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Toxicology of nanoparticles ☆

Andreas Elsaesser, C. Vyvyan Howard *

Nano Systems Biology Group, Centre for Molecular Biosciences, University of Ulster, United Kingdom

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ABSTRACT

While nanotechnology and the production of nanoparticles are growing exponentially, research into the toxicological impact and possible hazard of nanoparticles to human health and the environment is still in its infancy. This review aims to give a comprehensive summary of what is known today about nanoparticle toxicology, the mechanisms at the cellular level, entry routes into the body and possible impacts to public health.

Proper characterisation of the nanomaterial, as well as understanding processes happening on the nanoparticle surface when in contact with living systems, is crucial to understand possible toxicological effects. Dose as a key parameter is essential in hazard identification and risk assessment of nanotechnologies. Understanding nanoparticle pathways and entry routes into the body requires further research in order to inform policy makers and regulatory bodies about the nanotoxicological potential of certain nanomaterials.

Contents

1. Introduction—Nanotoxicology

Toxicology traditionally addresses adverse poisoning effects of chemicals to humans, animals and the environment. Historically, toxicology is often associated with Paracelsus and the concept of dose and dose response. He is attributed with having coined the phrase "the dose makes the poison" [1], implying a linear relationship. However toxicological dose responses can be complex and decidedly non-linear especially in the low and high dose range [2].

Key parameters for conventional toxicology are concentration and time. These factors can be easily measured for single chemicals and, after having established the nature of the dose response of a certain chemical, threshold levels can be determined under which a chemical compound may be considered either "safe" or "dangerous".

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* Corresponding author at: Centre for Molecular Biosciences, University of Ulster, Cromore Road, BT52 1SA, Coleraine, United Kingdom.
E-mail address: v.howard@ulster.ac.uk (C.V. Howard).

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Nanotoxicology has emerged only recently, years after the first boom of nanotechnology, when various nanomaterials had already been introduced into a number of industrial processes and products [3]. While we largely understand the properties of bulk materials and/or chemicals at the molecular level, there are new properties of materials being discovered in the zone between "molecule" and "bulk"—that is the nanoscale. When bulk materials are made into smaller and smaller pieces of matter their surface chemistry changes and chemical reactivity increases [4]. It is the reaction between the highly increased reactive surfaces of nanomaterials, due to the increased surface-to-volume ratio, and "wet" biochemistry that is the focus of attention in nanotoxicology. The urgency to react to a growing unregulated use of new nanomaterials, leading inevitably to some degree of environmental and human exposure, was apparent. Since then, investigations into the toxicological potential of nanomaterials have been constantly trying to catch up with the rapid growth of nanotechnology [5].

Growing concerns about possible adverse health effects of nanoparticles and nanostructures were derived from prior experience with, for example, asbestos [6] and air pollution [7]. Although carbon nanotubes, for instance, are much smaller in general than asbestos fibres, early fears were expressed that they might have asbestos-like effects on cells. Soot particles, produced by combustion processes, have also served as analogues for suggesting that nanoparticles could be potentially harmful to health [8].

Apart from their atomic composition, nanomaterials have been categorised according to whether the nanostructure is immobilised within a bulk material, for example as part of a surface of a bulk material or, alternatively, comprised of free, unbound particles capable of mobility within the environment and the body, with diameters in the nanometre range [9]. It is the latter which are the most concerning, from a toxicological point of view. Apart from the high mobility, it is their high surface to volume ratio which is a main property that makes them interesting from a research and product development point of view. Nanoparticles can be further sub-classified with regards to their location in a heterogeneous environment as being: within a volume or on the surface of a solid material or, alternatively, within a volume of a liquid or a gas. Amongst these subcategories, nanoparticles suspended in liquids and mixed with gases need the utmost attention when assessing toxicological hazards due to the danger of these nanoparticles escaping and becoming airborne.

Why should we be worried about airborne particles in particular? Asbestos has taught us that it is better to investigate potential health risks at an early stage of technological development. Increasing evidence that poor air quality coincides with an increase in mortality is showing us that exposure to particles via the airways does pose a major public health risk [10,11]. Little is known about the effects of long-term exposure to engineered nanoparticles and epidemiological data are not available yet, because of the relative novelty of nanotechnology. However, human epidemiological data on dust and air pollution consisting of ultrafine particles (< 100 nm) is available and could help to indicate epidemiological trends and showcase possible long term hazardous effects of man-made nanoparticles, when released into the environment [12]. Although the database for human health effects from ultrafine air pollution is still rather sparse (e.g. [13]) it is the only surrogate that we currently have for predicting the potential negative human health effects of engineered nanoparticles, if they become widespread in the environment.

Interaction mechanisms between nanoparticles and living systems are not yet fully understood. The complexity comes with the particles' ability to bind and interact with biological matter and change their surface characteristics, depending on the environment they are in. Scientific knowledge about nanoparticle–cell interaction mechanisms has been accumulating in recent years, indicating that cells readily take up nanoparticles via either active or passive mechanisms. Intracellularly however, mechanisms and pathways are more difficult to understand. Even particles of the same material can show completely different behaviour due to, for example, slight differences in surface coating, charge or size. This makes the categorisation of nanoparticle behaviour, when in contact with biological systems, intricate and thus nanoparticle hazard identification is not straightforward. This is one of the main distinctions between nanotoxicology and classical toxicology where, in the latter, characterisation of toxicants is, in general, protocolised with a well established set of methodologies available, employing a mass-based dose metric. However, with nanoparticles the dose metric is not straightforward, as discussed below. Furthermore the protocolisation of bioassays involving nanomaterials is still under development and has, in general, not yet been internationally validated. In addition there are many more variables to consider when working with nanomaterials and these include material, size, shape, surface, charge, coating, dispersion, agglomeration, aggregation, concentration and matrix.

The complexity increases when moving from in-vitro to in-vivo models. Hazard identification on the in-vivo level, with regards to nanomaterials, is still at an early stage. Major entry routes (lung, gut and possibly skin) as well as putative targets (lung, liver, heart and brain) have been identified. However, more research is required to understand mechanisms and pathways in the body. What seems clear is that exposure to insoluble nanoscale particles of <50 nm appears to be "new", when compared to the evolutionary history of the preindustrial world. Furthermore such nanoparticles appear to be able to highjack a pre-existing transport mechanism through the body using endocytotic mechanisms, in the same manner that viruses employ. Therefore, because widespread translation of nanoparticles within the body seems to be likely if the body is exposed, we need to take any toxicological risks seriously.

2. Nanoparticle characterisation—size, shape, charge, …

In order to measure toxicological endpoints, the starting point, here the nanomaterial, needs to be fully understood and characterised [14]. Otherwise, possible toxic effects cannot be easily attributed to a certain property of the nanomaterial or even the nanomaterial itself because, for example, impurities and other components could be held responsible [15]. Therefore it is absolutely critical to know the starting material and its properties. In the past this has not always been straightforward for industrially produced nanoparticles due to crude production processes and therefore huge variations in material properties e.g. size, shape, etc. [16]. The more nanotechnology advances, more refined manufacturing processes are developed leading to nanomaterials with uniform and consistent properties.

Apart from the nanomaterial classification according to the nanostructure location, nanomaterials and nanoparticles in particular have been categorised with respect to their "softness" and/or "hardness" [17]. While conductors and semi-conductors (metals, metal-non-metal compounds, non-metals such as carbon based structures) are considered "hard", "soft" materials such as dendrimer-, latex-, polymer- or protein-based nanoparticles are becoming increasingly interesting for bionano and nanomedicine applications. Another trend is the combination of "soft" with "hard" as for example particles with a hard core and a soft shell. As mentioned above, it is only through knowledge of the properties of the particles under study that meaningful toxicological evaluation is possible. This is of equal importance for industrial and environmental particles as well as for nanoparticles designed for nanomedical applications [18].

