Nano particles: Surface area as the metric in risk assessment

Eef Voogd
3052982
Eef.Voogd@tno.nl
Toxicology & Environmental Health, IRAS-UU

Supervisors:
Dr. D.H. Brouwer (TNO)
Dr. Ir. R. Houba (IRAS, NKAL)

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Summary

Nanoparticles are particles with a maximum size of 100 nm in at least one dimension. Nanoparticles are released by both natural and anthropogenic sources. Up until now only exposure limit values are present for bulk size substances. However, nanoparticles might have different health effects and or dose-response relationships. It is believed that small particles might have greater reactivity potential, e.g. inflammatory effects per unit mass. Since nanoparticles might have different health effects and or dose-response relationships compared with bulk size (micrometer size and above) particles, there is a need to evaluate the feasibility of deriving exposure limit values for these type of particles.

Inhalation exposure to nanoparticles can cause, among others, local inflammatory effects. These effects are partly dependent on location of deposition of the particles in the lungs, which dependents on particle size. Physical phenomena like agglomeration and aggregation of nanoparticles can change the particle size and thereby the location deposition. Agglomeration/aggregation can also include several different substances, increasing the complexity of the exposure, but also the complexity of the health effect.

Exposure limits are determined by combining exposure and hazard parameters. It is essential that both factors are available. Hazardous substances are identified and the effect is quantified in a dose-response relationship. Exposure assessment focuses on the possible concentrations present during exposure scenarios. The research of both exposure assessment and toxicology for nano particles is in the developing stage. First attempts are made to derive exposure limit values for nano sized materials.

Surface area is a metric which is believed to be important when nanoparticles are involved. However, more than one type of surface area metrics can be defined, which may vary in biological relevancy and complexity, dependent on several biological processes. Lung deposited surface area concentration and lung fluid available surface area seem to be the most relevant metrics. The concept of surface area is only believed to be relevant for insoluble particles, as soluble particles will dissolve in the lung fluid, making mass concentration the only relevant metric.

At this moment, there is no consensus about surface area as a dose metric or an exposure metric. In toxicology, surface area as a dose metric is not yet accepted to be the most relevant dose metric, leaving only few studies with surface area as the dose metric. Toxicological studies using this type of dose metric often do not use a surface area per volume metric, but only total or calculated surface area, or surface area, normalized for lung weight.

Surface area as the dose metric in exposure limits has potency as efforts are made to increase knowledge on dose-response relationships and exposure assessment methods based on surface area. At present however, data lacks in order to derive exposure limits with surface area as the dose metric.

Introduction

Nanoparticles are described as particles with a maximum size of <100 nm (0.1 μm) in at least one dimension (2). Nanoparticles can be released into the air by several natural processes e.g. volcano eruptions, but also anthropogenic activities, such as combustion contribute significantly to the release of nanoparticles. Nano sized particles can intentionally be produced in various forms (e.g. tubes, fibres, spheres etc.) and are incorporated in (consumer) products and in pharmacological products, because of their unique characteristics. Unintentional nano-sized particles are emitted by combustion engines, welding, etc. (1). Although the exposure to nano sized particles can result in dermal uptake and intake via oral consumption, this thesis focuses on inhalation exposure to airborne particles only.

Arbitrarily a size of 100nm (0.1 μm) is considered to be the upper boundary of nano sized particles. But physical processes are not likely to follow the same borders as researchers arbitrarily appoint them. Mechanisms like deposition show differences between bulk and nano sized particles (3), but researchers argue that differences between nano sized particles and bulk particles of the same substance are not to be seen above 30 nm (4). Due to the size, nano particles are reported to be able to penetrate tissues and cells more easily (3).

Strategies in toxicological testing as well as exposure assessment strategies are being developed at this moment. Most studies still appear to have an explorative character and focus on effect finding (5). As it is still uncertain which dose metric is the most appropriate to estimate the effect of nanoparticles to (human) health, it might be obvious that with respect to this issue there is some incongruity between exposure assessment and toxicology. Not only may dose metrics differ from exposure metrics, also exposure scenarios used by toxicologist seem to differ from relevant real-life exposure scenarios, questioning the extrapolation to of the toxicological end-points found (6) (7).

