

Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology

■ G. Oberdörster

From the Department of Environmental Medicine, University of Rochester, 601 Elmwood Avenue, Medical Center, Rochester, NY, USA

Abstract. Oberdörster G (University of Rochester, Rochester, NY, USA). Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology (Review). *J Intern Med* 2010; **267**: 89–105.

Nanotechnology, nanomedicine and nanotoxicology are complementary disciplines aimed at the betterment of human life. However, concerns have been expressed about risks posed by engineered nanomaterials (ENMs), their potential to cause undesirable effects, contaminate the environment and adversely affect susceptible parts of the population. Information about toxicity and biokinetics of nano-enabled products combined with the knowledge of unintentional human and environmental exposure or intentional delivery for medicinal purposes will be necessary to determine real or perceived risks of nanomaterials. Yet, results of toxicological studies using only extraordinarily high experimental doses have to be interpreted with caution. Key concepts of nanotoxicology are addressed, including significance of dose, dose rate, and biokinetics, which are exemplified by specific findings of ENM toxicity, and by discussing the importance of detailed physico-chemical characterization of nanoparticles, specifically surface properties. Thorough evaluation of desirable versus adverse effects is required for safe applications of ENMs, and major challenges lie ahead to answer key questions of nanotoxicology. Foremost are assess-

ment of human and environmental exposure, and biokinetics or pharmacokinetics, identification of potential hazards, and biopersistence in cells and subcellular structures to perform meaningful risk assessments. A specific example of multiwalled carbon nanotubes (MWCNT) illustrates the difficulty of extrapolating toxicological results. MWCNT were found to cause asbestos-like effects of the mesothelium following intracavitary injection of high doses in rodents. The important question of whether inhaled MWCNT will translocate to sensitive mesothelial sites has not been answered yet. Even without being able to perform a quantitative risk assessment for ENMs, due to the lack of sufficient data on exposure, biokinetics and organ toxicity, until we know better it should be made mandatory to prevent exposure by appropriate precautionary measures/regulations and practicing best industrial hygiene to avoid future horror scenarios from environmental or occupational exposures. Similarly, safety assessment for medical applications as key contribution of nanotoxicology to nanomedicine relies heavily on nano-specific toxicological concepts and findings and on a multidisciplinary collaborative approach involving material scientists, physicians and toxicologists.

Keywords: biokinetics, dose, exposure, hazard, inhalation, risk.

Introduction

Nanotoxicology and nanomedicine

This article will discuss concepts of nanotoxicology that are relevant for safety assessment and develop-

ment of industrial and medical applications of nanotechnology. Ideally, toxicologists and physicians will work close together to assure the safety of nano-enabled medical procedures. NIH [1] characterized nanomedicine as an offshoot of nanotechnology which

refers to highly specific medical interventions at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle or nerve. The European Science Foundation (ESF) [2] had defined nanomedicine as the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving human health using molecular tools and molecular knowledge of the human body. ESF identified five main areas: (i) analytical tools, (ii) nanoimaging, (iii) nanomaterials and nanodevices, (iv) novel therapeutics and drug delivery systems and (v) clinical, regulatory and toxicological issues. Nanotoxicology was tentatively defined as the science of engineered nanodevices and nanostructures that deals with their effects in living organisms [3], emerging from the toxicology of airborne ultrafine particles and gaining increasing importance with the growth of nanotechnological applications. When adapting the existing definition of 'toxicology' of the Society of Toxicology [4] to nanomaterials one would describe nanotoxicology as the study of the adverse effects of engineered nanomaterials (ENMs) on living organisms and the ecosystems, including the prevention and amelioration of such adverse effects. Thus, the importance of nanotoxicology for nanomedicine is obvious, as is that of toxicology for medicine in general. This article focuses mainly on the respiratory tract as portal of entry for environmental or occupational airborne nanomaterials or delivery of nano-enabled drugs as medicinal aerosols, yet comparisons to parenteral (intravenous) administration are also addressed.

A need for nanotoxicology

The ever increasing manufacturing and use of ENMs [5], specifically in the form of nanoparticles (NPs) of spherical and fibre-like shapes, for diverse industrial and biomedical applications as well as in consumer products, has raised serious concerns about their safety for human health and the environment. Their potential to cause undesirable health effects, in particular adversely affect susceptible parts of the population, contaminate the environment resulting in unforeseen deleterious consequences is seen as alarming and calls for preventive actions continue to be

repeated (e.g., [6–8]). As well, concepts of nanotoxicology aimed at identifying potential hazards are also applicable for safety evaluation in nanomedicine for applications of nanotechnology-based therapeutics and diagnostics. The small sizes of engineered NPs (<100 nm in at least one dimension) are associated with highly desirable properties (e.g., mechanical, electrical, chemical) for specific uses; yet these same desirable properties are also likely to be associated with unwanted greater biological/toxicological reactivity. Although an increased toxicity of some NPs in comparison with chemically identical larger particles has been recognized almost two decades ago [9], it is only more recently that the urgency for devoting greater efforts to assess the safety of ENMs has been called for by a major scientific organization [7]. Indeed, more information about the potential toxicity of ENMs and about intentional and inadvertent human and environmental exposures will be necessary to determine real or perceived risks of ENMs.

Table 1 summarizes some of the differences between NPs (<100 nm) and larger particles (>500 nm) that impact their biological/toxicological effects when taken up via the respiratory tract. Because there is no biologically plausible reason for a strict borderline of 100 nm that separates NPs from larger particles, a 'grey' zone between 100 and 500 nm is left open in this table. For example, data show that even 240 nm polystyrene particles can act like NPs and translocate across the alveolo-capillary barrier in the lung when they are coated with phospholipids [10].

Differences in physico-chemical properties between NPs and larger particles determine their behaviour as aerosol, their biodistribution in the body following translocation from the portal of entry, their cellular interactions, and their effects, as indicated in Table 1. Whereas many of the effects at the organ of entry, the respiratory tract, can be the same for both particle sizes, secondary organs are affected differently. However, translocation to secondary organs of larger particles from the respiratory tract via uptake into pulmonary lymphatics and blood circulation has been observed under conditions of lung particle overload and an associated inflammatory state. What

Table 1 Nanoparticles versus larger particles: characteristics, biokinetics and effects (respiratory tract as portal of entry)

	Nanoparticles (<100 nm)	Larger particles (>500 nm)
General characteristics		
Ratio: number/surface area per volume	High	Low
Agglomeration in air, liquids	Likely (dependent on medium: surface)	Less likely
Deposition in respiratory tract	Diffusion: throughout resp. tract	Sedimentation, impaction, interception; throughout resp. tract
Protein/lipid adsorption <i>in vitro</i>	Yes; important for biokinetics	Less effective
Translocation to secondary target organs		
Clearance	Yes	Generally not (to liver under 'overload')
Mucociliary	Probably yes	Efficient
Alv. macrophages	Poor	Efficient
Epithelial cells	Yes	Mainly under overload
Lymphatic circulation	Yes	Under overload
Blood circulation	Yes	Under overload
Sensory neurons (uptake + transport)	Yes	No
Protein/lipid adsorption <i>in vivo</i>	Yes	Some
Cell entry/uptake		
	Yes (caveolae; clathrin; lip. rafts; diffusion)	Primarily phagocytic cells
Mitochondria	Yes	No
Nucleus	Yes (<40 nm)	No
Direct effects (caveat: chemistry and dose!)		
At secondary target organs	Yes	No
At portal of entry (resp. tract)	Yes	Yes
Inflammation	Yes	Yes
Oxidative stress	Yes	Yes
Activation of signalling pathways	Yes	Yes
Primary genotoxicity	Some	No
Carcinogenicity	Yes	Yes

distinguishes NPs from their larger counterparts are new concepts of dose, a major influence of physico-chemical properties and unusual biokinetic behaviour which will be discussed in the next sections, followed by some closing comments on NP safety assessment.

