ERA-NET SIINN
Safe **I**mplementation of **I**nnovative
Nanoscience and **N**anotechnology

Deliverable D3.6

Inventory of knowledge gaps

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Executive summary

There are increasing numbers of products that have engineered nanomaterials (ENM) as components. Such products belong to a diverse group of categories, including medical devices and materials, electronics, appliances, cosmetics, solar panels etc.

In parallel to the increased production and use of materials that are termed “nanomaterials”, there is an increased awareness of potential safety issues, for environmental and human health. This is also reflected in substantial amounts of research efforts, which has led to publication of a high number of research reports (scientific “papers”) in the public data domain. The studies cover a wide range of safety related themes, including nanomaterial characterization, exposure assessment, toxicology and ecotoxicology, clinical findings, and risk assessment.

Despite the large number of studies, there are still many questions remaining regarding our understanding of how ENM can influence biological systems (including cells and tissues in humans and in environmental species), and have consequences for the safety of ENM. Thus, performing proper risk assessments regarding possible effects of ENM on environmental and health safety is still a difficult undertaking, due to lack of essential knowledge.

The present document aims at presenting major impediments for ENM risk assessment due to specific knowledge gaps. The ambition is not to put focus on all areas where additional knowledge is needed or wanted, but to stress the most crucial gaps in knowledge and approaches, where possible accompanied by specific research recommendations and advice. The resulting content is based on referenced SIINN documents, other referenced literature sources, and the authors´ own interpretations and experiences of the subject area.

We have highlighted crucial knowledge gaps which are valid for all types of nanomaterials, present as well as future. The key areas where research is strongly recommended include:

- identification and mapping of product value chains and life cycles
- determination of fate and behaviour of ENM in organismal as well as extra-organismal environments
- development of proper dose metrics and dose assessment models
- investigations into long-term low dose effects, both for humans and the environment
- further considerations of environmental ENM exposure

Introduction

There are increasing numbers of products that have engineered nanomaterials (ENM) as components. Such products belong to a diverse group of categories, including medical devices and materials, electronics, appliances, cosmetics, solar panels etc. The exact number of ENM containing products available for customers on the general market is unclear, although estimates have been produced by different actors. Thus, the Woodrow Wilson Center (Project on Emerging Nanotechnologies 2013) has listed more than 1600 manufacturer-identified nanotechnology based consumer products that are introduced to the market. An Austrian investigation (Gessler and Gaszó 2014) documented 492 entries to a national databank, spanning 19 categories of products.

In parallel to the increased production and use of materials that are termed “nanomaterials”, there is an increased awareness of potential safety issues, for environmental and human health. This is also reflected in substantial amounts of research efforts, which has led to publication of a high number of research reports (scientific “papers”) in the public data domain. The studies cover a wide range of safety related themes, including nanomaterial characterization, exposure assessment, toxicology and ecotoxicology, clinical findings, and risk assessment. The number of studies has increased significantly during the last decade, with each year being more “productive” than the previous ones (see also Krug 2014 for another view on number of publications). Our own search for entries of studies on the NCBI PubMed website (http://www.ncbi.nlm.nih.gov/pubmed) revealed that more than 3600 scientific articles with the term “nano* ” (indicating all words beginning with nano, irrespective of ending) combined with “toxic*” (and thus having a focus on toxicological effects of nanomaterials and nanotechnologies) were published in the biomedical periodical literature during the period 2000 to 2014 (see also Table 1 below). This kind of generic search does not provide the absolute number of relevant articles, but it indicates that there is a large amount of studies that should have relevance for the discussion of nanomaterials and safety aspects. The safety is then referring to the occupational situation (manufacture, transport, waste and disposal), the environment, and the consumers that come into contact with, and use the products in question.

A possibly more inclusive search was published by Samet (2014), who presented number of publications related to nanomaterials toxicology, human health, or environment for the period 2000-2013. Studies including toxicology and/or human health are on the same number level as the numbers presented here or in the work from Krug (2014). However, studies focusing on environmental impact far exceed the other two categories, with publication numbers in the thousands from 2008 and onwards. It is unclear which search terms have been used, so a direct comparison with the other two mentioned studies is not possible.

