Nanotechnology: assessing the risks

Nanotechnology is seen as a transformative technology, which has the potential to stimulate scientific innovation while greatly benefiting society. However, the enthusiasm with which the scientific and technical communities are embracing the technology is being tempered by concerns over possible downsides, including risks to human health. "Are these concerns valid?" is a question being asked by many, but frequently from differing perspectives. Given the increasingly complex interface between nanotechnology and society, relevant answers will be built on solid science and framed within a societal context.

Andrew D. Maynard

Chief Science Advisor, Project on Emerging Nanotechnologies, One Woodrow Wilson Plaza, 1300 Pennsylvania Ave. NW, Washington DC 20004, USA

E-mail: Andrew.Maynard@wilsoncenter.org

Nanotechnology has variously been described as a transformative technology, an enabling technology, and the next technological revolution. Even accounting for a certain level of hype, a heady combination of high-level investment, rapid scientific progress, and exponentially increasing commercialization point toward nanotechnology having a significant impact on society over the coming decades. However, enthusiasm over the rate of progress is being tempered increasingly by concerns over possible downsides of the technology, including unforeseen or poorly managed risk to human health[1-4]. Real and perceived adverse consequences in areas such as asbestos, nuclear power, and genetically modified organisms have engendered increasing skepticism over the ability of scientists, industry, and governments to ensure the safety of new technologies. As nanotechnology moves toward widespread commercialization, not only is the debate over preventing adverse consequences occurring at an unusually early stage in the development cycle, it is also expanding beyond traditional knowledge-based risk management to incorporate public perception, trust, and acceptance[5-8].

Within this context, the long-term success of 'nanotechnologies' (referring to the many specific applications and implementations of nanotechnology) will depend on rational, informed, and transparent dialogue aimed at understanding and minimizing the potential adverse implications to human health and the environment. A central question within this dialogue[2,3,9-13], one that has been raised in the popular media and the peer-reviewed press, is "how safe is nanotechnology?". However, such a general and unbound question is unlikely to yield useful information on the safety of specific nanotechnologies without further contextual information. Rather, appropriate contexts need to be defined and boundary conditions set if information on the safety of specific nanotechnologies is to be developed.

This review considers the current state of knowledge on the potential risk to human health presented by nanotechnologies, and explores the robustness of current research strategies and directions to

ISSN:1748 0132 © Elsevier Ltd 2006

ensure the development of 'safe' and publicly accepted nano-based products and technologies. Three broad areas are addressed that focus the discussion on those materials and technologies more likely to present a significant health risk. These cover materials of likely relevance to human health, nanomaterials' behavior on and in the body (loosely relating to hazard), and nanomaterials' behavior outside the body (loosely relating to exposure).

Engineered nanomaterials of relevance to human health

Nanotechnologies will likely be so diverse as to defy generic classification when it comes to evaluating potential health impact. It is therefore important to be able to define criteria that distinguish between technologies and products more or less likely to present a health risk, if we are to avoid inappropriate and possibly deleterious sweeping conclusions regarding potential impact. For example, complementary metal-oxide-semiconductor devices with sub-100 nm features, or high-resolution electron microscopes, will present a fundamentally different potential risk to human health than products containing unbound nanostructured particles, such as nanophase zinc oxide-based sunscreens. It is anticipated that nanotechnology standards being developed by organizations such as the International Standards Organization (ISO) and ASTM International will arrive at appropriate criteria in due course†. In the meantime, a number of published works have hinted at or proposed working criteria. The 2004 report on nanotechnology from the Royal Society and Royal Academy of Engineering[3] highlighted nanotechnologies associated with unbound sub-100 nm diameter particles as being of particular interest to human health. Oberdörster *et al.*[13] support this emphasis on sub-100 nm diameter particles in a discussion on the emerging field of nanotoxicology. However, it is clear from published toxicity studies that particle size alone is not a good criteria for differentiating between more or less hazardous materials and technologies. For instance, inhalation studies using rodents have demonstrated that 20 nm diameter TiO_2 particles have a greater impact on the animals' lungs than pigment-grade particles with the same composition, even though both particle sizes were administered as micrometer-diameter agglomerates[14].

Oberdörster *et al.*[15] have suggested that it is perhaps more appropriate to address the potential health impact of nanostructured particles – those having sub-100 nm scale structures – than nanometer-diameter particles. Maynard and Kuempel[16] explore this idea further, noting that the scale-dependent properties of nanomaterials are not necessarily associated with particle diameter, but with material structure. As an example, they use open agglomerates of single-walled carbon nanotubes (SWNTs), which may be micrometers in diameter, but exhibit structure at the nanoscale that is likely to influence their behavior (Fig. 1).

Within the context of inhalation exposure, Maynard and Kuempel[16] propose two criteria for identifying nanomaterials that may present a unique potential risk to human health:

1. The material must be able to interact with the body in such a way that its nanostructure is biologically available;
2. The material should have the potential to elicit a biological response that is associated with its nanostructure.

(a)

(b)

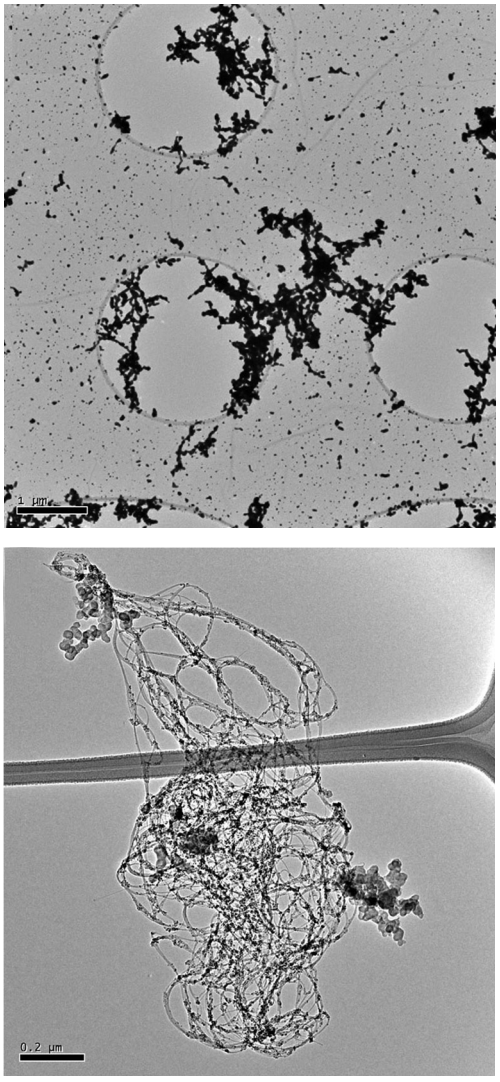

Fig. 1 Examples of airborne nanostructured agglomerates: (a) agglomerated 20 nm diameter Ag particles generated using a tube furnace[88]; (b) agglomerated SWNTs, released while agitating as-produced material. In each case, the agglomerates are micrometers in diameter, but are respirable[29] (i.e. are potentially able to deposit in the alveolar region of the lungs if inhaled) and have a distinctive structure at the nanometer scale. The significance of this nanostructure on toxicity is as yet unclear.

†ISO Technical Committee TC229 was established in 2005 and is developing standards and guidance on nanotechnology terminology and nomenclature, measurement and characterization, and environment, safety, and health. ASTM International Technical Committee E56 (nanotechnology) was established in the same year. Current work items include nomenclature and terminology, as well as characterization, risk management, and product stewardship.