Dose concepts

Dose-metric

Dose-response relationships for particulate materials require special attention in nanotoxicological research in part because the use of mass as a conventional and traditionally used metric of dose may be insufficient. Exposure and air quality standards for particles are

conveniently based on and expressed by mass. For example, the 24 h US National Ambient Air Quality Standard for fine particulate matter (PM_{2.5} = airborne particles <2.5 µm) is 35 µg m⁻³. This relatively low concentration would represent a huge concentration for ambient ultrafine particles (~20 nm) with number concentrations far exceeding 1 × 10⁶ particles cm⁻³. Thus, whilst it is appropriate that ambient air pollution standard for PM or limit values for occupational exposures to particulate compounds are given as mass concentrations, it may not be meaningful for NPs or ultrafine particles. For airborne ultrafine or NPs the accuracy of measuring the generally to be expected low mass concentrations down to a level of a few µg m⁻³ can be rather poor, even a small amount of

larger particles can easily distort the measurement. For example, a mass concentration of $10 \mu\text{g m}^{-3}$ of 20 nm particles of unit density contains 2.4×10^6 particles cm^{-3} of air (Table 2), and contamination with just seven $5 \mu\text{m}$ additional particles (unit density) per cm^3 already doubles this concentration to $20 \mu\text{g m}^{-3}$. Thus, in this case, a dose-metric expressed as particle number would make more sense. In contrast, conclusions from toxicological studies are that neither NP mass nor NP number but NP surface area is the most appropriate dose-metric for comparing the effects of NPs of different sizes and different types [3, 11–13], although some investigators dispute this conclusion [14, 15]. Considering that it is the particle surface properties that interact with tissue and cellular components provides meaningful biological plausibility to NP surface area as dose-metric. However, at the moment surface area may just be a surrogate for a better metric, i.e., biologically available surface area or surface reactivity. Additional studies are required to define this metric more precisely. In this context, the role of biosolubility also needs to be considered.

Dosimetry

Toxicological studies most often use mass doses of NPs that the investigators describe as being low; they are then excited when they observe significant effects without realizing that the applied doses have no relevance to real-world conditions. This is of particular concern when *in vitro* studies are performed with cells of secondary target organs and concentrations are used that exceed doses that are deposited under realis-

tic exposure conditions even in the respiratory tract as the primary organ of exposure. A case in point is a study on oxidative stress induction of microglia by doses of well characterized nano TiO_2 at $25 \mu\text{g mL}^{-1}$ and higher [16]. Whilst this result represents an interesting hypothesis forming finding, an extrapolation to real-world exposure scenarios is not possible given that the dose administered to the microglia cells was already greater than will be received by alveolar macrophages in the lung following 24 h of inhalation at a high concentration of 1 mg m^{-3} [17]. Considering furthermore that only 1% or 2% of the NPs deposited in the lung may translocate to the blood circulation, and of that none, or <1%, may translocate to the CNS [18], the relevancy of results from unrealistic high *in vitro* doses for real-world *in vivo* conditions should be seriously questioned.

A similar argument can be made for *in vivo* studies where high NP doses are used, particularly if these doses are administered as a bolus, for example, instillation- or aspiration-type delivery to the respiratory tract. The dose rate in such cases is extremely high, i.e., the dose is delivered within about a second in contrast to realistic inhalation exposure which may take hours, days, or even weeks to deliver the same dose. Nevertheless, results from such studies can be very valuable as proof of principle studies, yet results should subsequently be confirmed by realistic inhalation studies. Still, highly excessive bolus-type doses should be avoided, results are likely to be misinterpreted by the popular nonscientific press. An example is an intranasal instillation study with a total dose of 7.5 mg of nano TiO_2 instilled intranasally into mice (equivalent to instilling $\sim 17.5 \text{ g}$ into the nose of humans!) that resulted in significant oxidative stress and inflammation in the brain [19]. This study was subsequently featured as a scientific highlight (NPs damage brain cells) and misrepresented as an inhalation study [20].

Incubating epithelial cells of the respiratory tract (primary or cell-lines) *in vitro* with high doses of NPs is often justified with the presence of ‘hot spots’ of deposition of inhaled particles at bifurcation sites of the conducting airways [21]. Indeed, such ‘hot spots’

Table 2 Particle mass, number, and surface area: comparing different particle sizes of airborne concentration of 10 pg cm^{-3} (unit density particles)

Particle diameter (nm)	Particle number (N cm^{-3})	Particle surface area ($\mu\text{m}^2 \text{ cm}^{-3}$)
5	153 000 000	12 000
20	2 400 000	3016
250	1200	240
5000	0.15	12

for larger particles in the tracheobronchial region can increase local deposited doses several hundredfold (Fig. 1); however, for NPs enhanced deposition at these bifurcational ‘hot spots’ ranges from about 5-fold (for an area of $3 \text{ mm} \times 3 \text{ mm} = 9 \text{ cm}^2$) to about 60-fold maximally (for an area of $0.1 \text{ mm} \times 0.1 \text{ mm} = 0.01 \text{ cm}^2$) [22]. These limits of deposition enhancement at these ‘hotspots’ should be considered when several orders of magnitude higher *in vitro* doses are used and justified by the presence of ‘hotspots’ *in vivo*. For a small 96 well plate *in vitro* system with a total area of $\sim 0.4 \text{ cm}^2$ the enhancement would be only about 2-fold. Very high doses for studies with alveolar epithelial cells are even less justified, because for the alveolar region, inhaled NPs do not experience any enhanced deposition as diffusional deposition of the NPs at the low flow rate in the alveolar region is independent of spatial orientation of alveoli (Fig. 2) whereas larger particles experience uneven deposition in the alveoli due to gravitational settling and thereby may form ‘hot spots’ [23].

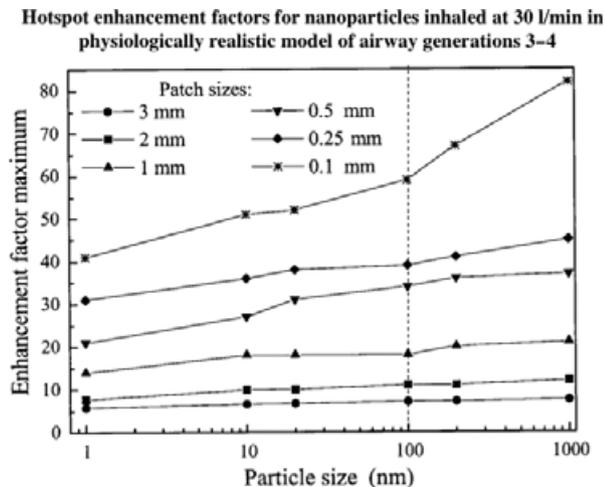


Fig. 1 Increased deposition of inhaled particles at carinal ridges of the generations 3–4 of the human tracheobronchial airways. Depending on the size of small square ‘hotspots’ with side-lengths ranging from 0.1 to 3 mm, the enhanced deposition for 1–100 nm particles amounts to about 5-fold (smallest patch size for 1 nm NPs) to about 60-fold (largest patch size for 100 nm NPs) of the average deposition in these airways. In contrast, enhancement factors for larger particles can reach values close to 500-fold (Reproduced from Baláshazy *et al.* 2003 [22] with permission, original figure 3).

Physico-chemical properties

Table 3 lists some physico-chemical properties of NPs that impact on their biological/toxicological activity. Only some of these are discussed here. Agglomeration and aggregation (Fig. 3) both in liquids or in air determine the actual particle size for NP-biointeractions or the deposition-site and -efficiency in the respiratory tract upon inhalation. Their measurement is essential for the interpretation of toxicological results because size is a key factor with respect to translocation across cell barriers or along neuronal pathways (Table 1). Likewise, mechanisms of cell entry seem to range from diffusion across cell membranes for the smallest NPs [24] via caveola, clathrin-coated pit or lip raft mediated uptake [25]. Entry via the nuclear pore complex (NPC) into the cell nucleus is restricted to the functional NPC diameter of 39 nm [26].

A study by Jiang *et al.* [27] showed that crystal structure can have a significant impact on NP reactivity, using as model TiO_2 NPs. Different sizes of anatase (3–200 nm), rutile, and amorphous TiO_2 as well as TiO_2 of different ratios of mixed crystallinity were compared with regard to their capacity to induce ROS in a cell-free phosphate buffer assay. Results showed a ranking from highest to lowest activity as amorphous > anatase > anatase/rutile mixture > rutile.