3. Nanoparticle "dose" and dose metric

As outlined above, dose is one of the key parameters in toxicology. In nanotoxicology it is important to evaluate relevant and realistic dose regimes in order to draw meaningful conclusions from in-vitro

and in-vivo experiments for public health risk assessment. This means that nanotoxicologists should test nanoparticle toxicity based on real-world doses rather than unrealistically high doses in order to achieve a biological response. The latter (e.g. Donaldson et al. [19] using instillation experiments) can be useful in elucidating mechanisms but is unlikely to be predictive of human pathology from environmental exposure. On the other hand, low dose inhalation experiments [20] are likely to be much more predictive of human harm.

In general high dose acute exposures are likely to be detected [21] and corrective/protective measures put in place. However the main public health concerns with nanomaterials will be with chronic low dose exposures over a life time possibly leading to increased incidences of degenerative diseases, as is the case with ultrafine particle exposure in aerosols.

There is considerable discussion currently on the most appropriate metric for assessing the "dose" [22] of nanoparticles, clearly a critical consideration in the field of nanomedicine. In classical toxicology dose is almost invariable related to mass. However with nanoparticles the toxicological effect does not fit with these concepts and has given rise to some level of disbelief amongst classically trained toxicologists. Because of the particulate nature of nanoparticles, a logical dose metric [23,24] will be related to the number of particles reaching each cell or cellular sub-compartment of relevance. Nanoparticle number can be complex to estimate [25]. However there are some indications from the literature that the total surface of nanoparticles, where many of the chemical reactions of interest occur, may be a more discriminatory metric, in some circumstances [24]. While mass alone cannot predict surface area, the number of nanoparticles within a certain size and shape range is predictive of surface area and is therefore likely to be a dose metric which is more predictive of harm than simple mass.

Since, if not somehow "stabilised", most nanoparticles show tendencies to form larger agglomerates, in-vitro and in-vivo assessment of nanotoxicological hazards have to take into account the nanoparticles' behaviour when in contact with biological systems.

4. Nanoparticle surface—increased reactivity

The smaller the diameter of a spherical particle the more the surface-to-volume ratio increases. Increased surface area is accompanied by increased chemical reactivity. This is particularly important for nanobiological interactions [26]. Therefore greater attention has to be paid to the surface material of a nanoparticle rather than its core material. This of course can be made use of in order to "design" suitable surface properties to promote, for example, certain nanoparticle pathways when in contact with biological systems. However, it has been widely recognised in the scientific community that nanoparticles in a biological or environmental context never consist of "bare" particles [27]. As soon as particles come into contact with heterogeneous environments, liquid or gaseous, smaller structures such as atoms, atom clusters, single molecules and/or macromolecules attach to the surface of the particle, binding either strongly or weakly. In a biological environment with biomolecules such as proteins and polymers present, this surface layer has been named the "corona" [28,29]. Research has shown that it is not the nanomaterial itself but the "corona" that mainly defines the properties of the "particle-plus-corona" compound (Fig. 1). This makes it necessary to understand not only the nanomaterial but also the nanoparticle environment when testing for nanotoxicity [30].

5. Nanoparticles and the environment

Nanoparticles released into the environment interact with air, water and soil. This often changes the surface properties of the particles which can result in particle aggregation or changes in particle charge and other surface properties [31]. These effects have been studied in water ecosystems and soil [32,33] and show the importance of understanding nanoparticles and their environmental setting as a "complex" that needs to be looked at in its entirety in order to understand particle behaviour in the environment [34]. A current debate addresses whether nanoparticles can cause toxicity as a contaminant in, for example, soil or water, via a "piggyback" mechanism on natural organic matter [35].

6. Nanoparticles and cells—in-vitro nanotoxicology

Unicellular organisms, which ruled the Earth for approximately the first 3 billion years of life, exist by engulfing matter, usually particulate, from their immediate environment. There are the two basic mechanisms, phagocytosis and endocytosis. The former is generally for large particles and requires a "recognition" step while the latter is for the trans-membrane transport of liquids and molecules. The evolution of multicellular organisms has not seen the loss of the ability of constituent cells to endocytose and indeed it appears that viruses, a form of naturally occurring "nanoparticle", use endocytosis to spread from cell to cell. There is considerable evidence that engineered nanoparticles do the same [36–38] and therefore once they have gained entry to the body we should expect them to translocate to distant sites within the body.

In all in-vitro nanotoxicology studies, particle dose has to be carefully considered. This applies to the initial concentration when

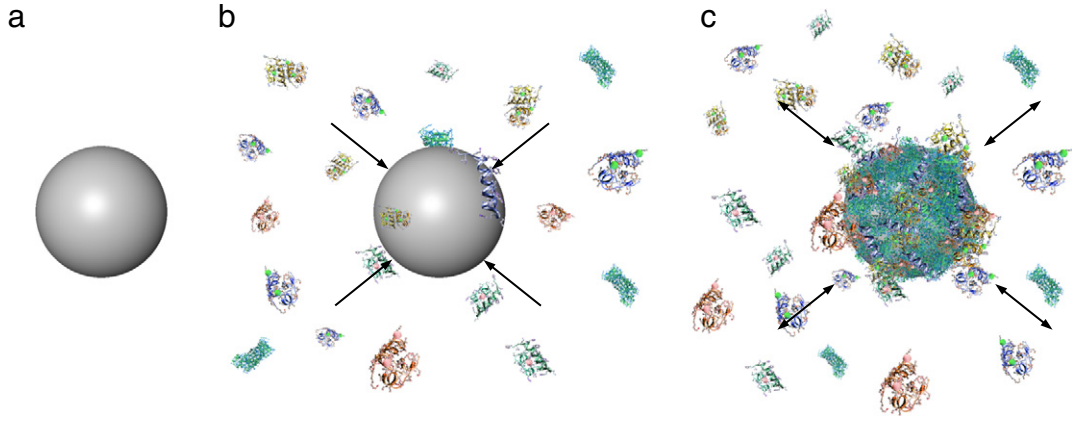

Fig. 1. Formation of nanoparticle corona: a) "bare" particle, b) nanoparticle in contact with proteins, c) corona formation. The corona can consist of a "hard corona", proteins firmly attached to the surface, or a "soft corona" of proteins which are only weakly bound to the nanoparticles and form an equilibrium layer with the surrounding matrix.

exposing cells to nanoparticles but also to the actual amount of nanoparticles taken up by a single cell. Various techniques are available to measure particle uptake and intracellular distribution [25]. Only by understanding particle uptake dynamics and pathways in a quantitative way, can nanotoxicological conclusions be drawn.

Mechanisms on the nano-bio interface can be either chemical or physical [26]. Chemical mechanisms include the production of reactive oxygen species (ROS) [39], dissolution and release of toxic ions [40], disturbance of the electron/ion cell membrane transport activity [41], oxidative damage through catalysis [42], lipid peroxidation [43] or surfactant properties [44]. ROS is considered as being the main underlying chemical process in nanotoxicology, leading to secondary processes that can ultimately cause cell damage and even cell death [45]. Moreover, ROS is one of the main factors involved in inflammatory processes. This is believed to happen via up-regulation of genes involved in the pro-inflammatory response triggered by the activation of certain transcription factors (NF-κB, AP-1) [46,47]. However, free radical formation can also have direct impacts on cell integrity [48,49].

Physical mechanisms at the nano-bio interface are mainly a result of particle size and surface properties [50]. This includes disruption of: membranes [51,52], membrane activity [27], transport processes [53], protein conformation/folding [54,55] and protein aggregation/fibrillation[56].