The request for specific ENM risk assessment emanates from the unusual properties of ENM (small particle size, large surface to volume ratio, many different materials) which may lead to a modified distribution model in the organism. In turn, this could cause a novel type of biokinetic behaviour, thereby producing different and unexpected effects, when comparing with the same elements or compounds in the form of chemicals in solution.

Table 1. Number of scientific publications accessed on the NCBIPubMed website using search terms as given in the first column. The search includes the period 1 Jan 2000 to 31 Dec 2014.

Search term	No of PubMed hits
Nano* AND toxic*	3648
Nano* AND environment	5939
Nano* AND environment NOT sensor	5760
Nano* AND risk	750
Nano* AND safety	807
Nano* AND health	2106

With such a large number of studies, are there still any questions remaining regarding our understanding of how ENM can influence biological systems (including cells and tissues in humans and in environmental species), and have consequences for the safety of ENM? The short answer to the question seems to be "yes", according to statements from a number of competent bodies that evaluate the knowledge basis and compare it to the relevant safety issues. Thus, performing proper risk assessments regarding possible effects of ENM on environmental and health safety is still a difficult undertaking, due to lack of essential knowledge. This has been stated by national and transnational organizations (see e.g. SCENIHR 2006, 2008, 2010; National Nanotechnology Initiative 2011; SOU 2013, among others), as well as by a number of independent scientists (e.g. Maynard et al. 2006; Borm et al. 2006; Savolainen et al. 2010; Simkó and Mattsson 2010, 2014; Klaine et al. 2012; Kuempel et al. 2012; Krug 2014). A very comprehensive overview of the current research landscape is also given in the NanoSafety Clusters treatise "Nanosafety in Europe 2015-20125 (Savolainen et al. 2014).

The presence of essential knowledge gaps is furthermore the driver behind directed funding of appropriate safety-oriented research, of which the funding mechanisms and goals of ERA-Net SIINN is an example. A component of this project is also to identify and list such knowledge gaps (this deliverable, D3.6), with special consideration of their impact on risk assessments.

Other tasks in the ERA-Net SIINN project have also contributed to analysing the knowledge base and to identify knowledge gaps. Furthermore, activities within several tasks have been decisive for formulating the Call Topics of the SIINN Calls for research. (See also Table 2 for a summary overview of relevant SIINN-activities). These activities and this document are complementary to each other and have different focus and perspectives. The present document aims at presenting **major impediments for ENM risk assessment due to specific knowledge gaps**.

The ambition is not to put focus on all areas where additional knowledge is needed or wanted, but to stress the most crucial gaps in knowledge and approaches, where possible accompanied by specific research recommendations and advice. The resulting content is based on referenced SIINN docu-

ments, other referenced literature sources, and the authors´ own interpretations and experiences of the subject area.

Table 2. Activities within ERA-Net SIINN that provide overview of the knowledge base, inventories of knowledge gaps, and/or recommendations for research.

