NATO Science for Peace and Security Series - C:
Environmental Security

Nanomaterials: Risks and Benefits

Edited by
Igor Linkov
Jeffery Steevens

Springer

This publication is supported by: The NATO Science for Peace and Security Programme

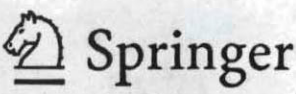
Nanomaterials: Risks and Benefits

Edited by

Igor Linkov
US Army Engineer Research
and Development Center
Concord, Massachusetts
U.S.A.

and

Jeffery Steevens
US Army Engineer Research
and Development Center
Vicksburg, Mississippi
U.S.A.



Published in cooperation with NATO Public Diplomacy Division

NATO Science for Peace and Security Series

This Series presents the results of scientific meetings supported under the NATO Programme: Science for Peace and Security (SPS).

The NATO SPS Programme supports meetings in the following Key Priority areas: (1) Defence Against Terrorism; (2) Countering other Threats to Security and (3) NATO, Partner and Mediterranean Dialogue Country Priorities. The types of meeting supported are generally "Advanced Study Institutes" and "Advanced Research Workshops". The NATO SPS Series collects together the results of these meetings. The meetings are co-organized by scientists from NATO countries and scientists from NATO's "Partner" or "Mediterranean Dialogue" countries. The observations and recommendations made at the meetings, as well as the contents of the volumes in the Series, reflect those of participants and contributors only; they should not necessarily be regarded as reflecting NATO views or policy.

Advanced Study Institutes (ASI) are high-level tutorial courses intended to convey the latest developments in a subject to an advanced-level audience

Advanced Research Workshops (ARW) are expert meetings where an intense but informal exchange of views at the frontiers of a subject aims at identifying directions for future action

Following a transformation of the programme in 2006 the Series has been re-named and re-organised. Recent volumes on topics not related to security, which result from meetings supported under the programme earlier, may be found in the NATO Science Series.

The Series is published by IOS Press, Amsterdam, and Springer, Dordrecht, in conjunction with the NATO Public Diplomacy Division.

Sub-Series

A.	Chemistry and Biology	Springer
B.	Physics and Biophysics	Springer
C.	Environmental Security	Springer
D.	Information and Communication Security	IOS Press
E.	Human and Societal Dynamics	IOS Press

http://www.nato.int/science
http://www.springer.com
http://www.iospress.nl

Series C: Environmental Security

HUMAN HEALTH RISKS OF ENGINEERED NANOMATERIALS

Critical Knowledge Gaps in Nanomaterials Risk Assessment

A. ELDER
Department of Environmental Medicine
University of Rochester
575 Elmwood Avenue, Box 850
Rochester, NY 14642, USA
Alison_Elder@urmc.rochester.edu

I. LYNCH
Centre for BioNanoInteractions
School of Chemistry and Chemical Biology
University College Dublin
Belfield, Dublin 4, Ireland

K. GRIEGER
Technical University of Denmark
Department of Environmental Engineering
Building 113
Kongens Lyngby 2800, Denmark

S. CHAN-REMILLARD
Golder Associates Ltd./HydroQual Laboratories Ltd.
#4 6125-12th Street S.E.
Calgary T2H 2K1, Canada

A. GATTI
University of Modena & Reggio Emilia
Lab of Biomaterials
Via Campi 213 A
Modena 41100, Italy

H. GNEWUCH
Naneum Ltd.
Canterbury Enterprise Hub
Canterbury CT2 7NJ, UK

E. KENAWY
Polymer Research Group, Department of Chemistry
Faculty of Science, University of Tanta
Egypt

I. Linkov and J. Steevens (eds.), *Nanomaterials: Risks and Benefits*,

A. SHVEDOVA
CDC/NIOSH
1096 Willowdale Road
Morgantown, WV 26505, USA

Abstract. There are currently hundreds of available consumer products that contain nanoscale materials. Human exposure is, therefore, likely to occur in occupational and environmental settings. Mounting evidence suggests that some nanomaterials exert toxicity in cultured cells or following in vivo exposures, but this is dependent on the physicochemical characteristics of the materials and the dose. This Working Group report summarizes the discussions of an expert scientific panel regarding the gaps in knowledge that impede effective human health risk assessment for nanomaterials, particularly those that are suspended in a gas or liquid and, thus, deposit on skin or in the respiratory tract. In addition to extensive descriptions of material properties, the Group identified as critical research areas: external and internal dose characterization, mechanisms of response, identification of sensitive subpopulations, and the development of screening strategies and technology to support these investigations. Important concepts in defining health risk are reviewed, as are the specific kinds of studies that will quickly reduce the uncertainties in the risk assessment process.[1]

1. Introduction

Nanomaterials are commonly described as having at least one dimension smaller than 100 nm. A broader definition, though, refers to those materials that are manipulated at the atomic, molecular, or macromolecular scales in order to achieve functionality that is different from that found in the bulk or molecular form [106].

Many consumer items are already available that contain nanomaterials, such as electronics components, cosmetics, cigarette filters, antimicrobial and stain-resistant fabrics and sprays, sunscreens, and cleaning products [115]. According to a recent survey of the Wilson Institute web site [29], there are at least 580 consumer products on the market, including four with FDA approval for therapeutic use. Although the potential for human exposures has not been fully evaluated and is likely to be low in many cases, the safety of nanomaterials at a wide range of doses and throughout the product life cycle should be characterized to ensure consumer, occupational, and environmental health.

Critical components of a systematic safety assessment for engineered nanomaterials include: evaluation of exposure concentrations in occupational and

[1] Summary of the NATO ARW Working Group discussions.

R. KORENSTEIN
Marian Gertner Institute for Medical Nanosystems
Department of Physiology and Pharmacology, Faculty of Medicine
Tel Aviv University
69978 Tel-Aviv, Israel

T. KUHLBUSCH
Institute for Energy and Environmental Technology
Bliersheimer Street 60
Duisburg 47229, Germany

F. LINKER
Occupational Health Care Services, DSM
ARBODienst DSM, Alert & Case Centre
Kerenshofweg 200
NL-6167AE Geleen, The Netherlands

S. MATIAS
Instituto Superior Téchnico
Universidade Téchnica de Lisboa
Av. Rovisco Pais
1049-001 Lisboa, Portugal

N. MONTEIRO-RIVIERE
Center for Chemical Toxicology Research and Pharmacokinetics
Department of Clinical Sciences, College of Veterinary Medicine
North Carolina State University
4700 Hillsborough Street
Raleigh, NC 27606, USA

V.R.S. PINTO
Rua Capote Valente 710
São Paulo 05409-002, Brazil

R. RUDNITSKY
Office of Space & Advanced Technology
US Department of State
OES/SAT, SA-23, 1990 K Street, NW, Suite #410
Washington, DC 20006, USA

K. SAVOLAINEN
Finnish Institute for Occupational Health, New Technologies and Risks Topeliuksenkatu 41 aA
GI-00250 Helsinki, Finland

environmental settings; the physicochemical characteristics of the material at the portal of entry; the structure and function of epithelial barriers at the portals of entry; interactions of materials with biomolecules (proteins, nucleic acids, lipids); biodistribution and elimination kinetics and identification of possible target organs; characterization of dose-response relationships; elucidation of mechanisms of response; identification of target tissues for nanomaterials effects; and identification of human subpopulations with unique susceptibility to the effects of nanomaterials. These concepts are summarized in Figure 1. New products are rapidly emerging in the nanotechnology industry without a parallel development of critical information regarding their safety. Furthermore, risk assessments are currently proceeding in many cases without adequate methodologies to define risk.

It should be noted that the assumptions used in assessing risks at the early stages of most emerging technologies are designed to be protective (precautionary principle) and to emphasize potential problems so that more attention is focused on managing or mitigating such risks. As the technology progresses through the product life cycle, more data becomes available and, thus, the assumptions used in risk assessment become more realistic [10, 94]. This article focuses on the critical knowledge gaps that impede the risk assessment process as well as strategies for rapid reductions in those uncertainties.

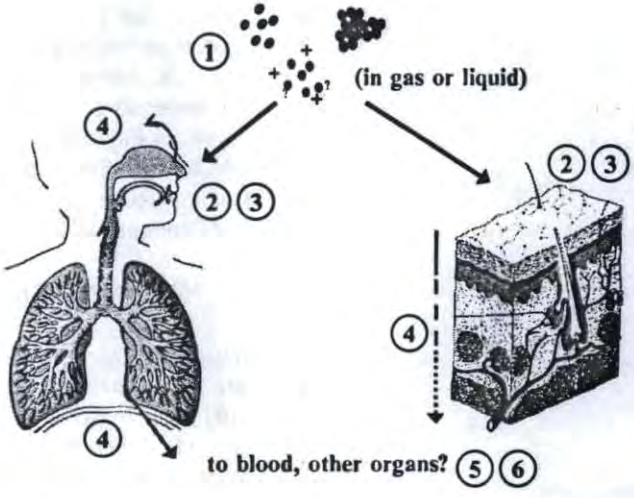


Figure 1. Key issues in assessing human health risk following nanomaterials exposures. (1) What is the nature of the nanomaterial at the portal of entry (e.g. agglomerated, charged, soluble, size?)?; (2) How do the physicochemical characteristics of nanomaterials change after deposition in the body (specific changes likely to depend on portal of entry)?; (3) Do nanomaterials penetrate epithelial barriers?; (4) Are nanomaterials transported away from the portal of entry to other organs (how much is transported? What are the target tissues?)?; (5) How do the nanomaterial properties changes as they are transported in the body (dissolution; protein/lipid binding)?; (6) How do responses at the cellular/tissue level affect transport of nanomaterials?