Both chemical and physical interactions lead to a number of follow-up processes in the cell that constitute the biological "response". These cellular responses can occur before or after internalisation of particles, or as a response to the uptake mechanism itself leading to, for example, "frustrated phagocytosis", a process were a cell tries but fails to totally engulf a particle due to its size or shape, as seen with some multi-walled carbon nanotubes [57]. Certain intracellular compartments are more susceptible and therefore vulnerable [58] to the above mentioned processes, which will be discussed in the following section.

6.1. Membranes—interface between nanoparticles and cells

In a cellular context, membranes, which are phospho-lipid bilayers, partition different intracellular compartments which each have specific functions. They also encapsulate the whole cell. In order to facilitate exchanges between compartments and/or cells, membranes have to be permeable. The outer cell membrane is the cell's interface to its external environment and allows selective transport of ions, molecules and also nanoparticles. Intracellular membranes separate distinct compartments (mitochondria, vesicles, nucleus, etc.) from the cytosol [59]. Membrane stability can be affected by nanoparticles either directly (e.g. physical damage) or indirectly (e.g. oxidation) which can lead to cell death. It is the ability of membranes to control intracellular homeostasis, through selective permeability and transport mechanisms, which makes them a vulnerable target for possible damaging effects of nanoparticles. Interactions of nanoparticles with membranes depend largely on the nanoparticles' surface properties. This is the reason why surface modifications are crucial in the design of drug delivery systems in order to enhance nanoparticle uptake into cells [59]. Nanoparticle size also plays an important role as it influences surface pressure and adhesion forces [60].

Research has shown that different nanomaterials can damage membranes by various processes (Fig. 2) leading to a compromise of membrane integrity and stability as well as the formation of nano-sized holes [61]. Gene expression analysis, for example, suggests that

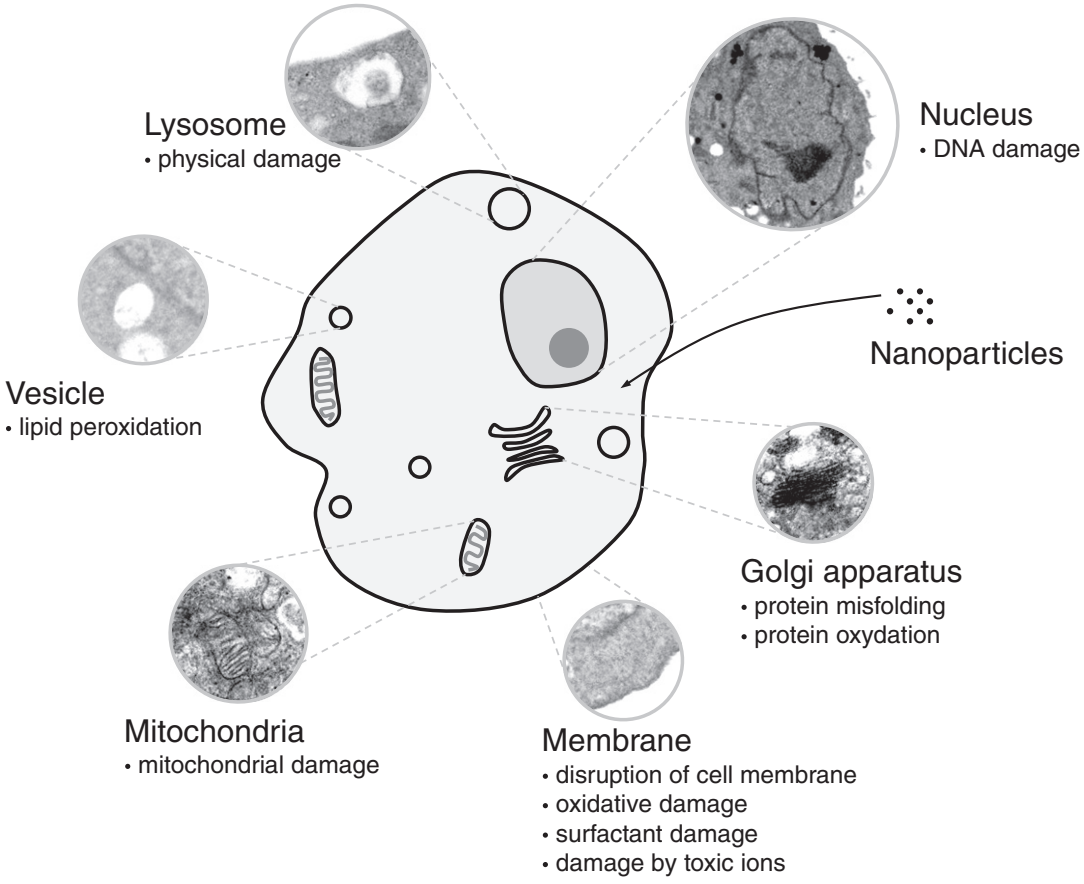

Fig. 2. Nanoparticle interaction with cells: intracellular targets and nanotoxicological mechanisms.

membrane damage is nanomaterial as well as concentration dependent [45]. Although biochemical factors such as reactive oxygen species can damage membranes [62], physicochemical properties of nanoparticles seem to be predominantly responsible for changes in membrane morphology and stability [61]. Mitochondria, as the cell's power plants, appear to be a major target for fullerenes [42] and carbon nanotubes [63]. However other nanoparticles (e.g. titanium dioxide, carbon nanotubes, polystyrene and silver) also seem to be able to affect mitochondrial function, leading to apoptosis [52,64,65]. Another preferential intracellular compartment is that of lysosomes, the cell's digestive system. Research has shown that nanoparticles generally end up in lysosomes where the cell tries either to digest or excrete them [38,66]. The impact of size, shape and nanomaterial on the cell's ability to digest or excrete nanoparticles after accumulation in lysosomes is not fully understood. Finally, the nuclear membrane uses nuclear pores to transport substances in and out the nucleus. Some, generally smaller, nanoparticles appear able to either diffuse through these pores [67,68] or be transported via receptor meditated transport mechanisms [69] to gain access to the intra-nuclear area [70–72].

6.2. How do nanoparticles affect proteins and macromolecules?

The cell apparatus relies to a large part on proteins and other macromolecules. These exist in the form of enzymes (e.g. gastrin), cell signalling molecules (e.g. hormones) or structural proteins (e.g. tubulin). Their normal functioning is therefore essential for all vital cellular activities. Correct molecular conformation is essential for proteins to work as intended and slight conformational changes can alter or destroy the protein's function. During their assembly process chaperones play an important role in controlling the manner in which proteins fold [73], in order to obtain a certain conformation. As mentioned above, nanoparticles, which can be of the same size magnitude of protein molecules, are able to interfere with cell signalling processes [74] or interact with proteins [75–77], either by chaperone-like activity [78–80] or by changing the configuration of, for example, peptides in forms of aggregation and fibrillation [81–83]. Protein misfolding and peptide fibrillation leading to, for example, amyloid-like structures are associated with neuro-degenerative diseases. Investigating possible miss-formation and overproduction of proteins and macromolecules at the cellular level is important for nanotoxicological considerations [75,84].

6.3. Nanoparticle interaction with DNA

From the earliest studies in nanotoxicology, DNA has attracted special attention when assessing potential toxicological risks caused by nanomaterials. Since researchers have reported that nanoparticles are able to enter the nuclear envelope, interest in possible genotoxic effects of nanoparticles has moved into the focus of attention. Various nanoparticles have been tested for genotoxicity [85,86]. However these studies have not been able to identify clearly which nanoparticle parameter is responsible for either positive or negative outcomes. Also, the mechanism of potential DNA damage is not fully understood. Apart from direct intercalation or the physical and/or electrochemical interaction with nanoparticles [87,88], ROS is again believed to play a key role in DNA damage. This means that particles do not necessarily have to reach the nucleus but could for example induce genotoxicity via oxidative stress [89,90].