Table 3 Physico-chemical NP properties of relevance for toxicology

Size (airborne, hydrodynamic)	} Properties can change – with method of production preparation process storage. – when introduced into physiol, media, organism.
Size distribution	
Shape	
Agglomeration/aggregation	
Surface properties	
Area (porosity)	
Charge	
Reactivity	
Chemistry (coatings, contaminants)	
Defects	
Solubility (lipid, aqueous, <i>in vivo</i>)	
Crystallinity	

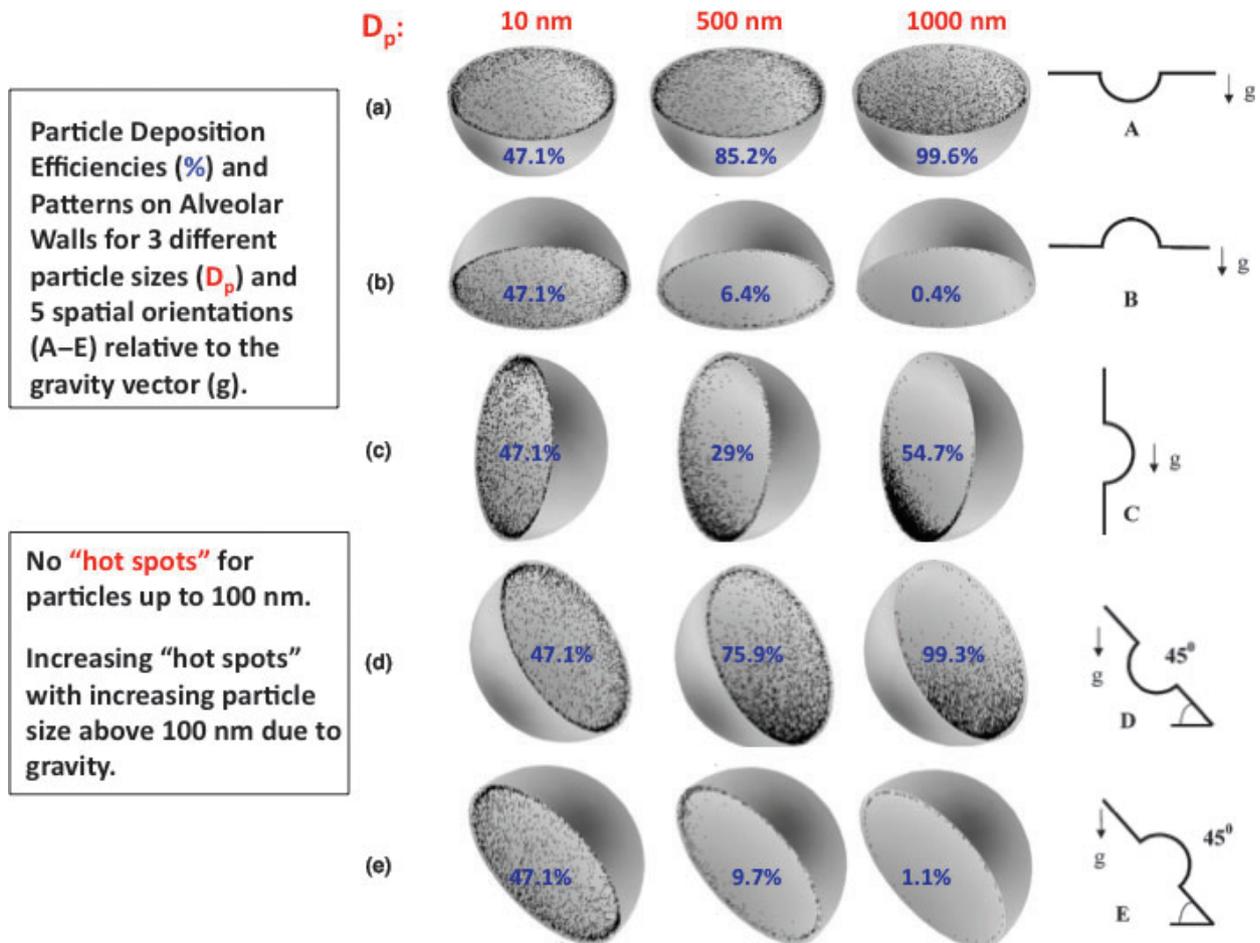


Fig. 2 Deposition efficiencies (%) and deposition patterns on alveolar walls for inhaled 10, 100 and 1000 nm particles (D_p) and five different spatial orientations (a–e) of alveoli relative to the gravity vector (g). Due to efficient diffusional deposition, there are no ‘hot spots’ for particles up to about 100 nm, whereas there are increasing ‘hot spots’ with increasing particle sizes above 100 nm (Reproduced from Baláshazy *et al.* (2008) [23] adapted with permission from Informa Healthcare [Inhalation Toxicology]).

Furthermore, the ROS-inducing capacity normalized to NP surface area showed a surprising dependence on size: anatase NPs between 3 and about 10 nm had about the same ROS-inducing capacity per unit surface area, followed by a steep increase between 10 and 30 nm and then a constant but greater ROS induction per unit surface area between 50 and 200 nm (Fig. 4). The authors suggested that this finding is due to the number of defects per unit surface area which is greater for the larger as compared to the smaller anatase NPs. A subsequent study by Han *et al.* [28] showed in an *in vivo* assay in rats that the pulmonary inflammation inducing capacity of these anatase NPs showed a similar pattern when inflamma-

tion was expressed per unit of anatase surface area. The concept of using a surface area based response-metric for prediction of *in vivo* NP hazard by a simple *in vitro* assay will be discussed later in this paper.

NP biokinetics

Portal of entry and translocation pathways

This article is restricted to the respiratory tract as portal of entry for airborne NPs. Knowledge about the biokinetics of NPs is an important component of dosimetry, it provides information about internal

exposure and doses for secondary organs including accumulation, retention and clearance rates. Information about NP biokinetics is in particular also necessary for the design of *in vitro* studies to identify sensitive target organs and to justify selection of NP concentrations in cell culture media when studying effects and mechanisms in cells of a specific target organ. For example, when exposing cultures of neuronal or cardiac cells to NPs. The tendency of NPs to translocate from the primary site of deposition to secondary organs has been well established, although there appears to be a misconception that such translocation is rapid and involves large amounts of all NPs deposited at the portal of entry. On the contrary, inhalation studies in rats using 15 and 80 nm iridium NPs have shown that only ~1–2% of NPs translocated from the lung to extrapulmonary organs [29–31]. As discussed by Kreyling *et al.* [32] it is possible though that greater fractions of smaller NPs (1–4 nm) translocate to secondary target sites, based on information from earlier studies with actinide oxide NPs [33, 34].

Another misconception is the assumption that the biodistribution of intravenously administered NPs is the same as that of NPs entering the blood circulation via translocation from the respiratory tract or other portals of entry (GI-tract, skin). Rinderknecht *et al.* [35] administered to rats either by intratracheal microspray or intravenous injection different sizes of gold NPs (5; 50; 200 nm) with different surface modifications [citrate; albumin; polyethyleneglycol (PEG)]. They showed that biodistribution to extrapulmonary organs is modified by all three factors: particle size, surface modification and the portal of entry. For example, 5 nm albumin-coated gold NPs when intravenously injected were retained preferentially in the liver, whereas after intratracheal administration of the same NPs more of them were retained in the bone marrow rather than the liver. Also, as discussed above, a minimal translocation from the lung to the blood of less than 1% over a 24 h period was confirmed; blood concentrations were only between 3 and 20 ng mL⁻¹ despite the administration of a high dose of 50 µg to the lung, which again underlines the need to consider realistic low doses for the design of *in vitro* studies with cells from extrapulmonary target organs.

Fig. 5 illustrates fundamental differences in NP transfer routes to blood and body organs when NPs are administered either into the respiratory tract or intravenously. Whereas dose and dose rates associated with NP administration by direct intravenous injection are obviously very high, both dose and entry rate of NPs from lung deposits into the blood compartment (arterial) are low. Thus, NPs delivered to the respiratory tract enter the blood compartment not only at different dose levels and different dose rates than directly i.v. injected NPs, but also into blood of different oxygenation states (arterial versus venous); furthermore, NPs will adsorb proteins and/or lipids at the site of entry, i.e., constituents of the epithelial lining fluid (ELF) in the respiratory tract and different plasma proteins in the blood compartment. Such secondary coating of NPs occurring at several stages of entry into and biodistribution to secondary body compartments will affect their kinetics. This can in part explain the different organ distribution and retention patterns observed in the aforementioned studies by Rinderknecht *et al.* [35].

Protein corona and nanoparticles

The underlying concept of differential adsorption for the biodistribution of NPs has been suggested by Müller and Keck [36]. It states that physico-chemical properties of NPs interacting with constituents at the site of entry into the body determine protein/lipid adsorption and desorption patterns on and off NPs – which are dynamic processes – and this in turn determines the biodispersion of NPs across barriers and into target tissues and cells. Indeed, recent studies have identified adsorption of different serum/plasma proteins on NPs, their affinity and exchange rates so that their biological identity through the formation of a protein ‘corona’ could be defined [37–42]. Determining the affinity of proteins and lipids for NPs will be a useful addition for their routine physico-chemical characterization. One of the higher affinity and slower exchanging plasma proteins found associated with NPs by Cedervall *et al.* [38] is apolipoprotein-E (Apo-E), to be discussed below.

