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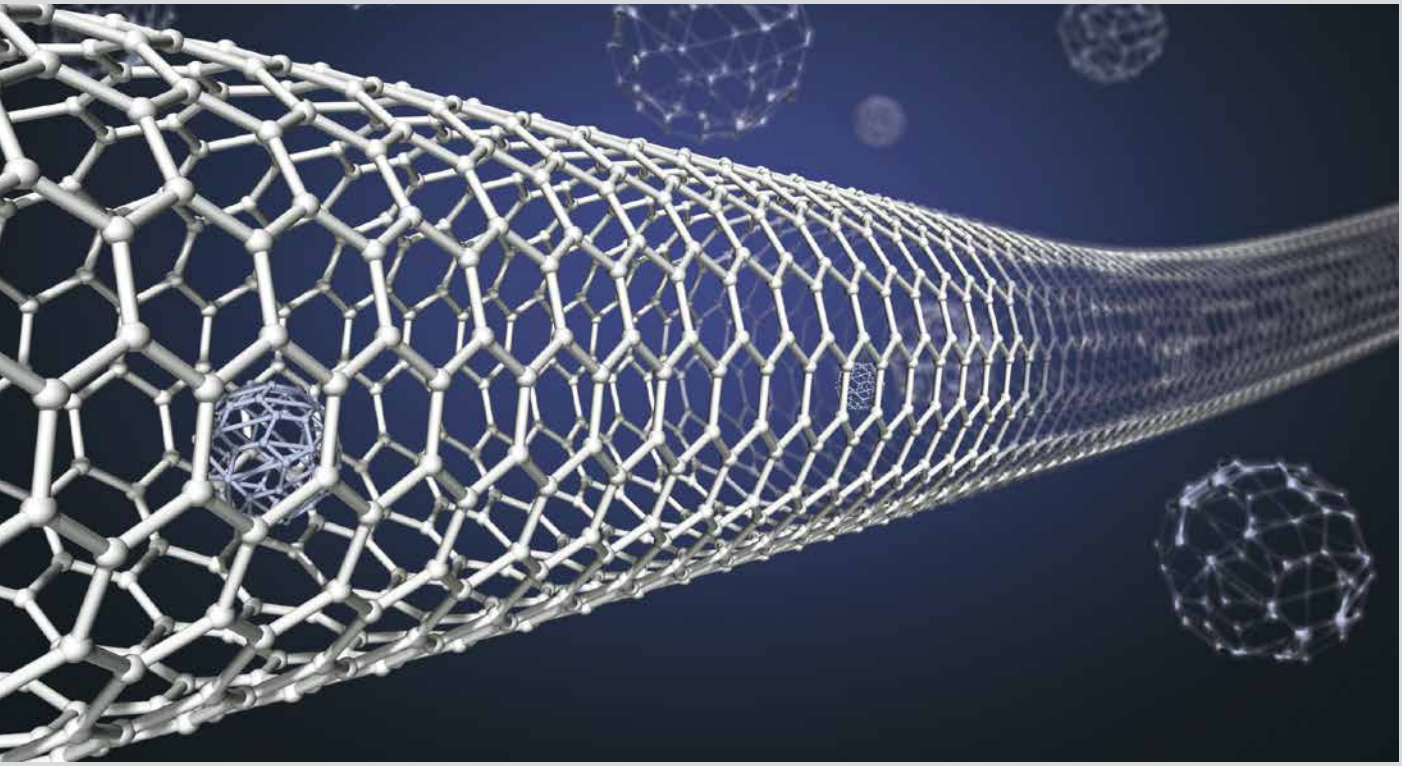

Nanotechnology and human health: Scientific evidence and risk governance

Report of the WHO expert meeting
10–11 December 2012, Bonn, Germany

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ABSTRACT

Nanotechnology, the science and application of objects smaller that 100 nanometres, is evolving rapidly in many fields. Besides the countless beneficial applications, including in health and medicine, concerns exist on adverse health consequences of unintended human exposure to nanomaterials.

In the 2010 Parma Declaration on Environment and Health, ministers of health and of environment of the 53 Member States of the WHO Regional Office for Europe listed the health implications of nanotechnology and nanoparticles among the key environment and health challenges.

The WHO Regional Office for Europe undertook a critical assessment of the current state of knowledge and the key evidence on the possible health implications of nanomaterials, with a view to identify options for risk assessment and policy formulation, and convened an expert meeting to address the issue.

Current evidence is not conclusive. As complexity and uncertainty are large, risk assessment is challenging, and formulation of evidence-based policies and regulations elusive.

Innovative models and frameworks for risk assessment and risk governance are being developed and applied to organize the available evidence on biological and health effects of nanomaterials in ways to inform policy.

Keywords

NANOPARTICLES — NANOTECHNOLOGY — PHARMACEUTICALS AND BIOLOGICALS — RISK MANAGEMENT — TOXICOLOGY

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Content

Abbreviations

µg	microgram
µm	micrometre
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
CB	Control Banding
CLP	Classification, Labelling and Packaging of chemicals
CNT	carbon nanotube
CoRAP	Community Rolling Action Plan
EC	European Commission
ECHA	European Chemicals Agency
EINECS	European Chemical Substances Information System
EU	European Union
GAARN	Group Assessing Already Registered Nanomaterials
GMO	genetically modified organism
IOM	Institute of Occupational Medicine
IRGC	International Risk Governance Council
ITS	Integrated Testing Strategies
IUCLID	International Uniform Chemical Information Database
MWCNT	multiwalled carbon nanotube
NIOSH	National Institute for Occupational Safety and Health
nm	nanometre
OC	operational conditions
OECD	Organisation for Economic Co-operation and Development
OEL	occupational exposure limit
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REL	recommended exposure level
RIP-oN	REACH Implementation Project on Nanomaterials
SAA	serum amyloid A
SDS	safety data sheets
SWCNTs	single-walled carbon nanotubes
TWA	time-weighted average
WHO	World Health Organization
WPMN	OECD Working Party on Manufactured Nanomaterials

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The meeting was chaired by Professor Michael Depledge, University of Exeter Medical School, and the rapporteur was Dr Steffen F. Hansen, Technical University of Denmark.

The report was edited by Steffen F. Hansen, Charles V. Howard, Marco Martuzzi and Michael Depledge. Participants in the expert meeting reviewed the text and provided comments.

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1. Introduction

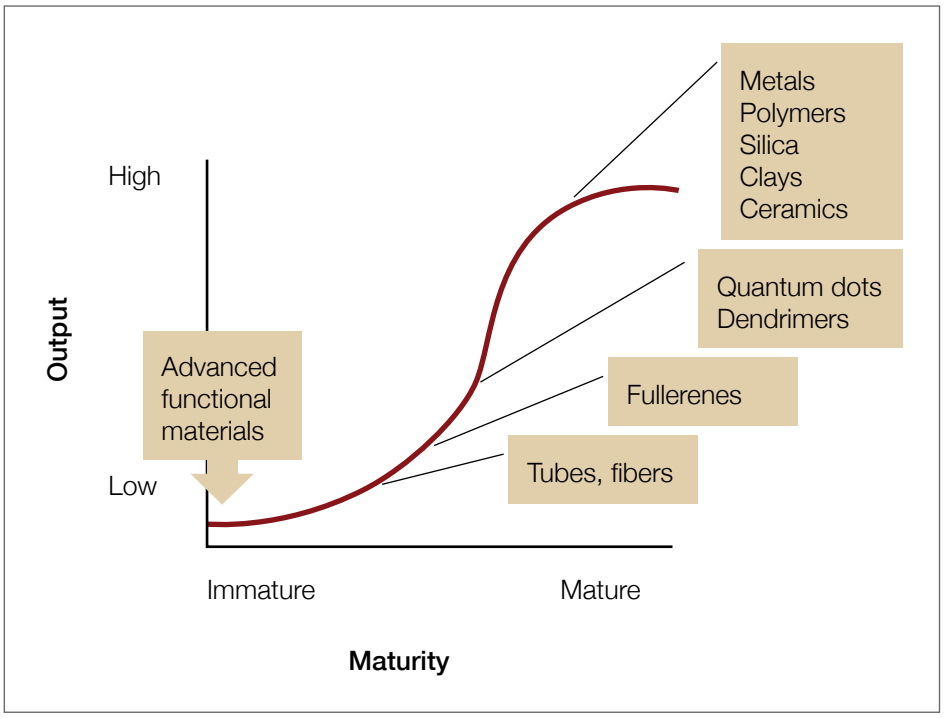
Nanomaterials and products based on nanotechnological applications are being commercialized and used at an increasing pace. In the Parma Declaration on Environment and Health,[1] the health implications of nanotechnology and nanoparticles are listed among the key environment and health challenges that ministers are committed to act on. Along with a call for increased research on the use of nanoparticles in products and nanomaterials, the ministers pledged to develop and use improved health risk and benefit assessment methods. Research into the environmental, health and safety aspects of nanomaterials is extensive and growing rapidly. The World Health Organization (WHO) Regional Office for Europe has been reviewing recent and current research, with a view to clarifying the connections between nanotechnology and health. Findings from this exercise suggest that a rigorous risk assessment is not feasible and that a pragmatic model of "risk governance" seems desirable. In order to explore how the Regional Office can contribute to progressing risk governance of nanotechnologies, a two-day workshop was held in December 2012. Participants with a wide range of expertise were invited to present their work, with the purpose of providing input to WHO. A background document on nanotechnology and health (not representing a comprehensive or systematic overview of the issue) was used as the basis for this meeting and is included in this report. Abstracts of the presentations made by participants are included in the annex. The workshop's discussion focused on four key areas (i) exposure assessment of nanomaterials, (ii) nanotoxicology, (iii) risk assessment, and (iv) regulation and risk governance.