7. Nanoparticles and humans—in-vivo nanotoxicology

In order to assess nanoparticle toxicity, in-vitro models are insufficient alone to predict possible hazards to humans [91]. In-vivo studies are necessary to elucidate mechanisms, pathways and entry routes of nanoparticles in a complex multicellular organism [92]. This is required not only for nanomaterials used in industrial processes, where human exposure could occur via the environment but also for nanomaterials where human exposure is part of the design, e.g. nanomedicines. For nanoparticles produced on an industrial scale, the extent to which factory workers, specific population subgroups or the public in general are exposed needs to be established. Several nanomaterials currently fall into this category: titanium dioxide [93], cerium dioxide [94], silicon dioxide [95], zinc oxide [96], silver [97] and carbon nanotubes [98], to name the most prominent ones. Gold nanoparticles are and will be increasingly used for nanotherapeutical applications due to their outstanding bioconjugation properties [99]. Research shows that, apart from the intrinsic nanotoxicological potential of the “bare” particle, coating and surface properties have to be taken more seriously in order to understand and predict toxicological effects at the in-vivo level.

7.1. Entry routes and vulnerabilities—how do particles get into the body?

It has been demonstrated that nanoparticles gain access to the body mainly via the airways, the skin or via ingestion [100] (Fig. 3). They are also able to translocate to secondary organs, however this has only been demonstrated in small quantities [22,101].

The main entry route for airborne particles to the body is through the respiratory system. Substantial data on ultrafine particles (dust, carbon black and other pollutants) and their effects to the airways and lungs is available [102]. Data on translocation of nanoparticles via the lungs are increasing. The main question is whether particles can cross the air-blood-barrier in the lungs and therefore gain access to the rest of the body [103]. The body has certain defence mechanisms against particles (mucus and mucociliary escalator [104]), however nanoparticles seem to be able to translocate from the lung into liver, spleen, heart and possibly other organs [105]. The main mechanism for nanoparticle translocation appears to be via endocytosis of alveolar epithelial cells [37]. Apart from exposure to the lungs, inhaled nanoparticles can also gain access to other organs via the olfactory bulb [106]. This is potentially hazardous from a neurotoxicological point of view, as particles would also be able to gain direct access to the central nervous system via this route [107].

Another potential exposure route in humans is via the skin [108]. Titanium dioxide nanoparticles are, for example, often used in sunscreen products and may gain access through hair follicles or wounds and lesions [108]. However, the literature on dermal absorption and translocation into the body of titanium dioxide is inconclusive and further research is required. Other particles such as fullerenes [99] and quantum dots [100] seem to be able to penetrate the dermis [109,110], dependant on size and surface coatings [111].

Since nanoparticles are increasingly used as food additives or in food processing and packaging, there are concerns that nanoparticles could gain access to the blood stream via gastro-intestinal assimilation. It has been demonstrated that nanoparticle uptake via the gut [112] is possible and seems to be size dependent [113]. However, further research is required to shed more light on gastro-intestinal assimilation [114].

The major concern however is that nanoparticles could gain access to other organs once having entered the body and reached the bloodstream [115]. Great importance is attached to natural barriers in the body, for example the air-blood barrier in the lung, the blood-brain barrier or the materno-foetal barrier [116]. Biodistribution studies of nanoparticles have found low concentrations of them in liver, spleen, heart and the brain [117–120]. Further concerns are the bioaccumulation of nanoparticles in certain organs [121]. It is not yet clear to what extent the body is able to excrete nanoparticles via urine [122] or whether residual nanoparticles bioaccumulate in certain organs and may even block the body's excretion systems. Clearance rates of 40%

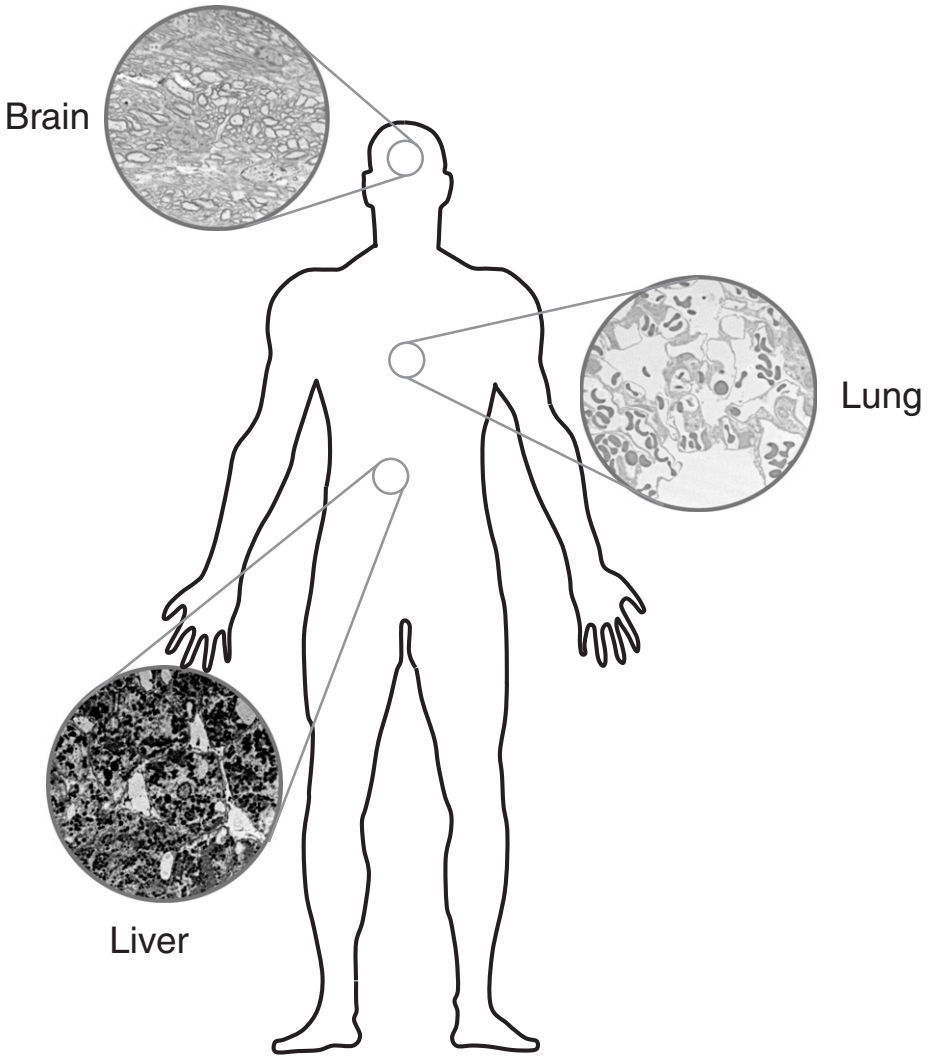

Fig. 3. Nanoparticle entry route into the body via the lung, particle accumulation in the liver and the most vulnerable site: the brain.

[123] up to around 50% [124] have been found in recent studies. However, given the slow translocation rates and tendency of nanoparticles to form agglomerates and stick to bio-surfaces, further research is required investigating chronic exposure and bioaccumulation versus bioclearance [125].

7.2. In vivo toxicity—which are the major mechanisms?

Recapitulating known mechanisms of nanoparticle toxicity at the cellular level, allows predictions regarding what damage nanoparticles could cause in certain parts of the body. Protein misfolding and protein fibrillation [126] induced by nanoparticles could cause major problems in the brain [127], however studies so far on this issue are based on in-vitro experiments and in-vivo confirmation needs to be performed. In the light of the asbestos disaster [128], chronic inflammation as a result of nanoparticle exposure, for example in the lung and other organs, via frustrated phagocytosis or production of reactive oxygen species [129] needs further attention. Another vulnerable target for possible toxicological effects of nanoparticles is the foetus. Research has shown that gold nanoparticles can cross the materno-foetal barrier [130] and fullerenes were found to have a fatal effect on mouse embryos [131]. The full mechanisms behind certain in-vivo toxicological findings are not yet fully understood and require further research.