When substances are harmful, risk reduction measures might be needed. In this process, data is required on the hazard and the presence of the substance. Risk assessment is a multidisciplinary process which combines the scientific knowledge of several disciplines for a conclusion about the safe use of a substance. This thesis will focus on the relevancy of data available on surface area as a metric in toxicology as well as exposure assessment for the derivation of exposure limits concerning nano sized substances. Although surface area is thought to have potential to be of importance in (quantitative) risk assessment of nanoparticles (3, 5, 8-12), no consensus has been reached about this point (13-15). In risk assessment, exposure limits play an important role. Exposure limits are set up to protect humans and the environment against toxic effects from all kinds of exposures. For nano sized materials, science is in the early stage of processes to collect and collate information on toxic effects and exposure related to nano sized materials and no specific exposure limits are available yet. The aim of this thesis is to summarise knowledge on what parameters are of influence for inhalation exposure to nanoparticles and what information is needed for the derivation of an exposure limit, based on surface area and whether this information is currently present in exposure assessment and toxicology involving nanoparticles.

Research Questions

To gain understanding about the aspects involved in the development of an exposure limit, it is first examined what the requirements are for setting up an exposure limit. Second, aspects of inhalation exposure to airborne nanoparticles important for the development of an exposure limit based on surface area are discussed. Next, the information focused on surface area in nano toxicology and exposure assessment is evaluated in view of its comparability. Finally, the feasibility of an exposure limit, based on surface area as a metric is discussed.

Material and methods

A cross-reference method is chosen to obtain relevant literature for this thesis. Several high influence reviews are used as base publications (6, 9, 15) and references of these reviews are evaluated on usefulness in this thesis. To include most recent publications (2009 and later) in this thesis an additional literature search was done, using scientific secure literature databases (PubMed and Omega (respectively http://www.pubmed.gov and http://omega.library.uu.nl) as Omega includes also more technical journals).

Derivation of exposure limit

Up until now, only exposure limit values for bulk substances are present, although exposure limit values for specific nano sized materials are being developed (16). However, it is still uncertain what properties of nano particles affect absorption, transport and effect and if they differ from non-nano particles of the same substance. There is evidence that nano sized particles have different effects than bulk sized particles of the same material (1, 17), making separate nano specific exposure limit values necessary.

The concept of derivation of an exposure limit will not be different for nano sized substances than for bulk sized substances. As shown in Figure 1 hazard identification first aims to recognize health effects. This effect finding is primarily focused to show whether the substance has the potential of any adverse health effect. In addition to effect finding, physico-chemical parameters are examined to assess the biologic processes involved with the found health effect. These can include ADME (Absorption, Metabolism, Distribution and Excretion) (18). ADME includes all processes from entry to removal from the body. As for some of these processes size is an important parameter, ADME characteristics of nanoparticles are likely to differ from bulk size particles. Understanding of the relationship of the physico-chemical characteristics of nano sized particles and the behaviour in the body is still limited. The above mentioned steps are not yet quantifiable. Toxicological assays can be used to identify dose-response relationships which can be used to quantify related health effects. When the dose response relationship is determined with the use of animal testing, susceptibility extrapolation has to be performed. These uncertainty factors are designed to adjust for differences in susceptibility between animals and humans and additionally low susceptible humans and high susceptible humans. Through the acquired dose response relationship risk calculations can be made (see Figure 1). With these calculations the exposure limit can be determined. In the right side of the figure exposure limits is represented by risk characterization and risk management (respectively “Risk calculation” and “Regulations and exposure standards”).