Of considerable concern were findings from biokinetic studies that inhaled NPs can translocate via olfactory

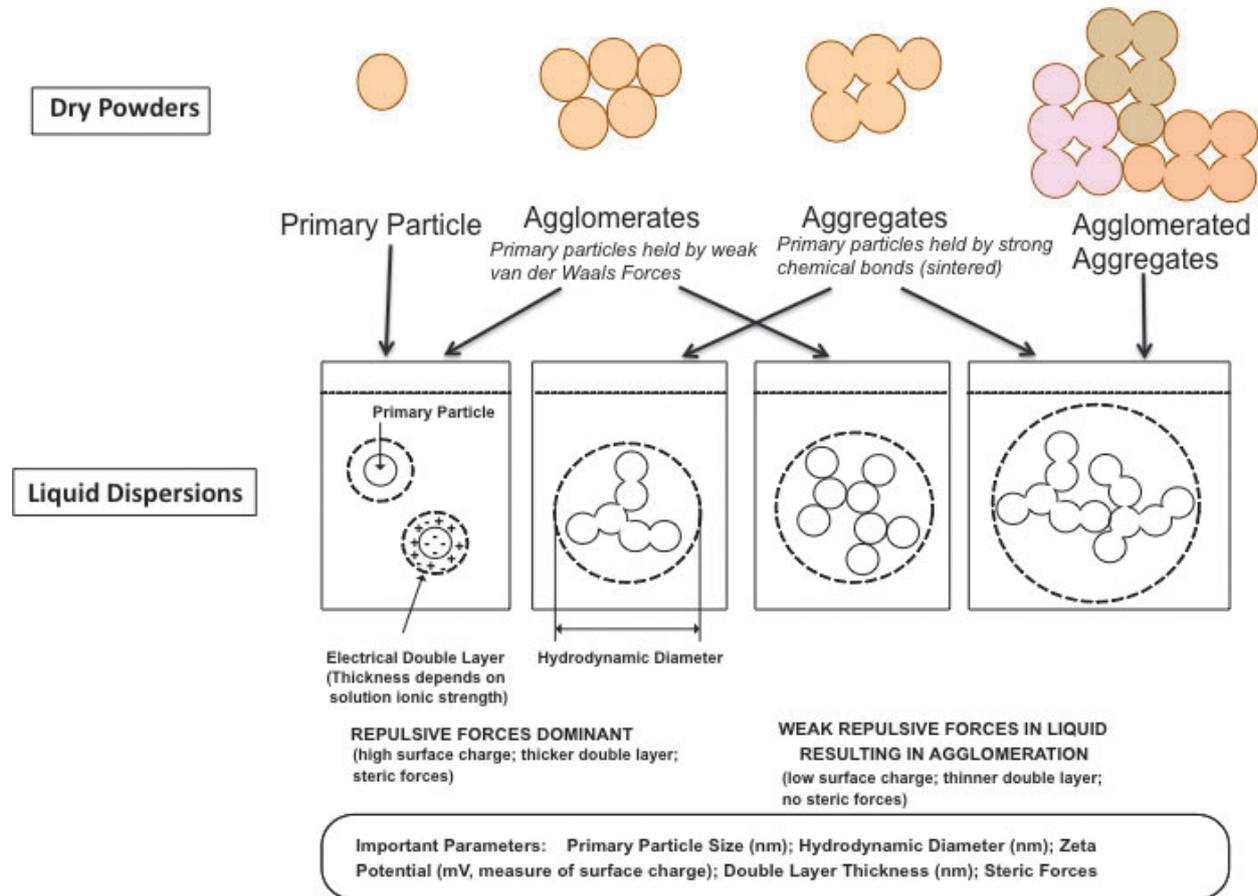


Fig. 3 In air: thermodynamic and aerodynamic (larger agglomerates and aggregates) and in liquid: hydrodynamic sizes of primary NPs, agglomerates (held together by weak van der Waals forces) and aggregates (held together by strong chemical bonds or sintered) and of agglomerated aggregates (modified from Jiang *et al.* [71]).

neurons from the nose to the CNS [43], and, depending on their chemistry, can induce significant inflammatory CNS effects [44]. Hypotheses are proposed that repeat exposures to inhaled NPs, including ambient ultrafine particles, may accelerate onset of neurodegenerative diseases due to the presence of translocated NPs [45]. Translocation pathways from the respiratory tract to the CNS are summarized in Fig. 6, and include neuronal, perineural, lymphatic and blood circulation routes. Each of these routes is likely to involve coating of NPs with proteins that are probably different and thereby affect their kinetics. No data are available that would allow more definite conclusions.

Apolipoprotein-E, mentioned above as one of the higher affinity proteins for NPs, was identified as medi-

ating delivery of drugs bound to NPs from the blood circulation to the CNS [46]. It was suggested that coating of NPs with Apo-E interact with the LDL receptor on brain capillary endothelium and thereby facilitates NP endocytosis. We performed intravenous injection studies with Apo-E coated 20 nm gold NPs in rats to test this hypothesis. Preliminary results are consistent with a greater CNS translocation in rats of i.v. administered Apo-E coated gold NPs, yet only very small amounts of <0.01% of the injected dose were translocated, and further confirmatory studies are needed.

Effects and biokinetics of nanotubes

The role and importance of NP translocation and quantifying biokinetics is highlighted by recent findings

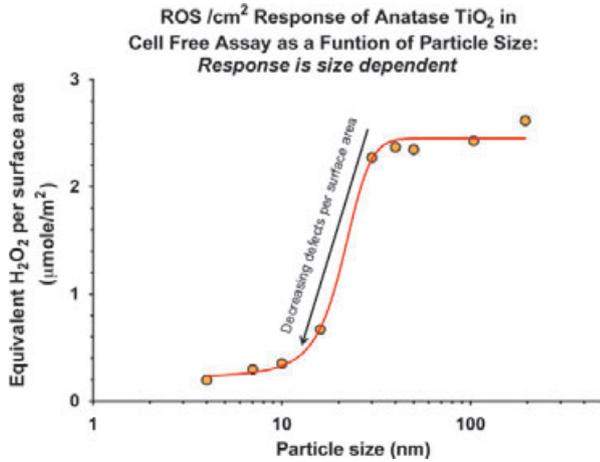


Fig. 4 Crystal structure of NPs influence their surface properties and affect their biological and chemical reactivity. For TiO₂ NPs, ranking of the ROS-inducing capacity in a cell-free phosphate buffer assay is from amorphous (greatest) to anatase to mixed rutile/anatase to rutile (lowest). In addition, size dependent response becomes apparent when activity is expressed per unit surface area [23]. The shape of the ROS per cm² activity (expressed as H₂O₂ equivalents per cm²) of different sizes of anatase NPs in the cell-free assay is due to decreasing defects per unit surface area which is likely to exist also with other NPs (modified from Jiang *et al.* [27]).

of the toxicity of fibrous shaped NPs, in particular carbon nanotubes (CNTs). Concerns had been raised earlier that NPs of fibrous shape and dimensions and of high biopersistence, resembling asbestos, may induce adverse effects similar to those known to be caused by asbestos exposure, *i.e.*, lung fibrosis, lung carcinoma, and mesothelioma [47]. Three recent studies in mice and rats indeed found that injections of multiwalled carbon nanotubes (MWCNTs) into mesothelial cell lined body cavities induced asbestos-like pathology. The first study showed that intraperitoneal injection of 3 mg per mouse of MWCNTs and of the positive control crocidolite asbestos in p53+/- mice induced mesothelioma and increased mortality, in contrast to no effects in a negative control group injected with fullerenes [48]. This study was criticized because of the use of extremely high doses and poor material characterization [49]. The second study compared the effects of long MWCNTs and long amosite asbestos with those of short MWCNTs and short amosite asbestos after intraperitoneal injection of 50 μg in mice [50]. Seven days after dosing peritoneal inflammation and granuloma formation was seen only with the long but not