8. Conclusions

Engineered nanoparticles represent a novel toxicological challenge. They are completely novel in evolutionary terms, the evidence shows that they gain can access to the body, particularly through inhalation, and then translocate within the body to distant sites at low doses.

A number of putative mechanisms of toxicological damage have been identified, including reactive oxygen species generation, protein misfolding, membrane perturbation and direct physical damage.

A critical step in nanotoxicology is to characterise the nanomaterial under examination and this is much more difficult than is the case in classical toxicology because of the multitude of variables in the parameter space. These include: particle size, roughness, shape, charge, composition and surface coating. The latter can change depending upon the matrix into which it is introduced.

Estimation of nanoparticle “dose” is complex, requiring a number of direct and/or indirect technologies to determine how many particles are reaching defined targets. The weighting of “dose” to mass, number or surface of nanoparticles is still the subject of intense research. Testing nanoparticle toxicity based on estimates of realistic human exposure is required instead of testing unrealistically high nanoparticle doses with no relevance to real-world exposures. However, chronic low-dose exposure is probably difficult to model in short lived laboratory rodents. It is probably necessary to develop

toxicological testing models in longer lived species to adequately model human lifelong exposures.

There is a need to develop a regulatory framework based on objective scientific research which will limit human exposure to unwanted engineered nanomaterials in the environment to safe levels. However, the therapeutic use of nanomaterials in medicine requires a different framework which balances the therapeutic benefit against the potential risk of harm.

References

[1] J.F. Borzelleca, Paracelsus: herald of modern toxicology, Toxicol. Sci. 53 (2000) 2–4.
[2] K.K. Rozman, J. Doull, Paracelsus, Haber and Arndt, Toxicology 160 (2001) 191–196.
[3] K. Donaldson, V. Stone, C.L. Tran, W. Kreyling, P.J. Borm, Nanotoxicology, Occup. Environ. Med. 61 (2004) 727–728.
[4] D.A. Jefferson, The surface activity of ultrafine particles, Philosophical Transactions of the Royal Society of London, Series A: Mathematical, Phys. Eng. Sci. 358 (2000) 2683–2692.
[5] V. Stone, K. Donaldson, Nanotoxicology: signs of stress, Nat. Nanotechnol. 1 (2006) 23–24.
[6] A.B. Kane, R.H. Hurt, Nanotoxicology: the asbestos analogy revisited, Nat. Nanotechnol. 3 (2008) 378–379.
[7] D.W. Dockery, C.A. Pope, X. Xu, J.D. Spengler, J.H. Ware, M.E. Fay, B.G. Ferris, F.E. Speizer, An association between air pollution and mortality in six U.S. cities, N. Engl. J. Med. 329 (1993) 1753–1759.
[8] K. Donaldson, L. Tran, L.A. Jimenez, R. Duffin, D.E. Newby, N. Mills, W. MacNee, V. Stone, Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure, Part. Fibre Toxicol. 2 (2005) 10.
[9] S.F. Hansen, E.S. Michelson, A. Kamper, P. Borling, F. Stuer-Lauridsen, A. Baun, Categorization framework to aid exposure assessment of nanomaterials in consumer products, Ecotoxicology 17 (2008) 438–447.
[10] N. Kunzli, R. Kaiser, S. Medina, M. Studnicka, O. Chanel, P. Filliger, M. Herry, F. Horak Jr., V. Puybonnieux-Texier, P. Quenel, J. Schneider, R. Seethaler, J.C. Vergnaud, H. Sommer, Public-health impact of outdoor and traffic-related air pollution: a European assessment, Lancet 356 (2000) 795–801.
[11] N. Kunzli, M. Jerrett, W.J. Mack, B. Beckerman, L. LaBree, F. Gilliland, D. Thomas, J. Peters, H.N. Hodis, Ambient air pollution and atherosclerosis in Los Angeles, Environ. Health Perspect. 113 (2005) 201–206.
[12] G. Oberdorster, M.J. Utell, Ultrafine particles in the urban air: to the respiratory tract—and beyond? Environ. Health Perspect. 110 (2002) A440–A441.
[13] H.-E. Wichmann, A. Peters, Epidemiological evidence of the effects of ultrafine particle exposure, Philosophical Transactions of the Royal Society of London, Series A: Mathematical, Phys. Eng. Sci. 358 (2000) 2751–2769.
[14] D.J. Burleson, M.D. Driessen, R.L. Penn, On the characterization of environmental nanoparticles, J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 39 (2004) 2707–2753.
[15] C.M. Sayes, D.B. Warheit, Characterization of nanomaterials for toxicity assessment, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 1 (2009) 660–670.
[16] H. Park, V.H. Grassian, Commercially manufactured engineered nanomaterials for environmental and health studies: important insights provided by independent characterization, Environ. Toxicol. Chem. 29 (2010) 715–721.
[17] D.A. Tomalia, In quest of a systematic framework for unifying and defining nanoscience, J. Nanopart. Res. 11 (2009) 1251–1310.
[18] S.C. Brown, M. Palazuelos, P. Sharma, K.W. Powers, S.M. Roberts, S.R. Grobmyer, B.M. Moudgil, Nanoparticle characterization for cancer nanotechnology and other biological applications, Methods Mol. Biol. 624 (2010) 39–65.
[19] K. Donaldson, V. Stone, P.S. Gilmour, D.M. Brown, W. MacNee, Ultrafine particles: mechanisms of lung injury, Philosophical Transactions of the Royal Society of London, Series A: Mathematical, Phys. Eng. Sci. 358 (2000) 2741–2749.
[20] G. Oberdürster, Toxicology of ultrafine particles: in vivo studies, Philosophical Transactions of the Royal Society of London, Series A: Mathematical, Phys. Eng. Sci. 358 (2000) 2719–2740.
[21] Y. Song, X. Li, X. Du, Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma, Eur. Respir. J. 34 (2009) 559–567.
[22] G. Oberdorster, Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology, J. Intern. Med. 267 (2010) 89–105.
[23] K. Wittmaack, In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? Environ. Health Perspect. 115 (2007) 187–194.
[24] G. Oberdörster, Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology, J. Intern. Med. 267 (2010) 89–105.
[25] A. Elsaesser, A. Taylor, G.S. de Yanes, G. McKerr, E.M. Kim, E. O'Hare, C.V. Howard, Quantification of nanoparticle uptake by cells using microscopical and analytical techniques, Nanomedicine (Lond.) 5 (2010) 1447–1457.
[26] A.E. Nel, L. Madler, D. Velegol, T. Xia, E.M. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, Understanding biophysicochemical interactions at the nano-bio interface, Nat. Mater. 8 (2009) 543–557.
[27] E. Navarro, A. Baun, R. Behra, N. Hartmann, J. Filser, A.-J. Miao, A. Quigg, P. Santschi, L. Sigg, Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi, Ecotoxicology 17 (2008) 372–386.
[28] I. Lynch, T. Cedervall, M. Lundqvist, C. Cabaleiro-Lago, S. Linse, K.A. Dawson, The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century, Adv. Colloid Interface Sci. 134–135 (2007) 167–174.
[29] T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K.A. Dawson, S. Linse, Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 2050–2055.
[30] I. Lynch, A. Salvati, K.A. Dawson, Protein–nanoparticle interactions: what does the cell see? Nat Nano 4 (2009) 546–547.
[31] R.D. Handy, F. von der Kammer, J.R. Lead, M. Hassellov, R. Owen, M. Crane, The ecotoxicology and chemistry of manufactured nanoparticles, Ecotoxicology 17 (2008) 287–314.
[32] J.T. Quik, I. Lynch, K. Van Hoecke, C.J. Miermans, K.A. De Schamphelaere, C.R. Janssen, K.A. Dawson, M.A. Stuart, D. Van De Meent, Effect of natural organic matter on cerium dioxide nanoparticles settling in model fresh water, Chemosphere 81 (2010) 711–715.
[33] M.A. Kiser, H. Ryu, H. Jang, K. Hristovski, P. Westerhoff, Biosorption of nanoparticles to heterotrophic wastewater biomass, Water Res. 44 (2010) 4105–4114.
[34] B. Nowack, The behavior and effects of nanoparticles in the environment, Environ. Pollut. 157 (2009) 1063–1064.
[35] M.R. Wiesner, E.M. Hotze, J.A. Brant, B. Espinasse, Nanomaterials as possible contaminants: the fullerene example, Water Sci. Technol. 57 (2008) 305–310.
[36] M.C. Garnett, P. Kallinteri, Nanomedicines and nanotoxicology: some physiological principles, Occup Med (Lond) 56 (2006) 307–311.
[37] N.R. Yacobi, N. Malmstadt, F. Fazlollahi, L. DeMaio, R. Marchelletta, S.F. Hamm-Alvarez, Z. Borok, K.J. Kim, E.D. Crandall, Mechanisms of alveolar epithelial translocation of a defined population of nanoparticles, Am. J. Respir. Cell Mol. Biol. 42 (2010) 604–614.
[38] C. Greulich, J. Diendorf, T. Simon, G. Eggeler, M. Epple, M. Koller, Uptake and intracellular distribution of silver nanoparticles in human mesenchymal stem cells, Acta Biomater. 7 (2011) 347–354.
[39] A. Nel, T. Xia, L. Mädler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (2006) 622–627.
[40] T. Xia, M. Kovochich, M. Liong, L. Mädler, B. Gilbert, H. Shi, J.I. Yeh, J.I. Zink, A.E. Nel, Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties, ACS Nano 2 (2008) 2121–2134.
[41] M.I. Auffan, W. Achouak, J.r. Rose, M.-A. Roncato, C. Chanéac, D.T. Waite, A. Masion, J.C. Woicik, M.R. Wiesner, J.-Y. Bottero, Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward *Escherichia coli*, Environ. Sci. Technol. 42 (2008) 6730–6735.
[42] S. Foley, C. Crowley, M. Smaihi, C. Bonfils, B.F. Erlanger, P. Seta, C. Larroque, Cellular localisation of a water-soluble fullerene derivative, Biochem. Biophys. Res. Commun. 294 (2002) 116–119.
[43] J.P. Kamat, T.P. Devasagayam, K.I. Priyadarsini, H. Mohan, Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications, Toxicology 155 (2000) 55–61.
[44] M.G. Cottingham, M.S. Hollinshead, D.J. Vaux, Amyloid fibril formation by a synthetic peptide from a region of human acetylcholinesterase that is homologous to the Alzheimer's amyloid-beta peptide, Biochemistry 41 (2002) 13539–13547.
[45] N. Gou, A. Onnis-Hayden, A.Z. Gu, Mechanistic toxicity assessment of nanomaterials by whole-cell-array stress genes expression analysis, Environ. Sci. Technol. 44 (2010) 5964–5970.
[46] M.C. Elaine, B.G. Matthew, Inflammation, free radicals, and antioxidants, Nutrition 12 (1996) 274–277 Burbank, Los Angeles County, Calif.).
[47] N. Li, T. Xia, A.E. Nel, The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles, Free Radic. Biol. Med. 44 (2008) 1689–1699.
[48] N. Li, C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines, A. Nel, Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, Environ. Health Perspect. 111 (2003) 455–460.
[49] T. Uchino, H. Tokunaga, M. Ando, H. Utsumi, Quantitative determination of OH radical generation and its cytotoxicity induced by TiO2-UVA treatment, Toxicol. Vitr. 16 (2002) 629–635.
[50] D. Walczyk, F.B. Bombelli, M.P. Monopoli, I. Lynch, K.A. Dawson, What the Cell "Sees" in Bionanoscience, J. Am. Chem. Soc. 132 (2010) 5761–5768.
[51] P.R. Leroueil, S.A. Berry, K. Duthie, G. Han, V.M. Rotello, D.Q. McNerny, J.R. Baker, B.G. Orr, M.M. Banaszak Holl, Wide varieties of cationic nanoparticles induce defects in supported lipid bilayers, Nano Lett. 8 (2008) 420–424.
[52] S.M. Hussain, K.L. Hess, J.M. Gearhart, K.T. Geiss, J.J. Schlager, In vitro toxicity of nanoparticles in BRL 3A rat liver cells, Toxicol. In Vitro 19 (2005) 975–983.
[53] J. Øvrevik, M. Låg, P. Schwarze, M. Refsnes, p38 and Src-ERK1/2 pathways regulate crystalline silica-induced chemokine release in pulmonary epithelial cells, Toxicol. Sci. 81 (2004) 480–490.
[54] T.S. Hauck, A.A. Ghazani, W.C.W. Chan, Assessing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells, Small 4 (2008) 153–159.
[55] P. Billsten, P.O. Freskgard, U. Carlsson, B.H. Jonsson, H. Elwing, Adsorption to silica nanoparticles of human carbonic anhydrase II and truncated forms induce a molten-globule-like structure, FEBS Lett. 402 (1997) 67–72.
[56] M. Chen, A. von Mikecz, Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles, Exp. Cell Res. 305 (2005) 51–62.
[57] C.A. Poland, R. Duffin, I. Kinloch, A. Maynard, W.A. Wallace, A. Seaton, V. Stone, S. Brown, W. Macnee, K. Donaldson, Carbon nanotubes introduced into the