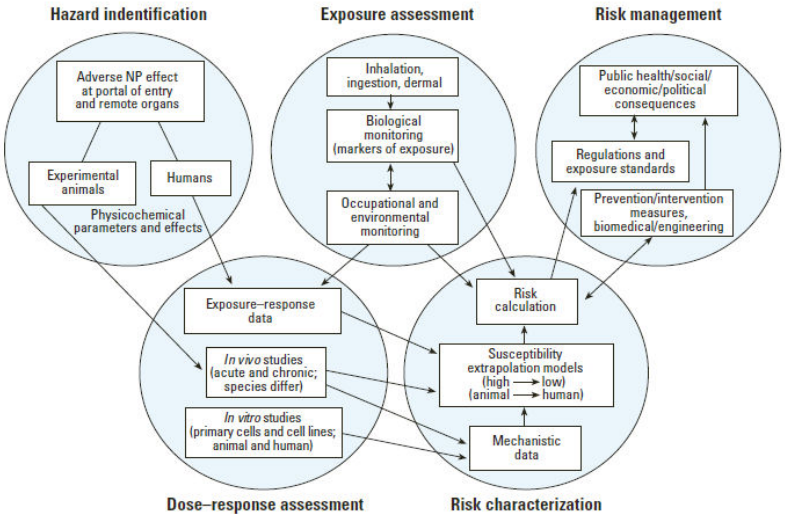

Figure 1 The process of risk assessment. This figure shows the multidisciplinarity of risk assessment (1).

Because of the lack of exposure limits, risk management is using standard precautionary principles, like ALARA (As Low As Reasonably Achievable) (19). Currently only a few attempts are made to derive exposure limits specifically for nano sized substances. One example is establishing an exposure limit for carbon nanotubes (16). First, physicochemical properties were determined to thoroughly characterize the substance. Furthermore, for several toxicological studies (with different end-points), dose response relations were determined. Several studies were done to evaluate the effects of the carbon nanotubes after inhalation. Acute and repeated dose studies were performed. In this example, the (conservative and time weighted average) OEL is derived from the NOAEL, regarding the retained fraction inside the lungs, based on physiologic properties of ventilation kinetics. The proposed OEL is based on mass concentration (16). In this case, the researchers did not find evidence to assume that surface area would be an appropriate dose metric.

Exposure to and surface area of airborne particles

The smaller particles become with the same total mass concentration, the larger the surface area and total number concentration will be. For diesel exhaust fumes, it is found that particles up to 50 nm contribute to only 0.1%-10% of the total mass concentration, but contribute to more than 90% of the total number concentration (20). Due to this property, it is believed that nanoparticles have a greater inflammatory potential per mass concentration than larger particles of the same substance (1, 9, 17).

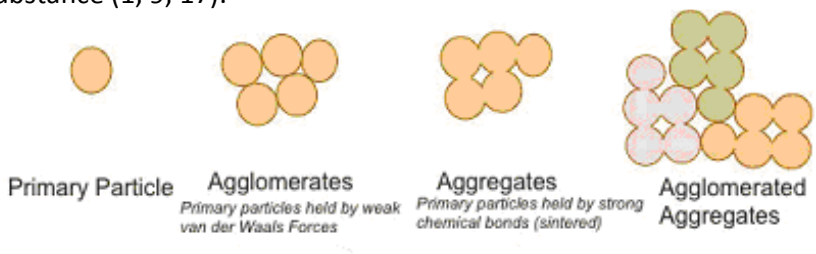

Figure 2 Agglomerates and aggregates in air and fluids (6)

Particle size, but also surface area itself is among others dependent on the fraction of aggregates and agglomerates. It is recognized that nanoparticles have the tendency to form aggregates and agglomerates, which in fact represent the major form of presence of nanoparticles (1, 8, 9, 15) (21). Most toxicological studies however focus solely on the effects of pristine nanoparticles. It can be questioned whether pristine particles have the same effect as bigger agglomerates and aggregates. As seen in Figure 2 agglomerates can be described as a collection of particles held together by Van der Waals forces and aggregates are the result of chemical bonding of particles. Agglomerated aggregates are the outcome of aggregated particles beginning to agglomerate. In the case of aggregates, due to the chemical bonding, fewer sites are available to interact with the target site. Agglomerates have weaker bonds, enabling the agglomerates to interact with the target site, although the particles are not pristine. Agglomeration and aggregation processes are continuous processes, as aggregates/agglomerates are formed and broken down constantly. Consequently, once deposited, particles can deagglomerate or deaggregate inside the lungs, making it very difficult to predict the fate of the particles in the body.