Deliverable or activity	Content	Comment
Deliverable D2.7 Roadmaps for the safe handling of nano-objects, safe processes, safe products and safe transportation of nano products addressing identified gaps.	Overview document based on input from Nanosafety Cluster, knowledge gaps identified in SIINN WP1, FP7 projects ITS-Nano and Nanofutures, own survey of nanosafety.	Research needs and research priorities are given from short, medium, and long-term perspectives.
Deliverable D1.4 Knowledge Gaps 1. Identification of knowledge gaps of in-vitro assays, on toxicity and translocation of nanomaterials, such as size, shape, surface reactivity, surface area, adsorption and their transportation through the human body.	Overview of in vitro studies using carbonaceous NMs, metals and metaloxide nanoparticles, and silica nanoparticles.	Knowledge gaps are identified form referenced literature sources.
Deliverable D1.5 Knowledge Gaps 2. Identification of knowledge gaps on the correlation between epidemiological health reports and possible safety and handling of nanomaterials	Overview of health effects studies.	Knowledge gaps are identified form referenced literature sources.
Deliverable D3.3 Health Data Selection	Procedures and criteria for health data selection.	Contains also a sample collection of references to relevant published scientific articles.
Deliverable D3.7 Guidelines for EHS Assessment	Risk assessment of ENM for human health and the environment	Challenges for ENM risk assessment includes knowledge gaps
First, second and third joint transnational Calls within the ERA-Net SIINN (2012, 2013, 2014)	Calls for transnational research projects to close knowledge gaps with respect to EHS (Environment and Human Safety) issues	Topic categories: - Establishment and development of models and methods for analytical tools, theoretical prediction, and characterization - Exposure assessment - Studies on Impacts of MNMs on environment - Studies on properties and effects of MNM on human health - Toxicity mechanisms - Over-arching aspects of

		nanosafety research

What can be learned from other research areas?

The current discussion of possible safety issues related to the introduction and use of nanomaterials and nanotechnologies is by no means the first example of how a new and emerging technology can influence the research agenda. There are currently similar type of activities and discussions related to the ever growing use of wireless technologies that emit electromagnetic fields (EMF) in the radiofrequency range. Developments in genetic engineering and an increased understanding of the molecular biology of the cell have led to many applications and a tremendous technological development in the field of biotechnology. Especially the use of these methods for genetic modification of organisms has been the subject of a large body of research with focus on safety aspects. Recently, the advent of synthetic biology and the potential for modifying and even creating biological structures and processes has raised safety concerns as well. In addition, the introduction and use of novel chemicals has received interest also from the point of view of possible risks. Safety related research focusing on ENM is thus by no means unique.

We, the authors of this document, have ourselves several decades of experience working on safety related aspects of the use of technologies that emit various types of EMF. The EMFs emanate from different types of appliances and have various physical properties. It is well known in what way the fields interact with biological systems at certain ("high") exposure levels. Exposure guidelines and directives for protection of the general public as well as workers exist since many years. However, exposure below these levels is receiving a vast interest due to possible health effects at exposure levels that can be experienced in the everyday life. A recent comprehensive overview of possible health effects of EMF at these levels was recently published by SCENIHR (SCENIHR 2015).

What is then the connection between EMF and ENM? Our view is that the nanosafety research currently is experiencing the same "stage of confusion" regarding what the results from studies really are telling us, as EMF research experienced 15-20 years ago. A substantial number of publications are available, but the studies are to a large extent not useful for risk assessment. This characterized EMF research as well. There were (and still are to some extent) many explanations for this state of affairs regarding EMF, of which some with larger impact are listed below:

- Experimental studies (both *in vivo* and *in vitro*) aiming at hazard identification and hazard characterization lack proper exposure characterization, appropriate controls, dose-response relationship studies, realistic exposure scenarios, relevant choice of biological end-points, statistical power, and have poor dosimetry.

- Clinical studies suffer from lack of proper exposure characterization, have questionable blinding procedures, and poor dosimetry.

- Epidemiological studies suffer from small effect sizes and need thus very large sample sizes that actually are missing, exposure assessment is poor, confounding factors are missing.

Furthermore, many findings were over-interpreted and have never been independently replicated. Plausible mechanisms for certain effects have never been established, and studies have often lacked a hypothesis base. Another factor of possible importance is publication bias, where studies reporting no effects of exposure may not be published.

Many of these (or similar) shortcomings in the EMF studies are found also in studies of ENM. However, the awareness that has been developing over time in the EMF area has substantially increased the quality of the studies that are performed.