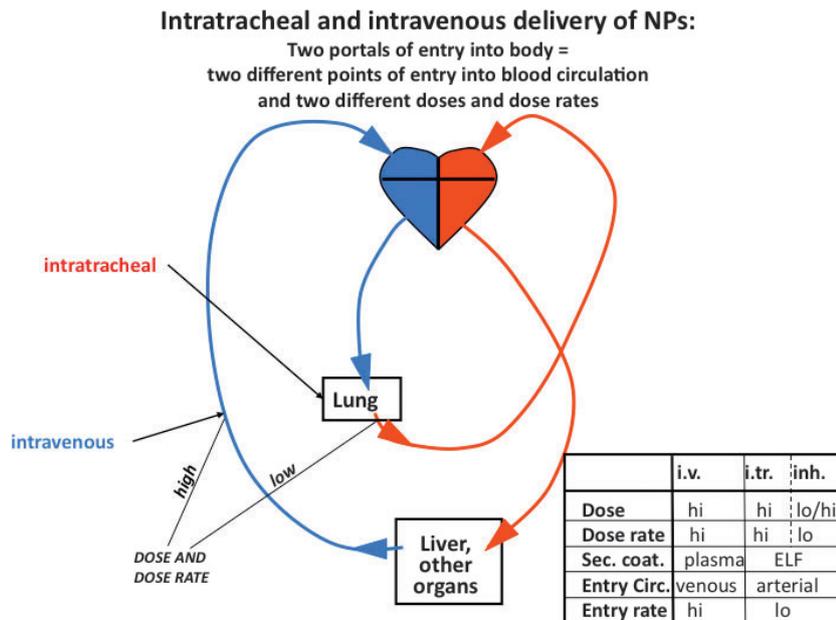


Fig. 5 The biokinetics of NPs in the body differs depending on the portal of entry. The same NPs administered to the lung (inhalation or intratracheal instillation) or intravenously interact with different biological media and will receive different secondary coatings. Their entry into the blood circulation is at different dose and dose rates and into blood of different oxygenation states, all of which affect NP biodistribution to secondary target organs.

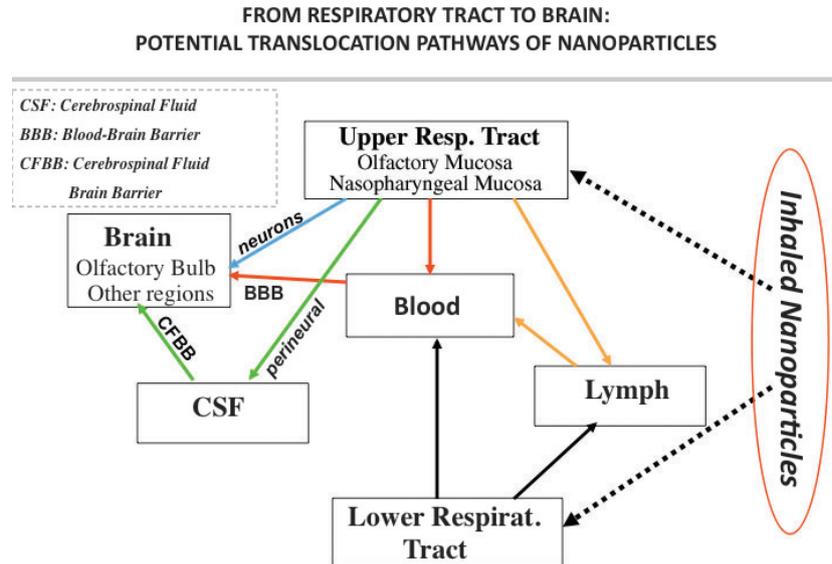


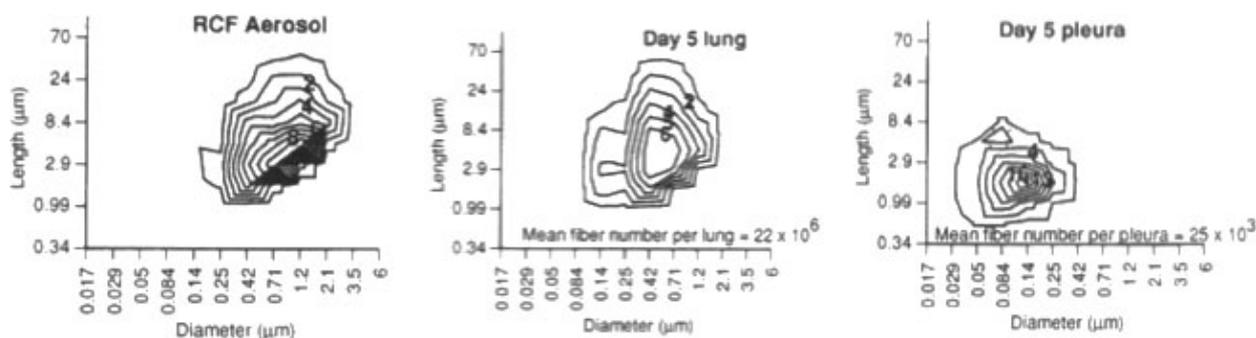
Fig. 6 Translocation of inhaled NPs to the brain involves different routes from deposition-sites in both the lower and upper respiratory tract. Routes include lymphatic, blood, neuronal and perineural pathways. Amounts translocated are low to extremely low because of the tight barrier function of BBB and CFBB. The physiologically low access of NPs to the CNS by circulatory routes prevents an easy targeting of this vital organ by potentially harmful NPs, provided that barriers remain intact; modifying NP surface properties can increase NP translocation to CNS structures for therapeutic purposes. However, physiological neuronal translocation pathways in the upper respiratory tract could be of concern with respect to induction of CNS toxicity.

the short length MWCNTs and amosite, confirming for MWCNTs a well established asbestos-like pathogenicity that is associated with long fibres but not with shorter ones [51]. The third study reported the induction of peritoneal mesothelioma in rats 37–40 weeks after intrascrotal injection of 1 mg MWCNTs per kg bodyweight. The positive crocidolite asbestos (2 mg kg^{-1} intrascrotally) did not induce a tumour response in this study [52]. Collectively, the results of these studies identify a serious potential carcinogenic risk of MWCNTs if they reach the pleural cavity after inhalation exposure. This is disturbing, based also on our knowledge about the decades long latency period for the manifestation of pleural mesothelioma that is associated with exposure to asbestos [53].

However, although the studies described above are important proof of principle studies which clearly point to a carcinogenic hazard of MWCNTs, there are a number of open questions that need to be resolved before a final assessment can be made. Key is to determine *in vivo* the kinetics and effects of inhaled

MWCNTs: do they translocate from the deposition-site in the lung to the pleura? If so, what is the efficiency of such translocation in terms of the dose retained in pleural tissues? What are the dimensions (in particular length) of the translocated MWCNTs? Do they cause inflammatory, granulomatous, carcinogenic effects, and what are the mechanisms? For example, the impact of impurities (transition metals) can be a significant source of oxidative stress induction, as has been shown for iron present in crocidolite [54].

Answering these questions poses many methodological challenges. Regarding biokinetics, only one study could be found that quantified lung to pleura translocation of fibrous particles. Gelzleichter *et al.* [55] exposed rats to an aerosol of refractory ceramic fibres for 5 days and compared fibre length distribution of the inhaled aerosol to those of the deposited fraction in the lung and of the translocated fraction in the pleura. They found that $\sim 0.11\%$ of the alveolar fibre burden reached the pleura at 5 days of exposure, and that these fibres were no longer than $\sim 8 \mu\text{m}$, whereas



Size distribution of Refractory Ceramic Fibers isolated from aerosol and after 5 days of inhalation exposure in the lung and pleura of rats.

Fig. 7 Size distribution of refractory ceramic fibres isolated from aerosol and lung and pleura of rats after 5 days of inhalation exposure. Only short fibres (<10 µm) translocated from pulmonary deposits of all fibres (up to ~50 µm long) to the pleura, suggesting that short fibres – if not cleared from the pleural space – also contribute to pleural pathology (Reproduced from Gelzleichter *et al.*, 1996 [55] with permission; Oxford University Press; original Fig. 2).

in both the aerosol and in the lung fibres up to 50 µm long were present (Fig. 7). This result may indicate that we need to consider also short fibres as potential contributors to an inflammatory response in pleural tissues. This would be consistent with findings of mainly short asbestos fibres in autopsied human lung and pleural tissues by Dodson *et al.* [56] who concluded from their analyses that fibres of all lengths contribute to pathological responses. With respect to CNTs, this conclusion is at variance with the findings of Poland *et al.* [50] and Muller *et al.* [57] who did not observe inflammation, granuloma formation or mesothelioma in rodents following i.p. injection of short (<5 or 1 µm) MWCNTs. One explanation could be that open lymphatic channels in the parietal pleura and the diaphragm serve as efficient clearance pathways for short fibrous particles and that their occlusion in pleural disease states prevents such efficient clearance and results in prolonged short fibre retention and interactions with mesothelial cells [58–60]. Even nonfibrous particles are translocated from the lung to the pleura, as demonstrated by Davis *et al.* [61] with TiO₂ and quartz inhaled together with asbestos by rats. They may contribute to granuloma formation if pleural clearance pathways are blocked.

