Highlight

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From nanoobject release of (Bio)nanomaterials to exposure

Abstract: An increasing variety of different nanostructured materials including bionanomaterials are used. During synthesis, but also during use of nanostructured materials along their life-cycle, nanostructured materials and engineered nano-objects (ENO) – may be released into the environment. They will follow different exposure pathways and create an exposure concentration at the point of different biological systems, especially human beings. The inhalation pathway is of greatest importance with regard to health issues. The exposure concentration together with the breathing conditions integrated over time leads to the dose of the deposited material, which is of greatest interest for different effect studies. We discuss in this paper the kind of nanostructured material released from bionanomaterials into the environment. A large part of existing exposure studies in the literature is critically considered. A strategy is proposed to investigate in a more effective way the ENO-release from nanostructured materials as the first step of the exposure pathway. The release – exposure relationship as well as exposure – dose relationship for the case of inhalation is described leading to the possibility of tracing and ideally a complete balancing from ENO-release to dose. In the end the still needed activities for ENO-control methods in the environment are summarized.

Keywords: ENP/ENO tracing and balancing; ENP/ENO exposure-dose relationship (inhalation); ENP/ENO release determination methods; ENP/ENO release-exposure relationship; ENP/ENO risk banding; exposure pathway.

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Introduction

During production and use of any kind of material unwanted release of energy and of material produced and of by-products may occur. The material is distributed in the environmental compartments air, water and soil and transported to biological systems, where it contributes to the exposure situation. This is the starting point for safety considerations, since the exposure of nature and human beings may cause negative effects. The study of negative and also positive effects in biological systems starts with different doses, which are time integrated values of exposure concentrations of different metrics times flow rates at a relevant point outside or in the biological system. To perform a realistic study, exposure concentrations in form of number, surface area and/or volume (mass) concentrations are needed to determine the relevant dose. This is obvious since the effect does not have to be determined if there is no exposure and therefore no dose. Even if there are small exposure concentrations, they may not be large enough to overcome an effect threshold [1].

Bionanobiotechnology involves the use of (individual) biomolecules for technical applications involving devices with at least two dimensions in the submicrometer regime, and/or vice versa, the use of technical nanometer-scaled devices to exploit biological structure and reactivity [2]. Our focus is mainly on engineered materials, which are used to direct the course of any therapeutic or diagnostic procedure. Bionanomaterials are a special form of these materials which consist of or at least include nanostructured materials, which are used to improve the properties of the material in different applications. Nanostructured materials do have internal or surface structures with at least one dimension <100 nm [3]. They can occur in form of thin layers of solid material and especially in the form of differently shaped nanoparticles and their agglomerates and aggregates in gaseous (powders, aerosols, sprays), liquid (suspensions, colloids) and solid (composites) matrices. Silicon nanowires and DNA modified gold nanowires have been used as DNA sensors [4, 5]. Semiconductor nanocrystals can be utilized for biolabeling or lasers as well as for

the external magnetic field required to switch a magnetized particle, which is highly relevant in magnetotactic bacteria and hard disk drives [2]. Silver nanoparticles have been used as anti bacteria agents [6] and coatings on textiles to remove undesirable odors [7] the release of silver nanoparticles into environment during washing of textiles has been studied [7]. Iron nanoparticles are furthermore used for cancer treatment [8], a field in which also carbon nanotubes might be applied in the future [9].