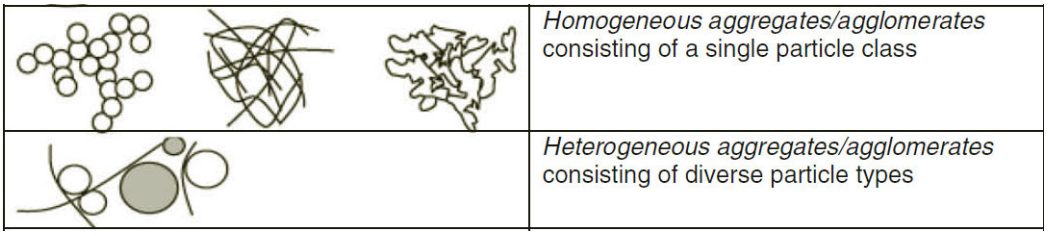

Figure 3 Homogeneous and heterogeneous agglomeration/aggregation, the latter is a combination of multiple substances (from Bouwmeester *et al.*)(18)

In addition, the activity of the particles is influenced by agglomeration and aggregation. In case of aggregation, the active surface area is reduced by chemical bonds between particles. The atoms involved in these chemical bonds are not available for further activity any longer, reducing the reactivity of the particles. In case of agglomerates the particles are linked in a loose manner, which keeps the particles surface area available for interactions.

Another feature in agglomeration and aggregation is heterogeneous agglomeration/aggregation as shown in Figure 3 (18). Heterogeneous aggregation and agglomeration can be combinations of different substances, but also different size particles. The combination of multiple nano sized substances delivers mixtures of substances and shapes at the target location. Here, again it is very difficult to predict what will happen to the particles over time.

Surface area reflects the surface of particles, but can be interpreted in several ways. Which surface area is the most relevant depends on which information is important for the user. The surface area concentration of particles present in ambient air (available for inhalation) does not necessarily reflect the surface area concentration of particles present at the target site (available for uptake). In case of inhalation exposure, this would be the alveoli. In ambient air, the surface area can be described by the geometric surface area, which includes all particles and the total surface of each particle. This parameter can mathematically be determined. The relevance of this parameter for health effects, however, is limited. It does not indicate reactivity of the particles, and it is the question whether the ambient air surface area concentration matches the concentration at the alveoli.

A more biological relevant metric might be the active surface area, which indicates the surface area, capable of interacting with atoms or ions and thus the potency to cause an effect. But the way active surface area concentration as such is commonly measured include the particles available for inhalation. Lung deposited active surface area is the active surface area of the fraction of particles which is deposited in the lungs (22). Modeling of deposition of particles shows uniform deposition in alveoli for particles under 100 nm, e.g. deposition of nanoparticles is independent on shape, size and direction of the alveolar walls (23). Figure 4 illustrates (with a differentiation of spatial orientation of the alveoli) the deposition fraction, dependent on particle size. An obvious difference can be observed between small and large particles. Particles smaller than 0.1 µm show no difference in deposition, indicating a uniform exposure in the entire lung with no ‘hot spots’ (23). Hot spots are locations were large fractions of particles are deposited (up to 100%).

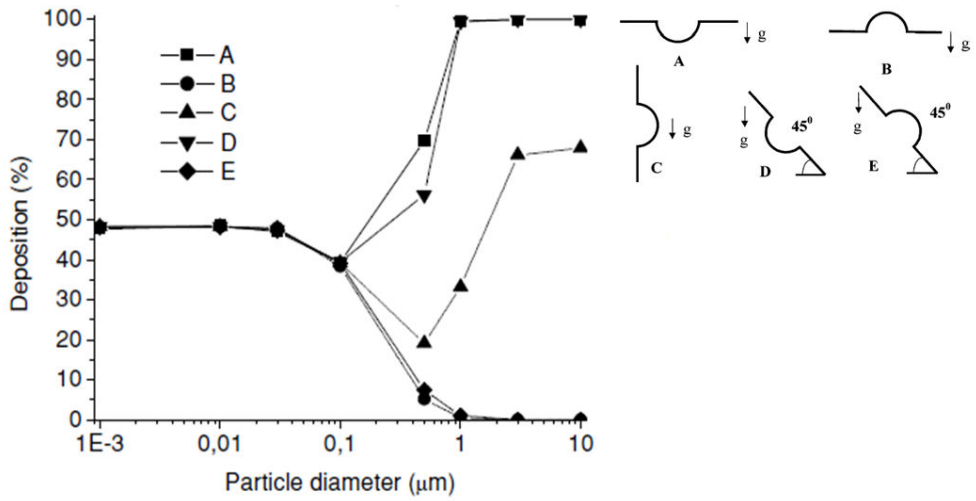

Figure 4 Outcome of the deposition model by Balásházy *et al.* Deposition is dependent on particle size and spatial orientation of the alveoli (23). 'g' is gravity direction.