2. Characterization of Nanomaterial Exposure

Although there is potential for occupational and environmental exposures to nanomaterials throughout their life cycle, very little is known about the concentrations of such exposures. Furthermore, the characteristics of nanoscale materials (e.g. size, shape, surface charge, agglomeration state, presence of secondary coatings from air or liquid carrier) as they might be encountered in the workplace or the environment are largely unknown.

Workplace exposure data for nanoparticles is scarce. However, Maynard et al. [59] reported peak airborne levels of respirable particles of single-walled carbon nanotubes up to 53 $\mu g/m^3$ in a small university laboratory. Han and colleagues [28] reported airborne levels of multi-walled carbon nanotubes during spraying, blending, and weighing operations in a research laboratory that ranged from undetectable levels to ~400 $\mu g/m^3$. However, these data are from total particulate samples at the breathing zone and, thus, the total mass concentration was not comprised exclusively of nanotubes. Nevertheless, incorporation of control measures reduced the nanotube-containing dust concentrations to background levels.

A recent leaflet from NIOSH regarding workplace exposures to nanomaterials states that current methods for controlling exposures are adequate, but that current measurement techniques "are limited and require careful interpretation" [69]. These somewhat contradictory statements reflect the need for personnel with extensive experience and specialized training in the handling and sampling of nanomaterials. Although NIOSH cites a lack of sufficient evidence as the basis for not recommending specific surveillance of nanoparticle-exposed workers, a framework for the safe exploitation of nanotechnology has recently been described that includes recommendations for methods and instrumentation to assess exposure levels, characterize particle size and surface area distributions, and to identify sources of nanoparticle release [58, 67, 68].

2.1. NANOMATERIALS CHARACTERIZATION

One critical research need is the development of methods and equipment for adequate nanomaterial characterization, as has been previously cited [4, 84, 95, 109, 110]. Nanomaterial properties may also be altered in both biotic and abiotic environments. Therefore, tools to detect and characterize chemical or physical modifications of nanomaterials in such environments are needed. There is also a pressing need to develop standardized assessments of particle characteristics including size, shape, size distribution, structure and surface area [70]. This would ensure that the same set of characteristics is described across studies, ultimately facilitating a comparison between materials and subsequent exposure. Another critical need is viewed to be the development of a set of reference nanomaterials that can serve as benchmarks for the investigation of other nanomaterials, thereby providing a basis for comparison. Reference materials are commonly used in traditional risk assessment frameworks for effects and exposure analyses. Significant efforts are being made in this regard, both by the National Institute of Standards

and Technology (US) and the Institute of Reference Materials and Measurements (EU), although the initial focus is on reference materials for calibration of instrumentation with respect to size determination, rather than reference materials for benchmarking of potential toxicity. At present, the scientific community lacks a set of commonly accepted reference materials, including consensus on suitable positive and negative control nanoparticles for different testing systems.

2.2. CHARACTERIZATION OF EXPOSURES

Assessing external human exposure to nanomaterials requires knowledge regarding the likelihood of exposure, changes in particle concentration over time, and identification and characterization of exposure directly prior to uptake. Workplace or ambient exposures to air- or liquid-suspended nanomaterials may occur. Although estimates have been reported for selected nanosized compounds [66], no data is available about actual levels of engineered nanomaterials in ambient environments, mainly due to the limitations of current measurement methods. There is clearly a need for a comparative exposure assessment which differentiates the routes and forms of exposure as well as the morphology of the nanomaterials. This section will mainly address inhalation exposures in the workplace, because this is currently seen as the most likely exposure scenario. However, skin and gastrointestinal tract exposures to gas- or liquid-suspended particles are also possible. Further details are provided in Kuhlbusch et al. [43] in this same edition.

2.2.1. Measurement Methods

Measurement methods for detection of airborne (nano-) particles can be characterized as (1) online/offline detection methods that distinguish environmental from product materials, (2) methods for different matrices (gas/liquid/solid), (3) personal or fixed sampling methods, (4) methods for different exposure metrics (mass, surface area or number concentration (total and size-resolved), chemical composition, etc.), and (5) methods that predict lung regional deposited dose.

No optimal method is currently available for measuring nanomaterials exposures, since, for example, the ideal metric is still a matter of debate. Certainly, the best method would be a personal sampler that determines all relevant physical and chemical properties in real time or near-real time within discrete particle size bins. This is, however, currently unavailable. Nevertheless, first steps towards simultaneously determining these properties are ongoing and are of extreme importance for realistic exposure assessment.

Most exposure measurements have either used an online technique to determine particle size distribution [42, 46, 63, 114] or offline techniques like thermal or electrostatic precipitation or diffusion/impaction and subsequent particle characterization [23, 82]. The choice of using particle number-weighted, as opposed to mass-weighted, size distribution measurements is driven by the expense and availability of the equipment, the high sensitivity of number concentration measurements towards nanosized particles, the possible relevance of particle number concentration for health effects, and the requirement for speciation. Of

similar importance with regard to linking particle properties to health may be the particle surface area, either as inhalable (Matter LQ 1-DC) or lung deposited fraction (TSI NSAM). An overview on measurement methods for nanoparticle detection can be found in Kuhlbusch et al. [44].

2.2.2. *Measurement Strategies*

One measurement challenge is the differentiation of environmental (background) from engineered nanoparticles. When deciding on measurement strategies and methods, the following points have to be taken into account. First, there is a need for a dynamic detection range, from a single particle to high number concentrations. Secondly, there is a need for particle physical and chemical characterization. Lastly, the time resolution (online/offline) must be considered.

There are three particle concentration ranges in terms of number that can currently be evaluated [43]: single particle detection, a concentration of 1,000–100,000 particles per cm^3, and a concentration of more than 100,000 particles per cm^3. Detection of single particles can be achieved using either single particle aerosol mass spectrometry (AMS) [72] or filter sampling with subsequent single particle analysis by TEM/EDX. Both techniques have their advantages and limitations, for example, the degree of chemical analysis that is possible. These methods would allow a differentiation of background from engineered nanoparticles

Detection of the source of particle concentrations >100,000 particles per cm^3 should generally be easy since the source must be in the vicinity of the point of measurement. The source can either be visually identified or detected by determining spatial particle number concentration profiles.

The difficulty in assessing nanoparticle exposure at levels between 1,000–100,000 particles per cm^3 is that background particle concentrations can be in the same concentration range. A first assessment of possible nanoparticle exposure can be conducted by concurrent measurements of ambient and workplace particle number concentrations and calculation of ambient particle penetration into the work area. This approach is possible for concentrations down to a few thousand particles per cubic centimeter [45]. Hence, clear differentiation of nanoparticles from environmental nanoscale particles can only be done by the methods described for single particle analysis.

2.2.3. *Levels of Exposure*

The limited exposure measurements conducted thus far in the workplace generally show low levels or levels below the detection limits for exposure during normal production and handling of nanomaterials. However, the adequacy of existing detection instrumentation needs to be considered. The exposure-related measurements were conducted in all steps of production and handling from the reactor, to processing and handling/bagging of, for example, carbon black and titanium dioxide [38, 45]. Measurements conducted in the presence of a leak within the palletizing line showed high exposure values indicating that exposure can be

possible, especially in cases where engineering controls fail or during cleaning and maintenance work in large scale nanomaterial production.

Measurements of dustiness of powders containing nanomaterials were conducted by Dahman and Monz [14] in the framework of the NanoCare Project. This investigation showed that engineered particles below 100 nm were not normally released using a counter flow system. However, there were exceptions depending on the material investigated. This example shows that extrapolations from few measurements and generalizations to other materials should be done carefully.

2.2.4. Future Tasks

Results are eagerly awaited from ongoing investigations focusing on possible human exposure during the life cycle of nanomaterials, from production, to their use in products, and during recycling. Several scenarios exist with different degrees of likelihood of possible release of nanomaterials into the environment and subsequent exposure. The following tasks are seen to bring advances in exposure assessments for nanoscale materials: the development of cost-effective screening methodologies for assessing exposure, the development of devices that measure personal exposure, evaluation of the adequacy of health surveillance protocols, strengthening current methods for assessing agglomerate stabilities in order to predict the potential for nanoparticle release during handling, the evaluation of nanoparticle aging during transport (e.g. airborne, in water), and improvements in the link between exposure assessments and dose metrics.

3. Barrier Function of Skin, Gastrointestinal Tract, and Respiratory Tract

If it can be assumed that most exposures to nanomaterials will occur in air or via the food chain/drinking water, then the respiratory tract, skin, and gastrointestinal tract are the primary routes of exposure to nanomaterials. However, other routes such as intravenous, intradermal, and ocular are important to consider for specialized applications. A critical component in evaluating the health risks associated with nanomaterials exposure is knowledge regarding barrier function at the portal of entry.

3.1. GASTROINTESTINAL TRACT

The gastrointestinal (GI) tract is not likely to be a primary route of exposure to nanomaterials. However, particles that deposit in the respiratory tract and taken up by alveolar macrophages are cleared via the mucociliary escalator and then expectorated or swallowed. Some of the particulate matter, then, that deposits in the lungs could be cleared to the GI tract (see following discussion about macrophage-mediated clearance of nanosized particles). However, the barrier function of the GI tract with respect to nanoparticles is somewhat equivocal.