Another concern, which also has been stated by Krug (2014), is that a substantial part of the studies of ENM and safety is considered as "nanotoxicoloy", but without the inherent quality criteria that characterize conventional toxicology studies. This lack of technical quality makes results from many studies questionable for risk assessment purposes. Of particular concern is the sparse use of standardized protocols for the experimental studies, the lack of dose-response studies, unrealistic exposure scenarios, and uncritical interpretations of the results. Due to the complexity of the field of nanomaterial safety, it seems reasonable to adopt a "systems toxicology" approach to the hazard identification and characterization part of ENM risk assessment (see Sturla et al. 2014 for a recent overview of the subject). This is an approach which integrates classical toxicology with quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization, and would thus seem appropriate for ENM toxicology.

Critical knowledge gaps areas

The following section outlines a number of knowledge gaps covering several aspects of risk assessment. Research that aims for mechanistic explanations of interactions, or that has a screening purpose is not included in this context, although such activities surely are valuable, although possibly not directly having bearing on risk assessment.

Value chains and life cycles

Initially the concept of a **value chain** (VC) was described as a chain of activities that a firm operating in a specific industry performs in order to deliver a valuable product or service for the market (Porter 1985). In the field of economics, a value chain is understood as a structure that can be used to categorize and organize factors related to industrial organization; the activities, places and firms involved in making a product or service. It includes the full range of activities that companies and workers do to bring a product or service from its conception to its end use and beyond. This includes the activities related to producing and transporting the product (**supply chain**) as well as other value-adding activities such as research, design, marketing, and support services (see Fig. 1 below). Thus this can be described as:

- the **full range** of value-adding activities/business functions: R&D, design, production, logistics, marketing, services

- **supply chain**/product life cycle stages: inputs, components, final products, distribution/sales, disposal/recycling

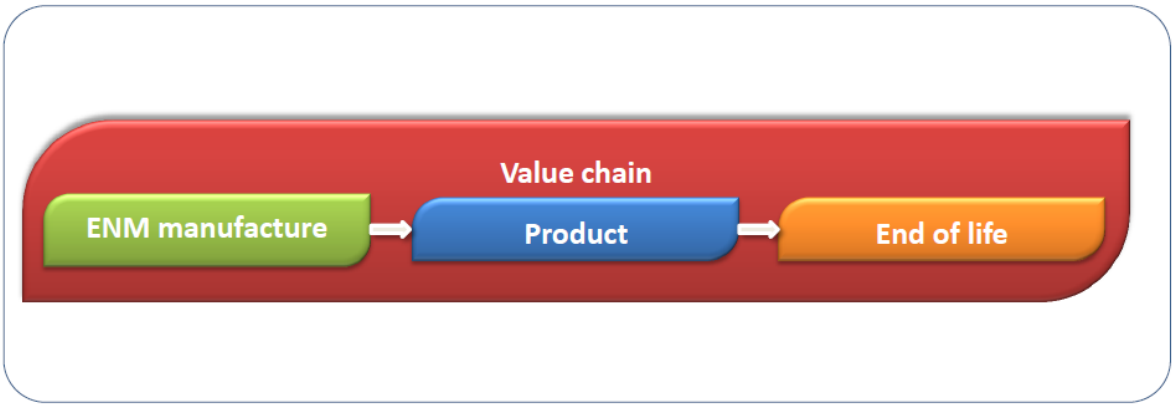

Figure 1. Schematic representation of a generic value chain, which goes from idea via product and use to end-of-life.

Life Cycle Analysis (LCA), on the other hand, 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

In many cases, there is considerable overlap between value chains and life cycles. For ENM risk assessment purposes, it is of vital importance that the material´s life cycle is comprehensively mapped, and put in the context of the value chain. As can be seen from Figure 2, 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.