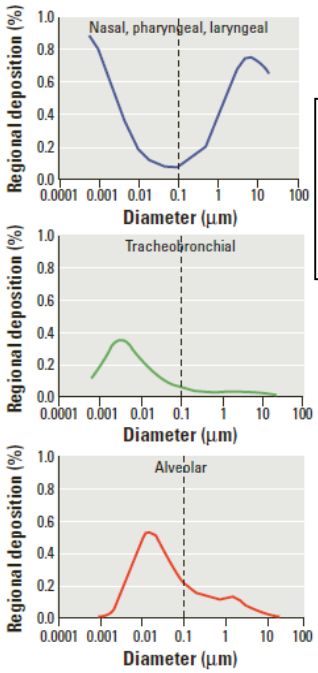

Figure 5 Deposition depending on particle size for three major components of the respiratory tract (1).

Figure 5 illustrates the different deposition rates per component of the respiratory tract. It shows that the particle size (distribution) heavily affects deposition efficiencies. Particle size indirectly can influence the health effect by effects on deposition and directly by the possibility of the particle to enter the nervous system, bloodstream or pass other cellular boundaries, due to the small size. For instance, there is evidence that some nano sized particles can intrude in both the bloodstream as the nervous system (1). Deposition in the nasal cavity is of interest when focused on effects in the nervous system, as olfactory bulbs are in the nasal cavity wall, directly accessible for particles that deposit there. Several factors including respiration rate, particle size, and air velocity are all of influence on location an amount of deposited fraction (1, 23). Deposition and thus effect can occur in the

higher regions (nasal- and larincheal cavities), but also further down the respiratory tract (tracheo-bronchial region and alveolar region).
Lung fluid available surface area is a biological concept which might be highly relevant for exposure to nanoparticles. This parameter only includes the surface area of the fraction of deposited particles that is in direct contact with the lung fluid (20). The concept of lung fluid available particles is based on the fact that it is impossible for (parts of) particles which do not contact lung fluid to react with the cellular surface. The respiratory tract is covered with lung fluid internally. The lung fluid is seen as the 'contact zone' (24) in which the particles have the possibility to come into contact with the target surface. A consequence of the presence of the lung fluid is that exposure to particles via inhalation is dependent on both the physicochemical properties in air as in liquid. Inhalation uptake actually occurs in fluid, (liquid phase) although exposure is normally measured in air (gas phase) (15). But the particles travel through air before reaching the target, and the particle size influences deposition, making both aerodynamic and hydrodynamic diameters important in the processes of exposure and ADME.
The usability of the biologically most relevant concept of Lung fluid available surface area is limited, as no unit for this concept is available. The lung deposited fraction of the active surface area concentration however might be a more appropriate metric. But this only is valid for insoluble particles. Soluble particles have the capability of dissolving in the lung fluid, which makes the potential dose in the lung fluid equal to the mass of all particles which are deposited on the location of interest (20). Giechaskiel and colleagues described the total exposure of Diesel exhaust consisting of two major fractions, a soluble and insoluble fraction. The soluble fraction has the potential to dissolve in the lung fluid. The particles are therefore, independent from size and shape, available for the target cells. The only appropriate metric for soluble particles is mass, as surface area is not of interest when the particles are dissolved. As this thesis focuses only on surface area as metric, the soluble fraction is of less interest at this moment.
As described earlier, the forming of agglomerates and aggregates (shown in Figure 2) can influence the particle size and thereby a variety of physical processes like deposition. It is found that in real world exposure situations particles hardly are present in their primary and pristine form, but have the tendency to coagulate into agglomerates and/or aggregates (1, 8, 9, 15) (21). Indirectly, this process influence deposition and location of deposition, as well as translocation when particles enter the cell and eventually the health effect may be altered. As exposure to solely pristine nanoparticles in normal (occupational) exposure conditions (for instance the use of more than one substance or various background aerosols) is not likely to occur as most exposure is a mixture of several substances and sizes, the results of such toxicological studies can be discussed. Moreover, it is argued that aggregates and agglomerates build up or brake down after entering the lungs, and the characteristics they had in air may change after deposition (15). This would suggest that at the site where toxic effects take place, the characteristics of the substance might not be the same as in free air.