The released material from nanostructured materials may be in form of atoms, molecules and – of main interest – nanoparticles (particles with different shapes and all three dimensions in the nanoscale), nano-objects (particles with at least one dimension in the nanoscale) as well as agglomerates or aggregates of such nanoparticles and nano-objects. The occupational risks of engineered materials that consist of nano-objects such as nanoparticles, nanofibers, nanotubes and nanowires, as well as aggregates and agglomerates of these materials (NOAA) have attracted increasing attention [10]. In case of nanostructured materials and their products there exists special concern, because they contain small entities, which, once released, do have a high mobility causing a fast distribution in the environment and living beings [11]. From many earlier studies on particles in the environment it is known that the hazard potential of particles in certain exposure situations is larger than that of the corresponding bulk materials. This is especially true for the inhalation of particles [12]. A large amount of knowledge exists about particles emitted from technical processes into air causing exposure followed by inhalation by mainly human beings and their effects in the human body [13, 14]. Currently, numerous types of novel nanoparticles with different properties are being produced for widespread applications in consumer products as well as bionanomaterials to be used in therapeutic, diagnostic and other novel medical applications. In the wake of this development literally hundreds of thousands ‘new’ types of nanoparticles are already or will be manufactured differing in size, surface area and mass per particle, agglomeration stage, shape, surface charge, composition as well as surface functionalization, layered structure and many more properties [15].

The effects of nanoparticles, nano-objects and nanostructured materials are still mostly unknown and therefore also the dose – effect relationship, which is the main topic of this special issue of the journal. The dose represents the amount of material collected by the biological system. In case of particles the amount can be described by the total number, mass (volume), surface area collected depending on the kind of effect investigated and its relevant measure. To be able to perform a study with a

realistic dose range, possible dose ranges occurring in real environment have to be determined [14]. Since the dose is difficult to measure directly, the exposure at an appropriate entrance port, a concentration measure of the entering material, is determined instead. The dose is then calculated with known flow rate, exposure time and, if necessary, factors describing the transmission process.

This exposure measurement is very difficult especially for nanostructured materials for several reasons. First of all we deal with a large variety of different released materials depending on the nanostructured materials and the treatment processes leading to the release. Any exposure measurement thereafter along the exposure pathway may lead to different results because of transformation processes changing the released nanostructured material in the environment. The best exposure measurement point is at the entrance to the biological system (personal sampler) [16, 17]. Another difficulty is the fact that – because of the many different biological effects – the toxicologists ask for various concentration measures and additionally for a large set of properties of the exposed nanostructured material.

Many exposure measurements have been performed at different points along the exposure pathway [18], which are of limited value for dose estimation. Although they may describe the actual exposure situation, it seems to be difficult to justify the dose estimation, because the exposure is influenced by many uncontrolled processes. From the rule “no exposure, no effect” we derive the rule “no material release into the environment, no effect”. This puts the emphasis on the beginning of the exposure pathway, describing the starting point through properties of the production process and the produced nanostructured materials during their whole lifetime. The produced nanostructured materials will deliver material to the environment in modified form during handling, use and at end of life, when they are recycled or deposited in a landfill [19]. Even if we restrict the task to only nanoparticle release of production processes and materials as well as their products, the number of processes causing release and their conditions is high. It seems to be impossible to investigate all the different material stress situations which may cause release. We will discuss a strategy to reduce the effort by identifying a reduced set of representative treatment processes and their conditions, which may be standardized. They should allow at least estimating the release potential, which then together with information about the particle transformation processes along the exposure pathway leads to the wanted exposure risk estimation. Exposure risk together with the hazard risk allows the risk estimation of the nanostructured material. The amount of used materials together with their release potentials provide

quantitative input data for a released material balance along the exposure pathway to the exposure point and maybe further on to the dose point, if the process steps along the exposure pathway can be described.

Material release, exposure pathway and dose

Technical processes and their products release – besides energy – material into the environmental compartments water, soil and air, which may cause unwanted effects in the environment (Figure 1).

The whole life-cycle including the material production, the fabrication of master-batches, manufacturing of products, their use, and – at the end of their life – the recycling processes, the incineration and the landfill of materials as well as their littering in the environment has to be considered with respect to the possible unwanted release of materials. During the different life-cycle stages different stress conditions have to be considered. The released materials from processes and products are transmitted in the different environmental compartments air, water and soil. During this transmission changes of physical and/or chemical properties of the released materials may occur, leading to differences between the released material and the material at the point of exposure. For the determination of exposure the whole range between material release and entrance into the effect (biological) system is of interest (Figure 2).