1.1. Global nanomaterial uses and trends

Nanomaterials are making their way into all aspects of our lives; these materials are being increasingly used in pharmaceutical and medical applications, cosmetics and personal products, energy storage and efficiency, water treatment and air filtration, environmental remediation, chemical and biological sensors, military defence and explosives (Chaudhry, 2012), and in countless consumer products and materials. For instance, in the area of food, nanomaterials can be used to provide new tastes and flavours; functional foods; hygienic food processing and packaging; intelligent, lightweight and strong packaging; extended shelf-life; and reduced agrochemicals, colours, flavours and preservatives (Chaudhry, 2012). The reasons for using nanomaterials differ, according to application. For example, some applications can benefit from the increase in surface area per unit mass, which gives greater functionality. Other applications can profit from gaining better control of material properties, improved dispersion and stable formulations, or a reduced use of chemical ingredients. Again, others exploit the enhanced uptake of nutrients and supplements or increased chemical and biochemical activity (Chaudhry, 2012). According to Chaudhry (2012), nanometals and nanopolymers have been developed to a "mature stage", in the sense that they have a high manufacturing output. Nanotubes, nanofibres and fullerenes continue to increase in output. At the moment, advanced functional materials are at an "immature" stage with low manufacturing output, or at an early stage, i.e. recently put on the market or being final tested (for example, for targeted drug delivery systems). This is expected to change dramatically in the future (see red line in Figure 1).

1 The Parma Declaration on Environment and Health was signed at the Fifth Ministerial Conference on Environment and Health, Parma, Italy, 10–12 March 2010 (http://www.euro.who.int/__data/assets/pdf_file/0011/78608/E93618.pdf).

Figure 1. Manufacturing output by nanomaterial class



Note: "Immature" materials are those that are still at the research and development stage, whereas "mature" materials are already being produced and commercialized.

Source: Chaudhry (2012). Reproduced by permission.

2. Exposure assessment

2.1. Assessing direct and secondary exposures

There are multiple possible primary and secondary exposure pathways stemming from current and potential nanotechnology applications, leading to occupational and consumer exposure. This exposure can occur via inhalation, ingestion or skin absorption depending on the nanomaterial and the specific application (for treated patients, injection is also relevant) (Hansen, 2012; Poland, 2012). Therefore, there is an urgent need to assess the level of population exposure to nanomaterials, over time and for different population subgroups. Normally, exposure assessment would involve an estimation of the concentrations by mass to which workers, consumers and other environmental receptors are exposed, through all different pathways. Extensive data is required to complete a full exposure assessment including information about manufacturing conditions, level of production, industrial applications and uses, consumer products and behaviour, and environmental fate and distribution (Hansen, 2012). Unfortunately, such detailed information is lacking for virtually every type of nanomaterial or group of nanomaterials, and technical difficulties hamper accurate measurement of nanomaterials in the workplace as well as in the environment. Despite significant progress in recent years, the biological and environmental pathways taken by nanomaterials remain largely unexplored (Hankin, 2012; Hansen, 2012; Howard, 2012; Poland, 2012). Substantial efforts have been made to estimate, predict or assess occupational, consumer and environmental exposures; the applicability

of current exposure assessment methods and guidelines for chemicals has also been reviewed in the light of what we know about nanomaterials. These efforts have been hampered by both the lack of information, in general, and poor access to information needed to complete exposure assessment. It is clear that there are a number of challenges related to assessing exposure and these need to be overcome if we are to progress towards a better assessment of possible health and environmental risks.

2.2. Determination of exposure metrics

When it comes to nanomaterials and nanoparticles, it has been repeatedly observed that mass concentration might not be the most significant metric for exposure assessment in relation to health effects. Determination of the most appropriate metric for reporting exposure is restricted not only by limited knowledge of possible mechanisms of action, but also by a number of technical difficulties, such as the lack of consistent sampling and analytical methods to detect and quantify concentrations of nanoparticles by dose or by particle number, which can be used to characterize exposure in real time (Poland, 2012. An important caveat with regard to determining the optimal metric to report exposure is that it has to be consistent with classical toxicological dose metrics. For nanotoxicology, however, the usual recommendation is to report more than one dose metric.

2.3. Exposure in children and other vulnerable subgroups

There is an urgent need to consider and assess exposure to nanomaterials in children and other vulnerable subgroups. It is well known that children are disproportionately more sensitive than adults when it comes to hazardous chemicals[2] and that children have a larger relative body surface area (WHO, 2008). In addition, it is important to note that some nanoproducts are intended for use by specific subgroups, such as children and the elderly, for example with baby bottles, pacifiers, and health-care products containing nanosilver for antimicrobial activity (Chaudhry, 2012).

2.4. Exposure assessment and toxicological data generation

Arguably, research to date has focused primarily on generating toxicological data and investigating toxicological mechanisms and modes of action, with less attention paid to exposure assessment. This may be due to the relatively early stage of engineered nanomaterials production and little opportunity to conduct extensive exposure studies. However, given the knowledge gaps, exposure assessment is vitally important and needed in order to orient and prioritize the collection of toxicological data for use in risk assessment. In order to design and conduct informative toxicological studies, knowledge is needed, for instance, on the most relevant routes of exposure for different subgroups, e.g. workers, consumers, children and the elderly. In addition, information is needed to determine what the realistic exposure ranges might be for different subgroups (Chaudhry, 2012).

2 For instance, due to unique exposure pathways (transplacental, breastfeeding), high metabolic rate, high rates of cell division, poor ability to metabolize, immature excretory capacity and exploratory behaviours leading to exposures (hand-to-mouth, object-to-mouth, non-nutritive ingestion).

3. Nanotoxicology

3.1. General toxicological methods

Several studies on various forms of nanomaterials (C60, single- and multiwalled carbon nanotubes, among others) have observed that the toxicological response was related to "dose by mass", i.e. the higher the dose that laboratory animals are exposed to, the more severe the adverse effect observed. From the available research, it is evident, however, that the toxicity of nanoparticles is not only mass-dependent but might also dependent on physical and chemical properties that are not routinely considered in toxicity studies (Howard, 2012; Loft, 2012; Vogel, 2012). For instance, for low-solubility, low toxicity particles, one hypothesis is that the surface area of the nanoparticles is a better predictor of toxicity in terms of inflammation (Howard, 2012; Vogel, 2012). Other studies found that particle number was the best dose metric; in others, toxicity was related to the number of functional groups on the surface of nanoparticles.

What are the properties that determine or influence the inherent hazards of nanoparticles? This is still an open question, partly because of the general lack of characterization of those nanoparticles tested (Howard, 2012). Questions have furthermore been raised about the appropriateness of current health and safety test protocols, guidelines and animal models, used as a basis for assessing the risks to humans. Specifically, there are doubts about whether the most appropriate animal species and models are being used to identify long-term, low-dose effects (Howard, 2012; Kearns, 2012; Loft, 2012). So far, no scientific research has univocally demonstrated that new biological endpoints or toxicological effects are triggered by exposure to nanomaterials (CCA, 2008, Hankin et al., 2011).

3.2. Possible adverse health effects

Since the early 2000s, concerns have been raised about whether carbon nanotubes (CNTs) might be hazardous. These concerns were initially based on the physical similarities with asbestos fibres, and indeed since 2004 a series of experimental studies have indicated that some CNTs are able to cause asbestos-like effects (Poland et al., 2008). This was one of the factors, among others, that influenced the United States National Institute for Occupational Safety and Health (NIOSH) to issue a recommended exposure level (REL) of 1 microgram (µg) per cubic metre of elemental carbon as a respirable mass 8-hour time-weighted average (TWA) concentration (NIOSH, 2011). For titanium dioxide nanoparticles it has been shown that 20–30 nanometre (nm) particles are considerably more toxic when it comes to respiratory health than their microparticle (>100 nm) counterpart (Vogel, 2012). For humans, it is known that nanoparticles deposit in the alveoli, where they are predominantly cleared via normal macrophage mediated mechanisms. A proportion of particles can translocate and this appears dependent on physicochemical properties; but whether chronic exposure leads to sufficient particle accumulation to trigger disease is unclear (Howard, 2012; Poland, 2012). For titanium dioxide, NIOSH has proposed an occupational exposure limit (OEL) of 0.3 mg/m^3 for nano-titanium dioxide, compared to 2.4 mg/m^3 for fine titanium dioxide particles (Vogel, 2012). In general, healthy skin is a better barrier than the respiratory tract, but further research is needed to assess the effect of formulations and coatings, as well as effects on damaged skin, (such as burned or stretched skin). Transfer and systemic distribution of nanoparticles has been reported via the gut after oral exposure in several studies, with accumulations typically in the liver and other organs of the reticuloendothelial system. Further research is needed on the dosimetry, as well as long-term effects of such accumulations (Poland, 2012).