Surface area as metric in exposure assessment and nanotoxicology

Although for this thesis is focused on surface area as a dose metric, there are several types of surface area described in literature, mostly because of differences in methods. Table 1 shows an overview of metrics found in peer-reviewed literature.

Table 1 Overview of available and used surface area metrics in nanotoxicology and exposure assessment to nanoparticles.
(N=nitrogen, Kr=krypton, GSA=Geometric Surface Area, d_p=diameter of the particle)

Used for	Metric	Properties	Method (example)	Unit (standard)
Characterization	BET surface area or specific surface area of a powder	Using gaseous absorption method, often N, but also Kr.	BET method (25)	m^2/g substance
Exposure assessment	Calculated surface area (often geometric surface area) of aerosols	Obtained via number concentration and particle size	GSA=d_p^2	m^2
Exposure assessment	Active or 'Fuchs' surface area (concentration) of aerosols	Surface area which is available for interaction with atoms or ions	(Epiphaniometer (26), using binding of α-radiant ^{221}Pb atoms) (diffusion charge monitor, using positive ions)	$\mu m^2/cm^3$
Exposure assessment	Lung deposited surface area (concentration) of aerosols	Active surface area of the deposited fraction (9)	Diffusion charger, including deposition and ion trap to manipulate the particle size distribution	$\mu m^2/cm^3$
Exposure assessment / Toxicology	Lung fluid available surface area	Active surface area of the deposited fraction, contacting lung fluid (20)	None available	None appropriate
Toxicology	BET based total administered surface area	Calculated surface area	Total mass x BET based specific surface area	m^2
Toxicology	BET based surface area	Calculated surface area	Mass concentration x BET based specific surface area	m^2/m^3
Toxicology	Surface area normalized for lung weight	Surface area per lung weight	Surface area normalized for lung weight	m^2/g lung

Table 1 shows an overview of currently available and used methods to determine the surface area concentration of aerosols and the surface area of a powder The methods vary from simple (surface area = (diameter of the average sized particle)^2) to complicated concepts as 'the surface area of the accessible fraction of deposited particles in the lung fluid'. In the latter biological availability of the exposure is accounted for, associated with the health effect.

The calculated surface area is a mathematical method, which includes other metrics to calculate the surface area. Simple methods like GSA=d_p^2 assuming an overall (median) particle diameter can be used (3), but methods including the aerosol size distribution or size bins are more appropriate (11). Although this calculated surface area is a relative fast method which can be used when particle size distributions are known, there are indications that active surface area concentrations measured by diffusion chargers can differ from calculated surface area concentrations (8, 10, 27). By calculation mostly overestimation is present, due to assuming that particles are spherical and do not lose any surface by contact between particles. Moreover, the biologic relevance of the exposure metric is of high importance. The relevance of total geometric surface area is limited, because it includes all particles and the entire surface present.

Active surface area concentration indicates the availability of locations at the particle where atoms or ions can interact. This parameter can be measured on several ways, for instance by attaching ions or atoms to the particles. There are several methods available to measure aerosol surface area concentrations, e.g. epiphaniometer (26) or diffusion charge monitors, like the LQ1-DC (Matter engineering). These measurement devices are based on the principle of the binding of a radioactive labelled atom or the addition of an ion. The total radioactive radiation or respectively the charge of the nano sized particle can then be determined as a measure for surface area concentration.

To take the deposition of the particles into account, the lung-deposited active surface area, defined as the active surface area of fraction of particles which theoretically would be deposited in the lungs, can be determined. An example of a device, capable of measuring such parameter is the NSAM (=Nanoparticle Surface Area Monitor) (3). This device is based on an ion diffusion charger and captures the deposited fraction. The active surface area of only this deposited fraction is then measured.