Most of the time, a transmission process with changes of concentration and properties of the exposure material occurs also in the effect system up to the point where the effect takes place and where the dose determination is of interest. The dose allows the determination of the effect, if the dose – effect relationship is known. This is the task of human- and eco-toxicology. Material release, exposure at the intake point of the effect system (personal samplers)

and the dose at the effect point are the most important control points along this exposure pathway from release to dose to effect.

Nanomaterials, released materials

Bionanomaterials can be considered as a subgroup of nanomaterials. They are only different from other engineered nanostructured materials with respect to their application and therefore their specific properties.

Nanomaterials are defined as materials with any external dimension in the nanoscale (approximately 1 nm–100 nm) or having internal structure or surface structure in the nanoscale [3]. Most important building blocks are nano-objects with one or more external dimension in the nanoscale, e.g., spherical nanoparticles, isometric nanoparticles, nanofibers, nanotubes or nanoplates. Often they occur as assembled nanomaterial, as a subgroup of nanostructured material with nanoscale internal or surface structures in form of loosely connected agglomerates or strongly by chemical bonds connected aggregates. Since they are designed for a specific purpose or function, they are called engineered nanoparticles (ENP) or engineered nano-objects (ENO), depending on their size and shape. The term NOAA (nano-objects and their agglomerates and aggregates) is also becoming a common expression, it is defined in an ISO technical specification [10].

Besides shape and structure differences, ENOs may also differ with respect to their composition and their surface properties. All properties may also change during their lifetime, because of different material stress situations. After synthesis in the solid, liquid or gaseous phase they may be introduced into other gaseous, liquid or solid matrix material. We can differentiate between several forms of nanostructured materials: aerosols, powders, suspensions and composites. Coatings can be considered to be a thin layered form of composites on a substrate.

Figure 1 Material release into the environment during production of materials and during the life-cycle of resulting products.

Figure 2 Process chain, exposure pathway and effect studies for materials released into the environment.

The primary interest in released materials from nanostructured materials within different matrix materials should be the embedded nanostructured material itself, because these materials are added to the bulk material. The release requires the separation of the nanostructured material from the matrix material, which is only possible, if enough energy is introduced locally to overcome the binding forces between the nanostructured and the matrix material.

This process is very often incomplete and additionally results in release of other forms of nanostructured materials in parallel. Besides engineered nanoparticles (ENP), nano-objects (ENO) and their agglomerates/aggregates (NOAA), other particles like

- matrix particles containing ENOs,
- nanoscaled particles (NP) newly formed out of the gas phase containing ENO-materials in molecular form,
- newly formed NPs without ENO-materials

can be released. For example, [20] observed release of discrete carbon nanotubes (CNTs) as well as CNTs embedded in particles of the matrix material from an abrasion process of epoxy/CNT composites. Göhler et al. [21] found no free nanoscale pigments from sanding, dynamic friction or wind erosion of nanocomposites. From the measurement point of view the – always existing – nanoscaled particle background also contributes to the measurement object [22].

Existing nano-object release and exposure studies

It is assumed that there is a release potential for ENOs from synthesis processes and from the different material forms

during life cycle leading to an exposure potential and followed eventually by a hazard. Both exposure and hazard contribute to the risk of the nanostructured material. Up to now there does not exist any study which considers all these steps for one nanostructured material during its whole lifetime. Instead, exposure (sometimes replaced by release) and hazard are treated independently. The exposure studies [18] are justified mainly based on arguments like:

- Any material consisting out of or containing ENOs is a potential source for ENO/ENP-release – a tremendous amount of different materials have to be investigated.
- Any treatment or stress of ENOs or nanostructured materials may cause ENO/ENP-release – a tremendous amount of different treatment and stress processes under different conditions have to be investigated.

Often only a few ENOs are occurring in exposure situations; we cannot detect them because of their small contribution disappearing in a large background. The result is a big measurement effort for eventually few hazard relevant ENOs.