The transfer of nanoparticles into blood and subsequent tissue distribution is likely to be very dependent on particle surface characteristics because of the

extreme shifts in acidity and the negatively charged mucous layer in the small intestine. Early work described the process of persorption, whereby micron-sized insoluble particles are transported from the intestinal lumen to the blood via paracellular pathways [113]. This process has been shown in in vivo studies to be size-dependent, with smaller particles (polystyrene microspheres, colloidal gold) being absorbed to a greater degree than larger ones [32, 35]. However, studies with highly insoluble radioactive metal nanoparticles have shown extremely low transfer into blood following GI tract exposures [41, 103], with some evidence for an inverse relationship between particle size and percent transfer as well as for negatively-charged particles having higher transfer rates [97]. Recent studies employing electron microscopy and elemental analysis have identified nanosized particulates, possibly from combustion sources or food, in human tissues such as liver, kidney, and colon [20–22]. Although it is not clear how the particles accumulated in these organs, both digestive and respiratory tact exposures are possible explanations. In vitro model systems are likely to have limited predictive power due to the absence of a mucous layer, which traps charged particles and potentiates their clearance via the feces.

3.2. SKIN

Skin is the largest organ of the body. Its permeability to engineered nanomaterials with respect to depth of penetration and interactions with structural components as well as nanoparticle absorption into blood are not well understood. Recent in vitro studies have employed flow-through diffusion cells to assess nanoparticle penetration and absorption through skin.

3.2.1. Potential for Nanomaterials Skin Penetration

Nanomaterials must penetrate the stratum corneum layer in order to exert toxicity in the lower cell layers. The quantitative prediction of the rate and extent of percutaneous penetration (into skin) and absorption (through skin) of topically applied nanomaterials is complicated due to many biological complexities, such as the diversity of the skin barrier function across species and body sites. The stratum corneum affords the greatest deterrent to absorption. Although the dead, keratinized cell layer itself is highly hydrophobic, the cells are also highly water-absorbing, a property that keeps the skin supple and soft as water is evaporated from the surface. Sebum appears to augment the water-holding capacity of the epidermis; however, its hydrophobic nature cannot be assumed to retard the penetration of xenobiotics, including nanomaterials. The rate of diffusion of topically-applied materials across the stratum corneum is directly proportional to the concentration gradient of the material across the membrane, the lipid/water partition coefficient of the material, and the diffusion coefficient of the material. It should be noted that organic vehicles may themselves penetrate into the intercellular lipids of the stratum corneum, thus affecting diffusion. Depending on the specific characteristics of the skin barrier, there is a functional molecular size/weight cut-off that prevents very large molecules from being passively absorbed across any membrane. The total

flux of any material across the skin is also dependent upon the exposed area, with dose expressed as mass per square centimeter. In vitro studies of nanomaterial penetration of skin may only approximate the in vivo situation since a long period of time may be required to achieve steady state conditions and, thus, exceed the time constraints of in vitro evaluations.

Transdermal flux (penetration) with systemic absorption of topically applied nanomaterials has obvious implications in toxicology and therapeutic drug delivery. However, knowledge of the depth and mechanism of particle penetration into the stratum corneum barrier is crucial. The skin provides an environment within the avascular epidermis where particles could potentially lodge and not be susceptible to removal by phagocytosis, yet be available for immune recognition through interaction with resident Langerhans cells. In fact, it is this relative biological isolation in the lipid domains of the epidermis that has allowed for the delivery of drugs to the skin using liposomal preparations.

Several studies have evaluated the hypothesis that nanoparticles can get through or get lodged within the lipid matrix of skin. Zinc oxide (ZnO, 80 nm) and agglomerates of titanium dioxide (TiO_2) smaller than 160 nm did not penetrate the stratum corneum of porcine skin in static diffusion cells [19]. Likewise, in vitro application of ZnO nanoparticles (26–30 nm) in a sunscreen formulation to human skin led to accumulation of nanoparticles in the upper stratum corneum with minimal penetration [13]. However, a pilot study conducted in humans about to undergo surgery showed penetration to the dermis of "microfine" TiO_2 that was applied over a period of 2–6 weeks [105]. Block copolymer nanoparticles (40 nm) that were topically applied to hairless guinea pig skin in diffusion cells were able to penetrate the epidermis within 12 h [99]. Additional studies with spherical (QD565, the number refers to the fluorescence emission maximum) and elliptical (QD655) CdSe-ZnS semiconductor nanocrystals that were applied to porcine skin in flow-though diffusion cells showed that penetration is dependent on surface coating or charge. Polyethylene glycol (PEG)- and carboxylic acid-coated QD565 were localized primarily in the epidermis by 8 h, while the QD565 PEG-amine penetrated to the dermis. However, shape was also shown to be a determinant of nanocrystal localization by the fact that the carboxylic acid-coated elliptical crystals (QD655) did not penetrate into the epidermis until 24 h of exposure [88]. Studies have also reported that nanocrystal surface coatings and charge can influence their toxicity in human epidermal keratinocytes [89]. These results highlight the diversity in terms of size and composition of particles that could possibly penetrate the stratum corneum to reach the deeper, viable layers of skin.

3.2.2. *Factors that Affect Penetration Through Skin*

Recent studies have demonstrated that mechanical action and perturbations of the skin barrier can affect the penetration of nanoparticles. For example, Tinkle et al. [108] reported that even large (0.5 μm) FITC-conjugated dextran beads could penetrate the stratum corneum of human skin and reach the epidermis following 30 min of flexing. However, the particles did not penetrate the skin at all if it was not mechanically flexed. Smaller amino acid-derivatized fullerene nanoparticles

(3.5 nm) were able to penetrate to the dermis of porcine skin that was flexed for 60 min and placed in flow-through diffusion cells for 8 h; non-flexed control skin showed penetration that was limited to the stratum granulosum layer of the epidermis [65, 87]. QD655 and QD565 coated with carboxylic acid (hydrodynamic diameters of 18 and 14 nm, respectively) were studied for 8 and 24 h in flow-through diffusion cells with flexed, tape stripped and abraded rat skin. No penetration occurred with the nonflexed, flexed, or tape-stripped skin. However, penetration to the viable dermal layer occurred in abraded skin. In some cases, retention of QD in hair follicles was observed in the abraded skin [117].

Another important consideration is the possible retention of nanoparticles in hair follicles, as has been alluded to above. Lademann and colleagues [48] showed that TiO_2 microparticles and polystyrene nanoparticles could be localized near orifices in human hair follicles. Agglomerates of iron oxide and maghemite nanoparticles with organic coatings (primary particle sizes ~5 nm) have been shown to penetrate hair follicles and the epidermis of previously frozen human skin surgical samples, suggesting a potential capacity for nanoparticles to traverse the dermal barriers [6]. Other studies with TiO_2 and methylene bis-benzotriazoyl tetramethylbutylphenol showed only 10% of the formulation remained in the furrows of the stratum corneum and infundibulum of the hair follicle of human skin [57]. QD621, nail-shaped PEG-coated CdSe-CdS nanocrystals that were topically applied to porcine skin in flow-through diffusion cells for 24 h penetrated the upper layers of the stratum corneum and were primarily retained in hair follicles and in the intercellular lipid layers, a situation also seen with carbon fullerenes [118]. Although it appears that only a small amount of the applied nanomaterial is retained in hair follicles, the kinetics of this retention and the possibility of subsequent systemic distribution must be evaluated.

3.2.3. Potential for Nanomaterials Absorption into Blood from Skin

The evaluation of nanomaterial absorption into blood is a complex matter, so results from in vitro systems that do not have intact microcirculation should be carefully interpreted. Furthermore, human and porcine skin may react differently with respect to nanoparticle penetration as compared to smaller organic chemicals and drugs where, as described above, human and porcine skin are very similar. Nevertheless, most recent work has demonstrated that absorption into blood would not be predicted following topical application of nanomaterials to skin. For example, QD621 nanocrystals that were applied to porcine skin in flow-through diffusion cells were not found in the perfusate at any time point or concentration [118]. Likewise, studies with QD565 coated with PEG, PEG-amine, or carboxylic acid that were topically applied to human skin in diffusion cells for 8 or 24 h showed that all three QD preparations remained on the surface of the stratum corneum or were retained within hair follicle invaginations, but were not detected in the perfusate [64]. Similar observations were made by this same group with porcine skin exposed to the same particles [88]. A recent in vivo study, though, showed that nanosized TiO_2 that was applied topically to pig skin in sunscreen

formulation did not accumulate in lymph node or liver tissue following exposures for 5 days per week for 4 weeks [90].

These studies demonstrate the complexity of skin and the stratum corneum lipid barrier with respect to assessing nanoparticle penetration and absorption into blood. In most cases studied to date, topically applied nanoparticles have not been shown to be absorbed into the systemic circulation. However, penetration into the stratum corneum can occur in all animal species studied. This penetration could be significant relative to immunological and carcinogenic endpoints. Current findings suggest that surface coatings as well as nanoparticle geometry also seem to modulate penetration. All of these factors must be studied further if realistic risk assessments of manufactured nanomaterials are to be made.

3.3. RESPIRATORY TRACT

3.3.1. The Pulmonary Epithelial Barrier

Nanoparticles that are inhaled as singlets have high predicted deposition efficiencies via diffusional processes in all regions of the respiratory tract [34]. For singlet particles of ~20 nm, the highest fractional deposition occurs in the alveolar region, where bulk air flow is low or absent [93]. Nanosized particles are not efficiently taken up by resident phagocytic cells (alveolar macrophages) [1, 27] unless they are agglomerated, thus promoting their retention in the lung and increasing the likelihood of interactions with the epithelial barrier. The alveolar epithelial barrier has a large surface area (80–140 m^2 in humans) [92] and is extensively vascularized. In a healthy lung, there are only a few cell types with which nanomaterials might interact in the alveolus: type I epithelial cells (which cover ~95% of the alveolar surface), type II epithelial cells, and macrophages. The basement membranes of the type I epithelial cells are continuous with those of endothelial cells in the pulmonary capillaries, so the total thickness through which nanoparticles have to travel to reach the blood is 0.3–2.5 μm, including the interstitial space [80].