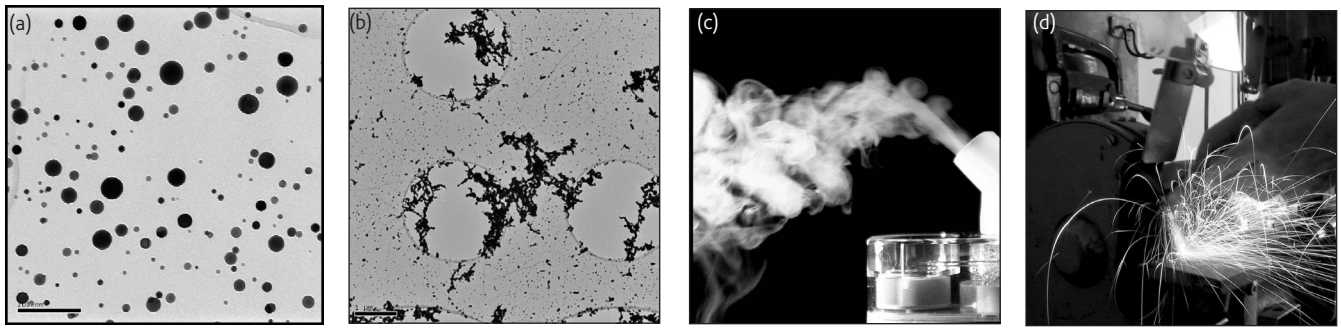

Fig. 2 Examples of engineered nanomaterials likely to be of concern to human health. (a) Unbound nanometer-diameter particles (in air or liquids). (b) Agglomerates of nanometer-diameter particles (in powders, air, and liquids). (c) Aerosols of nanometer-structure particle suspensions, solutions, or slurries. (d) Particles released while working with or using nanostructured materials, through machining, grinding, or wear and tear.

Although these two criteria relate to inhalation exposure, they are sufficiently broad to encompass all potential routes of exposure, and provide a useful working framework for distinguishing between materials and products that are less likely to present a health risk and those that are more likely to have some potential for adversely affecting health. When these criteria are linked to potential exposure to the skin, respiratory system, and gastrointestinal (GI) system, categories of materials and sources begin to emerge that may present a greater risk under some circumstances. These include unbound nanometer-diameter particles (in powders, aerosols, and liquid suspensions); agglomerates and aggregates of nanometer-diameter particles, where nanostructure-based functionality is retained; aerosolized liquid suspensions of nanomaterials; and the attrition (or comminution) of nanomaterial composites through various mechanisms (Fig. 2)[16].

Engineered nanomaterials in the body

While quantitative risk analysis considers many factors[13], the potential for a material to cause harm (hazard potential), and the amount of material able to reach target organs within the body (exposure potential) are critical to understanding potential health impact. Paracelsus (1493-1541) – widely regarded as the father of modern toxicology – is credited with the statement that "all things are poison and not without poison; only the dose makes a thing not a poison". As true now when dealing with emerging technologies as it was 500 years ago, his statement emphasizes the need to understand both how harmful a substance is, and how much of it can get into the body (and to specific organs), if risk is to be understood and managed.

Routes of entry

Three routes of entry into the body are likely to be of primary significance for engineered nanomaterials – inhalation, ingestion, and dermal penetration[13,15]. Two additional routes become important when considering nanotechnology-based medical devices and drugs – injection[15] and release from implants. Focusing on nonmedical exposure, the literature on impact associated with inhalation exposure vastly outweighs the alternative exposure routes, reflecting a current research emphasis on the health impact of airborne nanostructured materials[17]. Whether this represents relative risk, rather than the current interests of the research community, is unclear. Certainly, the health impacts of inhaling airborne particles have long been recognized: associations between exposure to 'very fine particles' and lung disease were recognized by Ramazzini in the 17th century, and documented links between aerosol exposure and ill health date back to the 4th century BCE[16].

Gastrointestinal tract

Particles deposited in the respiratory system that are cleared via the mucociliary escalator may be swallowed, leading to exposure to the GI tract. Additional ingestion routes include the use of nanostructured materials in food, water, and drugs. Relatively few studies have investigated nanostructured materials in the GI tract, and most have shown them to pass through and be eliminated rapidly[13]. However, compared with inhalation and skin exposure routes, there does not appear to be much research currently focused on this potential route of entry[17].

Skin

There has been a greater focus on the skin as a potential route of entry in recent years. The inclusion of nanoscale particles in sunscreens and cosmetics has raised concerns over possible dermal penetration of material, leading to ill health[3]. For example, nanoscale particles of materials such as TiO_2 and zinc oxide are being used as effective ultraviolet (UV) blocking agents in sunscreens[18,19], and nanoscale liposomes are currently used as delivery vehicles in skincare products[†]. Dermal exposure and penetration are also potential issues when handling engineered nanomaterials[20]. Whether engineered nanomaterials in contact with the skin represent a significant risk to health depends on their ability to penetrate through the outer protective layers and reach the epidermis or dermis, and the subsequent impact they may have on the body. Tinkle *et al.*[21] have shown latex particles smaller than 1 μm penetrate the outer layers of

[†]e.g. www.loreal.com/_en/_ww/research/innovations/nanosomes.aspx#application.

Nanotech on sale

Although nanotechnology promises great breakthroughs in areas such as energy generation and storage, high performance materials and medical treatments, many will first encounter engineered nanomaterials in everyday products such as cosmetics and personal goods (such as those shown), clothing, and sporting goods. A recently published web-based inventory of nano-enabled consumer products indicates that there are over 200 products on the market worldwide, ranging from computer processors to dietary supplements. The inventory, accessible at www.nanotechproject.org/consumerproducts, includes details of products identified by manufacturers as using nanotechnology.

(© 2006 David Hawxhurst, Woodrow Wilson Center.)

a skin sample during constant flexing. Other studies indicate that healthy, intact skin presents a good barrier against nanostructured particles[22]. However, there are indications that hair follicles could act as a repository of nanometer-diameter particles[23,24], and that the chemistry of carrier liquids may affect penetration potential[22]. Recently, Ryman-Rasmussen *et al.*[25] have shown that nanoscale quantum dots with different sizes, shapes, and coatings penetrate through the outer layers of pig skin samples in a flow cell, and enter the epidermal and dermal layers within 24 hours. The smallest particles – only 4.6 nm in diameter – showed localization in the epidermis and dermis within 8 hours, irrespective of the coating material used (polyethylene glycol, carboxylic acid, or polyethylene glycol-amine). Larger nonspherical particles (12 nm by 6 nm ellipsoids) showed a penetration rate that depended on the coating – but particles with all three coatings were found in the epidermis and dermis after 24 hours.

Even if nanoscale particles are able to penetrate through the outer layers of the skin, there is very little information on the hazard they might present. Research using subcutaneously introduced nanoscale particles suggests that they can be transported within the lymphatic system, raising questions about how they might influence immune responses, and there are some indications that neuronal uptake and transportation may occur[13]. However, discussions on the mechanisms of interaction and possible health outcomes are still rather speculative. Some concern has been expressed that the photogeneration of hydroxyl radicals by nanosized particles of materials like TiO_2 and zinc oxide may lead to oxidative damage in the skin, although the use of surface modification in such nanoparticles has been shown to suppress free-radical generation[26,27].

