure 4. Risk assessment of ENM requires knowledge about the hazard potential and of the actual exposure, for each stage in the value chain of the given ENM-containing product. There is thus possibly not appropriate to perform one single RA for a given material, but multiple ones, characterising a specific stage along the value chain.

The present specific tools used in characterization of ENM, in exposure assessment, in toxicological testing, and the risk assessment are appropriate, but not necessarily sufficient for RA. Methodological development in material characterization and detection, especially at low levels, better understanding of matrix interactions, improved understanding of tissue kinetics and effects of low level exposures, development of appropriate dose concepts, and development of quantitative RA will help decision makers when performing ENM relevant risk management.

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