The composition of lung lining fluid varies by region of the respiratory tract. In the alveolar region, the lining fluid consists of surfactants and an overlying aqueous phase. Pulmonary surfactant is ~90% lipids (mainly disaturated dipalmitoylphosphatidyl-choline and phosphatidylglycerol with smaller amounts of cholesterol) and 10% proteins, which are secreted by type II alveolar epithelial cells [26]. The alveolar lining fluid also contains plasma-derived proteins (e.g. albumin, transferrin, immunoglobulins) that are critical to host defense functions [39]. The degree to which nanomaterials might interact with these lipids and proteins in situ is largely unknown.

3.3.2. Fate of Nanoparticles that Cross the Alveolar Epithelial Barrier

An important factor in assessing the toxicity of nanomaterials is their distribution throughout the body and persistence in tissues following exposure. Obviously, this

is an issue that is difficult to fully address using in vitro model systems. Translocation to extrapulmonary tissues, including the liver and various brain regions (notably the olfactory bulb), has been demonstrated, albeit in small amounts, for inhaled nanosized poorly-soluble Mn oxide, ^{13}C, Ag, and ^{192}Ir [18, 41, 77, 78, 104]. In the case of the Mn oxide and ^{13}C nanoparticles, the observed targeting of the olfactory bulb was reported to be due to transport along the olfactory nerve, which has projections terminating directly into the nasal cavity. In regards to targeting of neuronal structures, though, deposition in the nose or alveoli is not an absolute requirement. Studies by Hunter and Undem [33] showed that nodose and jugular ganglia of the vagus nerve could be targeted by the intratracheal instillation of dye tracer particles.

Interestingly, Semmler and colleagues [96] showed that the retention and clearance kinetics of insoluble radioactive Ir nanoparticles (15–20 nm, count median diameter) was not different from reports in the literature for larger particles (polystyrene beads), although this was a mathematical exercise and not a direct comparison to larger particles with the same chemistry. However, later studies by this group showed that what was different was the degree of interstitialization of the nanosized ^{192}Ir particles [98]. Oberdörster et al. [75] also reported that the interstitialization rates were ~10 times higher for nanosized TiO_2 particles delivered to the lungs via intratracheal instillation as compared to larger particles of the same composition. More recently, Shvedova and colleagues [102] demonstrated that single-walled carbon nanotubes (SWCNT) delivered via inhalation exposure (deposited dose of 5 mg/mouse) resulted in the deposition of small SWCNT structures and the induction of cellular inflammation, LDH and protein release, and cytokine production that was two- to fourfold greater than responses that resulted from oropharyngeal aspiration exposure to larger agglomerated SWCNT structures. Morphometric evaluation of Sirius red-stained lung sections also showed that SWCNT inhalation caused a fourfold higher increase in fibrosis compared with that seen after pharyngeal aspiration, with collagen deposition in peribronchial and interstitial areas. Interestingly, Mercer et al. [60] demonstrated a fourfold greater fibrotic potency after pharyngeal aspiration of a well dispersed SWCNT compared to a less dispersed suspension. This potency difference was associated with a greater potential for smaller SWCNT structures to enter the alveolar walls and cause interstitial fibrosis. Overall, these results suggest that inhalation of dispersed SWCNTs leads to greater interstitialization and inflammation as compared to those delivered in an agglomerated bolus by aspiration. Thus, not only is the persistence or retention of the nanomaterials of importance, but so too is the distribution within an organ system.

The liver, kidneys, and spleen have been shown to be the organs with the highest retention of nanoparticles that cross the alveolar epithelial barrier [96, 104]. It is not entirely clear, though, how primary particle size or in vivo dissolution may affect the accumulation of materials in extrapulmonary organs. Some studies have reported very rapid accumulation of nanoparticles, as determined via chemical means, in liver, kidney, and olfactory bulb following respiratory tract exposures [17, 85, 104]. In comparison to the respiratory tract, nanomaterials that

are injected intravenously accumulate in almost all tissues that are harvested [12, 17], although this is somewhat size-and surface chemistry-dependent.

Not surprisingly, surface coating has been shown to be an important determinant of nanoparticle tissue distribution. At least two studies have shown that the attachment of polyethylene glycol (PEG) to the surface of the semiconductor nanocrystals increases their circulatory half-life after intravenous injection [2, 5] due to lowered uptake efficiency by the liver and spleen (reticulo-endothelial system). Reduced efficiency of liver uptake has also been shown for PEGylated nanosized magnetite particles [52]. At least for CdSe-ZnS nanocrystals, the particle size has also been shown to be an important determinant of tissue retention following intravenous injection. Particles with hydrodynamic diameters smaller than ~5.5 nm are almost completely eliminated via urine within the first 4 h [12]. Partly due to the effective cut-off size of the kidney filter, somewhat larger particles are exclusively eliminated over time via the feces [98].

4. Nanomaterials Interactions with Biomolecules

Data from in vivo and in vitro studies suggesting lipid and/or protein oxidation as a result of nanomaterials exposure provides indirect evidence of interactions with biomolecules. For example, Oberdörster et al. [74] demonstrated lipid peroxidation, but not protein oxidation, in brain tissue obtained from largemouth bass that were exposed to aggregated nC_{60} fullerenes in tank water. Should such interactions be a surprise, though? It has long been known that implanted materials acquire a protein coating that ultimately determines the fate of the implant in terms of biocompatibility. While this is likely to be the case at the nanoscale, too, the challenge will be to identify those proteins, lipids, and other biomolecules that interact with nanoparticles in the target organs and then to characterize the kinetic nature of those interactions [54]. Progress along these lines has been made recently with detailed identification of the proteins bound to nanoparticles [8, 9, 16] and the first indications of inappropriate folding leading to protein aggregation in the presence of nanoparticles [50]. A further challenge will be to understand the predictive value of this information in the context of human risk assessment.

4.1. INTERACTIONS WITH PROTEINS

Within the medical device community, it is now well accepted that material surfaces are modified by the adsorption of biomolecules such as proteins in a biological environment, and there is some consensus that cellular responses to materials in a biological medium reflect the adsorbed biomolecule layer, rather than the material itself [25, 55, 73]. Interestingly, recent studies suggest that nanomaterial surfaces, having much larger surface area than flat ones, are more amenable to studies to determine the identity and residence times of adsorbed proteins [9, 40]. The recently introduced concept of the nanoparticle-protein corona sees the adsorbed protein (biomolecule) layer as an evolving collection of

proteins that associate with nanoparticles in biological fluids, and suggests that this is the biologically relevant entity that interacts with cells [53].

A recent systematic study of interactions of polystyrene nanoparticles with no modification (plain) or modified with positive (amine) or negative (carboxylic) charges indicates that the surface and the curvature (particle size) both influence the details of the adsorbed proteins, although in all cases, a significant fraction of the proteins bound were common across all particles [51]. The significance of this for safety assessment is clear, as it implies that detailed characterization of the nanoparticles in the relevant biological milieu is vital.

Evidence is emerging in the scientific literature that coating of nanoparticles with specific proteins can direct them to specific locations – apolipoprotein E, for example, has been associated with transport of nanoparticles to the brain [61]. The binding of serum albumin to the surface of carbon nanotubes has also been shown to induce particle uptake and anti-inflammatory responses in a macrophage cell line [15].

However, there are several complicating factors, such as the fact that the biomolecule corona is not fixed, but is rather dynamic. The corona equilibrates with the surroundings, with high abundance proteins binding initially, but being replaced gradually by lower abundance, higher affinity proteins. Additionally, changes in the biomolecule environment, such as during particle uptake and distribution, will be reflected as changes in the corona. This makes for considerable difficulty in determining the nanoparticle biomolecule corona *in-situ*, as attempts to recover the particles for measurement by isolating them from their surroundings will by their very nature alter the subtle balance of the biomolecule corona. However, the situation is not all bad. A considerable portion of the biologically relevant biomolecules – the so-called "hard-corona" [51] – will remain associated with the nanoparticles for a sufficiently long time so as not to be affected by the measurement processes.

First indications of a potential role for nanoparticles in misfolding and aggregation events [7, 50] as well as inhibition of misfolding [83] are emerging. A range of different nanoparticles, including polymer particles, cerium oxide, carbon nanotubes and PEG-coated quantum dots, enhanced the rate of fibrillation of the amyloidogenic protein β-2-microglobulin under conditions where the protein was in the slightly molton-globular state at pH 2.5 [50]. A mechanism based on a locally high concentration of the protein in the vicinity of the nanoparticle surface, thus increasing the probability of formation of a critical oligomer, was proposed. A recent report from Bellezza and colleagues [7] demonstrated the interaction of myoglobin (Mb) with phosphate-grafted zirconia nanoparticles. Adsorption induced marked rearrangements of Mb structure, particularly loss of the secondary structure (α-helices). Two distinct structures were observed: (i) globular aggregates, similar to those for the native protein, and (ii) very extensive, branching structures of Mb, with morphological properties similar to Mb prefibrillar aggregates. In this case, the authors suggest that the prefibril-like aggregates were always observed next to the zirconia nanoparticles, suggesting that these structures develop from the bound protein. Studies in animals have shown that C_{60} hydrated fullerene may have anti-amyloidogenic capacity, as a single intracerebroventricular injection of a C_{60}

hydrated fullerene significantly improved the performance of a cognitive task in control rats, resulting from inhibition of the fibrillation of amyloid-beta 25-35 peptide [83]. These results may offer a significant therapeutic advantage towards diseases of the brain, which are often intractable, as well as raising the potential for risk.