abdominal cavity of mice show asbestos-like pathogenicity in a pilot study, Nat. Nanotechnol. 3 (2008) 423–428.
[58] K. Unfried, C. Albrecht, L.-O. Klotz, A. Von Mikecz, S. Grether-Beck, R.P.F. Schins, Cellular responses to nanoparticles: target structures and mechanisms, Nanotoxicology 1 (2007) 52–71.
[59] J.K. Vasir, V. Labhasetwar, Quantification of the force of nanoparticle–cell membrane interactions and its influence on intracellular trafficking of nanoparticles, Biomaterials 29 (2008) 4244–4252.
[60] C. Peetla, V. Labhasetwar, Biophysical characterization of nanoparticle–endothelial model cell membrane interactions, Mol. Pharm. 5 (2008) 418–429.
[61] V.V. Ginzburg, S. Balijepalli, Modeling the thermodynamics of the interaction of nanoparticles with cell membranes, Nano Lett. 7 (2007) 3716–3722.
[62] I. Rodea-Palomares, K. Boltes, F. Fernandez-Pinas, F. Leganes, E. Garcia-Calvo, J. Santiago, R. Rosal, Physicochemical characterization and ecotoxicological assessment of CeO2 nanoparticles using two aquatic microorganisms, Toxicol. Sci. 119 (2011) 135–145.
[63] Y. Zhu, Q. Zhao, Y. Li, X. Cai, W. Li, The interaction and toxicity of multi-walled carbon nanotubes with *Stylonychia mytilus*, J. Nanosci. Nanotechnol. 6 (2006) 1357–1364.
[64] T. Xia, M. Kovochich, J. Brant, M. Hotze, J. Sempf, T. Oberley, C. Sioutas, J.I. Yeh, M.R. Wiesner, A.E. Nel, Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm, Nano Lett. 6 (2006) 1794–1807.
[65] G. Jia, H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao, X. Guo, Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene, Environ. Sci. Technol. 39 (2005) 1378–1383.
[66] M. Al-Rawi, S. Diabate, C. Weiss, Uptake and intracellular localization of submicron and nano-sized SiO(2) particles in HeLa cells, Arch. Toxicol. 85 (2011) 813–826.
[67] A.S. Parfenov, V. Salnikov, W.J. Lederer, V. Lukyanenko, Aqueous diffusion pathways as a part of the ventricular cell ultrastructure, Biophys. J. 90 (2006) 1107–1119.
[68] W.T. Godbey, K.K. Wu, A.G. Mikos, Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 5177–5181.
[69] N. Pante, M. Kann, Nuclear pore complex is able to transport macromolecules with diameters of 39 nm, Mol. Biol. Cell 13 (2002) 425–434.
[70] Y. Williams, A. Sukhanova, M. Nowostawska, A.M. Davies, S. Mitchell, V. Oleinikov, Y. Gun'ko, I. Nabiev, D. Kelleher, Y. Volkov, Probing cell-type-specific intracellular nanoscale barriers using size-tuned quantum dots, Small 5 (2009) 2581–2588.
[71] I. Nabiev, S. Mitchell, A. Davies, Y. Williams, D. Kelleher, R. Moore, Y.K. Gun'ko, S. Byrne, Y.P. Rakovich, J.F. Donegan, A. Sukhanova, J. Conroy, D. Cottell, N. Gaponik, A. Rogach, Y. Volkov, Nonfunctionalized nanocrystals can exploit a cell's active transport machinery delivering them to specific nuclear and cytoplasmic compartments, Nano Lett. 7 (2007) 3452–3461.
[72] A. Hoshino, K. Fujioka, T. Oku, S. Nakamura, M. Suga, Y. Yamaguchi, K. Suzuki, M. Yasuhara, K. Yamamoto, Quantum dots targeted to the assigned organelle in living cells, Microbiol. Immunol. 48 (2004) 985–994.
[73] C.M. Dobson, Protein folding and misfolding, Nature 426 (2003) 884–890.
[74] F. Marano, S. Hussain, F. Rodrigues-Lima, A. Baeza-Squiban, S. Boland, Nanoparticles: molecular targets and cell signalling, Arch. Toxicol. 85 (2011) 733–741.
[75] K.A. Dawson, A. Salvati, I. Lynch, Nanotoxicology: nanoparticles reconstruct lipids, Nat. Nanotechnol. 4 (2009) 84–85.
[76] M. De, V.M. Rotello, Synthetic "chaperones": nanoparticle-mediated refolding of thermally denatured proteins, Chem. Commun. (2008) 3504–3506 (Camb).
[77] W. Shang, J.H. Nuffer, J.S. Dordick, R.W. Siegel, Unfolding of ribonuclease A on silica nanoparticle surfaces, Nano Lett. 7 (2007) 1991–1995.
[78] H. Takahashi, S. Sawada, K. Akiyoshi, Amphiphilic polysaccharide nanoballs: a new building block for nanogel biomedical engineering and artificial chaperones, ACS Nano 5 (2011) 337–345.
[79] D. Ishii, K. Kinbara, Y. Ishida, N. Ishii, M. Okochi, M. Yohda, T. Aida, Chaperonin-mediated stabilization and ATP-triggered release of semiconductor nanoparticles, Nature 423 (2003) 628–632.
[80] K. Akiyoshi, Y. Sasaki, J. Sunamoto, Molecular chaperone-like activity of hydrogel nanoparticles of hydrophobized pullulan: thermal stabilization with refolding of carbonic anhydrase B, Bioconjug. Chem. 10 (1999) 321–324.
[81] W.H. Wu, X. Sun, Y.P. Yu, J. Hu, L. Zhao, Q. Liu, Y.F. Zhao, Y.M. Li, TiO2 nanoparticles promote beta-amyloid fibrillation in vitro, Biochem. Biophys. Res. Commun. 373 (2008) 315–318.
[82] S.C. Wagner, M. Roskamp, M. Pallerla, R.R. Araghi, S. Schlecht, B. Koksch, Nanoparticle-induced folding and fibril formation of coiled-coil-based model peptides, Small 6 (2010) 1321–1328.
[83] D. Aili, K. Enander, J. Rydberg, I. Nesterenko, F. Bjorefors, L. Baltzer, B. Liedberg, Folding induced assembly of polypeptide decorated gold nanoparticles, J. Am. Chem. Soc. 130 (2008) 5780–5788.
[84] M. Bucciantini, E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J. Zurdo, N. Taddei, G. Ramponi, C.M. Dobson, M. Stefani, Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, Nature 416 (2002) 507–511.
[85] C.A. Barnes, A. Elsaesser, J. Arkusz, A. Smok, J. Palus, A. Lesniak, A. Salvati, J.P. Hanrahan, W.H. Jong, E. Dziubaltowska, M. Stepnik, K. Rydzynski, G. McKerr, I. Lynch, K.A. Dawson, C.V. Howard, Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity, Nano Lett. 8 (2008) 3069–3074.
[86] H.L. Karlsson, The comet assay in nanotoxicology research, Anal. Bioanal. Chem. 398 (2010) 651–666.
[87] W. Xie, L. Wang, Y. Zhang, L. Su, A. Shen, J. Tan, J. Hu, Nuclear targeted nanoprobe for single living cell detection by surface-enhanced Raman scattering, Bioconjug. Chem. 20 (2009) 768–773.