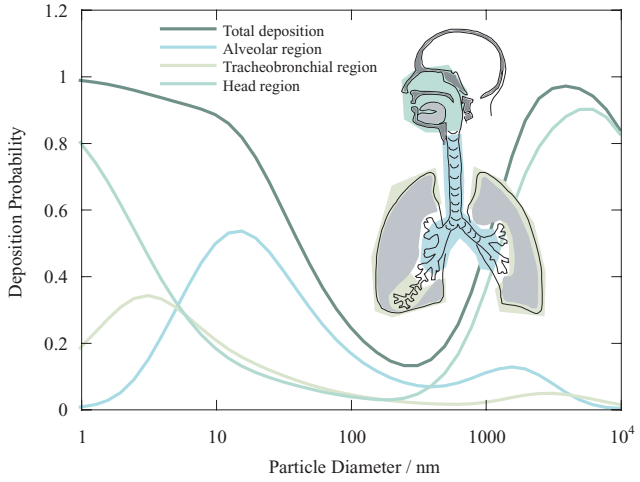

Fig.3 Modeled aerosol particle deposition within the respiratory tract[28]. Deposition has been modeled assuming an adult breathing through their nose at 25 l/min (light exercise), and exposed to spherical particles with a density of 1000 kg/m^3.

Ingestion, and possibly dermal penetration, are likely to become increasingly significant exposure routes as engineered nanomaterials are used in an ever-widening range of products. A recent survey of nanotechnology-based consumer products found that, out of over 200 manufacturer-identified 'nano' consumer products currently available, over 30% are applied directly to the skin or eaten[†]. In addition to products like these that are intentionally introduced to the body, little is known about the environmental accumulation of nanomaterials over product lifecycles, how this might affect exposure profiles.

Lungs

Inhalation of airborne material is clearly a significant potential exposure route[16]. Aerosol penetration into and deposition within the respiratory system has been studied and modelled extensively (Fig. 3)[16,28]. Most airborne particles smaller than a few tens of micrometers in diameter can be inhaled. Once in the respiratory system, particles will deposit in different regions according to their shape, diameter, and density. Diffusion-based deposition mechanisms

[†]www.nanotechproject.org/consumerproducts. This is a frequently updated inventory of nanotechnology-based consumer products. Cited numbers of products are from an analysis conducted on March 8th 2006.

lead to relatively high particle deposition probability in the alveolar region of the lungs for particles smaller than approximately 300 nm (although particles smaller than 4 μm have a greater than 50% probability of penetrating to this region[29]). Below approximately 30 nm, diffusion leads to high deposition probabilities throughout the respiratory system, including in the upper airways. At smaller diameters, deposition in the upper airways and especially the nasal region begins to dominate deposition in the alveolar region (Fig. 3).

Evaluations of health risk associated with aerosol exposure are generally based on the assumption that toxicity is associated with the mass and chemical composition of inhaled material. This mass-based approach has been very effective historically, leading to substantial reductions in respiratory disease with reduced exposures[30]. However, recent research has challenged the robustness of this approach for inhaled low-solubility particles. In one study, rats exposed to less than 60 μg/m^3 of freshly generated 26 nm diameter polytetrafluoroethene (PTFE) particles died of hemorrhagic pulmonary inflammation in less than 30 minutes[31]. To place these data in context, PTFE is a chemically inert polymer, and mortality was observed at mass concentrations comparable to the daily PM2.5 standard in the US, and one hundred times lower than occupational exposure limits for respirable 'nuisance dusts'[32]. In the same study, aged fume, which had been allowed to form larger agglomerates, did not exhibit the same potency, and the authors suggest that the toxicity observed was associated with the size and surface chemistry of the particles.

Oberdorster *et al.*[33] have further demonstrated a particle size-dependence on pulmonary inflammatory response in rats using TiO_2 particles. Although smaller particles were shown to be more potent than larger ones on a mass concentration basis, different sized particles showed a similar response when dose was interpreted in terms of particle surface area[34]. Similar studies have shown that response to low-solubility particles scales poorly with mass concentration, but closely with surface area concentration[35-38]. The dose-response relationship appears to be similar for chemically inert, low-solubility materials, suggesting a mechanism associated with the physical nature of the particles. However, insoluble particles that are chemically active, such as crystalline quartz, remain markedly more toxic than other insoluble materials, even when normalized for surface area (Fig. 4)[16].

Observed surface-associated material toxicity can be seen as an extension of macroscopic properties, rather than something unique to nanoscale materials. In comparison, a number of studies have shown that particle diameter may have a previously unrecognized role in determining the fate, and potential impact, of nanometer-diameter particles in the lungs. Particles smaller than a few hundred nanometers in diameter may be able to enter the lung interstitium following deposition in the lungs[39], and there is increasing evidence that nanometer-diameter particles can pass from the lungs into the bloodstream[40]. Particle penetration into cells has been observed, and *in vitro* studies using ambient ultrafine particles have shown evidence of particle localization in mitochondria, where they induce major structural damage[41]. In addition, recent studies have indicated that particles depositing in the nasal region may be transported to the olfactory bulb via the olfactory nerves[42]. Systemic transport of nanoparticles may also depend on the chemical properties of the nanoparticles[40,43]. The size-specific (and possibly chemistry-specific) transport of nanoparticles in the respiratory system and to other parts of the body would indicate a potential for health impacts not observed with larger particles.

Particle shape is also a factor when addressing potential hazard. Exposure to anisotropic particles such as fibers (e.g. asbestos) has long been associated with increased risk of fibrosis, lung cancer, and mesothelioma[44,45]. This raises additional concerns over the role of particle morphology when considering some complex nanostructured materials, including nanometer-diameter tubular and fibrous structures[46-55]. Warheit *et al.*[47] have shown that SWNTs can elicit transitory inflammation in rats and lead to multifocal granulomas, when introduced to the lungs using intratrachial instillation. Shvedova *et al.*[51]

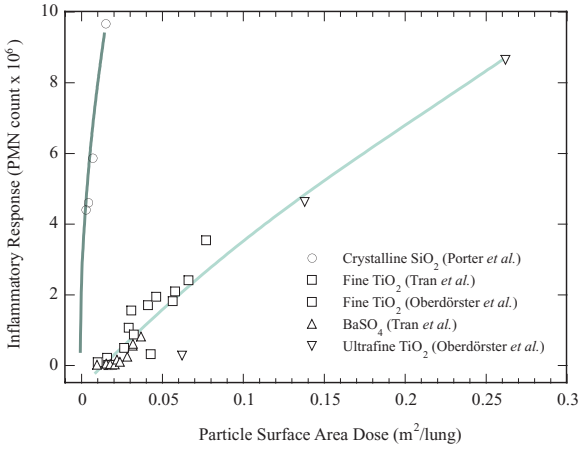

Fig. 4 Pulmonary inflammation (PMN count) of high toxicity dust (crystalline silica)[95] particles compared with low toxicity dust (TiO_2 and $BaSO_4$)[33,96] of both fine and ultrafine size, based on particle surface area dose in rat lungs. (Based on Maynard and Kuempel[16].)