3.3. Vulnerable subgroups

Although scarce, evidence from animal studies suggests that some nanoparticles might be toxic to vulnerable subgroups, such as fetuses. For instance, for 35 nm titanium dioxide nanoparticles Yamashita et al. (2011) have observed smaller uteri and smaller fetuses after pregnant mice had been injected intravenously, with nanoparticles being found in the placenta, fetal liver and fetal brain. Because humans are a long-lived species, the impact of early exposure to nanoparticles with possible, continued, long-term, chronic, low-dose exposure remains unknown. At present, acute toxicity seems not to be a problem for many nanoparticles. However, the effect of exposure during the development and imprinting of a number of systems, which may be susceptible to processes such as protein misfolding and immunological reactions, remains largely unknown. More generally, the nature and effects of proximal and distal exposure in organisms has yet to be robustly studied in toxicological research or understood in terms of the relevance on outcome and subgroups.

4. Risk assessment

4.1. Assessing risks of nanomaterials one by one

Ideally, case-by-case risk assessment of nanomaterials should be performed in order to take the unique properties of specific nanomaterials into consideration (Kobe, 2012). Traditional chemical risk assessment is based on the notion that the chemical identity governs the risk of a chemical. However, for nanomaterials a number of other characteristics may be relevant for assessing human health risks, and it is known that a large number of nanoparticle characteristics may influence the overall hazard (Chaudhry, 2012; Hankin, 2012). On the one hand, this argues in favour of a case-by-case risk assessment approach because this is the only manner in which one can obtain a univocal determination of the risk of a given nanoparticle and its specific properties. Adopting a case-by-case risk assessment, changes in the specific properties (size, surface area, etc.) can lead to totally different risks, requiring separate estimation.

On the other hand, the number of potential combinations of various material properties makes case-by-case risk assessment of nanomaterials so demanding as to be impracticable, unless the key specific properties driving the critical outcome of interest are well known. It has for instance been suggested that there are up to 50,000 potential combinations of single-walled carbon nanotubes (SWCNTs), depending on structural types, length, manufacturing and purification processes, and surface coatings. Each one of these SWCNTs has different chemical, physical and biological properties that may determine their overall hazard. Not all of these SWCNT varieties are expected to be of commercial relevance, but there are numerous other kinds of nanoparticles, such as fullerenes, quantum dots, metals and metal oxide nanoparticles, resulting in effectively countless types of nanomaterials, which may pose different types of risk.

Thus, identifying patterns and similarities between various sets and families of nanomaterials, which can be addressed collectively by applying grouping and read-across with regards to their toxicological profile and possible health implications, would be extremely beneficial. It is also a practical necessity if risk governance of nanotechnology is to be pursued (Chaudhry, 2012). This requires reporting of case-by-case specific material properties and their implication for toxicological and environmental behaviour, to allow the recognition, description and evaluation of such families of nanomaterials and/or applications.

4.2. Options for evolution of risk assessment

Given the challenges related to risk assessment, supplementary methods and tools to describe and manage the (potential) risk of nanomaterials need to be further explored, developed and applied. Examples of such methods are the Swiss Precautionary Matrix, NanoRiskCat and control/risk banding nano tools, outlined below.

Swiss Precautionary Matrix

As part of the 2008 Swiss Action Plan on "Synthetic Nanomaterials", the Swiss Precautionary Matrix has been developed to assist in the creation of a strategy to control the risk of human engineered nanomaterials, within the framework of existing legislation. The matrix is based on a set of core principles. As a first principle, the developers decided that the scope of the matrix should encompass producers and consumers, as well as the environment. Second, input needs were kept low and only include a few particle properties, little information about the quantities of materials and some information on products and processes. Finally, the output of the matrix is based on worst case estimates of nano-specific risk potential. To address the question "How big is the need to assess nano-specific risks?" via the matrix, a score is derived based on; (i) information about whether the material is indeed a nanomaterial; (ii) the intrinsic properties of the nanomaterial (e.g. reactivity, stability); and (iii) potential human exposure and potential emissions to the environment. If the score is 0–20, the precautionary need is classified as "A", i.e. the need for action is rated as low, whereas if the score is > 20, the precautionary need is classified as "B", i.e. specific action is needed and existing measures should be reviewed (Riediker, 2012). The matrix has a dual purpose as it is intended to be used by industry as an early warning system during product development as well as a tool to fulfil the obligation of self-supervision according to the chemicals and environmental law (Riediker, 2012).

NanoRiskCat

Similarly, Hansen (2012) presented a concept called NanoRiskCat (NanoRiskCat) that has been developed at DTU Environment in order to provide support to companies and regulators in regard to assessing and communicating what they know about the hazard and exposure potential of a nanomaterial used in consumer products. The final outcome of NanoRiskCat is communicated as a short title describing the intended use, and five coloured dots. The first three dots refer to potential exposure of (i) professional end-users, (ii) consumers, and (iii) the environment. The last two dots refer to the hazard potential for (i) humans and (ii) the environment. Each dot can be assigned one of four different colours, i.e. red, yellow, green and grey indicating high, medium, low, and unknown risk, respectively. The potential human health and environmental hazard of a given nanomaterial in a given nanoproduct is evaluated by considering a number of different criteria, such as whether the nanomaterial is classified as a high aspect ratio nanoparticle, whether the bulk form of the material has been classified as hazardous and whether it is persistent or bioaccumulative in progressive sequence. Depending on whether a particular criterion is or is not fulfilled, a colour code is eventually assigned.

Control/risk banding nano tools

NanoRiskCat is applicable to consumer products but does not consider occupational uses of nanomaterials; Brouwer (2012) presented a number of control/risk banding nano tools with a focus on occupational use of nanomaterials and nano-enabled products and articles. Most control banding tools are mainly based on qualitative risk assessment (risk is a function of hazard/severity and exposure/probability) and are not necessarily quantitative, due to scientific uncertainty. Control/risk banding provides a simplified approach to systematically collect, process and evaluate information on hazard (severity) and exposure (probability) by grouping into stratified risk (or control) bands. Risk bands are linked to the level of control e.g. CL1 (ventilation), CL2a/b (LEV/fume hood), CL3 (containment) and CL4a/b (full containment/review by specialist). There are currently several web-based control banding tools available for risk management purposes, which differ in regard to

the parameters addressed and how hazard and exposure bands are assigned. Furthermore, most of the tools address first generation nanomaterials, relying partially on information on the "parent" (bulk) material, and there is a serious need for calibration and performance checking, fine tuning of hazard banding and extension of the "validity" domain for exposure.

4.3. Life-cycle perspective of nanoproducts

So far most scientific and regulatory attention has been paid to assessing the risks of individual nanomaterials, considered in high purity and uniform formulations. Only recently has attention been given to the nature and toxicity of nanomaterials as they occur in various commercial products; to the overall resources used to manufacture, process and incorporate nanomaterials into products; to abrasion and aging of nanoproducts; and to waste containing nanomaterials. Assessing the overall risk of a nanoproduct is challenging as it requires knowledge of its general characteristics, how it is produced, where and how the nanomaterial is used and at which concentration, and how it is disposed of. Also, it is important to gather extensive information about the product itself, its changes during the life-cycle and the varying properties of nanomaterials due to different environmental surroundings and media. All this information is needed to paint a full picture of the health and environmental risks associated with the production and use of nanomaterials and nanoproducts during their life-cycle.

4.4. Detection, tracking and monitoring

Detection and tracking of nanomaterials pose significant challenges when it comes to possible human exposure situations (e.g. through inhalation, ingestion, skin absorption, etc.), as does understanding of the role of physicochemical characteristics such as surface modifications, vehicles and particle transformations during body penetration and distribution (Poland, 2012). This challenge also applies to investigating nanomaterials in different environmental media (i.e. air, water and soil) as well as throughout their life-cycles (i.e. manufacturing, processing, use and disposal). Current instruments and methods are too time-consuming and not robust enough to permit routine analysis of large numbers of environmental samples, even for a limited range of manufactured nanomaterials. As noted by the Royal Commission on Environmental Pollution in their 2008 report, *Novel materials in the environment: The case of nanotechnology*, techniques will need to be extremely sensitive and able to distinguish different physicochemical forms of nanomaterials, usually against a background of natural nanoparticles with similar structure and chemistry (RCEP, 2008). Furthermore, effective monitoring of production, uses, exposures and overall health conditions among workers and the general population is key in order to develop early warning systems that enable identification of unexpected effects from the production and use of nanomaterials. An effective monitoring system would require the recording over time of factors such as: which manufacturers produced what, where, when and how much; how different occupations were involved and might be exposed; what protection equipment has been used; etc.

5. Regulation and risk governance

5.1. Regulatory initiatives under way

Very few pieces of existing legislation in Europe have nano-specific provisions. Examples of legislation that do have such provisions are the European Union (EU) Biocidal Products Regulation and the Cosmetic Products

Regulation (Kobe, 2012). In principle, the European legislation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) covers nanomaterials, as it is applicable to substances in any size or form and for all their identified uses, although it does not include explicit nano-specific provisions (Fabrega Climent, 2012; Kobe, 2012). In practice, however, inapplicable provisions (relating for example to specific tonnage triggers and phase-in status) render this regulation ineffective for nanomaterials, according to some stakeholders (Azoulay, 2012). Thus, there are issues and challenges that need to be addressed. For instance, so far only a few registration dossiers received by the European Chemicals Agency (ECHA) under REACH have contained nanomaterials-specific information. These dossiers were identified by the Nano Support Project carried out by the European Commission (EC) Joint Research Centre and ECHA. This project aimed to provide an analysis and assessment of the information provided in REACH registration dossiers, develop options for specific nanomaterial provisions in REACH and examine the human health, environmental and socioeconomic consequences of these options (Aschberger, 2012).