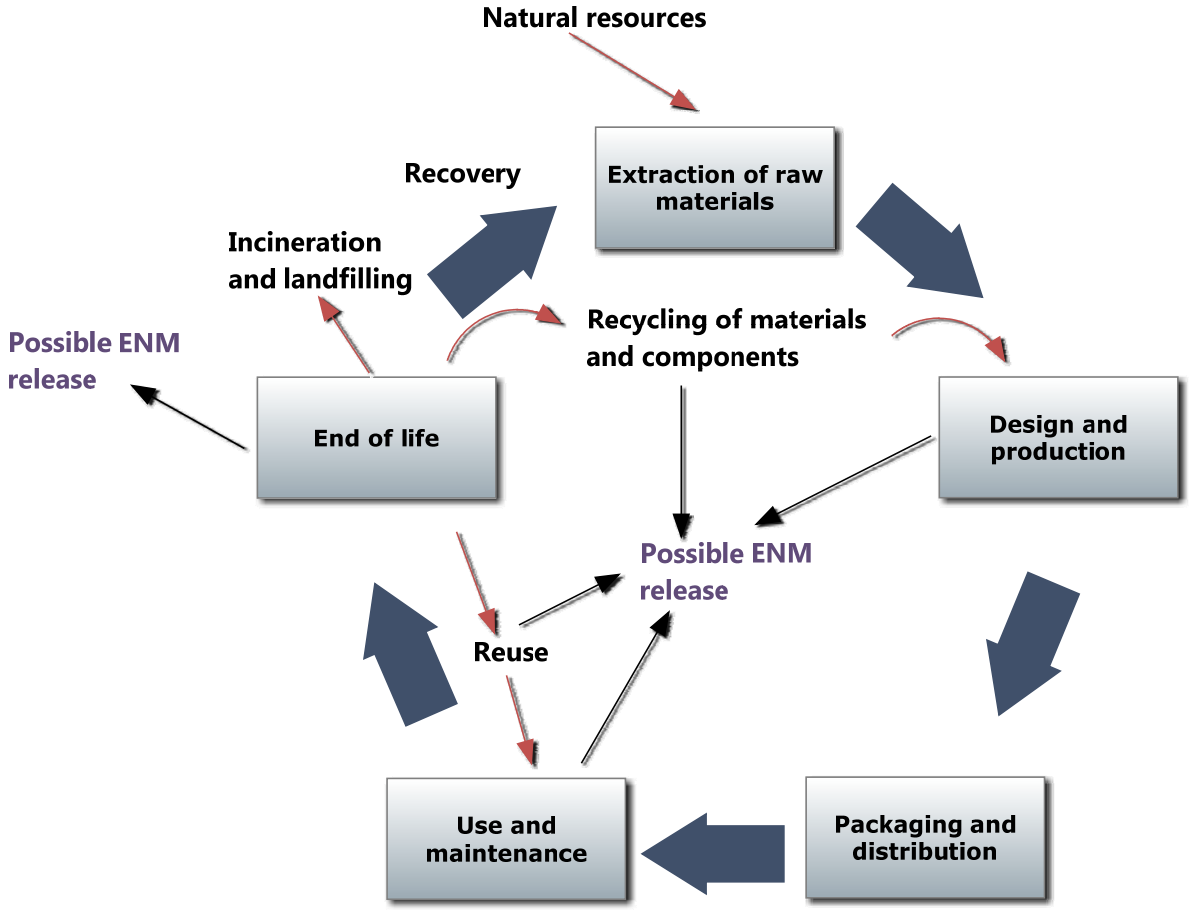

Figure 2. Generic life cycle of an ENM which is integrated into a product.

A large knowledge gap exists regarding almost all ENM-containing products and their respective life cycles. A consequence of this is that it is unknown in what form the specific ENM takes at different stages, and furthermore that there are question marks regarding the possible ENM release. This lack of knowledge precludes a relevant exposure assessment, and thus one of the corner stones for risk assessment.

Most studies so far related to specific safety aspects of a given ENM have used the pristine form of the ENM for at least hazard identification studies (toxicological studies using *in vivo* or *in vitro* approaches). Such pure materials are useful for studies of mechanisms and mode of actions, but are not necessarily the best choices for understanding the toxicological potential of the ENM in a specif-

ic, “real world” setting. For that purpose, the LCA provide information that can be used for further analysis giving knowledge about which form(s) an ENM is adopting at different stages of its life.