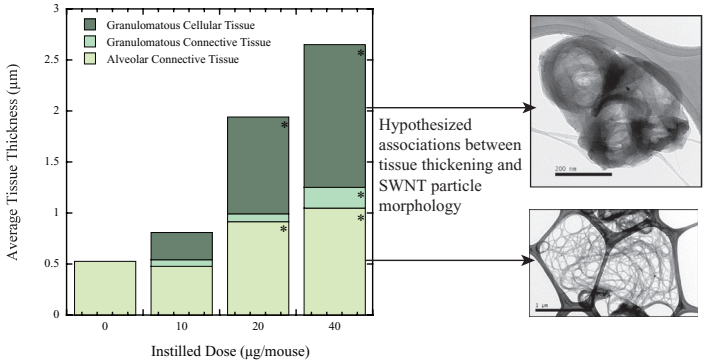

*Fig. 5 Hypothesized association between SWNT morphology and tissue thickening in proximal and distal regions of the lung, following pharyngeal aspiration in mice[51]. Morphometric measurement of connective and cellular tissue is shown 28 days post-pharyngeal aspiration of SWNTs. *P < 0.05.*

have demonstrated that pharyngeal aspiration of SWNTs in mice leads to acute inflammation with early onset yet progressive fibrosis and granulomas. Fibrosis was associated dense clumps of deposited SWNT material, but was also observed in distal regions of the mice lungs where dense clumps of SWNTs were not seen. Observations of fibrosis in the absence of clearly visible SWNTs led to the hypothesis that different SWNT agglomerate morphologies are responsible for the two distinct responses observed (Fig. 5).

Although multiwalled carbon nanotubes (MWNTs) are already used in commercial products (predominantly encapsulated in composite materials), relatively few studies have investigated their potential toxicity. In a recently published *in vitro* study, MWNTs were found to penetrate into human epidermal keratinocyte cells and to elicit production of an inflammatory cytokine[55]. Bottini *et al.*[52] have shown MWNTs to be cytotoxic to human T-cells, with oxidized nanotubes being significantly more potent than pristine nanotubes.

Toxic mechanisms

A mechanistic understanding of nanostructured material behavior in the body and ill health is still some way off, although a number of review articles address possible mechanisms of interaction[13,16,56]. Particles that enter the bloodstream may affect the blood vessel lining or function and promote blood clot formation[57], they may also be associated with cardiovascular effects linked to inhaling ambient ultrafine particles[58,59]. A cardiovascular response initiated by lung inflammation has also been proposed[60] that does not depend on particles entering the bloodstream. Although somewhat speculative, computer modeling has indicated that C_{60} molecules may bind to and deform nucleotides, if they are able to come into contact with DNA molecules[61]. While subcellular exposure to free underivatized C_{60} molecules (which are hydrophobic) is unlikely, C_{60} can form stable nanometer-diameter colloidal particles in water[62], and may potentially penetrate cells in this form.

Oxidative stress is considered to be an important mechanism, and certainly a diverse range of nanoscale materials have been shown to generate reactive oxygen species (ROS) in biological environments[13,63]. Even so, there remains considerable uncertainty over the processes underlying ROS generation from particles, and the precise impact on organ-level, cellular, and subcellular systems. Examples of apparently divergent studies (e.g. do C_{60} molecules lead to oxidative stress[62] or protect against it?[64]) only serve to underline the complexity of the issued being addressed.

The importance of material characterization

The dependence of engineered nanomaterials' behavior on physical and chemical structure significantly increases the difficulty in developing a sound understanding of material toxicity. Without detailed material physicochemical characterization, toxicity studies become difficult to interpret, and inter-comparison of studies becomes near impossible. Factors such as agglomeration state, surface chemistry, material source, preparation method, and storage take on a significance that has often been overlooked, potentially leading to inappropriate conclusions being drawn. For example, the toxicity of a material such as a SWNT is likely to be affected significantly by production process, atomic structure, surface modification, purity, aggregate morphology, preparation method, and method of delivery. Without information at this level, comparing toxicity evaluations becomes highly qualitative. A workshop on nanotoxicology held in 2004[65] underlined the need for detailed characterization when evaluating engineered nanomaterials, stating in the final report that "It is essential that the physical and chemical characterization of nanoscale materials be much more complete than has been the case in the sparse toxicology literature appearing to date". Oberdörster *et al.*[15] further underlined the need for rigorous characterization, proposing extensive measurement and documentation requirements as elements of nanotoxicology screening strategies.

Engineered nanomaterials outside the body

The behavior of engineered nanomaterials outside the body will have a significant impact on exposure, dose, and ultimately health risk. Material behavior, together with significant characteristics, will also

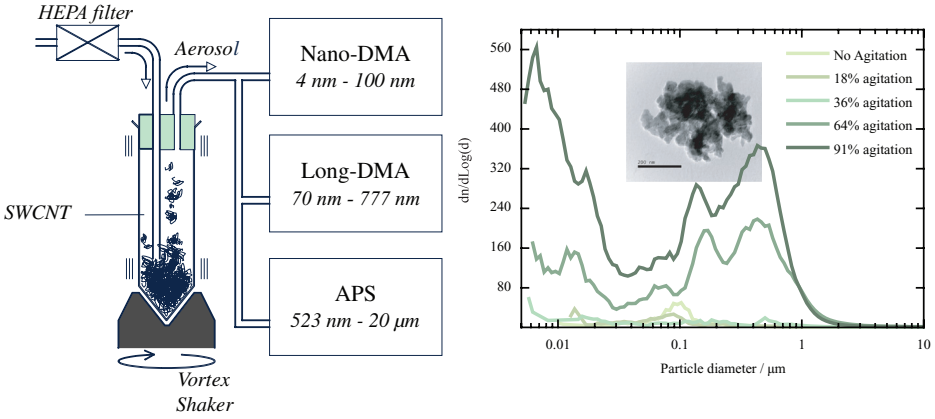

Fig. 6 Measurements of particle size distribution and concentration released when agitating as-produced SWNT material[75]. Released particles with a mobility diameter of 100 nm (shown in inset transmission electron micrograph) appeared to comprise predominantly nontubular material, with little evidence of carbon nanotubes being present. Physicochemical information on particles smaller than 50 nm is currently not available.

determine how exposure is most appropriately measured. Not only is it necessary to consider the potential for engineered nanomaterials to be released in a form that leads to exposure; chemical and structural transformations between the point of release and the point of exposure will also determine likely health impact. This becomes particularly significant where hazard is dependent on structural and surface properties, as changes in these properties may lead to significant differences between the released (or basic) material, and the material people are exposed to. For example, hydrophobic C_{60} molecules can assemble into stable nanometer-diameter colloidal particles in water[62,66], and individual SWNTs may be dispersed in aqueous suspension in the presence of some proteins[67,68]; both transformations may lead to substances that present a very different hazard to the untransformed material.

Release of nanostructured materials

In the workplace, the release of engineered nanostructured particles will be associated with specific processes and products[16,69,70]. The potential release of engineered nanomaterials in an accessible form outside the workplace is more difficult to pin down. Releases may an inevitable consequence of use, e.g. during the use of nanoparticles for groundwater remediation[71], as additives to fuels†, or as a component of personal care products[18]. Or they may be unintentional, e.g. industrial emissions, wear and tear on nano-enabled products, disposal of nano products, and even excretion of nonmetabolized nanomedicines. In each case, there is little or no information currently available on the nature and magnitude of potential releases, or material dispersion and transformation in the environment.

Given the high value of most engineered nanomaterials currently being developed and produced, together with the frequent need for pristine manufacturing conditions, it is unlikely that what might be considered 'wasteful' releases will be commonplace. Industrial material releases will probably be predominantly associated with production system leaks/spills, maintenance, product handling and transport, and disposal[69]. Releases may also occur during product finishing – during grinding burrs and imperfections from molded nanocomposite materials, for example. Of these potential release routes, it is perhaps the potential release of airborne nanomaterial from powders that intuitively presents the greatest exposure hazard[16] .