A recent review has summarized much of the current state-of-the-art in protein-nanoparticle interactions [54]. A major hope of this field of research is that it will be possible in the future to predict biological impacts of nanoparticles based on a screening of the proteins for which they have the highest affinity, and an understanding of the role of these proteins in nanoparticle uptake, trafficking and subcellular localization.

4.2. INTERACTIONS WITH LIPIDS

There are almost no reports of the interaction between nanoparticles and lipids to date, although considerable work has been done to develop solid lipid nanoparticles for targeted drug delivery [36, 81] or using lipids such as phosphorylcholine or oleic acid to stabilize nanoparticles, including enabling their transfer from organic solvents to aqueous solutions [11, 24]. Several reports on the use of lipid coatings to reduce protein binding have also been published recently. Ross and Wirth [86] reported that laterally diffusible phosphocholine bilayers inside the pores of colloidal silica nanoparticles suppressed 93% of the binding of avidin relative to the unmodified silica colloidal crystals. Another recent report shows that gold nanorods can be coated with lipid bilayers and used as sensors for protein binding, but that the process is complex and requires issues such as membrane curvature and adhesion properties [3].

Some studies with the original aim of quantifying the binding of lipids to nanoparticles have been used as controls within broader studies of protein binding to nanoparticles. For example, a recent study of human serum albumin (HSA) binding to polymeric nanoparticles found that the thermodynamics of binding was very different in the presence and absence of oleic acid, which is a major binding ligand of HSA. Using isothermal titration calorimetry, the authors found that HSA binding to the polymeric particles is exothermic, whereas in the presence of oleic acid the adsorption is endothermic. Binding of oleic acid to the particles was found to be endothermic [49].

On the basis of the discovery that lipoproteins have a large affinity for nanoparticles of many different surface compositions, an obvious question that arises is whether the particles are actually binding the lipoprotein complexes. Thus, apolipoproteins in blood associate with lipoprotein particles, e.g. chylomicrons (>100 nm) and high density lipoproteins (8–10 nm), with diameters that are similar to engineered nanoparticles [56]. These lipoprotein complexes are composed of triglycerides and cholesterol esters in the core surrounded by proteins and a monolayer of phospholipids. A study of the binding of cholesterol and triglycerides to polymeric nanoparticles has shown that the ratio of bound cholesterol to bound triglyceride corresponds to the ratio in high density lipoprotein, suggesting that the nanoparticles bind the whole lipoprotein complex [31].

Binding of lipoprotein complexes to nanoparticles could potentially explain why many of the nanoparticles that bind these proteins and complexes are not recognized by the body as foreign and as such do not elicit a toxic or immune response. However, it is early days yet, and considerably more research into nanoparticle-biomolecule interactions is needed.

5. Mechanisms of Response to Nanomaterials

There is a plethora of studies in the literature regarding the in vitro and in vivo effects of engineered nanomaterials. However, much of this data is difficult to interpret because of inadequate particle characterization, exposure doses that are not well-justified in terms of realistic exposure conditions, or the elution of substances (impurities) of known toxicity (e.g. transition metals). Nevertheless, several studies have pointed to oxidative stress as an important mechanistic process related to nanomaterials toxicity.

For example, Sayes et al. [91] showed that as nC_{60} fullerenes became more water-soluble through derivatization of the particle surface, toxicity was dramatically reduced. The reduction in cytotoxicity was correlated with a lowered oxygen radical production by the fullerenes. Nanoparticle oxidative capacity, as determined using acellular methods, has also been shown to correlate well with oxidant-sensitive reporter activity in cultured cells and acute in vivo inflammatory responses [76]. As mentioned above, Oberdörster's study in bass [74] reported evidence of brain tissue lipid oxidation and a trend towards reduced glutathione depletion. Glutathione is an abundant tripeptide with broad antioxidant capacity and is gradually depleted in favor of the oxidized form as the severity of oxidative stress increases [71]. Shvedova and colleagues [101] exposed mice to single-walled carbon nanotubes (SWCNTs) via oropharyngeal aspiration and showed dose-related increases in granuloma formation (in association with SWCNT aggregates in tissues), interstitial fibrosis (in areas where SWCNTs were not visible), neutrophilic inflammation, glutathione depletion, increases in 4-hydroxynonenal, and increases in soluble inflammatory mediators. Furthermore, in vitro studies using cultured human keratinocytes and murine macrophages supported the role of oxidant production in response to nanotubes, as evidenced by the intracellular formation of lipid peroxidation products and antioxidant depletion. The same studies also highlighted the role of trace amounts of iron from the synthetic process in the observed responses [37, 100]. This latter study, in particular, highlights the need to identify transition metals, either as contaminants or structural components, in nanomaterial preparations as part of a safety evaluation.

In addition to the oxidative stress hypothesis, there is also compelling data regarding the role of surface coating or charge as a determinant of particle toxicity. Early studies using near micron-sized polystyrene micellar particles (~750 nm) demonstrated the principle that a negative surface charge was responsible for membrane depolarization and inflammatory cytokine induction in bronchial epithelial cells [112]. Likewise, a negative surface charge of micron-sized ambient particulate matter from diverse sources was correlated with

increases in intracellular calcium and cytokine induction [111]. These responses were thought to be related to the activity of acid-sensitive receptors on the cell surface, suggesting cell type specificity of response (e.g. neuroepithelial). Ryman-Rasmussen and colleagues [89], though, recently showed that negatively-charged CdSe-ZnS semiconductor nanocrystals were more cytotoxic in human epidermal keratinocytes than positively-charged particles of the same size and composition. The extent to which these mechanisms may be involved in the responses of diverse cell types to nanosized particles remains to be determined.

Following in vivo exposures, a combination of factors will ultimately determine the toxicity of a given material: oxidative capacity is likely to be related to acute responses and in vivo solubility; interactions with proteins and lipids may modify these processes (either increase or decrease toxicity) and also determine the biodistribution of the particles; and the persistence of the material will affect the long-term clearance and effects.

6. Sensitive Subpopulations

Knowledge regarding the biodistribution of nanomaterials as well as the mechanisms of response to them will lead to reasonable hypotheses regarding subpopulations that might experience adverse effects following exposure where other individuals will not. For example, individuals with underlying cardiopulmonary disease are more susceptible to the effects of ambient particulate air pollution [47, 79, 107]. Pre-existing bacterial or viral infections or disease states (e.g. diabetes) can contribute to oxidant-antioxidant imbalance or the activation status of inflammatory cells such that nanomaterials exposure could lead to persistent and overwhelming oxidative stress and tissue injury. In addition, inflammatory disease states can affect epithelial barrier function [30, 62, 116], thus altering the distribution of nanomaterials that are deposited in the respiratory tract or that are circulating in the blood. Depending on the route of exposure and the characteristics of the nanoparticles, many studies have demonstrated accumulation in major organ systems and passage through epithelial barriers. This raises the possibility that nanosized particles can also accumulate in germ line cells or the placenta and perhaps be transferred to the developing fetus, although this is an issue that has not received a great deal of attention.

7. Summarizing Concepts

7.1. ACCEPTABLE SCREENING STRATEGIES

In general, there are no commonly accepted screening assays for nanomaterials health effects. The American Society for Testing and Materials recently adopted a set a screening tests for the safety evaluation of nanomaterials intended for therapeutic use, including blood cell hemolysis, cytotoxicity in porcine kidney and human hepatocarcinoma cells, and the formation of mouse granulocyte-macrophage

colonies. For nanomaterials that may be encountered in the workplace or the environment, though, any screening strategy needs to be related to known mechanisms of response and/or aspects of the material physico-chemistry that predict in vivo responses. Some examples could include measurements of the oxidative capacity of the particle surface and assessment of protein binding. However, these kinds of assays have not yet been validated.

7.2. SUMMARY OF URGENT RESEARCH NEEDS

The most pressing research needs for the purpose of reducing the uncertainties in nanomaterials risk assessment are apparent from the preceding text. They include characterizations of external and internal exposures and identifications of mechanisms of response and sensitive subpopulations, all of which must be supported by thorough physicochemical characterization of test materials. This knowledge is likely to lead to useful screening approaches, as illustrated in Figure 2.

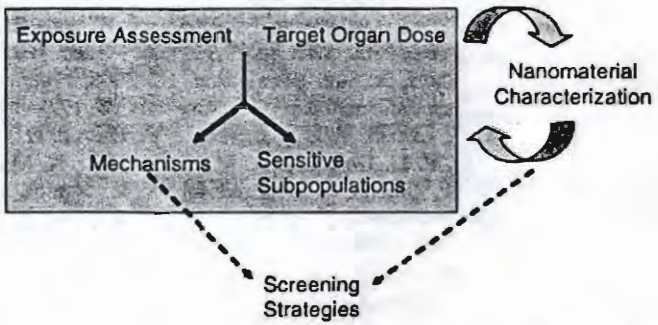


Figure 2. Overview of the immediate research needs in regards to human health risk assessment of nanomaterials.

A full understanding of external exposure includes measurements of particle concentrations and physicochemical properties over time in gas or liquid carriers. In particular, the impact of agglomeration/deagglomeration behavior and soluble forms of the material need careful attention. Critical information for determining internal dose of nanomaterials includes an evaluation of the ability of the material to breech physiological barriers, the dose to and retention in target organs and cellular/subcellular structures, changes in the physicochemical properties of the material as it is distributed in the body, and how the interactions of the material with endogenous biomolecules ultimately affect target organ dose. Some of these efforts will require the development of new technologies, particularly for nanoparticle-containing aerosol characterization.