Recently a literature review [18] about workplace situations was published, covering the work published by that time to determine the exposure of ENOs from different synthesis and treatment processes of ENPs/ENOs as well as treatments of nanostructured materials like composites. In all studies, more or less defined exposure measurements for different materials in combination with different practical treatment or stress processes were performed. The investigated materials and treatment processes are listed in Figure 3 based on the review in [18]. The arrows indicate combinations, which have been considered. Although a lot of investigations have been made already, the picture is still very incomplete. There are many more materials, processes and operational

Figure 3 List of materials, processes and the investigated combinations based on the studies reviewed in [18].

conditions. The number of possible combinations, which have to be investigated, is huge.

According to this review different approaches have been pursued to derive exposure relevant information in workplaces: (case a) process based studies in simulated workplaces and of simulated work processes and (case b) studies based on real workplaces. Many exposure studies have been made under laboratory conditions with different nanostructured materials and simulating different treatments of the nanostructured materials. They are listed in [18] in Table 5. Most often they are interpreted as an example for real workplace conditions (case b), although they measure nanoparticle concentration very close to the investigated material. If no property changes occur between release and measurement point except for defined dilution, they can be considered to be release studies (case a). In only a few cases engineered nanoparticles or nano-objects were detected. In most cases the released particles consisted out of matrix material containing ENP/ENOs or newly formed nanoparticles (out of the gas phase) containing either vaporized ENP/ENO-material or no ENP/ENO-material at all. The question arises, whether the latter two releases can be considered to be ENPs from the nanostructured material or if they should be considered as background particles.

Clear differentiation of ENO released by an investigated material from background aerosols (either coming from other sources or being formed during the release process) is easier under laboratory conditions with particle free surrounding air than in real workplace measurements. If ENOs are of interest in real work places, the analysis has to allow for a separate measurement of background particles, which have to be subtracted from the ENO plus background measurement [22]. Process based studies in simulated workplaces and of simulated work processes also enable investigations on how variances in handling or process conditions influence release rates. Release rates are important pieces of information for workplace modelling approaches as investigated and described in detail by Schneider et al. [23].

In Table 5 in the review article [18] the investigated nanomaterials have been grouped according to material groups (powders, suspensions, coatings and composites), very similar to the earlier introduced material structure we propose. According to these groups the experimentally simulated treatment processes are different like rotating drum processes for powders or sanding processes for coatings. Unfortunately the process parameters used are different in the studies even of the same process.

The outcome is that in principle as many studies have to be performed as there are possible combinations of

materials, processes and process parameters. Because of different materials, different synthesis and different treatment processes the results of the studies discussed in [18] are not comparable and do not allow any summarized and transferable conclusions. The authors in the review article made the following statement: “The review of the laboratory test procedures shows that many workplace-related processes are currently simulated in the laboratory. While some approaches are quite advanced we still lack a coherent, systematic approach over all work related processes as well as studies on single simulations. This area, important for modelling of work processes and subsequent possible exposure, has to be further developed on the basic research level as well as in view of a standardized method.”

Strategy for nanoparticle release potential determination

There is a need for reducing the tremendous work which has to be done, if we want to cover the huge and still increasing matrix of different nanostructured materials and processes under many different conditions. It does not make sense to reduce the number of different materials to begin with. Later on, after we gain more experience, we might want to do that. But we should start to think about how to reduce the number of synthesis and especially treatment cases. The number of different treatment processes and their conditions may be even larger than the number of different materials. A more systematic approach has to be taken. Since it is obvious that without ENO-release there is no risk, the interest is first in the ENO-release potential of nanostructured materials, which can be considered to be material properties for a limited number of defined (standardized) material stress situations.

In case of synthesis we may not look for a material property in the first place, but for a process property. Here only release and exposure studies in real situations do make sense [24, 25]. However, in case of material treatment we may be able to reduce the effort quite a bit.