The likelihood of an aerosol being generated when handling or otherwise agitating a powder is governed by many factors, and is currently not well understood. The concept of particle release from powders is aptly named 'dustiness', and has been explored in relation to the release of inhalable and respirable particles[72,73]. Dustiness characterizes the potential of a material to release particles into the air when handled, and provides a basis for estimating potential health risk from inhalation exposure. As yet, the concept has not been directly extended to the release of aerosols from nanostructured materials. However, Baron *et al.*[74] have developed a novel method of qualitatively evaluating aerosol release from nanopowders. Using the method, which is based on a laboratory test tube shaker, they demonstrated measurable but low aerosol release rates from SWNT powder (Fig. 6). These measurements were supported by field studies showing airborne concentrations to be less than 50 µg/m^3 when the same material was removed from a reaction vessel[75].

Dispersion and physical transformation

Dispersion following release will depend on many factors. The dynamics of airborne nanometer-diameter particles suggest that they will generally follow airflows and not be influenced by mechanisms such as settling and inertial deposition[76]. However, nanometer-diameter particle motion in the presence of electrostatic, magnetic, and

†Envirox™ fuel-borne catalyst: www.oxonica.com

thermal fields may be significant in determining transport, transformation, and fate[16].

Diffusion is unlikely to lead to nanostructured particles deviating significantly from gas flow streamlines: even at 1 nm, the diffusion coefficient of airborne particles is less than a twentieth of that for air[76]. However, diffusion does strongly influence nanometer-diameter particle coagulation, leading to dynamic transformations in physical and chemical structure. The coagulation rate depends on the square of the particle concentration, and thus dominates the behavior of airborne particles at high concentrations but is negligible at low concentrations. Simple estimates[16] of variations in aerosol number concentration resulting from coagulation show that a 50% reduction in concentration is expected within 20 s at concentrations of 10^{14} particles/m^3, and within 55 hours at concentrations of 10^{10} particles/m^3. In addition, Preining[77] has calculated that the number concentration half life for 10 nm diameter particles is 8.1 days at a mass concentration of 1 ng/m^3, 11.7 minutes at 1 μg/m^3, and 0.7 seconds at 1 mg/m^3.

Coagulation is likely to influence exposure, deposition, and translocation of nanomaterials in the body, as well as biological response following exposure. Solid nanometer-diameter particles form fractal-like agglomerates in the absence of coalescence, with fractal dimensions of typically between 1.75 to 2.5[78]. Open-structured agglomerates with fractal-dimensions below two have specific surface-areas close to that of the constituent particles[79,80]. If toxicity is driven by surface characteristics, it is therefore likely that diffusion-limited coagulation will not have a significant impact on the hazard presented by airborne nanometer-diameter particles beyond influencing deposition region in the respiratory tract (assuming negligible physicochemical restructuring following coagulation). This would appear to be borne out by toxicity studies using diffusion-limited agglomerates of TiO_2[14,34].

Following coagulation, restructuring of agglomerates is somewhat dependent on particle chemistry and structure. Sintering, which depends on particle composition and size as well as temperature, is influential in determining physical structure and structural stability[81]. The binding forces between individual particles within agglomerates will also influence deagglomeration, and the subsequent release of smaller particles, either in the air, or in the body. Preliminary research has indicated that TiO_2 nanometer-diameter particles generated as a fume do not fully deagglomerate when dispersed in simulated lung fluid, but separate into agglomerates with a modal diameter of approximately 100 nm[82]. A study of PM2.5 particles in lung lining fluid has suggested particle aggregation rather than deagglomeration in the lungs[83].

Measuring exposure

Exposure measurement is essential to quantifying risk related to any potential hazard, and is closely associated with material behavior outside the body. Engineered nanomaterials present a particularly complex challenge, given potential associations between nanoscale structure and hazard. Although current research is far from conclusive, it is clear that conventional exposure metrics of mass concentration and chemical composition alone will not suffice in some cases. Oberdörster *et al.*[15], for instance, have identified 17 parameters potentially relevant to toxicity screening tests. Devising and applying methods sensitive to every possible characteristic of potential relevance is clearly not realistic in most situations, requiring compromises to be made in measuring exposure until further information is available on critical parameters. One approach, indicated in the recommendations from Oberdörster *et al.*, is to measure exposure in terms of potentially relevant physical metrics – number, length, surface area, and mass. Based on the limited toxicology studies published thus far, arguments can be made for measuring exposure against number, surface area, and mass concentration. However, in each case the particle size range within which measurements are made is clearly critical, inasmuch as size determines deposition and translocation within the body, and is potentially associated with specific biological interactions. While physical metrics do not directly provide information on some material characteristics (such as surface chemistry), they may be associated with relevant characteristics, and thus provide useful and viable surrogate measurements.

Mass concentration measurements

Mass concentration measurements offer continuity with historic and current monitoring approaches, but are relatively insensitive to nanometer-diameter particles[16]. An attractive option as measurement technologies are readily available and relatively inexpensive, the appropriateness of mass concentration measurements will depend on nanomaterials' mechanisms of action in the body and the feasibility of making measurements within specific particle size ranges. Personal mass concentration measurements are currently possible for particles smaller than 250 nm in diameter, using the bottom stage of a cascade impactor[84]. Bulky standalone cascade impactors can provide size-segregated mass concentration information below 100 nm. However, there are currently no aerosol mass concentration monitors that enable personal size-selective exposure measurements to particles smaller than 100 nm to be made. Having said that, the relevance of a 100 nm cut-off is still unclear. If agglomerates of nanometer-diameter particles are more potent on a mass-basis that agglomerates of larger-diameter particles (as is indicated for some nanoscale TiO_2 particles), mass concentration measurement methods capable of differentiating between particles of the same size, but differing nanostructure, are required. There are currently no instruments on the market that can achieve this directly.

Number concentration measurements

Number concentration is relatively easy to measure in air for particles larger than 10 nm using condensation particle counters (CPCs), and the technique can be extended to particles as small as 3 nm in diameter

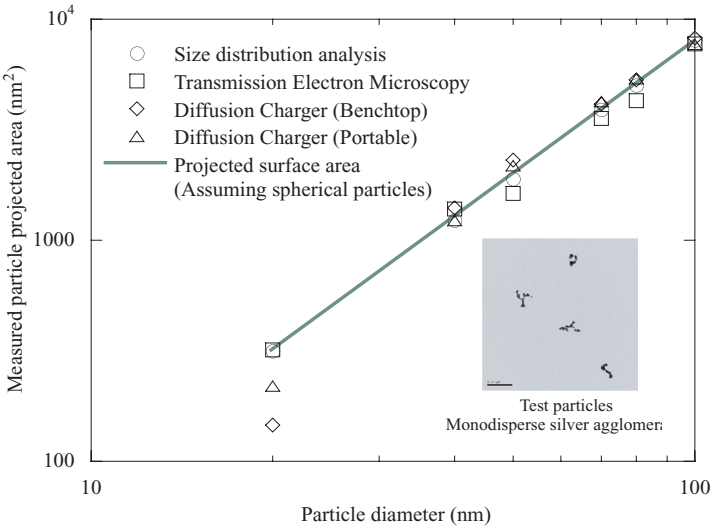

Fig. 7 Response of three aerosol surface-area methods to monodisperse particles between 20 nm and 100 nm mobility diameter[88]. Size distribution analysis was based on measurements using a scanning mobility particle sizer (SMPS, Model 3094, TSI). Benchtop diffusion charger: LQ1-DC (Matter Engineering, Switzerland). Portable diffusion charger: DC2000CE (EcoChem, USA).

with relative ease[16,85]. Number concentration measurements on their own are not particle-size- or chemistry-specific, making it difficult to distinguish between process-specific and background sources. This becomes critical when background aerosol concentrations are orders of magnitude higher than process-specific concentrations. Despite this drawback, the use of number concentration measurements has been proposed for 'sniffing out' nanoscale particle aerosol emission sources in workplaces, by carrying out measurements close to potential or suspected sources[85].