[88] M. Mehrabi, R. Wilson, Intercalating gold nanoparticles as universal labels for DNA detection, Small 3 (2007) 1491–1495.
[89] G. Bhabra, A. Sood, B. Fisher, L. Cartwright, M. Saunders, W.H. Evans, A. Surprenant, G. Lopez-Castejon, S. Mann, S.A. Davis, L.A. Hails, E. Ingham, P. Verkade, J. Lane, K. Heesom, R. Newson, C.P. Case, Nanoparticles can cause DNA damage across a cellular barrier, Nat Nano 4 (2009) 876–883.
[90] P. Myllynen, Nanotoxicology: damaging DNA from a distance, Nat. Nanotechnol. 4 (2009) 795–796.
[91] P. Rivera Gil, G. Oberdorster, A. Elder, V. Puntes, W.J. Parak, Correlating physicochemical with toxicological properties of nanoparticles: the present and the future, ACS Nano 4 (2010) 5527–5531.
[92] A. Seaton, L. Tran, R. Aitken, K. Donaldson, Nanoparticles, human health hazard and regulation, J. R. Soc. Interface 7 (Suppl 1) (2010) S119–S129.
[93] N.C. Mueller, B. Nowack, Exposure modeling of engineered nanoparticles in the environment, Environ. Sci. Technol. 42 (2008) 4447–4453.
[94] B. Park, K. Donaldson, R. Duffin, L. Tran, F. Kelly, I. Mudway, J.P. Morin, R. Guest, P. Jenkinson, Z. Samaras, M. Giannouli, H. Kouridis, P. Martin, Hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive—a case study, Inhal. Toxicol. 20 (2008) 547–566.
[95] D. Napierska, L.C. Thomassen, D. Lison, J.A. Martens, P.H. Hoet, The nanosilica hazard: another variable entity, Part. Fibre Toxicol. 7 (2010) 39.
[96] M.J. Osmond, M.J. McCall, Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard, Nanotoxicology 4 (2010) 15–41.
[97] M. Ahamed, M.S. Alsalhi, M.K. Siddiqui, Silver nanoparticle applications and human health, Clin. Chim. Acta 411 (2010) 1841–1848.
[98] R.J. Aitken, M.Q. Chaudhry, A.B. Boxall, M. Hull, Manufacture and use of nanomaterials: current status in the UK and global trends, Occup Med (Lond) 56 (2006) 300–306.
[99] R.A. Sperling, P. Rivera Gil, F. Zhang, M. Zanella, W.J. Parak, Biological applications of gold nanoparticles, Chem. Soc. Rev. 37 (2008) 1896–1908.
[100] S.T. Stern, S.E. McNeil, Nanotechnology safety concerns revisited, Toxicol. Sci. 101 (2008) 4–21.
[101] A. Elder, I. Lynch, K. Grieger, S. Chan-Remillard, A. Gatti, H. Gnewuch, E. Kenawy, R. Korenstein, T. Kuhlbusch, F. Linker, S. Matias, N. Monteiro-Riviere, V.R.S. Pinto, R. Rudnitsky, K. Savolainen, A. Shvedova, Human Health Risks of Engineered Nanomaterials, in: I. Linkov, J. Steevens (Eds.), Nanomaterials: Risks and Benefits, Springer, Netherlands, 2009, pp. 3–29.
[102] K. Donaldson, D. Brown, A. Clouter, R. Duffin, W. MacNee, L. Renwick, L. Tran, V. Stone, The pulmonary toxicology of ultrafine particles, J. Aerosol Med. 15 (2002) 213–220.
[103] W.D. Bennett, Rapid translocation of nanoparticles from the lung to the bloodstream? Am. J. Respir. Crit. Care Med. 165 (2002) 1671–1672 author reply 1672.
[104] M. Geiser, Update on macrophage clearance of inhaled micro- and nanoparticles, J. Aerosol Med. Pulm. Drug Deliv. 23 (2010) 207–217.
[105] H.S. Choi, Y. Ashitate, J.H. Lee, S.H. Kim, A. Matsui, N. Insin, M.G. Bawendi, M. Semmler-Behnke, J.V. Frangioni, A. Tsuda, Rapid translocation of nanoparticles from the lung airspaces to the body, Nat. Biotechnol. 28 (2010) 1300–1303.
[106] Y. Liu, Y. Gao, L. Zhang, T. Wang, J. Wang, F. Jiao, W. Li, Y. Li, B. Li, Z. Chai, G. Wu, C. Chen, Potential health impact on mice after nasal instillation of nano-sized copper particles and their translocation in mice, J. Nanosci. Nanotechnol. 9 (2009) 6335–6343.
[107] A. Elder, R. Gelein, V. Silva, T. Feikert, L. Opanashuk, J. Carter, R. Potter, A. Maynard, Y. Ito, J. Finkelstein, G. Oberdorster, Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, Environ. Health Perspect. 114 (2006) 1172–1178.
[108] M. Crosera, M. Bovenzi, G. Maina, G. Adami, C. Zanette, C. Florio, F. Filon Larese, Nanoparticle dermal absorption and toxicity: a review of the literature, Int. Arch. Occup. Environ. Health 82 (2009) 1043–1055.
[109] J.G. Rouse, J. Yang, J.P. Ryman-Rasmussen, A.R. Barron, N.A. Monteiro-Riviere, Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin, Nano Lett. 7 (2006) 155–160.
[110] J.P. Ryman-Rasmussen, J.E. Riviere, N.A. Monteiro-Riviere, Penetration of intact skin by quantum dots with diverse physicochemical properties, Toxicol. Sci. 91 (2006) 159–165.
[111] J.P. Ryman-Rasmussen, J.E. Riviere, N.A. Monteiro-Riviere, Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes, J. Invest. Dermatol. 127 (2007) 143–153.
[112] A.T. Florence, Nanoparticle uptake by the oral route: fulfilling its potential? Drug Discovery Today: Technologies 2 (2005) 75–81.
[113] P. Jani, G.W. Halbert, J. Langridge, A.T. Florence, Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency, J. Pharm. Pharmacol. 42 (1990) 821–826.
[114] J.J. Powell, N. Faria, E. Thomas-McKay, L.C. Pele, Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract, J. Autoimmun. 34 (2010) J226–J233.
[115] W.I. Hagens, A.G. Oomen, W.H. de Jong, F.R. Cassee, A.J.A.M. Sips, What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul. Toxicol. Pharmacol. 49 (2007) 217–229.
[116] K. Yamashita, Y. Yoshioka, K. Higashisaka, K. Mimura, Y. Morishita, M. Nozaki, T. Yoshida, T. Ogura, H. Nabeshi, K. Nagano, Y. Abe, H. Kamada, Y. Monobe, T. Imazawa, H. Aoshima, K. Shishido, Y. Kawai, T. Mayumi, S.-i. Tsunoda, N. Itoh, T. Yoshikawa, I. Yanagihara, S. Saito, Y. Tsutsumi, Silica and titanium dioxide nanoparticles cause pregnancy complications in mice, Nat Nano 6 (2011) 321–328.
[117] J.F. Hillyer, R.M. Albrecht, Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles, J. Pharm. Sci. 90 (2001) 1927–1936.