The first step in any ENP/ENO-release process from nanostructured materials is the separation of the ENPs or ENOs from the matrix material. This can be done by

- comminution (mech. energy input),
- evaporation and/or oxidation of matrix material (thermal energy input),
- dissolution of matrix material (chemical energy input),
- weathering, a combination of radiative, chemical and mechanical energy input in the environment.

The result may be ENOs in form of agglomerates, even if they were separated from the matrix material. If they are not separated from the matrix material they are not introduced into the environment in form of original ENPs or ENOs. The agglomerates can de-agglomerate in the environment, but this has to be described as an additional property of the agglomerates.

The greatest interest of industrial hygienists and toxicologists is in the release of synthesized ENPs/ENOs during production (synthesis), handling or application (treatment) of nanostructured materials and use, recycling and littering (treatment) of nanostructured materials into the environment. The case of ENP/ENO-release during synthesis is best described by an emission factor (EF), which is defined as number, surface area and/or mass (volume) released to the environment per unit of time. One can also consider relating the amount of ENP/ENO-release to the amount of nanostructured material produced. The ENP/ENO-release per unit of mass of produced or treated nanostructured material is best described by a release factor (RF) defined as number, surface area and/or mass (volume) per unit of mass of nanostructured material. This depends on properties of the nanostructured materials and the amount and kind of energy input during the different kinds of treatment of the material. The ENP/ENO emission and release factors can be considered to be important process and material properties, since with no release and emission there is no exposure and therefore no risk. That does not include any accidental release.

We propose to group the nanostructured materials according to their matrix phases:

- Powders: loosely packed nanoparticles or loosely packed aggregates of nanoparticles surrounded by gas
- Aerosol: independent nanoparticles in a gas
- Suspensions: nanoparticles in liquids
- Composites, coatings: nanoparticles in solids (Table 1).

The treatment processes or stress cases have been grouped according to the kind of energy input. The idea is to look for processes, and each of those represents a certain kind of energy input. This could reduce the huge number of different treatment processes. In the first necessary step, i.e. the separation of matrix and nanostructured material, nanomaterials are subjected to mechanical, thermal, chemical and/or environmental energy input. We call this different stress cases (Table 1). We try to sort the different kinds of treatments according to their energy input (stress cases). For the combination of the different material groups with energy input cases representative processes under relevant conditions have to be defined and later on,

Table 1 Grouping of nanomaterials and of treatment processes (stress cases) according to the kind of energy input.

Nanostructured materials	Processes	Energy input
Aerosols	Pouring	Mechanical
	Mixing	
	Sanding	
	Finishing	
Powders	Polishing	Thermal
Suspensions	Combustion	
	Evaporation	Chemical
Composites/coatings	Dissolution	
	Weathering	
	UV-radiation	

if possible, to be standardized. Examples of such combinations are:

- Mechanical stress – powders: dustiness test (e.g., rotating drum)
- Mechanical stress – liquids: nebulizer
- Mechanical stress – composites and coatings: abrasion
- Thermal stress – all material forms: Thermal-Gravimetric Analysis (TGA)
- Chemical stress – all material forms: Dissolution test
- Environmental stress – all material forms: Accelerated degradation test under simulated environmental conditions followed by other stress tests.

For each process a measure for release potential has to be found, which may be different for the different stress situations. In case of mechanical stress this could be the number of ENPs/ENOs released per unit of material mass determined with size distribution measurements and followed by size integration, derived from dustiness, abrasion or spray tests. The determination of these measures would allow at least a ranking of the materials for the given stress situation according to their release potential and maybe together with hazard information the determination of a maximum acceptable release factor.

The earlier defined release factors describe the amount of released material by number, surface area and volume (mass) concentrations. The possibilities to measure these quantities are still very limited.