Surface area concentration measurements

As has been seen, a number of studies have associated the surface area of insoluble particles (including nanoscale particles) with inflammatory response in the lungs, and in this respect surface area seems a promising exposure metric for airborne nanostructured particles. Although methods such as isothermal adsorption (BET analysis) and transmission electron microscopy (TEM) analysis provide high quality data on material surface area, emerging methods such as diffusion charging provide a more viable approach to measuring aerosol surface area *in situ*[16]. Diffusion-charger-based aerosol monitors measure the rate at which positive unipolar ions diffuse to neutral particles, and relate this to particle surface area[86,87]. Ku and Maynard[88] have shown good agreement between diffusion chargers from Matter Engineering (LQ1-DC, Switzerland) and EcoChem (DC2000CE, USA), TEM-derived surface area, and size-distribution-derived surface area for sub-100 nm particles (Fig. 7), although Jung and Kittelson[86] have published data showing the LQ1-DC to underestimate surface area between 30 nm and 150 nm. For particles larger than 100 nm mobility diameter, the diffusion chargers increasingly underestimate the aerosol surface area, as is anticipated from theory[86,87,89]. Research is still needed to establish whether this degree of underestimation is significant in relation to engineered nanomaterials' exposure and health impact.

Interestingly, instruments with a response approximating to aerosol surface area may provide a useful indication of potential risk where specific information on appropriate exposure metrics is not available, as may be shown using a simple thought experiment. It is reasonable to assume that impact may be related to particle diameter through the relationship

$$\text{Impact} \propto d^{\alpha} \quad (1)$$

where d is the particle diameter and has a value between zero (particle number-based response) and three (particle-mass- or volume-based response). With no *a priori* information on associations between particle structure and health impact, α could potentially take on any value between zero and three (assuming that a nonlinear relationship with mass is not observed), leading to the geometric mean of all possible values of 1.5. However, $\alpha = 0$ represents no association between particle structure and impact; assuming that $\alpha = 1$ represents a more realistic lower limit on associations between particle structure and impact, the geometric mean of likely values of α increases to 2.

This is a hypothetical thought experiment and is clearly flawed in a number of ways. However, it provides a basis for beginning to explore measurement approaches that might be applicable in the absence of clear and unequivocal nanostructured material dose-response relationships. Where specific guidance on exposure metrics is not available, it indicates that instruments having a particle diameter-dependent response with α between 1.5 and 2 are likely to be most useful in representing potential risk. Published data on diffusion charger

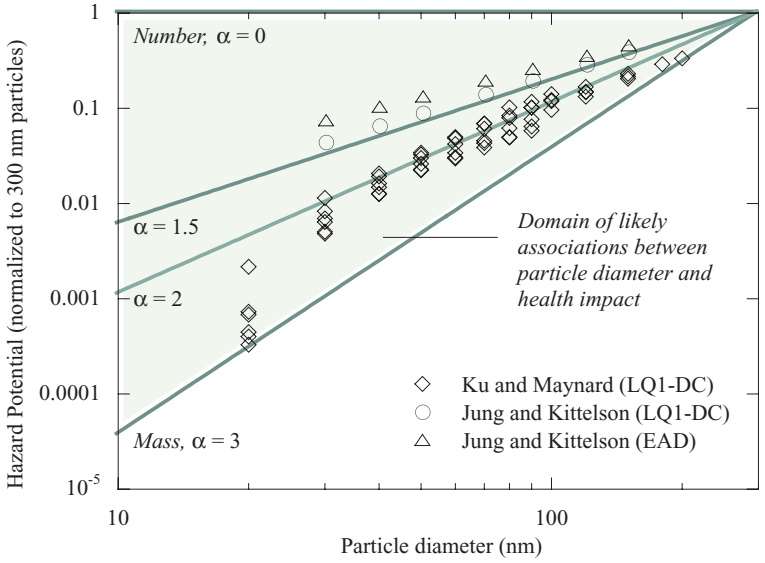

Fig. 8 Possible associations between particle diameter and hazard potential (eq 1) normalized to 300 nm diameter particles. α = 1.5 represents the geometric mean of all possible likely values, while α = 2 represents the geometric mean of values between 1 (length dependency) and 3 (mass dependency). Also shown are published data on diffusion charger responses[86,88] (normalized to 300 nm diameter particles), demonstrating the potential use of this method in characterizing airborne exposures to engineered nanomaterials, even in the absence of specific information on appropriate measurement metrics.

response[86,88] lie close to these values (Fig. 8), suggesting that this is a useful measurement technique to consider when assessing nanostructured particle exposure.

In an extension of the use of diffusion charging, Wilson *et al.*[90] have demonstrated that the response of a diffusion charger can be tuned to match the surface area of particles depositing in the lungs by adjusting the voltage on an ion trap. The resulting instrument (Model 3550 Nanoparticle Surface Area Monitor, TSI) estimates the surface area of particles depositing in either the respirable or tracheobronchial region of the lungs.

Managing potential risk in policy and social contexts

While the science of nanomaterials and human health impact is maturing, it is still at a stage of raising many more questions than answers. Current research demonstrates that some engineered nanomaterials can behave differently in the body than more conventional materials, and may present a health risk that is not captured within established risk assessment paradigms. However, we are still at a stage where the sparseness of published research leads to information being inconclusive, sometimes apparently conflicting and often speculative. There is little published research on the importance of characteristics unique to the nanoscale (as opposed to properties that scale with size), and most risk-based research appears to be focused on first-generation engineered nanomaterials, despite the concurrent development of second- and third-generation technologies[91]. Against this background, significant investments are being made in nanotechnology, commercial products are anticipated to increase[92], public awareness of the potential benefits and risks is growing, and discussions are beginning on oversight of the technology[4].

Quantitative risk assessment remains difficult for engineered nanomaterials. It is reasonable to speculate that there will be risks, and that conventional risk assessment paradigms will not always suffice. However, specific information on hazard, exposure, dose, response, and other compartments within risk assessment frameworks is lacking. At the same time, an increasingly influential public is shaking the reliance on science-based risk governance alone[6,93]. Failures of public trust in technologies such as nuclear power and genetically modified foods have demonstrated the power of perceived risk in determining success or failure – and in influencing risk in a broader context of economic risk, or risk related to the consequences of rejection. The inclusion of public perceptions in managing risk is seen as essential by groups such as the International Risk Governance Council[6], if effective risk governance models are to be developed in an increasingly complex and interdependent environment.