At the end of this section on biokinetics/translocation a few comments are in order about dosimetric implications when designing studies of and interpreting

results from intracavitary injection studies. As cautioned earlier, high doses and dose rates have to be viewed and compared *vis-a-vis* realistic *in vivo* dosing. Considering a study in which 3 mg MWCNTs are injected intraperitoneally in rats with a peritoneal surface of ~600 cm², that would be equivalent to 22 mg for the alveolar surface (~4400 cm² rat alveolar surface). Assuming a 20% deposition efficiency of inhaled MWCNTs over an 8 h exposure period, that would require an inhaled concentration of 1.1 g m⁻³, which is hardly achievable. Considering instead a still high, but more realistic, inhaled concentration of 1 mg m⁻³, this would result in a translocated pleural dose of only 23 ng, using a translocation efficiency of 0.11% of the alveolar burden [50]. This is quite obviously in stark contrast to bolus doses of 50–3000 µg injected intraperitoneally in rodent studies.

Nanotubes in nanomedicine

A tumourigenic potential of single-walled carbon nanotubes (SWNTs) has not been investigated as of now. Several studies with SWCNTs administered to the lung of mice by oropharyngeal aspiration (20 µg per mouse) and by inhalation (5 mg m⁻³; 5 h day⁻¹, 4 days) reported inflammation, granuloma formation and fibrosis in the lung, pleural responses were not determined [62]. SWCNTs are investigated for use in drug delivery in animal studies; they were shown to

be effective in delivering an anti-tumour agent (paclitaxel) to retard tumour growth successfully in a mouse model of breast cancer [63]. The investigators concluded that nanotube drug delivery is promising for cancer therapy with high treatment efficacy and minimum side effects. Biodistribution studies identified liver and spleen as main organs of SWCNT accumulation, without showing liver toxicity up to 25 days postadministration. Thus, although this study did not identify short-term toxicity potential long-term effects were not evaluated which should be part of testing for toxicity in nanomedicine as it is for safety assessment of ENMs in consumer products if exposures of consumers to ENMs is to be expected. Expected benefits have to be carefully weighed against potential risks.

Safety assessment

Hazard and risk characterization

Toxicity testing of ENMs using *in vitro* or *in vivo* assays is aimed at identifying a potential hazard by establishing dose–response relationships for characterizing such hazard. However, because a risk of adverse effects associated with ENMs is a function of hazard and exposure [risk = f (hazard; exposure)], the generally accepted approach is to incorporate both components into a risk assessment paradigm, consisting of Hazard Identification, Hazard Characterization, Exposure Assessment and Risk Characterization [64] so that appropriate risk management decisions can be made. As is emphasized throughout this article, toxicity studies using very high doses can certainly identify a NP as hazardous, in fact one can argue that any NP given at high enough doses will induce a significant ‘toxic’ effect. The question is, at what dose does it occur, what assay is used, in short, how realistic is the study for *in vivo* exposure conditions? Even rather benign TiO₂ NPs administered at high enough doses when given repeatedly by inhalation causes lung tumours in rats due to lung overload [65]. Therefore, it is desirable to develop and validate simple non *in vivo* assays for the purpose of predicting *in vivo* responses in order to reduce and avoid extensive testing using laboratory animals. In parallel, efforts should be made to obtain data on exposure levels

occurring for workers at NP manufacturing sites despite best occupational hygiene conditions, as well as for anticipated consumer exposures to nano-enabled products. With respect to nanomedicine, those applications that involve intentional administration of nanotechnology-based therapeutics and diagnostics to the body *in vivo* will always be mandatory.

A working group of the International Life Sciences Institute suggested a tiered testing system to assess NP toxicity [66]. Table 4 lists the different stages, which include an emphasis on detailed physico-chemical characterization prior to and during subsequent testing in cell-free, cellular and *in vivo* assays. Studies designed to determine whether *in vitro* assays are predictive for *in vivo* effects have come to opposite conclusions. Seagrave *et al.* [67] compared particulate emission samples of seven different types of diesel and gasoline engines in terms of their *in vitro* mutagenicity (bacterial assay) and *in vivo* inflammatory/histopathological responses and found generally parallel toxicity rankings between the *in vitro* and *in vivo* assays. They cautioned, however, that additional research is warranted to determine whether the findings of their study can be generalized. In contrast, little correlation between *in vitro* and *in vivo* results was found, when the toxicity of fine and nanoparticles was assessed in alveolar macrophages and a pulmonary epithelial cell line and then compared to the *in vivo* pulmonary inflammation induced by the same particles in rats [68]. These authors had applied a

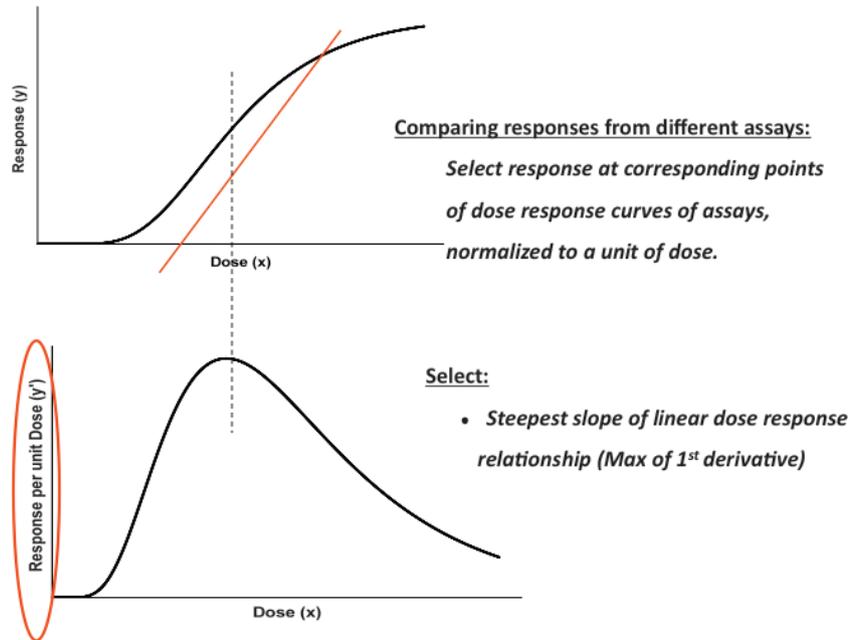
Table 4 Tiered testing system to assess NP toxicity (ILSI Report):[66]

- Physico-chemical characterization
- Cell-free assays (solubility; ROS generating potential; chem-reactivity; agglomeration/aggregation; zeta potential; other)
- Cellular assays [primary cells; cell-lines; (primary and secondary organs); co-cultures]
- *In vivo* assays [generally rodents; diverse methodologies (resp. tract; skin; GI-tract)]

Question

Can any of the *in vitro* tests be used to predict *in vivo* toxicity?

Fig. 8 Analysing complete dose–response relationships (from no-effect to supra-maximal effect doses, upper part of figure) of *in vitro* and *in vivo* assays of NPs to determine the steepest slope as an appropriate point for comparing responses across assays. This defines a response-metric in terms of the maximum response per unit dose (mathematically the first derivative, lower part of the figure). Using NP surface area as dose-metric for expressing the response-metric results in the best fit for correlating *in vitro* with *in vivo* responses. This concept needs to be validated with a broad range of NPs.



wide range of doses in their *in vitro* assays and two doses *in vivo* given intratracheally. They concluded that more sophisticated *in vitro* cell cultures are to be developed and validated to gauge the relative toxicity of inhaled particles *in vivo*.