Several activities have been initiated by, for example, ECHA, the EC, the Organisation for Economic Co-operation and Development (OECD), academics and civil society organizations. ECHA, for instance, is currently concentrating on how the registered nanoforms of a given substance have been characterized, as characterization is seen as a prerequisite for proper risk assessment. Specifically, ECHA's effort includes developing best practices by providing substantial feedback and advice to future registrants as well as requesting information that should have been provided (Fabrega Climent, 2012; Kobe, 2012). Various nanomaterials are currently being evaluated by different EU Member States, and consensus meetings and workshops are being held between ECHA, the EC, the EU Member States and representative registrants and stakeholders (Fabrega Climent, 2012; Kobe, 2012).

With regard to national initiatives, France has initiated a compulsory declaration of nanomaterials that applies for manufacturers that produce, import, distribute or formulate nanomaterials in quantities of more than 100 g/year. Belgium, Denmark, Germany and Italy, as well as the EC are exploring the option of having a harmonized database on the production and commercialization of nanomaterials.

Industry relies on the technical guidance provided by ECHA for chemicals in general. A number of key nano-specific guidance updates were implemented in 2012 through the outcomes and recommendations from two REACH implementation projects on nanomaterials. These include: sample preparation, reporting of key physicochemical properties, exposure measurements and modelling, appropriate use of metrics of hazard and exposure assessment, and applicability of nontesting approaches (Hankin, 2012).

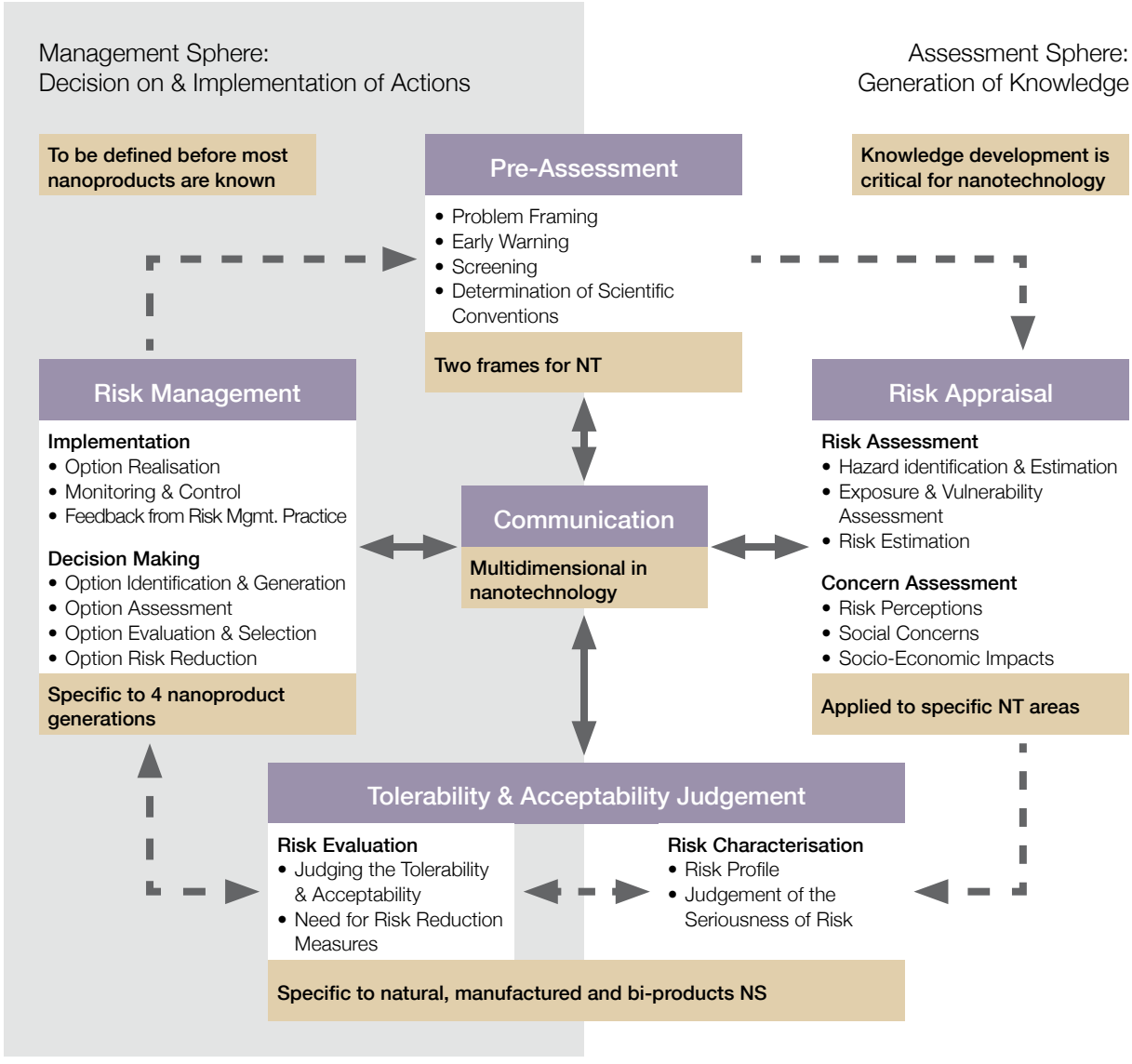
When it comes to chemical risk assessment, much of the technical guidance provide by ECHA to industry relies on the OECD's approximately 150 test guidelines on physicochemical properties, human toxicity, ecotoxicity, etc. The general approach developed to date by the OECD and various OECD Member States for testing and assessment of chemicals is applicable to nanomaterials; however, particular test guidelines may have to be further adapted to their specificities (Kearns, 2012). To this effect, the OECD has initiated a sponsorship programme that aims at reviewing existing OECD test guidelines for adequacy in addressing the nanomaterials' physicochemical properties, biotic systems, degradation and accumulation, and health effects (Kearns, 2012).

5.2. Risk governance

Due to data gaps, scientific uncertainty and ambiguity, traditional risk analysis, where regulation and risk management measures are based foremost on an independent and complete scientific risk assessment, may tend to lead to "paralysis by analysis" (EEA, 2001, 2013). Too much time can be spent waiting for the completion of risk assessment procedures and too little focus is put on implementing measures to prevent or reduce possible risks. Thus, many review exercises concur about the need for approaches that build on risk assessment but are better designed to handle complexity. The Risk Governance Framework developed by

the International Risk Governance Council (IRGC), provides an example of such an approach for nanomaterial risk analysis, as it combines aspects of the traditional risk assessment framework with societal and ethical characteristics (Figure 2). This framework was considered particularly relevant for nanotechnology due to the uncertainties surrounding the potential health and environmental effects, and hence the need to involve a wide range of stakeholders when making decisions regarding nanotechnology's use and implementation (Grobe, 2012).

Figure 2. The IRGC Risk Governance Framework



Source: IRGC (2007). Reproduced by permission.

5.3. Risk communication

Communication is a key element of this framework as one cannot choose "not to communicate" (Grobe, 2012). As noted by IRGC (2006) "lack of communication and understanding about the science, application and regulation of nanotechnology among all stakeholders may have negative effects on societal impressions and political/regulatory decision making". Hence communication plays a central role as it is of major importance throughout the entire value chain and the product's life cycle. Good communication serves a number of purposes. First of all, it should facilitate understanding among stakeholders, for example about the rationale behind the risk appraisal and risk management phases. Second, communication should help stakeholders make informed choices about risk when they are themselves involved in risk-related decision-making (IRGC, 2006). From a more holistic perspective, the heavy focus on communication between risk assessors and managers, between scientists and policy-makers, as well as civil society, is intended to "…foster tolerance for conflicting viewpoints and provides the basis for their resolution, and creates trust in the institutional means for assessing and managing risk and related concerns" (IRGC 2006, 2008).

5.4. Multistakeholder dialogue

The current state of knowledge about nanomaterials is pervaded with uncertainty and ambiguity and this requires a continuous dialogue between various scientific disciplines, risk assessors, regulators, stakeholders and civil society about how best to exploit the benefits of nanomaterials and how to avoid the risks. Examination of the benefits and risks together is essential for a realistic discussion about what is at stake and the current and future role of nanotechnology (IRGC, 2008). Consumer studies have identified the need for more information about the functionality, benefits and safety of nanomaterials along the product life-cycle. Efforts towards transparency and information sharing on the part of industry, regulators, consumers, interest groups and all relevant stakeholders, are essential; failure to promote and maintain a meaningful dialogue will result in problematic situations, similar, for example, to the debate around genetically modified organisms (GMOs) (Grobe, 2012).