Although it has presented a challenge for particle toxicologists in the past, in vivo-to-in vitro dose comparisons would be helpful not only in understanding the relevancy of in vitro test results, but also in the development of screening assays. Determinations of mechanisms of action also need to be clearly linked to realistic external and internal doses. However, it should be recognized that mechanistic

information will be critical in identifying sensitive subpopulations that may have lower thresholds for responding to nanomaterials because of, for example, alterations in repair of tissue damage or oxidant/antioxidant imbalance.

Lastly, it is imperative that there is strong global commitment to funding these essential research areas. It is more cost-effective in the long term to proactively address these critical knowledge gaps than to be reactive in regards to nanomaterials health risk assessment. Especially in light of significant scientific uncertainty and a lack of clear regulation, such an approach will allow the nanotechnology industry to flourish while increasing openness and transparency in decision-making processes.

References

1. Ahsan, F., Rivas, I. P., Khan, M. A., and Torres Suárez, A. I., 2002, Targeting to macrophages: role of physicochemical properties of particulate carriers - liposomes and microspheres - on the phagocytosis by macrophages, *J. Control. Release* **79**: 29–40.
2. Åkerman, M. E., Chan, W. C. W., Laakkonen, P., Bhatia, S. N., and Ruoslahti, E., 2002, Nanocrystal targeting *in vivo*, *Proc. Natl. Acad. Sci. USA* **99(20)**: 12617–12621.
3. Baciu, C. L., Becker, J., Janshoff, A., and Sonnichsen, C., 2008, Protein-membrane interaction probed by single plasmonic nanoparticles, *Nano Lett.*, **8**: 1724–1728.
4. Balbus, J. M., Maynard, A. D., Colvin, V. L., Castranova, V., Daston, G. P., Denison, R. A., Dreher, K. L., Goering, P. L., Goldberg, A. M., Kulinowski, K. M., Monteiro-Riviere, N. A., Oberdorster, G., Omenn, G. S., Pinkerton, K. E., Ramos, K. S., Rest, K. M., Sass, B., Silbergeld, E. K., and Wong, B. A., 2007, Meeting report: hazard assessment for nanoparticles - Report from an interdisciplinary workshop, *Environ. Health Perspect.*, **115(11)**: 1654–1659.
5. Ballou, B., Lagerholm, B. C., Ernst, L. A., Bruchez, M. P., and Waggoner, A. S., 2004, Noninvasive imaging of quantum dots in mice, *Bioconjugate Chem.* **15**: 79–86.
6. Baroli, B., Ennas, M. G., Loffredo, F., Isola, M., Pinna, R., and López-Quintela, M. A., 2007, Penetration of metallic nanoparticles in human full-thickness skin, *J. Invest. Dermatol.* **127**: 1701–1712.
7. Bellezza, F., Cipiciani, A., Quotadamo, M. A., Cinelli, S., Onori, G., and Tacchi, S. 2007, Structure, stability, and activity of myoglobin adsorbed onto phosphate-grafted zirconia nanoparticles, *Langmuir* **23**: 13007–13012.
8. Cedervall, T., Lynch, I., Foy, M., Berggård, T., Donnelly, S. C., Cagney, G., Linse, S., Dawson, K. A., 2007b, Detailed identification of plasma proteins adsorbed on copolymer nanoparticles, *Angew. Chem. Int. Ed.* **46**: 5754 –5756.
9. Cedervall, T., Lynch, I., Lindman, S., Berggård, T., Thulin, E., Nilsson, H., Linse, S., and Dawson, K. A., 2007a, Understanding the nanoparticle protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *PNAS* **104**: 2050–2055.
10. Chan-Remillard, S., Kapustka, L., and Goudey, S., 2008, Effects-based risk assessment of emerging and existing nanomaterials, *Proceedings of the Nano Science and Technology Institute NanoTech 2008 Conference*, Boston, MA.
11. Chen, M., Ding, W. H., Kong, Y., and Diao, G. W., 2008, Conversion of the surface property of oleic acid stabilised silver nanoparticles from hydrophobic to hydrophilic based on host-guest interaction, *Langmuir* **24**: 3471–3478.

12. Choi, H. S., Liu, W., Misra, P., Tanaka, E., Zimmer, J. P., Ipe, B. I., Bawendi, M. G., and Frangioni, J. V., 2007, Renal clearance of quantum dots, *Nat. Biotechnol.* **25(10)**: 1165–1170.
13. Cross, S. E., Innes, B., Roberts, M. S., Tsuzuki, T., Robertson, T. A., and McCormick, P., 2007, Human skin penetration of sunscreen nanoparticles: in-vitro assessment of a novel micronized zinc oxide formulation, *Skin Pharmacol. Physiol.* **20**: 148–154.
14. Dahman, D., and Monz, C., 2008, Determination of dustiness of nanostructured materials, *Gefahrstoffe - Reinhaltung der Luft*, submitted.
15. Dutta, D., Sundaram, S. K., Teeguarden, J. G., Riley, B. J., Fifield, L. S., Jacobs, J. M., Addleman, S. R., Kaysen, G. A., Moudgil, B. M., and Weber, T. J., 2007, Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials, *Toxicol. Sci.* **100**: 303–315.
16. Ehrenberg, M., and McGrath, J. L., 2005, Binding between particles and proteins in extracts: implications for microrheology and toxicity, *Acta Biomater.* **1**: 305–315.
17. Elder, A., Corson, N., Gelein, R., Mercer, P., Rinderknecht, A., Finkelstein, J., and Oberdörster, G., 2007, Tissue distribution in rats of surface-modified quantum dots following lung or intravenous exposure, *Am. J. Respir. Crit. Care Med.* **175**: A246.
18. Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J., and Oberdörster, G., 2006, Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, *Environ. Health Perspect.* **114(8)**: 1172–1178.
19. Gamer, A. O., Leibold, E., and van Ravenzwaay, B., 2006, The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin, *Toxicol. In Vitro* **20**: 301–307.
20. Gatti, A. M., 2004, Biocompatibility of micro- and nano-particles in the colon (part II), *Biomaterials* **25(3)**: 385–392.
21. Gatti, A. M., and Montanari, S., 2008, *Nanopathology: The Health Impact of Nanoparticles*, Pan Stanford, Singapore.
22. Gatti, A. M., and Rivasi, F., 2002, Biocompatibility of micro- and nanoparticles (Part I) in liver and kidney, *Biomaterials* **23(11)**: 2381–2387.
23. Gnewuch, H., Muir, R., Gorbunov, B., Priest, N. D., and Jackson, P. R., 2008, A novel size-selective airborne particle sampling instrument (WRAS) for health risk evaluation. In: Linkov, I. and J. Steeves (eds.), *Nanotechnology: Risks and Benefits*, Springer, Dordrecht.
24. Goto, Y., Matsumo, R., Konno, T., Takai, M., and Ishihara, K., 2008, Polymer nanoparticles covered with phosphorylcholine groups and immobilized with antibody for high-affinity separation of proteins, *Biomacromolecules* **9**: 828–833.
25. Gray, J. J., 2004, The interaction of proteins with solid surfaces, *Curr. Opin. Struct. Biol.* **14**: 110–115.
26. Griese, M., 1999, Pulmonary surfactant in health and human lung diseases: state of the art, *Eur. Respir. J.* **13**: 1455–1476.
27. Hahn, F. F., Newton, G. J., and Bryant, P. L., 1977, *In vitro* phagocytosis of respirable-sized monodisperse particles by alveolar macrophages, *ERDA Ser.* **43**: 424–435.
28. Han, J. H., Lee, E. J., Lee, J. H., So, K. P., Lee, Y. H., Bae, G. N., Lee, S-B., Ji, J. H., Cho, M. H., and Yu, I. J., 2008, Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility, *Inhal. Toxicol.* **20**: 741–749.
29. Hansen, S. F., Michelson, E. S., Kamper, A., Borling, P., Stuer-Lauridsen, F., and Baun, A., 2008, Categorization framework to aid exposure assessment of nanomaterials in consumer products, *Ecotoxicology*, **17(5)**: 438–447.