Conclusion

The big challenge regarding consequences for risk assessment deals with the exposure assessment aspects related to the different stages of a life cycle or value chain. Thus, it is necessary to establish:

- if there is any release of ENM at specific stages of the materials life cycle or the products value chain
- the specific physical form that the ENM is taking if there is any release
- the actual quantity (taking the most appropriate metric(s) into consideration) of the ENM
- any background of similar but naturally occurring materials

These data will then guide the work related to other aspects of risk assessment.

Fate and behaviour of ENM in different surroundings

As was brought up in the preceding section, the pure form of an ENM is seldom the form that will be part of the exposure to humans or to environmental species. This is due to that 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.

A good illustration of a generic pathway of ENM from source to adverse outcome is presented in Landsiedel et al. (2014). Without going into details, the pathway starts with the material´s form, either as a powder, or embedded in a matrix or on a surface. The physical-chemical properties of the material are then undergoing changes during the life cycle of the ENM. The resulting form of the material can subsequently be in the form of a suspension, or as an aerosol, which can be taken up into the body (via respiratory pathways, the gastro-intestinal tract, or through dermal absorption).

Within the organism, any material that is taken up will encounter various types of biological fluids and also extracellular components of epithelia. This interaction will equip the ENM with a modified surface, which in turn affects the way the ENM interacts with biological structures. This surface modification was initially found to have protein components typical for various extracellular environments (Cedervall et al. 2007; Lundqvist et al. 2008). This proteinaceous surface has recently been mapped in more detail for different types of body compartments (Gossmann et al. 2015; Kelly et al. 2015; Pozzi et al. 2015; Ritz et al. 2015), although these investigations are the first of their kind and by no means the final answers to what happens with different ENM once they enter the human body. Also lipids and lipoproteins (Hellstrand et al. 2009) as well as carbohydrate determinants (Wan et al. 2015) are deposited on an ENM surface. In conclusion, there are still significant question marks surrounding all aspects of this intra-organismal surface modification, and its´ impact on ENM effects.

Fate and behaviour of ENM in different environmental settings is another area where there is some data available, although substantial knowledge gaps are still remaining. It is reasonable that ENM behave differently than chemicals in solution in compartments such as air, water, or soil and sediments. The materials will undergo aging during the different stages of their lives, and will aggregate and agglomerate in a manner depending both on their inherent physical-chemical properties and on the specific characteristics of the physical environment. Similar to the situation in the human (or animal) body, the specific matrix where the ENM is located will determine the behaviour of the ENM. These aspects have received attention (see e.g. Lin et al. 2010; Nowack et al. 2012; von der Kammer et al. 2012; Gottschalk et al. 2013), but there is nevertheless significant knowledge gaps that need to be filled for the purpose of risk assessment.

Conclusion

The surface modifications of ENM in the environments that they occupy have great impact on the behaviour and thus the fate of these materials. It is very important to investigate and understand:

- how ENM are surface modified when encountering different body fluids and components of the extracellular matrix in an organism

- how surface modification influences transport, distribution, and persistence of ENM within the organism

- how surface modifications affect the potential for ENM to penetrate tissue barriers such as the blood-placenta, blood-brain, blood-cerebrospinal fluid, blood-neuron, blood-testis barriers, and also the epithelial linings of the gastro-intestinal tract

- how surface modifications impact cellular molecules and thus biological processes

- how environmental matrices (organic as well as inorganic) modify the surfaces of ENM that are released into the environment

- the behaviour of ENM in different environmental compartments, with special emphasis on aggregation/agglomeration and transport kinetics, including uptake into environmental organisms (bacteria, fungi, algae, plants, animals)

Dose

It is recognized that in conventional toxicology there are different drivers of the studies. This includes the intrinsic chemical, the material morphology, and the radiation driven toxicity, which all use clearly defined metrics. What should then be used as the “standard” dose metric for nanotoxicology? There are many candidate characteristics including the mass of the investigated material, which is the most conventional approach. However, also other properties have been considered, such as the size of the particle, the shape and the aspect ratio, as well as the surface charge. However, due to the observation that nanomaterials often exhibit greater toxicity than the bulk form of the material, it has been suggested that the exceptionally large surface area, or even the specific surface area (surface area-to-volume ratio) that ENM exhibit could be useful metrics.