5.5. Transboundary environmental, health and safety issues

More than 60 countries have government-led nanotechnology initiatives underway, but there is a need for more international coordination of these initiatives, along the lines, for example, of the EC Nanosafety Cluster. Also, more investment targeted towards achieving sustainable economic, social and environmental benefits for all is needed. It is important to remember that the environmental, health and safety issues that might be associated with the production and use of nanomaterials are transboundary in nature, e.g. with the physical transfer of nanomaterials, nanoproducts and nanowaste from one country to another via air, rivers and international trade. Rapid changes in manufacturing technologies and processes might also affect the need for and use of raw materials and natural resources, involving contrasting interests of developed and developing countries, as well as an uneven or inequitable risk–benefit distribution among different countries and economic groups (IRGC, 2006). As noted by the IRGC (2006, 2008), current regulatory structures and processes are fragmented with respect to jurisdiction, type of regulation, and the lack of harmonization of risk assessment and management procedures, both nationally and internationally. The IRGC stated already in 2006 that "decision makers worldwide need to work towards a system of risk governance for nanotechnology that is global, coordinated, and involves the participation of all stakeholders, including civil society" (IRGC, 2006).

6. Conclusion

Research on nanotechnology and health has steadily increased over the past 10 years and has generated extensive knowledge. However, as described in the WHO background document in Annex 2, there is a general agreement that the overall data and evidence on the health effects of nanotechnology is far from conclusive. The toxicity and ecotoxicity of some nanomaterials has been described, with attention principally focused on in vitro toxicological assessment and exposure in the occupational setting. There are indications, for example, that CNTs and titanium dioxide might be a hazard in occupational settings, and that nanosilver might be an environmentally relevant contaminant. However, the public health implications are unclear. Although first generation nanomaterials are being used in a variety of applications and products, they do not seem to present an imminent public health issue. On the other hand, in light of the rapidly growing production of nanomaterials (and thus possible exposure), several research questions need to be addressed, especially about long-term impacts.

Given the scientific uncertainty and still emerging evidence, and given the early indications of harm and possible adverse human health effects that have been hypothesised for some nanomaterials, a precautionary approach seems desirable. The possible extent of population-scale exposure, for example through consumer products, cosmetics, food additives, the speed of technological development and the projected proliferation of applications, has yet to be assessed. Accurate information about nanoparticle exposure and distribution in the human body, toxicological mechanisms and possible adverse health effects is needed, even though translating this information into an assessment of the risks for human health is challenging. Available methodology, current protocols and testing guidelines for chemical risk assessment, as applied in chemical safety, could be used as a basis for risk assessment of nanomaterials, but they need adaptation. Alternative or adapted models and frameworks are thus being developed and applied to organize the available evidence on biological and health effects of nanomaterials in ways to inform policy. Many of these approaches, developed by consortia of public and sometimes private agencies and expert groups, recognize the high degree of complexity and uncertainty; hence they are based on several steps (sequential or organized in flow charts), make provision for extended stakeholder consultation and, some, rather than focusing on one agent, involve comparisons of alternative ones.

Since it is currently difficult to formulate conclusive and reliable assessments of the health risks of nanomaterials, due to knowledge gaps, it is important that adequate mechanisms are put in place, at various levels, to ensure transparent and fair negotiation between stakeholders. Further initiatives must be undertaken to use available evidence on health implications, to the extent possible, to inform the risk governance of nanotechnology.

References

Aschberger K (2012). Nanosupport – Assessment of nanomaterials in REACH registration dossiers. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Azoulay D (2012). *Just out of REACH; How REACH is failing to regulate nanomaterials and how it can be fixed*. Geneva, Center for International Environmental Law.

Brouwer D (2012). Control/risk banding nano tools. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

CCA (2008). *Small Is Different: A Science Perspective On The Regulatory Challenges of the Nanoscale*. Ottawa, The Council of Canadian Academies.

Chaudhry Q (2012). Current and projected applications of nanomaterials. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

EEA (2001). *Late Lessons from Early Warnings: The Precautionary Principle 1896–2000*. Copenhagen, European Environmental Agency.

EEA (2013). *Late Lessons from Early Warnings: Science, Precaution, Innovation*. European Copenhagen, European Environmental Agency.

Fabrega Climent J (2012). European Chemicals Agency activities on nanomaterials. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Grobe A (2012). The IRGC Risk Governance Framework: Applications in food and cosmetics. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Hankin SM et al. (2011). *Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) – Final Project Report*. Brussels, European Commission (RNC/RIP-oN2/FPR/1/FINAL).

Hankin S (2012). REACH implementation projects on nanomaterials: Outcomes and implementation. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Hansen SF (2012). Exposure pathways of nanomaterials. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Howard V (2012). General toxicity of NM. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

IRGC (2006). *Nanotechnology risk governance (White Paper)*. Geneva, International Risk Governance Council.

IRGC (2007). *Nanotechnology risk governance: Recommendations for a global, coordinated approach to the governance of potential risks*. Geneva, International Risk Governance Council.

IRGC (2008). *Risk governance of nanotechnology applications in food and cosmetics*. Geneva, International Risk Governance Council.

Kearns P (2012). OECD programme on nanomaterials. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Kobe A (2012). Nanomaterials in European Union regulation. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Loft S (2012). Cardiovascular and other systemic effects of nanoparticles. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

NIOSH (2011). *Occupational exposure to carbon nanotubes and nanofibers*. Washington, DC, Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health (Current Intelligence Bulletin 65).

Poland CA et al. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, 3:423–428.

Poland C (2012). Nanoparticles: Possible routes of intake. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

RCEP (2008). *Novel materials in the environment: The case of nanotechnology*. London, Royal Commission on Environmental Pollution.

Riediker M (2012). The Swiss Precautionary Matrix. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

Vogel U (2012). Pulmonary and reproductive effects of nanoparticles. *WHO Workshop on Nanotechnology and Human Health: Scientific Evidence and Risk Governance. Bonn, Germany, 10–11 December 2012*.

WHO (2008). *Children are not little adults. WHO Training Package for the Health Sector*. Geneva, World Health Organization (http://www.who.int/ceh/capacity/Children_are_not_little_adults.pdf, accessed 23 June 2013).

Yamashita K et al. (2011). Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nature Nanotechnology*, 6(5):321–328.

Annex 1

Abstracts

NANO SUPPORT – Assessment of nanomaterials in REACH registration dossiers[1]

Dr Karin Aschberger, Scientific Officer, Nanobiosciences Unit, Joint Research Centre Ispra, European Commission

The Nano Support Project was conducted in close collaboration between the Joint Research Centre and ECHA for the EC Directorate-General for the Environment,. Its aim was to provide a comprehensive assessment of the availability and quality of data on the properties of nanomaterials in REACH registration dossiers due by December 2010. Based on the assessment, options to amend REACH to better address nanomaterials were developed. The final task comprised an assessment of the potential socioeconomic costs and benefits of these additional requirements (i.e. options).

From more than 26,000 submitted registration dossiers, covering 4,700 substances, 45 dossiers (referring to 33 distinct substances) possibly addressing nanoforms or nanomaterials were identified. The selection was based on a combination of automated searches of the International Uniform Chemical Information Database (IUCLID), on knowledge of the substances selected for assessment in the OECD Working Party on Manufactured Nanomaterials (OECD-WPMN); and other criteria to identify substances considered to include a nanoform. It is possible that other dossiers could be considered to cover nanomaterials or nanoforms, however, they could not be identified as it was often not clear whether nanoforms were included within the scope of the registered substance. In addition, the information provided on the forms or granulometry of substances often did not allow for identification of particles in the nano-range.

A first detailed analysis and assessment on substance identification, physicochemical properties and other relevant information in the 45 dossiers led to the exclusion of 20 dossiers (covering 15 substances) as they could not conclusively be considered to cover nanomaterials or nanoforms. A further assessment of the remaining 25 registration dossiers (covering 21 substances) led to the identification of three categories of registration dossiers: (i) the registrant recognized that nanomaterials or nanoforms were within the scope of the dossier (8 dossiers/5 substances), (ii) the substances were considered to exist only as nanomaterials without a bulk form (12/9), and (iii) the nanomaterials were identified on the basis of the presence of a “nanotail” in the provided particle size distribution (5/5). All 25 dossiers were subject to further detailed analysis and assessment of substance identity, physicochemical properties, human health, fate, ecotoxicity, classification and labelling and the chemical safety report. It should be noted that the assessment was not a compliance check nor any other formal REACH evaluation of the dossiers and substances analysed.

The examination of the substances’ identity and key physicochemical properties (e.g. granulometry, surface area) revealed that the information provided in the dossiers was, in general, insufficient to identify a nanomaterial or nanoform of a substance. The general observation for other endpoints, such as human health or environment, was that the test materials were usually not well characterized and different forms could not be distinguished.

Based on the result of this assessment, 21 options were developed for adapting REACH to better reflect the properties of nanomaterials. These options refer to five categories: (i) substance identification and physicochemical properties, (ii) general and specific options for human health hazards, (iii) environmental fate, (iv) environmental hazards and exposure assessment, and (v) risk characterization.

An assessment of the consequences of these options for industry, consumers, human health and the environment during 2012–2022 was carried out by a contractor. As a first step for the definition of the baseline, it was decided that only nine of these options should be addressed for the impact assessment. The remaining 12 options were, following intensive dialogue with ECHA staff, eventually considered part of the current requirements (i.e. the baseline) and therefore considered to represent compliance costs.