If oversight of nanotechnology is to nurture beneficial technologies rather than stifle them, it will be necessary to develop appropriate ways of working within a framework of scientifically sound information and public perception. Recent research into public perceptions has indicated enthusiasm over the potential uses of nanotechnology, but concern over the ability of industry and government to regulate it[7,94]. In a recent independent report[4], Davies acknowledges that regulation of nanotechnology will be difficult, but concludes that if nothing specific is done to manage the potential adverse effects of nanotechnology, "the public potentially would be left unprotected, the government would struggle to apply existing laws to a technology for which they were not designed, and industry would be exposed to the

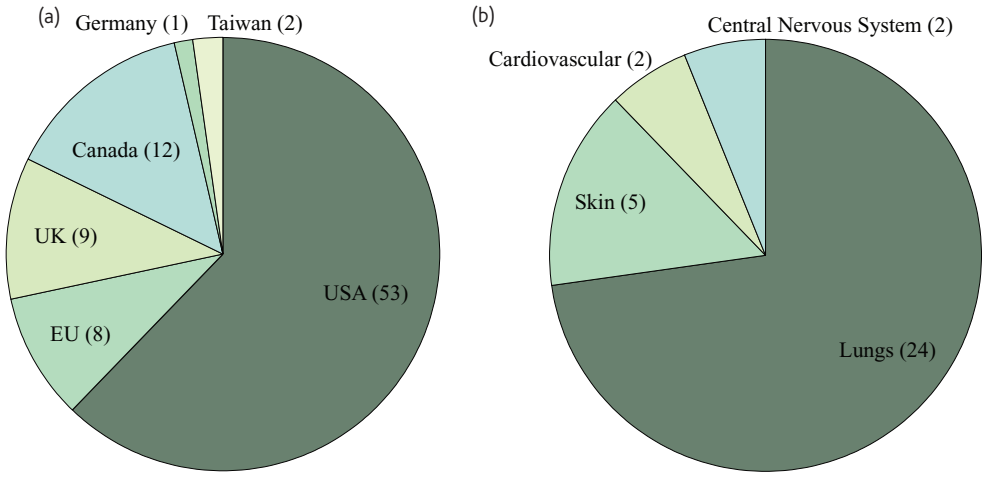

Fig. 9 Overview of current research into the potential health, safety, and environmental implications of nanotechnology[17], based on a publicly accessible inventory†. (a) Number of listed research projects that are highly relevant to the environmental, safety, and health implications of nanotechnology. (b) Number of listed research projects in the US specifically focused on the potential impact of engineered nanomaterials to the lungs, cardiovascular system, central nervous system, and the skin. Projects focused on the GI tract were searched for, but not found. †www.nanotechproject.org/index.php?id=18.

possibility of public backlash, loss of markets, and potential financial liabilities".

Despite current risk-based uncertainty and the complexity of overseeing the emergence of 'safe' nanotechnologies, one certainty is the need for further research and better data. A recent analysis of current environment, safety, and health-impact research indicated that there is a significant amount of preemptive work addressing potential risks of engineered nanomaterials (Fig. 9)[17]. However the same analysis concluded that there is a lack of overall strategy in the current research portfolio, and that without a clear strategic framework, greater resources, and increased partnerships and collaboration, critical questions are unlikely to be answered in a timely manner.

Summary

Nanotechnology will continue to be developed and used, despite concerns over the possible health risks associated with some materials. Whether it will achieve widespread acceptance and reach its full potential will depend critically on good risk-based science being carried out within a strategic coordinated framework, appropriate oversight, and the support of the people who use it and who will ultimately bear the burden of any emerging health risks. This, in turn, challenges the very way we do science – with cross-communication between disparate areas of expertise being fundamental to progress – and how we translate good science into good policy. Based on the current state of knowledge, the risk is real for some nanotechnologies, but as yet unquantifiable. That is not to say that we must assume significant risk in all cases – to do so would ignore the messages to be drawn from current and past knowledge and experience, and would potentially deflect attention from more significant and comparatively well-understood risks to human health. Rather, current knowledge enables us to place initial boundaries on the question of how 'safe' is nanotechnology. Now that we can begin to ask the right questions, it should be possible to develop strategic research and implementation plans that lead to scientifically sound, rational and responsible approaches to understanding and managing the possible impacts of nanotechnology on health. nt

REFERENCES

1. ETC Group, *No Small Matter II: The Case for a Global Moratorium. Size Matters!* ETC Group, Winnipeg, Canada, (2003), Occasional Paper Series Vol. 7, No. 1
2. Hood, E., *Environ. Health Perspect.* (2004) **112** (13), A741
3. The Royal Society and The Royal Academy of Engineering, *Nanoscience and Nanotechnologies*, The Royal Society and The Royal Academy of Engineering, London, UK, (2004)
4. Davies, J. C., *Managing the Effects of Nanotechnology*, Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Washington DC, USA, (2006), 2006-1
5. Hett, A., *Nanotechnology. Small Matter, Many Unknowns*, SwissRe, Zurich, Switzerland, (2004), 1501255_04
6. Renn, O., Risk Governance. *Towards and Integrative Approach*, International Risk Governance Council, Geneva, Switzerland, (2005) White Paper No. 1
7. McCoubrie, J., *Informed Public Perceptions of Nanotechnology and Trust in Government*, Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Washington DC, USA, (2005)
8. Roco, M. C., *J. Nanoparticle Res.* (2003) **5** (3-4), 181
9. Van, J., Nanotechnology industry puts focus on safety issues, *Chicago Tribune* (January 21, 2006), 3
10. Arthur, C., Does Scarlett need regulatory oversight? *The Guardian* (January 19, 2006)
11. Weiss, R., Nanotechnology precaution is urged, *Washington Post* (July 30 2004), A02