After collecting the particles on a substrate, samples of powders, of aerosols and of suspensions can be physically and chemically characterized offline by electron microscopy (SEM or TEM, EDX). Number size distributions of ENPs/ENOs in aerosols can be determined quasi online with a Scanning Mobility Particle Sizer (SMPS) [26]. The existing techniques for number concentration

distributions in air are limited to spherical particles, otherwise the measured size parameter is only an equivalent diameter. First steps towards agglomerate and aggregate measurement in air and water have been taken and an instrument, Universal Nanoparticle Analyzer (UNPA) has been developed [27–29]. Figure 4 lists the different materials and the needed transfer processes to aerosols, which include aerosolizers (dustiness test) and nebulizers. The conditions have to be chosen such that single, but stable agglomerates/aggregates are measured.

In case of thermal stress applied in the TGA-analysis it could be the number of ENOs released into air during heating and/or the left over nanostructured material after finishing the heating procedure evaporating only the matrix material. One can also make use of the observable temperature difference in evaporation or oxidation between the ENOs and the matrix material. Further information about the material behaviour could be derived from additional analysis of the gaseous effluents of the process. The criteria should contribute to the categorization of release potential of the different materials within groups of different matrix materials. Further controllable processes have to be investigated for the other stress cases.

With respect to hazard description based on the properties of the released ENOs, several parameters play a role within the different material groups. First parameter, which is relevant for all material forms, is the chemical composition of the released ENOs, which may be close to the composition after synthesis. In many cases information should be available already from the synthesis process.

For powders the release potential increases with increasing dustiness (mass concentration of ENOs in air), the transportability and toxicity may increase with decreasing primary particle size [30], the stability of

the released material in the environment increases with increasing evaporation or dissociation temperature. The last two properties are also relevant for suspensions. In case of composites the release potential increases during heating, depending on the differences between temperatures for evaporation and dissociation of matrix and nanostructured materials. Advantages of measuring the release potential of ENOs under defined stress (energy) conditions are:

- Reduction of the dependency of the release potential on treatment conditions
- Improving the comparability of the release potentials of different materials. For the different stress cases the release potential can be considered to be a material property.
- The needed effort compared to exposure measurements is smaller and the measurement is more accurate (background elimination is more reliable). For any balancing calculation between the ENO release and human uptake or environmental concentration it is important to have sound input data as well as a good model for balancing.

The release data can serve as input data for tracing and balancing of the processes along the exposure pathway. The release potential is the starting point for exposure potential estimation, which is used together with hazard estimations in control banding models [31].

The release–exposure relationship

The released ENOs are distributed in the environmental compartments air, water and soil. Some of them are released directly into these media and stay there; others – after release into one medium – are transferred to another medium, especially from air to soil and water. The processes occurring after release along the exposure pathway can be described in form of a process chain (see Figure 2) consisting out of the transmission processes in the environmental compartments.

In order to control these process steps after the ENO-release determination, the relationship between ENO-release and exposure has to be considered [32]. Of greatest interest is the situation at the point of intake by the effect system. In case of air and inhalation by human beings this is the situation close to mouth and nose of human beings. For this purpose so-called personal samplers are needed [16]. It would be even better to measure at the point of effect (in the lung), but that is practically impossible.

Figure 4 Release potential analysis based on aerosol size distribution measurements for powders, aerosols and suspensions.

The transfer process has to be described quantitatively. Of major concern is the release into air. Only if the number concentrations are very high (e.g., $10^7/\text{cm}^3$ during leak in synthesis process), coagulation processes near the source reducing the number concentration and increasing the ENP-agglomerate size, take place. Especially large background particles may collect preferably the small ENPs. Liquid particles can coalesce and form larger spherical droplets. During transmission ENP or ENO can be further modified by evaporation or by picking up other molecules via chemical reaction, condensation, adsorption and photochemical transformation. The mechanisms are depending on the thermodynamic state and the composition of the air and the residence time until the ENP or ENO are finally picked up by the effect system. The ENP- or ENO-concentration measures may be reduced due to losses to surrounding walls. But other than that the major process taking place after release is dilution due to mixing with the air flow. These processes are described in more detail for workplace exposure scenarios by Schneider et al. [23]. The transport behaviour of the small ENPs is very similar to molecules. Therefore measured and calculated flow fields can describe the transport behaviour of ENPs in air.