1 The opinions in this presentation are those of the authors and not necessarily those of ECHA or the EC.

The costs and benefits for human health were assessed quantitatively based on case studies and extrapolation to the whole market for nanomaterials. It was estimated that 500–2,000 substances (including different forms and surface modifications of the same substance) would be placed on the market, at more than 1 ton/year during 2012–2022.

The costs of the option scenario (nine options) for industry, including testing and updating registration dossiers, were estimated to be €11–73 million, as a cumulative effort until 2022. If grouping and read-across approaches were not taken into account extensively, the costs would rise to €100–600 million.

Human health benefits, e.g by improving the health of the general population were estimated to be €165 million, on average (with a range of €83–248 million) for cumulative savings for 2012–2042. As health benefits are expected to accrue with significant delays after implementation of the options, a direct comparison with the costs is not possible. All estimations are associated with high uncertainty. Qualitative impacts of the implementation of the options include more transparency on the use and safety of nanomaterials, greater innovation and better possibilities for risk reduction measures.

The report finally concludes that, based on an implementation of the options, additional costs for companies can lead to a reduced uncertainty about potentially adverse effects. These may lead to considerable health and environmental benefits, if combined with appropriate risk reduction measures.

The results of this project had a direct impact on the EC evaluation of the need to review REACH for nanomaterials within the 2nd Communication of the regulatory aspect of nanomaterials in October 2012. In addition it will have an impact on the forthcoming envisaged modifications of the REACH Annexes, to better address the properties and risks of nanomaterials.

Control/risk banding nano tools

Derk H Brouwer, Netherlands Organisation for Applied Scientific Research (TNO)

Control banding was developed in the pharmaceutical industry as a pragmatic tool to manage the risk resulting from exposure to a wide variety of potentially hazardous substances in the absence of firm toxicological and exposure data (Zalk and Nelson, 2008). It is a risk assessment approach, in a context of uncertainty, using the generally accepted risk paradigm, where risk is a function of severity of impact (hazard) and the anticipated probability of that impact (exposure). Both hazard and exposure are graded into 2–5 different levels, usually referred to as “bands”. The two sets of bands are combined, most often in a matrix, resulting in control or risk bands. The production and use of (manufactured) nanomaterials and nanomaterial-enabled products and articles, however, may introduce new and unknown risks. In such a context of uncertainty, the control banding approach can be very helpful in implementing a risk management strategy according to a precautionary method.

During the last five years, risk-management decision-support tools related to the use of nanomaterials have been developed and published; however, most of these are developed for occupational use of nanomaterials. In some cases, the application domain is extended to the use (application) of nano-enabled products. Risk categorization frameworks, such as the Precautionary Matrix (Höck et al., 2008), and NanoRiskCat (Hansen et al., 2011), include environmental exposure or impact, and consumer exposure.

The major tools used for the work environment are either control banding, where a proactive approach is used and control measures are not taken into account (indicating “potential” exposure); or risk banding, where a re(tro)active approach is used and control measures are taken into account (indicating “actual” exposure). Brouwer (2012) reviewed different “tools” for workplace risk management related to the production and

use of nanomaterials, among which was the Nano Control Banding Tool (Zalk et al., 2009); the Guidance (Cornelissen R et al., 2011); the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) control banding tool (Riediker et al., 2012); Stoffenmanager Nano (Duuren-Stuurman et al., 2012); and NanoSafer (National Research Centre for the Working Environment, 2013). The first three tools are typical control banding tools, i.e. the output is a level of control, whereas Stoffenmanager Nano and NanoSafer are risk prioritization tools, i.e. the output indicates a relative risk. Both of these latter tools are web-based, where the underlying exposure model and the hazard categorization approach are not shown to the user. Figure A1 provides an overview of control banding tools.

The methods used to assign a band differ between the various tools: the Nano Control Banding Tool uses a scoring system for both hazard and exposure banding; the Guidance, ANSES and Stoffenmanager Nano tools use a decision tree (binary system) to assign hazard band; and NanoSafer uses a mixed system to allocate a hazard band. Since specific hazard information on nanomaterials is often lacking, the hazard banding or categorization heavily relies on an evaluation of information on the "parent" (bulk) material.

The exposure banding aspect of Stoffenmanager Nano and NanoSafer is based on the conceptual model for inhalation exposure described by Schneider et al. (2011), which is a relatively simple near-field and far-field source–receptor model. The conceptual model distinguishes four "source domains", which refer to combinations of the process phase (or product chain stage) and the likelihood and form of aerosols emissions. For example, during the down-stream use a (nano)powder handling scenario is likely to generate agglomerated particles and only few unattached particles. In all tools, except NanoSafer, the hazard and the control bands are "independent" entities, i.e. the hazard and exposure bins are assigned based on their ranges. NanoSafer expresses the exposure banding as the ratio of the hazard-related pseudo-"occupational exposure limit" and the predicted exposure. The major characteristics of the tools are listed in Figure A1.1. Note that the Precautionary Matrix and NanoRiskCat are included for reason of comparison, and that the tool developed by the International Organization for Standardization (ISO) (declassification to be expected by March 2013) combines the ANSES tool and Stoffenmanager Nano.

In general, most tools lack "calibration" or a check on performance, and the hazard banding needs fine tuning, particularly for the next generation of nanomaterials, where no "parent" materials are present.

In a workshop held in Amsterdam October 1, 2012 on control and risk banding tools, at which many prominent tool developers were present, the following priority areas for further development were identified: (i) providing guidance for the selection of the tool that fits the intended use; (ii) providing training and guidance to users; (iii) embedding tools in a risk assessment framework (tiered approach); (iv) evaluation and validation using worked examples; (v) improving availability of information (material safety data sheet, suppliers, researchers); (vi) enhancing data on effectiveness of risk management measures and good practices; and (vii) creating a feedback loop to include new information in tools. The workshop concluded that all tools are valuable if used properly for their respective domains of application. Therefore, the first priority mentioned above is acknowledged to be a top priority that should result in a pre-selection of tools based on, for example, (i) the scope of the tool (proactive or retroactive); (ii) the stage of the product chain "covered" by the tool (e.g. nanomaterial versus nano-enabled articles); (iii) the domain (e.g. worker, consumer); (iv) the target user and/or scale (e.g. research or small and medium enterprises; (v) the needed (or available) knowledge level of the user (e.g. lay person or expert); or (vi) the required (or available) input data, or combinations of these parameters. This is intended to prevent inappropriate use of the tools and consequently ambiguous output, reducing the caveat of misinterpretation of tool performance.

Figure A1.1. Overview of the major characteristics of control banding tools

CB tool Short name	HAZARD BANDING			EXPOSURE BANDING							MATRIX	
	Allocation system			Source domains/ type of activities*							Number of Bands/ Levels	
	binary	score	*N*	Synthesis	Powder handling	Application Ready to use products	Abrasion	Emission potential	Exposure potential	*N*	CB	RL
Precautionary Matrix	-	+	[1]	(+)	(+)	(+)	(+)	+	-	[1]	2	-
CB Nano Tool	-	+	4	+	+	-	-	+	-	4	4	-
ANSES [ISO —(pro-active)]	+		5	(+)	+	+	+	+	-	4	5	-
Stoffenmanager Nano [ISO —(retro-active)]	+	-	5	+	+	+	(+)	-	+	4	-	3
NanoSafer	+	+	4	-	+	-	-	-	+	5		5
Guidance	+	-	3	+	+	+	+	+	-	3	3	-
nanoRiskCat	+	-	3			+	+			3	-	-

[1]Not appropriate since the Precautionary matrix has no separate hazard and exposure bands

Parenthesis:	implicitly addressed	RL	Risk level
N:	Number of bands	+	used/addressed by tool
CB:	Control Banding	-	not used/addressed by tool
RB:	Risk Banding	(+)	only implicitly addressed by tool

Source: adapted from Brouwer (2012). Reproduced by permission of Oxford University Press.

References

Brouwer DH (2012). Control banding approaches for nanomaterials. *Annals of Occupational Hygiene*, 56:506–514 (DOI: 10.1093/anhyg/MES039; http://annhyg.oxfordjournals.org/content/56/5/506.full.pdf, accessed 24 June 2013).

Cornelissen R et al. (2011). *Guidance working safely with nanomaterials and nanoproducts. The guide for employers and employees*. Amsterdam, Dutch Social Partners FNV, VNO-NCV and CNV.

Duuren-Stuurman B et al. (2012). Stoffenmanager Nano Version 1.0: A web-based tool for risk prioritization of airborne manufactured nano objects. *Annals of Occupational Hygiene*, 56:525–541.

Hansen FS, Braun A, Asptrup-Jensen K (2011). *NanoRiskCat – A conceptual decision support tool for nanomaterials*. Copenhagen, Danish Environmental Protection Agency.

Höck J et al. (2008). *Precautionary Matrix for Synthetic Nanomaterials*. Bern, Switzerland. Federal Office for Public Health and Federal Office for the Environment.

National Research Centre for the Working Environment (2013). Risk Evaluaton Tool and Good Practise for Safe Handling of Nanoparticles (NANOTOOL) [web site]. Copenhagen, National Research Centre for the Working Environment (http://www.arbejdsmiljoforskning.dk/en/projekter/arbejdsmiljoekatalog-om-haandtering-af-nanopartikler-nanotool, accessed 18 July 2013).