A new approach to predictive toxicity testing was suggested by Rushton *et al.* [69] by analysing *in vitro* and *in vivo* dose–response curves to determine their steepest slope as a point for comparison (Fig. 8). Using as dose-metric the NP surface area for establishing the dose–response relationship, the steepest slope is equivalent to the maximum response per unit NP surface area which was proposed as a novel response-metric. Using this concept, Rushton *et al.* [69] reported that results of *in vitro* cell-free and cellular assays had a good predictive power for *in vivo* inflammatory responses as determined in a pilot study involving eight NPs of different physico-chemical composition and reactivity. To test their approach further the investigators applied this response-metric concept to the results of the above-mentioned study by Sayes *et al.* [68] who had not found a satisfactory *in vitro*–*in vivo* correlation. When their *in vitro* and *in vivo* effect data were converted to responses per unit surface area (Fig. 8), the result showed a highly

significant *in vitro*–*in vivo* correlation [69]. The authors also suggested to use this response-metric for creating a hazard scale, based on the NP's reactivity per unit surface area, as derived from the analysis of the dose–response relationships. Such hazard category could be based on different end-points measured in specific assays as illustrated in Table 5. A hazard

Table 5 Example of categorizing NPs by a hazard scale [69] (based on maximum effect per unit NP surface as derived from dose–response curves, Fig. 8)

NP-type	Size (nm)	Hazard category
Carbon black	41 (aggregated)	Very low
TiO ₂ (anat.)	20 (aggregated)	
TiO ₂ (anat./rut.)	25 (agglomerated)	Low
Polystyrene	60 (positive charge)	
Au	50	
Ag	35 (aggl./aggreg.)	High
Cu	40 (aggl./aggreg.)	Very high

Example is based on pulmonary inflammatory response in rats (elicited neutrophils per cm²). Other *in vitro* or *in vivo* end-points can be selected, e.g., ROS per cm²; PMN per cm²; LDH per cm²; MN per cm²; Prot.Aggr. per cm².

defined this way would be of practical relevance because NPs are then categorized based on their biological activity rather than by a physico-chemical category (e.g., metal; metal oxides; polymers). This metric of reactivity per unit NP surface area may also be a better surrogate for a biologically available surface than just NP surface area alone as discussed in the dose-metric section.

As mentioned at the beginning of this section, obvious limitations of toxicity testing are that they identify and characterize a hazard, whereas for a quantitative risk assessment additional exposure data are required. Furthermore, *in vitro* assays provide information for acute toxicity only, and validated methods of extrapolating acute *in vitro* assay results for predicting chronic *in vivo* effects have not been developed.

Concluding remarks

Calls for more toxicological information that will help to protect workers' and the public's health reflect an increased concern about the potential dangers of nanotechnology. However, raising fears based on a perceived risk that originates from dubious data is not helpful. However, although many or even most ENMs with a potential for human exposure are not likely to induce adverse effects, it may well turn out that some could cause an asbestos-type disaster if uncontrolled. A tragic case in point appears to be a recent report about worker exposure to heated polystyrene fumes and polyacrylate NPs in an unventilated confined space for several months, resulting in progressive pulmonary fibrosis, pleural effusions and granuloma formation with fatal outcome [70]. Regardless as to whether the NPs had caused the severe pathology – which is unclear based on the information provided – holding on to extremely poor industrial hygiene conditions at the workplace was completely irresponsible. Preventing exposure is key, and that can readily be achieved today with appropriate engineering technology and personal protection equipment.

Efforts aimed at preventing a repetition of past mistakes include the identification of ENM properties that trigger toxicity (e.g., contaminants; impurities,

defects), and to remove them in order to design 'safe' ENMs. Even without being able to perform a quantitative risk assessment for ENMs due to the lack of sufficient data on exposure, biokinetics and organ toxicity, until we know better it should be made mandatory to prevent exposure by appropriate precautionary measures/regulations and by practicing best industrial hygiene to avoid future horror scenarios.

Nanotechnology, nanomedicine and nanotoxicology are complementary disciplines aimed at the improvement of human life: nanotechnology has a bright future with multiple applications in many areas including engineering, optics, energy, consumer products. Nanomedicine will develop applications for novel and superior diagnostic, therapeutic and preventive measures. Nanotoxicity provides for the necessary safety assessment of nano-enabled products. Exciting achievements based on nanotechnology and nanomedicine await us in the future; yet there are as many challenges to get it right and recognize and avoid potential risks associated with these new developments where nanotoxicology will have a crucial role. Essential for the successful present and future developments is a multidisciplinary team approach involving material scientists, physicians and toxicologists who work closely together.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

This work was supported by NIEHS grant ES01247, AFOSR grant FA9550-04-1-0430, EPA STAR PM Center grant RD-832415. The excellent assistance of Ms. Judy Havalack in the preparation of this article is greatly appreciated.

References

- 1 [NIH] National Institutes of Health. National Institute of Health Roadmap for Medical Research: nanomedicine. 2006. Available at: <http://nihroadmap.nih.gov/nanomedicine/>. Accessed May 15 2006.

- 2 [ESF] European Science Foundation. *Nanomedicine – An ESF – European Medical Research Councils (EMRC) Forward Look Report*. Strasbourg cedex, France: ESF, 2004.
- 3 Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Env Health Perspect* 2005; **113**: 823–39.
- 4 Society of Toxicology (SOT). 2005. Available at: http://www.toxicology.org/ai/fa/Definition_of_Toxicology.doc.
- 5 Woodrow Wilson Intl. Center for Scholars. News release: nanotech-enabled consumer products top the 1,000 mark. Release No. 64-09, August 25, 2009. Available at: (<http://www.nano-techproject.org>).
- 6 NNI: Strategy for Nanotechnology-related environmental, health, and safety research. 2008. Available at: http://www.ostp.gov/galleries/NSTC/NNI_EHS_Research_Strategy.pdf.
- 7 Royal Society and Royal Academy of Engineering (UK) 2004. Nanoscience and nanotechnologies: opportunities and uncertainties. Available at: <http://www.royalsoc.ac.uk>.
- 8 Alvarez PJJ, Colvin V, Lead J, Stone V. Research priorities to advance eco-responsible nanotechnology. *ACS NANO* 2009; **3**: 1616–9.
- 9 Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. *Nanotoxicology* 2007; **1**: 2–25.
- 10 Kato T, Yashiro T, Murata Y *et al*. Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries. *Cell Tissue Res* 2003; **311**: 47–51.
- 11 Donaldson K, Brown D, Clouter A *et al*. The pulmonary toxicology of ultrafine particles. *J Aerosol Med* 2002; **15**: 213–20.
- 12 Tran CL, Buchanan D, Cullen RT, Searl A, Jones AD, Donaldson K. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. *Inhal Toxicol* 2000; **12**: 1113–26.
- 13 Duffin R, Tran L, Brown D, Stone V, Donaldson K. Proinflammatory effects of low-toxicity and metal nanoparticles *in vivo* and *in vitro*: highlighting the role of particle surface area and surface reactivity. *Inhal Toxicol* 2007; **19**: 849–56.
- 14 Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL. Pulmonary instillation studies with nanoscale TiO₂ rods and dots in rats: toxicity is not dependent upon particle size and surface area. *Tox Sci* 2006; **91**: 227–36.
- 15 Wittmaack K. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? *Environ Health Perspect* 2007; **115**: 187–94.
- 16 Long TC, Tajuba J, Sama P *et al*. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons *in vitro*. *Environ Health Perspect* 2007; **115**: 1631–7.
- 17 Oberdörster G., Yu CP. The carcinogenic potential of inhaled diesel exhaust: a particle effect? *J Aerosol Sci* 1990; **21(S1)**: S397–401.
- 18 Kreyling WG, Semmler-Behnke M, Seitz J *et al*. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. *Inhal Toxicol* 2009; **21(S1)**: 55–60.
- 19 Wang J, Liu Y, Jiao F *et al*. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO₂ nanoparticles. *Toxicology* 2008; **254**: 82–90.
- 20 Benninghoff AD, Hessler W. Nanoparticles damage brain cells. *Environ Health News*, 2008. Available at: <http://www.environmentalhealthnews.org/ehs/news/science/nanoparticles>.
- 21 Phalen RF, Oldham MJ, Nel AE. Tracheobronchial particle dose considerations for *in vitro* toxicology studies. *Toxicol Sci* 2006; **92**: 126–32.
- 22 Balásházy I, Hofmann W, Heistracher T. Local particle deposition patterns may play a key role in the development of lung cancer. *J Appl Physiol* 2003; **94**: 1719–25.
- 23 Balásházy I, Hofmann W, Farkas Á, Madas BG. Three-dimensional model for aerosol transport and deposition in expanding and contracting alveoli. *Inhal Toxicol* 2008; **20**: 611–21.
- 24 Geiser M, Rothen-Rutishauser B, Kapp N *et al*. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect* 2005; **113**: 1555–60.
- 25 Smith AE, Helenius A. How viruses enter animal cells. *Science* 2004; **304**: 237–42.
- 26 Pante N, Kann M. Nuclear pore complex is able to transport macromolecules with diameters of ~39 nm. *Mol Biol Cell* 2002; **13**: 425–34.
- 27 Jiang J, Oberdörster G, Elder A, Gelein R, Mercer P, Biswas P. Does nanoparticle activity depend upon size and crystal phase? *Nanotoxicology* 2008; **2**: 33–42.
- 28 Han X, Finkelstein JN, Elder A, Biswas P, Jiang J, Oberdörster G. Dose and response metrics in assessing *in vitro* and *in vivo* nanoparticle toxicity. *Toxicologist* 2009; **108**: [SOT Abstract].
- 29 Kreyling WG, Semmler M, Moller W. Dosimetry and toxicology of ultrafine particles. *J Aerosol Med* 2004; **17**: 140–52.
- 30 Semmler M, Seitz J, Erbe F *et al*. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhal Toxicol* 2004; **16**: 453–9.
- 31 Semmler-Behnke M, Takenaka S, Feretsch S *et al*. Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent re-entrainment onto airways epithelia. *Environ Health Perspect* 2007; **115**: 728–33.
- 32 Kreyling WG, Semmler M, Erbe F *et al*. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health* 2002; **65**: 1513–30.
- 33 Cooper JR, Stradling GN, Smith H, Breadmore SE. The reactions of 1.0 nanometre diameter plutonium-238 dioxide particles with rat lung fluid. *Int J Radiat Biol* 1979; **36**: 453–66.
- 34 Stradling GH, Smith H, Cooper JR. Factors affecting the mobility of actinide oxides and their influence on radiological protection. In: Sanders CL, Cross FT, Lagle GE, Mahaffey JA, eds. *Pulmonary Toxicology of Respirable Particles (19th Annual Hanford Life Sciences Symposium)*. Washington, DC: US DOE. Tech. Info. Ctr. US DOE. CONF-791002, 1980; 209–23.