There are published attempts to answer 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.

Conclusion

It is recognized that reporting the mass or concentration of an ENM that is used in toxicological studies is not enough for interpretation of results that can be used for predictive toxicology purposes and/or risk assessment. The current state of affairs does not allow to clearly state which metric would be the most appropriate for describing dose in this kind of research. It is therefore needed to:

- investigate the appropriateness of other properties with special focus on the surface area and where possible the specific surface area
- develop further dose assessment models that take both the complexity of ENM into account, as well as possible differential sensitivities of exposed organs, tissues and cells

Long-term low dose effects

Most studies of potential harmful effects of ENM have dealt with acute and/or short term effects of exposure to more or less defined materials at high concentrations. These types of studies have the potential to primarily answer questions related to hazard identification, namely if a specific material has the potential to cause damaging effects, irrespective of condition. For risk assessment purposes, this is only one part of the entire picture, since these studies do not take into account either changes of the ENM over time, the long-term effects of exposure, and exposure to possibly (very) low doses. With exception of the possibility for certain occupational accidents, it is not very likely that either humans (as consumers, mainly) or the environment will be subjected to such exposure.

The most appropriate studies regarding the effects of long-term exposures to human health comes from epidemiological studies. An epidemiological study should ideally capture all major sources of exposure as a function of time during the relevant time period (considering latency) prior to occurrence of the outcome. For exposures from environmental and occupational sources, as well as personal use of devices, comprehensive construction of exposure history requires evaluation of exposure as a function of time.

In general, personal measurements are regarded as the gold standard for assessment of current short-term exposure, though spot measurements may not reflect long-term exposure. For studies on health risks from ENM, the relevant time period for which exposure data would be needed is a period of perhaps several years preceding the diagnosis. Typically, exposure assessment only encompasses either a short-term measurement with personal monitoring, or a spot measurement providing only a snapshot of instantaneous exposure levels at a single location. As a rule, retrospective exposure

assessment is more challenging and prone to errors than estimation of concurrent exposures. Study subjects are rarely an optimal source of information, due to potential errors in recall, particularly for case-control studies.

The minimum requirements for exposure assessment for an epidemiological study to be informative include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body. Valid exposure assessment allows a researcher to distinguish sub-groups of the population with contrasting exposure levels.

Whatever exposure metric is used, it is important to demonstrate its adequacy for the specific study hypothesis, for instance with the help of validation studies, comparison of different metrics aimed at predicting the same exposure, or sensitivity analyses using different error scenarios.

Previously there have been quite a number of epidemiological studies of fine and ultrafine particles effects on human health. These studies have uncovered effects on the cardiovascular system (Simkhovich et al. 2008; Miller et al. 2012), on respiratory capacity (e.g. Jacquemin et al. 2012), on haemostasis (Emmerechts and Hoylaerts 2012), as well as neuropsychological effects (Guxens and Sunyer 2012). There are also a few studies have focused on effects of combustion derived nanoparticles, and especially effects on the cardio-vascular system (Cassee et al. 2011; Donaldson et al. 2013).

There are few, if any, well performed epidemiological studies on health effects from the type of ENM that are integrated into consumer products, either with focus on the occupational setting, or with focus on the general public. This is due to the relative short period that such materials have been present on the market, and also on the fact that there are no exposure data available that can substantiate any significant exposure. In general, it is also to be considered that exposure assessment would be difficult from a retrospective point of view.