Riediker M et al. (2012). Development of a control banding tool for nanomaterials. *Journal of Nanomaterials* (DOI: 10.1155/2012/879671; http://www.hindawi.com/journals/jnm/2012/879671/, accessed 24 June 2013).

Schneider T et al. (2011). Conceptual model for assessment of inhalation exposure to manufactured nanoparticles. *Journal of Exposure Science and Environmental Epidemiology*, 21(5):450–463.

Zalk DM and Nelson DI (2008). History and evolution of control banding: A review. *Journal of Occupational and Environmental Hygiene*, 5:330–346.

Zalk DM, Paik SY, Swuste P (2009). Evaluating the control banding nanotool: A qualitative risk assessment method for controlling nanoparticle exposures. *Journal of Nanoparticle Research*, 11:1685–1704.

European Chemicals Agency activities on nanomaterials

Julia Fabrega Climent, Directorate of Evaluation, European Chemicals Agency

Nanomaterials are described as particles with structures of at least one dimension within 1–100 nm. The manufacture of nano-size particles can result in novel physicochemical properties that may differ from those of their bulk counterpart. This can be exploited for innovative technological applications which offer substantial benefits to society.

Given the rapid development of nanotechnology industries and the foreseen increase in production volumes of nanomaterials worldwide, it is expected that the number of products available in the European market will increase significantly in the coming years. Hence, it is important that comprehensive risk assessments are conducted for nanoforms, as the rapid increase in their manufacture and use raises inevitable questions regarding their potential effects on health and on the environment.

Although there are no explicit requirements for nanomaterials under the EC REACH or CLP[2] regulations; the ECHA, the EU Member State competent authorities consider that nanomaterials meet the REACH definition for substances, and therefore REACH requirements apply. Many substances exist in different forms (solids, suspensions, powders, nanomaterials, etc.), and under REACH different forms can appear within a single registration of a substance. However, the registrant must ensure the safety of all included forms and provide adequate information to address the different forms in the registration dossier, including the chemical safety assessment and its conclusions, as well as different classifications, where appropriate (EC, 2012).

ECHA and EU Member State competent authorities agreed to develop a common approach to address the current information requirements in dossiers containing nanoforms, taking into account the scientific and legislative uncertainties in the framework provided by REACH.

An assessment (performed on the ECHA database in 2011) on how nanomaterials have been addressed in REACH registrations showed that only seven substance registrations had selected “nanomaterial” as the form of the substance in voluntary fields. A further assessment identified additional substances with nanoforms. Many registrations for substances known to have nanomaterial forms do not mention clearly which forms are covered or how the information provided relates to the nanoform. Only limited information specifically addresses the safe use of the specific nanomaterials supposedly covered by the registration dossiers. These findings are partly explained by the absence of an adopted definition of the term “nanomaterials” at the time of the first registration deadline of December 2010, the lack of detailed guidance to registrants on registration for nanomaterials, and the general wording of the REACH annexes.

In 2012, ECHA began to examine dossiers registered under REACH containing nanoforms. When elements in a dossier indicate that the substance or forms of the substance may fall under the definition of a nanomaterial, ECHA has issued requests for information. The requests focussed on the characterization of nanomaterials, in particular on the size distribution and on surface treatment. The analysis of the information received from the registrants was still on-going at the editorial deadline. The registrants either did not react at all, answered

2 Classification, Labelling and Packaging of chemicals.

without providing the information requested, or did provide additional information on primary particle size and specific information on surface treatment.

Currently, most nanomaterials are phase-in substances (substances listed on the European Chemical Substances Information System [EINECS] and preregistered Thus, the applicable registration deadline (2010, 2013 or 2018) is triggered by the production volume (production volume per legal entity), as well as the hazardous classification of the substance. To date, only substances manufactured or imported to the EU at more than 1000 tonnes per year and substances classified as CMR1/2 (1 tonnes/year) or R50/53 (100 tonnesyear) were registered. Lower tonnages will be registered at a later stage. Thus, this might be a relevant factor in the low number of dossiers received to date, given that the manufacture of most nanomaterials has not yet grown to high production volumes (> 1000 tonnes/year per legal entity).

In October 2011, the EC adopted a recommendation on the definition of "nanomaterial". The purpose of the definition was to enable determining when a material should be considered a particulate nanomaterial or a nano-constituent material, and should be covered by several EU regulations (EC, 2011). ECHA is implementing the definition as a benchmark in assessing substances within REACH and invites registrants to proactively characterize their substances in light of this definition. The characterization of nanoforms of a registered substance is a prerequisite to the proper determination of hazards and subsequently risks of the substance in its nanoform. ECHA's current focus is seeking clarity on the physicochemical characteristics of nanomaterials. To this end, it will use the available REACH instruments to obtain available data (e.g. in accordance with Article 36) or request the generation of new data (Article 41). Such a gradual approach, combined with a collaborative and constructive interaction with registrants and stakeholders, forms the first step towards a full safety assessment of nanomaterials under REACH.

Nanomaterials risk assessment

One of the main obligations under REACH is that manufacturers, importers and downstream users have to ensure the safe use of the substance they place on the market (in whatever form it is synthesized). Given the current status of scientific developments in the area of exposure and risk assessment of nanomaterials, industry, regulators and other stakeholders face a substantial challenge in adequately characterizing the risks to humans and to the environment.

For regulatory purposes, appropriate characterization of manufactured nanomaterials is essential. Information on physicochemical parameters is important for synthesis, product formulation and toxicological testing. Hence, ECHA, in close collaboration with EU Member State competent authorities, the EC and stakeholders, is focusing substantially on assessing the type of information that is needed from industry in registration dossiers, in order to characterize the risks nanomaterials may pose to humans and to the environment. In initial stages, efforts concentrated on physicochemical parameters and processes that, when reported, can aid identifying nano-dossiers.

Activities on nanomaterials

There are a large number of REACH processes (e.g. registration, evaluation, authorization and restrictions) and CLP processes (e.g. classification and labelling) for which ECHA needs to be able to carry out its tasks for nanoforms, as for any other form of a substance. Therefore, since 2011, ECHA has gradually increased its activities in the area of nanomaterials, in order to properly address the regulatory, scientific and legal challenges and provide support and advice to industry and stakeholders. Some of the activities that have been conducted in 2012 include:

- ECHA has been providing feedback and advice (e.g. via webinars) to registrants that wish to register nanomaterials at the next registration deadline (2013).

- Guidance developments for nanomaterials have led to six new and eight corrigenda to appendices to chapters of the Information Requirements and Chemical Safety Report.
- IUCLID updates incorporate several nanomaterial flags, and the IUCLID manual is also being updated to align it with the guidance updates.
- Article 36 decisions have been sent to registrants to request further information on their substance. The decisions request information that the registrant should have already, and do not require further testing.
- Three substances have been selected for the Community Rolling Action Plan (CoRAP) List of Substances based on initial grounds of concern. These are: silicon dioxide, silver and titanium dioxide.
- A nanomaterials working group (ECHA-NMWG) has been created to discuss scientific and technical questions relevant to REACH and CLP processes. ECHA-NMWG is an informal advisory group consisting of experts from EU Member States, the EC, ECHA and accredited stakeholder organizations.
- A Group Assessing Already Registered Nanomaterials (GAARN), established by the EC Directorate-General for the Environment and chaired by ECHA, has the purpose of building a consensus in an informal setting on best practices for assessing and managing the safety of nanomaterials under the REACH regulation. This aims to increase confidence and mutual understanding among stakeholders so that nanomaterials can be sustainably developed.

References

EC (2011). *Commission Recommendation 2011/696/EU*. Brussels, European Commission (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF, accessed 24 June 2013).

EC (2012). *Second Regulatory Review on Nanomaterials. Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee. COM(2012) 572*. Brussels, European Commission (http://ec.europa.eu/nanotechnology/pdf/second_regulatory_review_on_nanomaterials_-_com(2012)_572.pdf, accessed 24 June 2013).

REACH implementation projects on nanomaterials: Outcomes and implementation

Dr Steve Hankin, Institute of Occupational Medicine

It is a continuing matter of debate whether current formal regulatory frameworks, including REACH, are well suited to identifying nano-specific issues. These issues include the relevance of notification triggers and information requirements, and important knowledge gaps in the toxicology, physicochemical characteristics and exposure data needed for highly-informed risk assessment and risk management. In the face of this uncertainty, a number of activities aim to improve REACH in relation to nanomaterials. One such activity, the REACH Implementation Projects on Nanomaterials (RIP-oN), sought to provide scientific and technical advice on key aspects of the implementation of REACH with regard to nanomaterials. The objectives of the RIP-oN 2 and RIP-oN 3 projects, undertaken by a consortium led by the Institute of Occupational Medicine (IOM), were to develop specific advice on:

i. how REACH information requirements on intrinsic properties of nanomaterials can be fulfilled, including the appropriateness of the relevant test methods (and dosimetry) for nanomaterials; and outlining, when relevant, possible specific testing strategies (RIP-oN 2);
ii. the information that is needed for safety evaluation and risk management of nanomaterials and, in particular, if information is needed beyond or in addition to the current information requirements listed in REACH annexes VI–X (RIP-oN 2);

iii. how to do exposure assessment for nanomaterials within the REACH context to cover (a) development of exposure scenarios, (b) evaluation of operational conditions (OC) and risk management and mitigation measures, and (c) exposure estimation (RIP-oN 3);
iv. how to conduct hazard and risk characterization for nanomaterials (RIP-oN 3).