The differences between an exposure situation and a dose situation will decrease with decreasing distance between the effect system and the location where the exposure control takes place. Studies have been performed to model the distribution and changes of ENPs close to the source as well as in rooms to detect critical situations for workers and to determine appropriate positions for control measurements [33, 34].

Exposure–dose relationship

ENPs or ENOs released into the air may affect the ecosystem as well as human beings. The field of nanotoxicology focuses on gaining a thorough understanding of the relationship between the toxicity of ENPs and ENOs depending on their dose levels in number, surface area and volume (mass) per organ, organ surface area, cell, etc. and particle properties such as: size, shape (spheres, agglomerates/aggregates, fibers, plates), material composition (total, at surface), their reactivity and bio-persistence, crystallinity, porosity, solubility, i.e., their physicochemical characteristics for human beings mainly.

General entrance ports for nanostructured materials into human beings are the skin, food ingestion and inhalation followed by deposition in the lung. Several skin studies have shown that the uptake via skin is negligible.

There are still some questions about injured skin [35, 36]. Food ingestion studies are rather seldom thus far [37].

The exposure material may also be changed during transport in the effect system. The transmission process between inhalation and deposition in different compartments of the lung has been investigated the most. The amount of ENOs contained in inhaled air is typically referred to as the exposure level for ENOs. The ENOs inhaled by human beings are deposited in the different compartments of the lung [38]. For inhalation exposure, as considered here, the dose refers to the amount of particles reaching the respiratory epithelium. Also deposition in the nose is possible. This location of deposition is especially important for possible translocation to the brain [39]. Once the dose is known one can infer the biological effect from toxicological dose – response measurements using either in-vivo (animal) or in-vitro (cell) models. In the lung airborne particles are mainly deposited onto the respiratory epithelium due to the combined effects of diffusion, sedimentation and impaction. For the nanosized ENPs diffusion is the main deposition mechanism, but for ENOs larger than about 200 nm sedimentation also starts to play a role. This may be the case for ENOs in form of agglomerates or aggregates. Since diffusion is an isotropic process, the deposition of nanoparticles is spatially quite uniform on the lung tissue for a given lung depth, but its regional deposition efficiency varies significantly depending on particle size, lung morphology and respiratory parameters. For typical breathing patterns, nanoparticles between about 10 and 50 nm deposit very efficiently (maximum of about 50% at 20 nm) in the alveolar region, which represents the potentially most vulnerable site of deposition (very thin tissue barrier towards the blood circulation) and accounts for more than 90% of the lung surface area [40]. Detailed discussions of the governing parameters of regional lung deposition have been presented elsewhere [38, 41, 42].

The determination of the dose can be replaced by exposure measures, if a direct exposure – effect study is performed, e.g., ROS determination [43]. The dose can be determined, if the exposure to ENO concentration at the entrance point and the breathing parameters breathing frequency, tidal volume, the sampling time as well as the deposition efficiency in different parts of the lung are known. Lung deposition models are available. Another possibility is to use instruments, which include the deposition efficiency in their size dependent response function (NSAM, AEROTRAC) [44–47].

With the known dose-effect relationship the effect can then be quantified. Besides for effect determination,

exposure concentration measurements are also of interest for estimating the relevant dose for studying the dose-effect relationship. For several reasons *in vivo* studies are not very popular. *In vitro* studies are preferred. For the investigation of lung toxicity, a strong preference was expressed for air – liquid interface (ALI) cell exposure systems (rather than submerged cell exposure systems) as they more closely resemble *in-vivo* conditions in the lungs and they allow for unaltered and dosimetrically accurate delivery of aerosolized nano-objects to the cells. Ultimately this will result in linking nano-object (physicochemical) properties with toxicological pathways and cellular uptake mechanisms, yield sufficient and appropriate information for risk assessment, and lead to societal acceptance of nanomaterials and products made using nanotechnology [48].