30. Heckel, K., Kiefmann, R., Dörger, M., Stoeckelhuber, M., and Goetz, A. E., 2004, Colloidal gold particles as a new in vivo marker of early acute lung injury, *Am. J. Physiol.* **287**: L867–L878.
31. Hellstrand, E., Lynch, I., Andersson, A., Drakenberg, T., Dahlbäck, B., Dawson, K. A., Linse, S., and Cedervall, T., 2008, Complete high density lipoproteins in nanoparticle corona. Manuscript submitted.
32. Hillyer, J. F., and Albrecht, R. M., 2001, Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles, *J. Pharm. Sci.* **90(12)**: 1927–1936.
33. Hunter, D. D., and Undem, B. J., 1999, Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea, *Am. J. Respir. Crit. Care Med.* **159(6)**: 1943–1948.
34. International Committee on Radiological Protection, 1994, Human respiratory tract model for radiological protection. A Report of Committee 2 of the ICRP.
35. Jani, P., Halbert, G. W., Langridge, J., and Florence, A. T., 1990, Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency, *J. Pharm. Pharmacol.* **442(12)**: 821–826.
36. Jayagopal, A., Sussman, E. M., and Shastri, V. P., 2008, Functionalised solid-lipid nanoparticles for transendothelial delivery, *IEEE Trans. NanoBiosci.* **7**: 28–34.
37. Kagan, V. E., Tyurina, Y. Y., Tyurin, V. A., Konduru, N. V., Potapovich, A. I., Osipov, A. N., Kisin, E. R., Schwegler-Berry, D., Mercer, R., Castranova, V., and Shvedova, A. A., 2006, Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron, *Toxicol. Lett.* **165(1)**: 88–100.
38. Kaminski, H., Fissan, H., Beyer, M., Mitura, H., Maier, M., Weinand, R., and Kuhlbusch, T. A. J., 2008, Exposure investigations related to engineered nanoparticles in Al_2O_3 and TiO_2 work place environments. In Proceedings of Nanotox 2008, Zurich, Switzerland.
39. Kim, K. J., and Malik, A. B., 2003, Protein transport across the lung epithelial barrier, *Am. J. Physiol.* **284(2)**: L247–L259.
40. Klein, J., 2007, Probing the interactions of proteins and nanoparticles, *PNAS* **104**: 2029–2030.
41. Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., and Schulz, H., 2002, Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low, *J. Toxicol. Environ. Health Part A* **65**: 1513–1530.
42. Kuhlbusch, T. A. J., and Fissan, H., 2006, Particle characteristics in the reactor and pelletizing areas of carbon black production, *J. Occup. Environ. Hyg.* **3(10)**: 558–567.
43. Kuhlbusch, T. A. J., Fissan, H., and Asbach, C., 2008a, Nanoparticles and exposure: measurement technologies and strategies. In: Linkov, I. and J. Steeves (eds.), *Nanotechnology: Risks and Benefits*, Springer, Dordrecht.
44. Kuhlbusch, T. A. J., Fissan, H., and Asbach, C., 2008b, Measurement and detection of nanoparticles in the environment. In: Krug, H. (ed.), *Nanotechnology, Volume 2: Environmental Aspects*, Wiley-VCH, Weinheim, pp. 229–267.
45. Kuhlbusch, T. A. J., Kaminski, H., Beyer, M., Jarzyna, D., Fissan, H., and Asbach, C., Measurement of nanoscale TiO_2 in workplace environments - method and results, unpublished data.
46. Kuhlbusch, T. A. J., Neumann, S., and Fissan, H., 2004, Number size distribution, mass concentration, and particle composition of PM_1, $PM_{2.5}$ and PM_{10} in bagging areas of carbon black production, *J. Occup. Environ. Hyg.* **1(10)**: 660–671.

47. Künzli, N., Jerret, M., Mack, W. J., Beckerman, B., LaBree, L., Gilliland, F., Thomas, D., Peters, J., and Hodis, H. N., 2005, Ambient air pollution and atherosclerosis in los angeles, *Environ. Health Perspect.* **113(2)**: 201–206.
48. Lademann, J., Weigmann, H., Rickmeyer, C., Barthelmes, H., Schaefer, H., Mueller, G., and Sterry, W., 1999, Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. *Skin Pharmacol. Appl. Skin Physiol.* **12**: 247–256.
49. Lindman, S., Lynch, I., Thulin, E., Nilsson, H., Dawson, K. A., and Linse, S., 2007, Systematic investigation of the thermodynamics of HSA adsorption to N-isopropylacrylamide-N-tert-butylacrylamide polymeric nanoparticles, *Nano Lett.* **7**: 914–920.
50. Linse, S., Cabaleiro-Lago, C., Xue, W.-F., Lynch, I., Lindman, S., Thulin, E., Radford, S. E., and Dawson, K. A., 2007, Nucleation of protein fibrillation by nanoparticles, *PNAS*, **104**: 8691–8696.
51. Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., Kenneth A., and Dawson, K.A., 2008, Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *PNAS*, **105(38)**: 14265–14270.
52. Lutz, J. F., Stiller, S., Hoth, A., Kaufner, L., Pison, U., and Cartier, R., 2006, One-pot synthesis of PEGylated ultrasmall iron-oxide nanoparticles and their in vivo evaluation as magnetic resonance imaging contrast agents, *Biomacromolecules* **7(11)**: 3132–3138.
53. Lynch, I., Cedervall, T., Lundqvist, M., Cabaleiro-Lago, C., Linse, S., and Dawson, K. A., 2007, The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st Century, *J. Colloid Interface Sci.* **134–135**: 167–174.
54. Lynch, I., and Dawson, K. A., 2008, Protein-nanoparticle interactions, *Nano Today*, **3**: 40–47.
55. Lynch, I., Dawson, K. A., and Linse, S., 2006, Detecting cryptic epitopes in proteins adsorbed onto nanoparticles, *Sci. STKE* **327** : 14.
56. Mahley, R. W., Innerarity, T. L., Rall, S. C., Jr., and Weisgraber, K. H., 1984, Plasma lipoproteins: apolipoprotein structure and function, *J. Lipid Res.* **25**: 1277–1294.
57. Mavon, A., Miquel, C., Lejeune, O., Payre, B., and Moretto, P., 2007, In vitro percutaneous absorption and in vivo stratum corneum distribution of organic and a mineral sunscreen, *Skin Pharmacol. Physiol.* **20**: 10–20.
58. Maynard, A. D., and Kuempel, E. D., 2005, Airborne nanostructured materials and occupational health, *J. Nanoparticle Res.* **7**: 587–614.
59. Maynard, A. D., Baron, P. A., Foley, M., Shvedova, A. A., Kisin, E. R., and Castranova, V., 2004, Exposure to carbon nanotube materials I: aerosol release during the handling of unrefined single walled carbon nanotube material, *J. Toxicol. Environ. Health* **67(1)**: 87–107.
60. Mercer, R. R., Scabilloni, J., Wang, L., Kisin, E., Murray, A. R., Schwegler-Berry, D., Shvedova, A. A., Castranova, V., 2008, Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **294(1)**: L87–97.
61. Michaelis, K., Hoffmann, M. M., Dreis, S., Herbert, E., Alyautdin, R.N., Michaelis, M., Kreuter, J., and Langer, K., 2006, Covalent linkage of apolipoprotein e to albumin nanoparticles strongly enhances drug transport into the brain, *J. Pharmacol. Exp. Ther.* **317**: 1246–1253.
62. Minami, T., Okazaki, J., Kawabata, A., Kuroda, R., and Okazaki, Y., 1998, Penetration of cisplatin into mouse brain by lipopolysaccharide, *Toxicology* **130**: 107–113.
63. Möhlmann, C., 2005, Vorkommen ultrafeiner aerosole an arbeitsplätzen, *Gefahrstoffe – Reinhaltung der Luft* **65(11/12)**: 469–471.

64. Monteiro-Riviere, N. A., and Inman, A. O., 2008, Evaluation of quantum dot nanoparticle penetration in human skin. *The Toxicologist* **102(S-1)**: 1029.
65. Monteiro-Riviere, N. A., Inman, A. O., and Ryman-Rasmussen, J. P., 2007, Dermal effects of nanomaterials. In: Monteiro-Riviere, N.A., Tran, C.L. (eds.), *Nanotoxicology: Characterization, Dosing, and Health Effects*, Informa Healthcare, New York, pp. 317–337.
66. Mueller, N. C., and Nowack, B., 2008, Exposure modeling of engineered nanoparticles in the environment, *Environ. Sci. Technol.* **42**: 4447–4453.
67. National Institute for Occupational Safety and Health, 2007, Current Intelligence Bulletin: *Interim Guidance for the Medical Screening of Workers Potentially Exposed to Engineered Nanoparticles*, docket NIOSH-115, http://www.cdc.gov/niosh/review/public/115.
68. National Institute for Occupational Safety and Health, 2008a, Approaches to safe nanotechnology: guidance on managing the health and safety concern with engineered nanomaterials, http://www.cdc.gov/niosh/topics/nanotech/.
69. National Institute for Occupational Safety and Health, 2008b, *Safe Nanotechnology in the Workplace: An Introduction for Employers, Managers, and Safety and Health Professionals*, Publication No. 2008-112.
70. National Nanotechnology Initiative: Second Assessment and Recommendations of the National Nanotechnology Advisory Panel, Report of the President's Council of Advisors on Science and Technology, April 2008.
71. Nel, A., Xia, T., Mädler, L., and Li, N., 2006, Toxic potential of materials at the nanolevel, *Science* **311**: 622–627.
72. Noble, C. A., and Prather, K. A., 2000, Real time single particle mass spectrometry: a historical review of a quarter century of the chemical analysis of aerosols, *Mass Spectrom. Rev.* **19**: 248 – 274.
73. Norde, W., and Lyklema, J., 1991, Why proteins prefer interfaces, *J. Biomater. Sci. Polym. Ed.* **2**: 183–202.
74. Oberdörster, E., 2004, Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass, *Environ. Health Perspect.* **112(10)**: 1058–1062.
75. Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S., and Finkelstein, J., 1992, Role of the alveolar macrophage in lung injury: studies with ultrafine articles, *Environ. Health Perspect.* **97**: 193–199.
76. Oberdörster, G., Rushton, E., and Finkelstein, J., 2008, Screening assays for assessing nanoparticle toxicity, *The Toxicologist* **102(S-1)**: 434.
77. Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., and Cox, C., 2002, Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats, *J. Toxicol. Environ. Health* **65**: 1531–1543.
78. Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C., 2004, Translocation of inhaled ultrafine particles to the brain, *Inhal. Toxicol.* **16(6–7)**: 437–445.
79. Peters, A., von Klot, S., Heier, M., Trentinaglia, I., Hörmann, A., Wichmann, H-E., Löwel, H., and for the KORA study group, 2004, Exposure to traffic and the onset of myocardial infarction, *New Engl. J. Med.* **351(17)**: 1721–1730.
80. Phalen, R. F., Yeh, H.-C., and Prasad, S. B., 1995, Morphology of the respiratory tract. In: McClellan, R. O. and R. F. Henderson (eds.), *Concepts in Inhalation Toxicology*, Taylor & Francis, Washington, DC, pp. 129–150.
81. Pines, L. A., Hegg, R., Valduga, C. J., Graziani, S. R., Rodrigues, D. G., and Moranhao, R. C., 2008, Use of cholesterol-rich nanoparticles that bind to lipoprotein