12. Hampton, T., *JAMA, J. Am. Med. Assoc.* (2005) **294** (20), 2564
13. Oberdörster, G., *et al., Environ. Health Perspect.* (2005) **13** (117), 823
14. Bermudez, E., *et al., Toxicol. Sci.* (2004) **77**, 347
15. Oberdörster, G. *et al., Part. Fiber Toxicol.* (2005) **2** (8), doi:10.1186/1743-8977-2-8
16. Maynard, A. D. and Kuempel, E. D., *J. Nanoparticle Res.* (2005) **7** (6), 587
17. Maynard, A. D., *Inventory of Research on the Environmental, Health and Safety Implications of Nanotechnology*, Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Washington DC, USA, (2005)
18. Popov, A. P., *et al., J. Phys. D: Appl. Phys.* (2005) **38** (15), 2564
19. Willander, M. *et al., Microelectron. J.* (2005) **36** (11), 940
20. Baron, P. A., *et al., Evaluation of aerosol release during the handling of unrefined carbon nanotube material*, NIOSH, Cincinnati, OH, (2003) DART-02-191/NTIS PB2003-102401
21. Tinkle, S. S., *et al., Environ. Health Perspect.* (2003) **111** (9), 1202
22. Tsuji, J. S., *et al., Toxicol. Sci.* (2006) **89** (1), 42
23. Lademann, J., *et al., J. Invest. Dermatol. Symp. Proc.* (2005) **10** (3), 301
24. Lademann, J. *et al., Skin Pharmacol. Appl. Skin Physiol.* (1999) **12** (5), 247
25. Ryman-Rasmussen, J. P., *et al., Toxicol. Sci.* (2006), doi: 10.1093/toxsci/kfj122
26. Lademann, J., *et al., Skin Pharmacol. Appl. Skin Physiol.* (2000) **13** (5), 258
27. Wakefield, G., *et al., Mater. Sci. Technol.* (2004) **20** (8), 985
28. *Human respiratory tract model for radiological protection*, International Commission on Radiological Protection (ICRP) Publication 66, Elsevier, Oxford, (1994)
29. *Air Quality – Particle size fraction definitions for health-related sampling*, International Standards Organisation (ISO), Geneva, (1995), ISO 7708:1995
30. Maynard, A. D., and Baron, P. A., Aerosol measurement in the workplace. In *Aerosols Handbook. Measurement, Dosimetry and Health Effects.* Ruzer, L. S., and Harley, N. H., (eds.) CRC Press, Boca Raton, (2004), 225
31. Oberdörster, G., *et al., Inhal. Toxicol.* (1995) **7**, 111
32. *Particulates not otherwise regulated*, National Institute for Occupational Safety and Health (NIOSH), (1998), NIOSH Manual of Analytical Methods 0600
33. Oberdörster, G., *et al., Environ. Health Perspect.* (1994) **102** (S5), 173
34. Oberdörster, G., *Philos. Trans. R. Soc. London, Ser. A* (2000) **358** (1775), 2719
35. Tran, C. L. *et al.*, Inhal. Toxicol. (2000) **12** (12), 1113
36. Lison, D., *et al., Arch. Toxicol.* (1997) **71** (12), 725
37. Brown, D. M., *et al., Toxicol. Appl. Pharmacol.* (2001) **175** (3), 191
38. Duffin, R. *et al., Ann. Occup. Hyg.* (2002) **46**, 242
39. Ferin, J., and Oberdörster, G., *J. Aerosol Med: Deposition and Clearance Effects in the Lung* (1992) **5** (3), 179
40. Oberdörster, G., *et al., J. Toxicol. Environ. Health, Pt. A* (2002) **65** (20), 1531
41. Li, N., *et al., Environ. Health Perspect.* (2003) **111** (4), 455
42. Oberdörster, G., *et al., Inhal. Toxicol.* (2004) **16** (6-7) 437
43. Kreyling, W. G., *et al., J. Toxicol. Environ. Health, Pt A* (2002) **65** (20), 1513
44. Doll, R., *Br. J. Ind. Med.* (1955) **12**, 81-86
45. Peto, J., *et al., Br. J. Ind. Med.* (1977) **34** (3), 169
46. Shvedova, A. A., *et al., J. Toxicol. Environ. Health* (2003) **66** (20), 1909
47. Warheit, D. B., *et al., Toxicol. Sci.* (2004) **77**, 117
48. Huckzko, A., *et al., Fullerene Sci. Technol.* (2001) **9** (2), 251
49. Huckzko, A., and Lange, H., *Fullerene Sci. Technol.* (2001) **9** (2), 247
50. Lam, C.-W., *et al., Toxicol. Sci.* (2004) **77**, 126
51. Shvedova, A. A., *et al., Am. J. Physiol.-Lung Cell. Mol. Physiol.* (2005) **289**, 698
52. Bottini, M., *et al., Toxicol. Lett.* (2006) **160** (2), 121
53. Manna, S. K., *et al., Nano Lett.* (2005) **5** (9), 1676
54. Jia, G., *et al., Environ. Sci. Technol.* (2005) **39** (5), 1378
55. Monteiro-Riviere, N. A., *et al., Toxicol. Lett.* (2005) **155** (3), 377
56. Donaldson, K., *et al., Philos. Trans. R. Soc. London, Ser. A* (2000) **358**, 2741
57. Nemmar, A., *et al., Toxicol. Lett.* (2004) **149** (1-3), 243
58. Pekkanen, J. *et al., Circulation* (2002) **106** (8), 933
59. Wichmann, H. E., and Peters, A., *Philos. Trans. R. Soc. London, Ser. A* (2000) **358** (1775), 2751
60. Seaton, A., *et al., Lancet* (1995) **345**, 176
61. Zhao, X., *et al., Biophys. J.* (2005) **89**, 3856
62. Sayes, C. M., *et al., Nano Lett.* (2004) **4** (10), 1881
63. Donaldson, K., *et al., J. Aerosol Med: Deposition and Clearance Effects in the Lung* (2002) **15** (2), 213-220
64. Gharbi, N., *et al., Nano Lett.* (2005) **5** (12), 2578
65. Roberts, S. M., *Developing experimental approaches for the evaluation of toxicological interactions of nanoscale materials*, Presented at Developing experimental approaches for the evaluation of toxicological interactions of nanoscale materials. Gainsville FL, USA (2005)
66. Oberdörster, E., *Environ. Health Perspect.* (2004) **112** (10), 1058
67. Zorbas, V. *et al., J. Am. Chem. Soc.* (2005) **126** (23), 7222
68. Zheng, M. *et al., Nat. Mater.* (2003) **2** (5), 338
69. Aitken, R. J., *et al., Nanoparticles: An Occupational Hygiene Review*, Institute of Occupational Medicine, Edinburgh UK (2004)
70. Luther, W., (ed.) *Technological Analysis. Industrial application of nanomaterials - chances and risks*, Future Technologies Division of VDI Technologiezentrum GmbH, Düsseldorf, Germany (2004)
71. Zhang, W.-X., *J. Nanoparticle Res.* (2003) **5**, 323
72. *MDHS 81: Dustiness of powders and materials*, Health and Safety Executive (HSE) UK (1996)
73. Heitbrink, W. A., *et al., Am. Ind. Hyg. Assoc. J.* (1992) **53** (10), 617
74. Baron, P. A., *et al., Evaluation of aerosol release during the handling of unrefined carbon nanotube material*, DART-02-191/NTIS PB2003-102401, Cincinnati OH, USA, NIOSH (2003)
75. Maynard, A. D., *et al., J. Toxicol. Environ. Health* (2004) **67** (1), 87
76. Hinds, W. C., *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*, 2nd Edition, John Wiley & Sons, New York NY, USA (1999)
77. Preining, O., *J. Aerosol Sci.* (1998) **29**, 481-495
78. Baron, P. A., *et al.*, Nonspherical particle measurements: shape factors, fractals and fibers. In *Aerosol Measurement. Principles, Techniques and Applications*, 2nd Edition, Baron, P. A., and Willeke, K., (eds.) John Wiley & Sons, NY (2001), 705
79. Stefaniak, A. B., *et al., AIHA J.* (2003) **64** (3), 297
80. Rogak, S. N., *et al., Aerosol Sci. Technol.* (1993) **18** (1), 25
81. Mukherjee, D., *et al., J. Chem. Phys.* (2003) **119** (6), 3391
82. Maynard, A. D., *Ann. Occup. Hyg.* (2002) **46** (Suppl. 1), 197
83. Kendall, M. *et al., Am. J. Physiol.-Lung Cell. Mol. Physiol.* (2002) **282** (1), L109
84. Singh, M., *et al., Atmos. Environ.* (2003) **37** (34), 4781
85. Brouwer, D. H., *et al., Ann. Occup. Hyg.* (2004) **48** (5), 439
86. Jung, H., and Kittelson, D. B., *Aerosol Sci. Technol.* (2005) **39**, 902
87. Keller, A., *et al., J. Vac. Sci. Technol., A* (2001) **19** (1), 1
88. Ku, B. K., and Maynard, A. D., *J. Aerosol Sci.* (2005) **36** (9), 1108
89. Fuchs, N. A., *The Mechanics of Aerosols*, Pergamon (Elsevier), Oxford, UK (1964)
90. Wilson, W. E., *et al.*, Use of the electrical aerosol detector as an indicator for the total particle surface area deposited in the lung. Presented at Symposium on Air Quality Measurement Methods and Technology, Research Triangle Park (2004)
91. Roco, M. C., *AIChE. J.* (2004) **50** (5), 890
92. *Sizing Nanotechnology's Value Chain*, Lux Research Inc., New York, USA (2004)
93. Durant, J., *et al.*, (eds.) *Biotechnology in the Public Sphere*, Science Museum, London (1998)
94. Cobb, M. D., and Macoubrie, J., *J. Nanoparticle Res.* (2004) **6** (4), 395
95. Porter, D. W. *et al., J. Toxicol. Environ. Health A* (1999) **14** (57(1)), 25
96. Tran, C. L. *et al.*, Investigation and prediction of pulmonary responses to dust. Part II. In *Investigations into the pulmonary effects of low toxicity dusts.* Parts I and II., Health and Safety Executive UK, Suffolk, UK, (1999) Contract Research Report 216/1999