An even more detailed metric concept is published by Giechaskiel *et al.* as the lung fluid available active surface area (20). This metric is only a concept at this stage, but is a biological very relevant metric. Lung fluid available active surface area is an advanced metric concept with a large complexity, which can be worth the effort in developing when a more detailed metric concept for surface area is needed. More complex metrics can be necessary if in the future there is evidence that more simple metrics like mass, particle size or even surface area seem unable to characterize dose response relationships properly.

Up until now there is no method available which is able to identify the particles simultaneously with measuring the concentration in air. In contrast, size distribution can be determined at the same time as the concentration (5). Offline identification is a major part of the assessment of exposure to nano-sized particles. An observed increase in concentration in combination with an offline identification as a specific

nano- particle is occasionally evaluated as potential for exposure to a/the nano-sized substance. But this method includes the assumption that the composition of the sample taken for offline analysis is representative for the entire exposure period.
Exposure methods at present include stationary measurements, rather than personal exposure measurements. Main reasons for this are size and weight of the devices (up to 35 kg), or the use of radio active sources. Currently, no personal instruments are on the market that detect particle concentration and/or surface area concentration However, in view of an appropriate exposure assessment it is very important to assess breathing zone concentration for the individual worker.
In toxicological assays, mostly including *in vivo* rodent studies, administered dose is expressed as calculated total surface area (m^2) (17), calculated surface area (m^2/m^3) (13) and surface area normalized for lung weight (m^2/g lung) (1, 19) and even in BET specific surface area (m^2/g substance) (14). A varied pool of metrics is used in toxicological studies, even when only surface area is used. Difference in the used dose metric will most likely mean a difference in dose response relationships also, as the metrics have fundamental dissimilarities. For a correct dose response relationship a biological relevant dose metric should be used. This includes the physico-chemical parameters and processes like absorption and internal distribution of the particles.

What is needed for an exposure limit, based on surface area as the dose metric?

To establish an exposure limit based on surface area, knowledge on dose response relationships must be available. In addition, an exposure limit implicates the feasibility of a method to measure exposure in an appropriate metric. A method to measure concentrations where the target organ is exposed to also needs to be available. An exposure limit for nano sized (insoluble) particles based on surface area as a dose metric would indicate a concentration below which it is safe to use the substance. This exposure limit would be set up, based on toxicological studies, which have determined a surface area dose-effect relationship. In *in vivo* inhalation studies where surface area is not used as dose metric, administered doses are often in mass concentration or particles concentration. But with the material specific surface area, the mass concentration can be recalculated to a surface area per volume concentration.

A suggestion for the most correct dose metric can be the lung deposited active surface area. This metric includes deposition of particles and also the active surface of the particles, which is able to initiate effects.

To test whether during exposure scenarios the measured exposure is below the exposure limit, appropriate measurement devices should be used, capable of measuring relevant surface area concentration. In the case that a direct surface area concentration measurement is not available or not possible, the use of a method based on another exposure metric which is highly correlated to surface area concentration might be feasible. However, presently it is not shown that surface area concentration is highly correlated to any other exposure metric (8).

In view of compliance with an exposure limit a validated assessment method should be used which is reliable, accurate and easy to use. Ideally, active surface area concentration in the breathing zone (24, 28) should be measured to define personal exposure. A conversion factor can than be used to calculate the fraction of lung deposited, however in some cases this specification step is already included in the measurement device, (3, 9). Currently exposure assessment can already use methods to measure exposure to nanoparticles with surface area concentration as a metric (9).

Discussion

There is evidence that health effects caused by nanoparticles are different or have other intensities than health effects caused by bulk size particles of the same substance (1, 17). Therefore, it is necessary that exposure limits are derived for nanoparticles specifically as well. It is likely that the differences in effects between bulk size particles and nanoparticles are caused by a difference in ADME characteristics (18), due to the reduced size of nanoparticles.