Another aspect of long-term exposure resides in the distribution of persistence of ENM within the organism. After uptake and possible translocation to secondary tissues, the ENM is taken up into cells, e.g. by means of phagocytosis. Although there are some in vivo studies addressing the long-term fate of administered ENM in rats (e.g. Andersson et al. 2014, 2015; Coccini et al. 2015) substantial knowledge gaps remain regarding what happens with the applied nanoparticles over time. This is also further complicated by that various ENM possibly undergo different rates of intracellular destabilization, and that the intermediate products can have different impact on cellular processes.

Regarding low-dose levels of exposure, toxicological studies classically determine e.g. no observed adverse effect levels (NOAELs) in acute experiments. The data from these studies are certainly valid for short-term exposures, but are not guaranteeing that low doses are without effect over longer periods of exposure time. Such studies regarding ENM are missing but would provide substantial input to risk assessment.

Conclusion

The majority of studies regarding effects of ENM are short-term studies where at most the NOAELs have been determined. There is on the other hand a lack of studies, epidemiological as well as in vivo

animal studies, that address health related long-term effects. The knowledge base would benefit substantially from:

- epidemiological studies with focus on occupational exposures of ENM that are produced in high volumes and/or used in many types of applications. This could also include activities related to medical care or research. Prospective cohort studies taking many types of health conditions and different types of exposure into account would be most useful.

- experimental studies (both *in vivo* and *in vitro*) that address the problems of persistency, material aging and low-dose accumulation effects in biological settings on several levels of organization (from cell to organism)

Environmental aspects

The two aspects of EHS (**environmental** and **health** safety) should not be considered as separate phenomena. They are interconnected and influence each other in multiple ways. Thus, knowledge about e.g. exposure to ENM in the environment do have direct implications for exposures to human, and for aspects of human health. Conversely, if exposure to humans occur in e.g. an occupational setting, it is also possible that it will have impact on the environmental exposure. Thus, the different aspects of important knowledge gaps that are outlined in the sections above also have relevance for any consideration of environmental aspects.

There is a possibility for release of ENM to the environment during any stage of a products life cycle. The over-all problems of how ENM move, deposit, and accumulate are important to solve. In addition, the possible transformation via biological and/or photochemical processes, or via contact with water or still other processes will determine how and to what extent the ENM impact on the environmental species. The environmental accumulation of ENM can have implications for a number of species, including humans, in the case of accumulation in the food chain.

A number of studies have focused on the environmental aspects of ENM exposures. These studies span all aspects, from characterization of ENM in matrices, to assessing exposure, and performing eco-toxicological studies (see Gottschalk et al. 2013; Marcoux et al. 2013; Froggett et al. 2014; Zhao et al. 2014; Walters et al. 2014; Schaumann et al. 2014 for recent reviews). Nevertheless, the environmental impact of releases of ENM to the environment, along the value chain of a product, still remains to be investigated.

Conclusion

Previous studies have focused on isolated parts of the value chain, and/or on limited aspects of the entire risk assessment process. What is missing is an integrative risk assessment approach, which takes:

- exposure assessment

- hazard identification

- hazard characterization

- ENM fate and behavior
- ecological aspects
- and uncertainty analysis into consideration.

General conclusions

Despite more than a decade of intense research efforts in the environmental and health safety aspects of ENM, there are still significant knowledge gaps that preclude integrative and predictive risk assessment. This is possibly a natural state due to the multitude and complexity of nanomaterials, and that such risk assessment activities require trans- and multidisciplinary actions that are difficult to initiate and manage.

However, a safe implementation of the innovative nanomaterials and nanotechnologies that our society is expecting will also require more clear answers regarding risk identification and risk management. This is certainly pressing due to the expected future development of even more sophisticated materials.

This document has highlighted crucial knowledge gaps which are valid for all types of nanomaterials, present as well as future. The key areas where research is strongly recommended include

- Identification and mapping of product value chains and life cycles
- Determination of fate and behaviour of ENM in organismal as well as extra-organismal environments
- Development of proper dose metrics and dose assessment models
- Investigations into long-term low dose effects, both for humans and the environment
- Further considerations of environmental ENM exposure

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