[118] A. Nemmar, P.H.M. Hoet, B. Vanquickenborne, D. Dinsdale, M. Thomeer, M.F. Hoylaerts, H. Vanbilloen, L. Mortelmans, B. Nemery, Passage of inhaled particles into the blood circulation in humans, Circulation 105 (2002) 411–414.
[119] Z.Q. Ji, H. Sun, H. Wang, Q. Xie, Y. Liu, Z. Wang, Biodistribution and tumor uptake of C60(OH) x in mice, J. Nanopart. Res. 8 (2006) 53–63.
[120] G. Oberdorster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, A. Lunts, W. Kreyling, C. Cox, Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats, J. Toxicol. Environ. Health A 65 (2002) 1531–1543.
[121] P.J. Borm, D. Robbins, S. Haubold, T. Kuhlbusch, H. Fissan, K. Donaldson, R. Schins, V. Stone, W. Kreyling, J. Lademann, J. Krutmann, D. Warheit, E. Oberdorster, The potential risks of nanomaterials: a review carried out for ECETOC, Part. Fibre Toxicol. 3 (2006) 11.
[122] S.S. Nigavekar, L.Y. Sung, M. Llanes, A. El-Jawahri, T.S. Lawrence, C.W. Becker, L. Balogh, M.K. Khan, 3H dendrimer nanoparticle organ/tumor distribution, Pharm. Res. 21 (2004) 476–483.
[123] G. Xie, C. Wang, J. Sun, G. Zhong, Tissue distribution and excretion of intravenously administered titanium dioxide nanoparticles, Toxicol. Lett. (2011).
[124] Q. He, Z. Zhang, F. Gao, Y. Li, J. Shi, In vivo biodistribution and urinary excretion of mesoporous silica nanoparticles: effects of particle size and PEGylation, Small 7 (2011) 271–280.
[125] M. Longmire, P.L. Choyke, H. Kobayashi, Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats, Nanomedicine 3 (2008) 703–717.
[126] S. Linse, C. Cabaleiro-Lago, W.F. Xue, I. Lynch, S. Lindman, E. Thulin, S.E. Radford, K.A. Dawson, Nucleation of protein fibrillation by nanoparticles, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 8691–8696.
[127] G. Oberdorster, A. Elder, A. Rinderknecht, Nanoparticles and the brain: cause for concern? J. Nanosci. Nanotechnol. 9 (2009) 4996–5007.
[128] G. Oberdorster, Toxicokinetics and effects of fibrous and nonfibrous particles, Inhal. Toxicol. 14 (2002) 29–56.
[129] K. Donaldson, F.A. Murphy, R. Duffin, C.A. Poland, Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma, Part. Fibre Toxicol. 7 (2010) 5.
[130] P.K. Myllynen, M.J. Loughran, C.V. Howard, R. Sormunen, A.A. Walsh, K.H. Vahakangas, Kinetics of gold nanoparticles in the human placenta, Reprod. Toxicol. 26 (2008) 130–137.
[131] T. Tsuchiya, I. Oguri, Y.N. Yamakoshi, N. Miyata, Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo, FEBS Lett. 393 (1996) 139–145.