Inhalation exposure to nanoparticles can be described in different metrics. Only surface area is discussed in this thesis, because it is believed that nanoparticles have a greater potential to cause harm per mass concentration than larger particles of the same substance, due to the larger surface area per mass (1, 9, 17). Consensus has to be reached on the appropriate metric for nanoparticles. As on the surface of particles reactivity takes place, the increased surface area per mass of nanoparticles is thought to be of influence on the effect. Surface area as metric is a broad definition. The relevancy of surface area is dependent on the biological parameters involved with the exposure.

Lung fluid available surface area (20) is a biological concept suggested as a first more complex metric which includes biological relevancy. The question is whether it is possible to describe inhalation exposure to nanoparticles in a metric as surface area or mass. It is possible that inhalation exposure to nanoparticles is too complex to be described by a metric as surface area. Because of the complexity of the concept of lung fluid available surface area, a more appropriate metric in exposure assessment should be lung deposited fraction of active surface area, which also includes several biological relevant parameters.

Also important for the exposure to particles is deposition in the lungs (when inhalation exposure is involved). Deposition is dependent on among others size, respiration rate and air velocity in the respiratory tract. When the deposition rate changes by for example a decrease in particle size, the effect can change as well, because of simply the lack of particles at a certain location. Nanoparticles have the tendency to form aggregates, agglomerates and even agglomerated aggregates, which makes exposure to pristine nanoparticles in ‘real life’ exposure scenarios unlikely (1, 8, 9, 15, 21). Size of the airborne particles can change by agglomeration and aggregation of particles and indirectly changing deposition rates (and location).

In aggregated particles, the reactive surface is decreased because of the chemical bonding between the particles. In effect active surface area of an agglomerate is higher than the active surface area of an aggregate when substance, particles size and number per aggregate/agglomerate is the same. The tendency to form aggregates rather than agglomerates will depend on the chemical structure of the pristine particles and will influence the reactivity of the substance at the target site.

Besides homogeneous aggregates and agglomerates it is also possible that heterogeneous aggregates or agglomerates are formed. Diverse particle types (size, substance) can form agglomerates or aggregates (18). Especially agglomerates of several substances can induce unexpected effects, as the toxicological effects of the single particles can be enhanced or reduced by the combination of both substances. In toxicology often only pristine particles are used only as exposure. In what extent this influences the outcome of the study must be evaluated case by case. However,

for effect finding the use of pristine particles can be a good indication of the health effects caused by the particles.
In exposure assessment it is possible to measure lung deposited active surface area concentrations (3, 9). However, offline identification of the substance in question remains necessary (5). It is important to realize that without identification of the substance it remain uncertain whether the source of interest is indeed the source of the measured substance. Disturbance by other (unexpected) sources can influence the measurements.
In toxicology dose can be expressed in several ways, but the choice of the dose metric can influence the result of the assay because of methodological differences. Relevancy of some metrics can be less when it is used out of proportion, for instance BET specific surface area (14) which can only be used as a substance property for further processing of data.
Surface area as the dose metric for exposure limits has potency. Both toxicology and exposure assessment are making efforts in the establishment of data on this subject. A major advantage is that currently, exposure assessment is already capable of measuring concentrations with surface area as a metric, although personal sampling remains difficult. However, offline identification is still necessary, increasing the complexity of exposure assessment. At the same time, toxicology is working on the identification of dose-response relationships. Further research is still needed to increase the knowledge on health effects associated with nanoparticles.
As the term ‘nano’ only applies to particle size all substances including nano sized particles should be evaluated if an exposure limit value is necessary. This includes great amounts of work, additional to the already existing bulk sized exposure limit derivations.
At present however, it is not yet possible to derive exposure limits for Nanoparticles specifically, as dose-response relationships are still to be identified. Scientific consensus on surface area as a dose metric is still not reached, but is very important for the acceptation of all exposure limits based on surface area. Although effort is being made in the collection of data on exposure to nanoparticles, the amount of data available at this moment is rather limited. Other methods of exposure assessment like exposure modelling are therefore not readily usable, making exposure measurements necessary to collect a considerable amount of data. To insure efforts are not made pointless, decisions need to be made on the choice of metric and thus also further evidence of dose-response relationships need to be found.
At this moment it is not yet possible to derive exposure limits based on surface area. But when agreement is found on the correct metric for the risk assessment of nanoparticles and more data is available on specific nanoparticles dose response relationships it will be feasible to derive exposure limits.

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