Tracing and balancing of ENO-release to dose

With known ENO-release as starting point the ENOs can be traced to the point where the dose has to be known. The final achievement should be a quantitative balance (number, surface area, mass) between ENO-release and dose. There are many different pathways for released ENOs in the environment as well as in the effect system with several endpoints. The inhalation pathway is described in Figure 5 in the form of a material flow diagram. The released material is dispersed in the environment contributing to the exposure situation of a subject. The inhaled material is partly deposited in the lung creating a certain dose related to the effect of interest. Other parts of the material flow are deposited in other parts of the lung or are exhaled.

A quantitative analysis starts with the ENO release-exposure relationship. The tracing can be performed for number, surface area or mass (volume) concentrations. Here it is described on the basis of number. Just like

Figure 5 Flow diagram for released material in case of inhalation.

the emission factor EF, the release factor RF (number of ENO divided by the treated material mass, see Figure 6) in combination with the treated material mass per time leads to number of released ENOs per unit of time. The change of this value along the exposure pathway has to be described by an aerosol change factor or – more complicated – by a function, which takes care of the number changes along the exposure pathway and the flow rate of the flow in which the ENOs are distributed. The resulting exposure concentration (number per volume) is the input data for the exposure – dose relationship. For dose calculations in case of inhalation this exposure concentration has to be multiplied with the breathing parameters (volume, frequency, time) and the lung deposition factor [49]. In the studies described in [18] such calculation was not considered up to now. Only qualitative risk judgements based on the presence of released ENOs has been made. Zhang et al. [50] computed the lung deposition based on the measured particles released in thermal cutting of polystyrene foams. The focus was not on ENOs, but the toxic flame retardant hexabromocyclododecane (HBCD). Zhang et al. estimated that the respiratory intake of HBCD by a worker thermally cutting polystyrene foam for 1 h is about 13 µg, equivalent to the total intake of HBCD by an adult during 1–3 months in the normal life. Dose calculations have also been performed in [51].

We started with ENO release from synthesis and from products described by emission factors or release factors. Finally we would like to get insight into and quantify the possible effects. The needed knowledge to perform such a balance is available. The quantitative balance can be based on ENO-number, surface area or mass (volume) depending on the considered effect. Quantitative effect estimations can be made.

Figure 6 Release factors for synthesis and treatment processes of nanostructured materials.

Future research activities for ENO-control in the environment

ENOs may be released from synthesis processes as well as from the produced nanostructured materials and their products during all life-cycle stages. ENO release or release potential can be considered as material properties for a limited number of material stress situations. For the determination of ENO release a limited number of processes with the potential to become reference or even standard processes have to be developed. Investigations have to be performed to define such processes and their operating parameters.

The effect research (toxicology) should influence this development to get data relevant for effect investigations. Most important is to define the needed concentration measures to get relevant data for dose estimation and further ENP-properties.

Models have to be developed and experimental experience has to be gained to describe the changes during transmission processes along the exposure pathway. Models are already available for cases which allow description by flow fields. Models have to be evaluated by exposure measurements.

For the needed size distribution measurements the existing techniques are not yet appropriate, because of a lack of instruments for agglomerates and aggregates.

Measurement techniques which relate the measured exposure parameter to dose or even an endpoint of the interesting effect may be a good solution avoiding – at least partly – the problems with the complicated exposure – dose relationship.

The work done with respect to ENO-release and release-exposure relationship should finally allow the balance of the released material to calculate the dose. With the dose-effect relationship determined by toxicologists the risk of nanostructured materials can be at least estimated.

Because of the fact that without a release of material there is no risk, health and safety considerations ask for independent activities especially by engineers towards development of sustainable nanostructured materials, avoiding release, if necessary avoiding the distribution of released materials at the release point.

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