- 35 Rinderknecht A, Oberdörster G, de Mesy Bentley K *et al.* Serum protein coated gold nanoparticles in the perfused human term placenta. *Toxicologist* 2009; **108**: [SOT Abstract].
- 36 Müller RH, Keck CM. Drug delivery to the brain realization by novel drug carriers. *J Nanosci Nanotechnol* 2004; **4**: 471–83. Referred to Müller and Heinemann (1989).
- 37 Cedervall T, Lynch I, Lindman S *et al.* Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *PNAS* 2007; **104**: 2050–5.
- 38 Cedervall T, Lynch I, Foy M *et al.* Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. *Angew Chem Int Ed* 2007; **46**: 5754–6.
- 39 Cedervall T, Lynch I, Lindman S *et al.* Novel methods to quantify binding rates and affinities of proteins to nanoparticles: effects of nanoparticle composition and size. *PNAS* 2007; **104**: 2050–5.
- 40 Ehrenberg M, McGrath JL. Binding between particles and proteins in extracts: implications for microrheology and toxicity. *Acta Biomater* 2005; **1**: 305–15.
- 41 Ehrenberg MS, Friedman AE, Finkelstein JN, Oberdorster G, McGrath JL. The influence of protein adsorption on nanoparticle association with cultured endothelial cells. *Biomaterials* 2009; **30**: 603–10.
- 42 Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc Natl Acad Sci USA* 2008; **105**: 14265–70.
- 43 Oberdörster G, Sharp Z, Atudorei V *et al.* Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 2004; **16**: 437–45.
- 44 Elder A, Gelein R, Silva V *et al.* Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect* 2006; **114**: 1172–8.
- 45 Calderón-Garciduenas L, Azzarelli B, Acuna H *et al.* Air pollution and brain damage. *Toxicol Pathol* 2002; **30**: 373–89.
- 46 Kreuter J, Shamenkov D, Petrov V *et al.* Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *J Drug Target* 2002; **10**: 317–25.
- 47 Donaldson K, Aitken R, Tran L *et al.* Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol Sci* 2006; **92**: 5–22.
- 48 Takagi A., Hirose A, Nishimura T *et al.* Induction of mesothelioma in p53^{+/−} mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci* 2008; **33**: 105–11.
- 49 Ichihara G, Castranova V, Tanioka A, Miyazawa K. Letter to the editor. *J Toxicol Sci* 2008; **33**: 38.
- 50 Poland CA, Duffin R, Kinloch I *et al.* Carbon nanotubes introduced into the abdominal cavity of icr show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol* 2008; **3**: 423–8. Available at: <http://www.nature.com/naturenanotechnology>.
- 51 Davis JMG, Jones AD. Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. *Br J Exp Path* 1988; **69**: 717–37.
- 52 Sakamoto Y, Nakae D, Fukumori N *et al.* Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J Toxicol Sci* 2009; **34**: 65–76.
- 53 Bianchi C, Bianchi T. Malignant mesothelioma: global incidence and relationship with asbestos. *Ind Health* 2007; **45**: 379–87.
- 54 Simeonova P, Luster MI. Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-alpha response from alveolar macrophages. *Am J Resp Cell Mol Biol* 1995; **12**: 676–83.
- 55 Gelzleichter TR, Bermudez E, Mangum JB, Wong BA, Everitt JI, Moss OR. Pulmonary and pleural responses in Fischer 344 rats following short-term inhalation of a synthetic vitreous fiber. *Fundam Appl Toxicol* 1996; **30**: 31–8.
- 56 Dodson RF, Atkinson MA, Levin JL. Asbestos fiber length as related to potential pathogenicity: a critical review. *Am J Ind Med* 2003; **44**: 291–7.
- 57 Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D. Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicol Sci* 2009; **110**: 442–8.
- 58 Moalli PA, MacDonald JL, Goodglick LA, Kane AB. Acute injury and regeneration of the mesothelium in response to asbestos fibers. *Am J Pathol* 1987; **128**: 426–45.
- 59 Goodglick LA, Kane AB. Cytotoxicity of long and short crocidolite asbestos fibers *in vitro* and *in vivo*. *Cancer Res* 1990; **50**: 5153–63.
- 60 Miserocchi G, Sancini G, Mantegazza F, Chiappino G. Translocation pathways for inhaled asbestos fibers. *Environ Health* 2008; **7**: 4.
- 61 Davis JMG, Jones AD, Miller BG. Experimental studies in rats on the effects of asbestos inhalation coupled with the inhalation of titanium dioxide or quartz. *Int J Exp Pathol* 1991; **72**: 501–25.
- 62 Shvedova AA, Kisin E, Murray AR *et al.* Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**: L552–65.
- 63 Liu Z, Chen K, Davis C *et al.* Drug delivery with carbon nanotubes for *in vivo* cancer treatment. *Cancer Res* 2008; **68**: 6652–60.
- 64 National Academy of Sciences (NAS). *Risk Assessment in the Federal Government: Managing the Process*. Washington, DC: NAS 1983.
- 65 Heinrich U, Fuhst R, Rittinghausen S *et al.* Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. *Inhal Toxicol* 1995; **7**: 533–56.
- 66 Oberdörster G, Maynard A, Donaldson K *et al.* and a report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol* 2005; **2**: 8.
- 67 Seagrave J, McDonald JD, Gigliotti AP *et al.* Mutagenicity and *in vivo* toxicity of combined particulate and semivolatile organic

- fractions of gasoline and diesel engine emissions. *Toxicol Sci* 2002; **70**: 212–26.
- 68 Sayes CM, Reed KL, Warheit DB. Assessing toxicity of fine and nanoparticles: comparing *in vitro* measurements to *in vivo* pulmonary toxicity profiles. *Toxicol Sci* 2007; **97**: 163–80.
- 69 Rushton EK, Jiang J, Leonard SS *et al.* Concept of assessing nanoparticle hazards considering nanoparticle dose-metric and chemical/biological response-mixes. *JTEH*, 2009, in press.
- 70 Song Y, Li X, Du X. Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *Eur Respir J* 2009; **34**: 559–67.
- 71 Jiang J, Oberdörster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. *J Nanopart Res* 2009; **11**: 77–89.

Correspondence: Günter Oberdörster DVM, PhD, Department of Environmental Medicine, University of Rochester, 601 Elmwood Avenue, Med. Ctr. Box 850, Rochester, NY 14642, USA.
(fax: 585 256 2631; e-mail: gunter_oberdorster@urmc.rochester.edu).