receptors as a vehicle to paclitaxel treatment of breast cancer: pharmacokinetics, tumor uptake and a pilot clinical study, *Cancer Chemother. Pharmacol.*, in press.
82. Plitzko, S., 2003, A thermal precipitator as a personal sampler, BIA-Report 7/2003, BIA-Workshop "Ultrafine aerosols at workplaces", 21./22.08.2002 at the BG Institute for Occupational Safety and Health - BIA, Sankt Augustin, Germany, http://www.hvbg.de/e/bia/pub/rep/rep04/pdf_datei/biar0703/bia0703e_gesamt.pdf, 127-129.
83. Podolski, I. Y., Podlubnaya, Z. A., Kosenko, E. A., Mugantseva, E. A., Makarova, E. G., Marsagishvili, L. G., Shpagina, M. D., Kaminsky, Y. G., Andrievsky, G. V., and Klochkov, V. K., 2007, Effects of hydrated forms of C60 fullerene on amyloid 1-peptide fibrillization in vitro and performance of the cognitive task, *J. Nanosci. Nanotechnol.* **7**: 1479–1485.
84. Rickerby, D. G., and Morrison, M., 2006, Nanotechnology and the environment: a European perspective, *Sci. Technol. Adv. Mater.* **8(1–2)**: 19–24.
85. Rinderknecht, A., Elder, A., Prud'homme, R., Gindy, M., Harkema, J., and Oberdoerster, G., 2007, Surface functionalization affects the role of nanoparticle disposition, *Am. J. Respir. Crit. Care Med.* **175**: A246.
86. Ross, E. E., and Wirth, M. J., 2008, Silica colloidal crystals as three-dimensional supports for supported lipid films, *Langmuir* **24**: 1629–1634.
87. Rouse, J. G., Yang, J., Ryman-Rasmussen, J. P., Barron, A. R., and Monteiro-Riviere, N. A., 2007, Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin, *Nano Lett.* **7**: 155–160.
88. Ryman-Rasmussen, J., Riviere, J. E., and Monteiro-Riviere, N. A., 2006, Penetration of intact skin by quantum dots with diverse physicochemical properties, *Toxicol. Sci.* **91**: 159–165.
89. Ryman-Rasmussen, J., Riviere, J. E., and Monteiro-Riviere, N. A., 2007, Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes, *J. Invest. Dermatol.* **127**: 143–153.
90. Sadrieh, N., Wokovich, A. M., Gopee, N. V., Siitonen, P. H., Cozart, C. R., Howard, P. C., Doub, W. H., and Buhse, L. F., 2008, Analysis of dermal penetration of titanium dioxide (TiO_2) from sunscreen formulations containing micron- and nano-scale particles of TiO_2, *Toxicologist* **102(1)**: A1023.
91. Sayes, C. M., Fortner, J. D., Guo, W., Lyon, D., Boyd, A. M., Ausman, K. D., Tao, Y. J., Sitharaman, B., Wilson, L. J., Hughes, J. B., West, J. L., and Colvin, V. L., 2004, The differential cytotoxicity of water-soluble fullerenes, *Nano Lett.* **4(10)**: 1881–1887.
92. Scheuch, G., Kohlhaeufl, M. J., Brand, P., and Siekmeier, R., 2006, Clinical perspectives on pulmonary systemic and macromolecular delivery, *Adv. Drug Deliver. Rev.* **58**: 996–1008.
93. Schlesinger, R. B., 1995, Deposition and Clearance of Inhaled Particles. In: R. O. McClellan and R. F. Henderson (eds.), *Concepts in Inhalation Toxicology*, Taylor & Francis, Washington, DC, pp. 191–224.
94. Schulte, P. A., Trout, D., Zumwalde, R. D., Kuempel, E., Geraci, C. L., Castranova, V., Mundt, D. J., Mundt, K. A., and Halperin, W. E., 2008, Options for occupational health surveillance of workers potentially exposed to engineered nanoparticles: state of the science, *J. Occup. Environ. Med.* **50(5)**: 517–526.
95. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), 2007, The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials, European Commission.
96. Semmler, M., Seitz, J., Erbe, F., Mayer, P., Heyder, J., Oberdörster, G., and Kreyling, W. G., 2004, Long-term clearance kinetics of inhaled ultrafine insoluble iridium

particles from the rat lung, including transient translocation into secondary organs, *Inhal. Toxicol.* **16**: 453–459.
97. Semmler-Behnke, M., et al., 2008, Strong size dependency of biodistribution of gold nanoparticles (NP) between 1.4 and 200 nm administered either to the lungs, blood or gut of rats, Nanotox 2008 Conference, Zürich, Switzerland, September 2008.
98. Semmler-Behnke, M., Takenaka, S., Fertsch, S., Wenk, A., Seitz, J., Mayer, P., Oberdörster, G., and Kreyling, W. G., 2007, Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent reentrainment onto airways epithelium, *Environ. Health Perspect.* **115(5)**: 728–733.
99. Shim, J., Seok, K. H., Park, W. S., Han, S. H., Kim, J., and Chang, I. S., 2004, Transdermal delivery of minoxidil with block copolymer nanoparticles, *J. Control. Release* **97**: 477–484.
100. Shvedova, A. A., Castranova, V., Kisin, E. R., Schwegler-Berry, D., Murray, A. R., Gandelsman, V. Z., Maynard, A., and Baron, P., 2003, Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells, *J. Toxicol. Environ. Health Part A* **66(20)**: 1909–1926.
101. Shvedova, A. A., Kisin, E. R., Mercer, R., Murray, A. R., Johnson, V. J., Potapovich, A. I., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., and Baron, P., 2005, Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice, *Am. J. Physiol.* **289**: L698–L708.
102. Shvedova, A. A., Kisin, E. R., Murray, A. R., Johnson, V. J., Gorelok, O., Arepalli, S., Hubbs, A., Mercer, R. R., Keohavonf, P., Sussman, N., Jin, J., Stone, S., Chen, B. T., Deye, G., Maynard, A., Castranova, V., Baron, P. A., and Kagan, V. E., 2008, 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.* **295(4)**: L552–565.
103. Stather, J. W., Harrison, J. D., Rodwell, P., and David, A. J., 1979, The Gastrointestinal absorption of plutonium and americium in the hamster, *Phys. Med. Biol.* **24(2)**: 396–407.
104. Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziensis, A., Heinzmann, U., Schramel, P., and Heder, J., 2001, Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats, *Environ. Health Perspect.* **109(Suppl. 4)**: 547–551.
105. Tan, M. H., Commens, C. A., Burnett, L., and Snitch, P. J., 1996, A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens, *Australas J. Dermatol.* **37**: 185–187.
106. The Royal Society and the Royal Academy of Engineering, Nanoscience and Nanotechnologies: Opportunities and Uncertainties, The Royal Society, 2004.
107. Timonen, K. L., Vanninen, E., de Hartog, J., Ibald-Mulli, A., Brunekreef, B., Gold, D. R., Heinrich, J., Hoek, G., Lanki, T., Peters, A., Tarkiainen, T., Tiittanen, P., Kreyling, W., and Pekkanen, J., 2005, Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: the ULTRA study, *J. Expo. Anal. Environ. Epidemiol.* **16(4)**: 332–341.
108. Tinkle, S. S., Antonini, J. M., Rich, B. A., Roberts, J. R., Salmen, R., DePree, K., and Adkins, E. J., 2003, Skin as a route of exposure and sensitization in chronic beryllium disease, *Environ. Health Perspect.* **111**: 1202–1208.
109. United States Environmental Protection Agency: Nanotechnology White Paper, Science Policy Council, February 2007.
110. United States Food and Drug Administration: Nanotechnology: A Report of the U.S. Food and Drug Administration Nanotechnology Task Force, July 2007.

111. Veronesi, B., de Haar, C., Lee, L., and Oortgiesen, M., 2002, The surface charge of visible particulate matter predicts biological activation in human bronchial epithelial cells, *Toxicol. Appl. Pharmacol.* **178(3)**: 144–154.
112. Veronesi, B., Wei, G., Zeng, J-Q., and Oortgiesen, M., 2003, Electrostatic charge activates inflammatory vanilloid (VR1) receptors, *Neurotoxicology* **24**: 463–473.
113. Volkheimer, G., 1975, Hematogenous dissemination of ingested polyvinyl chloride particles, *Ann. NY Acad. Sci.* **246**: 164–171.
114. Wake, D., Mark, D., and Northage, C., 2002, Ultrafine aerosols in the workplace, *Ann. Occup. Hyg.* **46(Suppl. 1)**: 235–238.
115. Woodrow Wilson International Center for Scholars, http://www.nanotechproject.org.
116. Xiao, H., Banks, W. A., Niehoff, M. L., and Morley, J. E., 2001, Effect of LPS on the permeability of the blood-brain barrier to insulin, *Brain Res.* **896**: 36–42.
117. Zhang, L. W., and Monteiro-Riviere, N. A., 2008, Assessment of quantum dot penetration into intact, tape stripped, abraded and flexed rat skin, *Skin Pharmacol. Physiol.* **21**: 156–170.
118. Zhang, L. W., Yu, W. W., Colvin, V. L., and Monteiro-Riviere, N. A., 2008, Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes, *Toxicol. Appl. Pharmacol.* **228**: 200–211.