These projects were carried out as an objective scientific review based on an informed and systematic gathering and consideration of evidence by experts who used their knowledge and professional judgement when considering the relevance and contribution of the scientific evidence towards delivering the projects' objectives.

The final project reports summarize the key specific issues related to nanomaterials in a REACH context. They also recommend updates to the guidance in a form compatible with possible future integration into the existing REACH Guidance on Information Requirements and Chemical Safety Assessment. Clear reference to the existing REACH guidance was provided. For issues which were considered to be insufficiently technically mature for developing detailed guidance, the need for further research and development is indicated. All task reports were subject to review by the project's EC Steering Group, constituting representatives of the EC Joint Research Centre, Directorate-General for the Environment, Directorate-General for Enterprise and Industry and the ECHA, and by a Stakeholder Consultation Group, consisting of the members of the REACH Competent Authorities Sub-Group on Nanomaterials and other relevant experts from EC Member States, industry and nongovernmental organizations nominated by the Competent Authorities for REACH and CLP (CARACAL).

The assessment of the scientific evidence and subsequent recommendations are the considered opinion of the authors and were submitted for consideration by the EC, who have subsequently published the recommendations as appendices to existing guidance documents.

The final reports for the RIP-oN projects[3] and nanomaterial-specific appendices to ECHA Guidance[4] are freely available to download.

Since the completion of the RIP-oN 2 and 3 projects, a number of additional and on-going activities continue to inform the discussion and development of nanomaterials regulation. These include publication of the EC definition of a nanomaterial, the EC Second Regulatory Review on Nanomaterials published in October 2012, and their forthcoming assessment of regulatory options for nanomaterials under REACH expected in December 2013. Additional evidence gathering activities include the NanoSupport project[5] and other EC sponsored projects underway including "Scoping possible modifications across the breadth of EU safety & health at work legislation for nanomaterials" and "Scoping the impact on industry, consumers, human health & the environment from possible options for changing the REACH regulation". These can be expected to be complemented by contributions to the evidence-base from national, European and international research projects of relevance to nanomaterials risk assessment.

Summary of findings from RIP-oN2

Of the existing information requirements reviewed, in general the guidance on physicochemical properties is considered to be applicable to nanomaterials, with the exceptions of the limited relevance and applicability of the properties and methods for surface tension, flash point and viscosity. Further evaluation of the suitability of existing methods for water solubility, partition coefficient, adsorption and desorption was also recommended.

The existing guidance on toxicological data information requirements was considered applicable for the assessment of nanomaterials, although it was highlighted that attention needs to be given to measuring, dosing,

3 http://ec.europa.eu/environment/chemicals/nanotech/index.htm#ripon
4 http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
5 http://ec.europa.eu/environment/chemicals/nanotech/pdf/jrc_report.pdf

delivery and tracking of nanomaterials in the test system. In general, the basic ecotoxicological properties and endpoints described in OECD test guidelines for the determination of potential effects of test substances in environmental compartments (aquatic, terrestrial, sediment) after acute or chronic exposure are considered adequate and relevant for nanomaterials. However, OECD acknowledge that the test guidelines were not specifically designed for the testing of nanomaterials, and the guidance provided on preparation, delivery of test substances to the test system, exposure quantifications, dose metrics, measurement, and metrology is considered to be insufficient for testing of nanomaterials.

The potential additional relevant specific intrinsic properties, which have been identified from an objective review of published scientific sources of information, include:

- Physicochemical properties
  - particle shape
  - surface area
  - surface energy
  - surface chemistry
  - surface charge
  - redox potential
  - cell-free reactive oxygen species (ROS) and reactive nitrogen species (RNS) production capacity
  - state of dispersion
  - state of agglomeration

- Toxicological endpoints
  - cell uptake
  - cell viability
  - oxidative stress
  - inflammation
  - fibrosis
  - immunotoxicity (sensitization)
  - cardiovascular toxicity

- Ecotoxicological endpoints
  - ventilation rate
  - gill pathologies
  - mucus secretion
  - brain pathology
  - animal behaviour
  - oxidative stress biomarkers – superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST).

The value and feasibility of incorporating the identified potential additional relevant specific intrinsic properties into the REACH guidance was considered on the basis of the available scientific evidence. Notably, the published scientific evidence demonstrates a consensus that representative sample preparation and thorough and accurate physicochemical characterization (using multiple techniques) is an essential component of assessing the potential (eco)toxicity of nanomaterials.

With regard to toxicity, a range of endpoints have been examined relating to some of the existing information requirements under REACH, including: acute toxicity, repeated dose toxicity, toxicokinetics, mutagenicity, carcinogenicity and reproductive toxicity. However, no material regarding other REACH information requirements (such as dermal and respiratory sensitization and irritation) was identified. Frequently, studies considered

the endpoints of cell viability, oxidative stress, and pro-inflammatory effects in vitro. Studies undertaken and reported to date have highlighted a number of key issues or gaps in existing testing strategies that may influence their outcome, and thus should be observed closely in the assessment of nanomaterials within the context of REACH. Factors such as the exposure method, dose selection, species used and cell type under investigation, all have the potential to impact on the assessed toxicity of nanoparticles, highlighting how experimental design can influence the resulting toxicological profile.

A limited body of scientific evidence is available to inform the provision of specific practical advice with regard to the ecotoxicological information requirements in REACH, but a number of issues have been found to influence the ecotoxicologic responses observed in the study of nanomaterials. These include: (i) coating and functionalization of the surface and particle impurities, (ii) suspension preparation methods, (iii) release of free metal ions, (iv) particle aggregation, and (V) relevance of dose (concentration)-response for ecotoxicological studies of nanomaterials. The extent of influence of these factors on the ecotoxicological impact of nanomaterials is still emerging. Although at the time of writing this report, results from the OECD-WPMN Sponsorship Programme had not emerged, the limited information currently available from OECD-WPMN has been considered. Documents published or classified by the International Organization for Standardization and European Committee for Standardization as being at “Final Draft International Standard” or “Draft International Standard” stage were reviewed and commented upon. Those at an earlier stage of development (i.e. “Committee Draft” stage or lower) were reviewed and commented upon, to the extent possible.

The gap analysis of relevant intrinsic properties for nanomaterials assembled and further developed the findings from (i) the examination of existing REACH guidance, (ii) the identification of additional relevant specific intrinsic properties for nanomaterials, and (iii) the assessment of relevance and applicability of testing, endpoints and methods described in the scientific literature and on-going international work. The framework used for the gap analysis considered physicochemical properties and toxicological and ecotoxicological endpoints. It integrated the existing and recognized additional properties and endpoints to identify those that may and may not be addressed by standard test guideline methods. It also sought to identify where further development of in vitro, in vivo or other methodologies is required. The gap analysis was structured by property and/or endpoint, and systematically assessed whether a property and/or endpoint is relevant and the respective characterization methods were applicable to nanomaterials. Commentary was provided on aspects including: whether the property and/or endpoint is applicable to substances, particles, or nanomaterials only; the method type (standard, nonstandard method or widely-accepted in research and development); the applicability and limitations of the method; information on the type of data provided by the method; and identification of research and development needs. The outcomes of the gap analysis have informed the development of specific guidance updates and recommendations for research and development.

The relevance and applicability of the current Integrated Testing Strategies (ITS) to nanomaterials for properties and endpoints in the existing REACH guidance have been reviewed and any limitations identified. For each ITS , the relevance and applicability to nanomaterials has been indicated along reviewed, and the need for any update to the existing guidance text has been indicated, where this is considered feasible given the current state of knowledge. In general, only minor updates are necessary to some of the existing ITS for the properties and/or endpoints considered. This includes the general testing strategy for physicochemical properties, water solubility (reflecting a need to distinguish between solubilization and dispersion), and the need to justify scientifically the use of quantitative structure–activity relationship models and/or read-across in the toxicological endpoints. A substantive update to the ITS for granulometry is suggested, reflecting the recommended substantive update to the guidance for this property. Advice is provided on the scientific basis for the categorization of nanomaterials and application of in silico methods, read-across and category approaches for deriving hazard information for nanomaterials from the information on bulk substances or from comparison between nanomaterials. Whilst the lack of data across a wide range of structural and compositionally different nanomaterials precludes a fully prescribed category-based approach being developed, the suggested approaches for possible development indicate where such groupings may be applied.

Summary of findings from RIP-oN3

OC, risk management measures and the "hierarchy of control" concept that underpins much of the REACH guidance in this area were considered to be equally valid for nanomaterials as for other substances. There is evidence that control and risk management methodologies that are already known can provide levels of protection for workers from exposure to engineered nanomaterials. Whilst it was not indicated that new nano-specific risk management measures need to be developed, the protection provided against specific nanomaterials needs to be evaluated. Evidence indicates that emissions in the workplace are substantially reduced if a process involving engineered nanomaterials is performed in a properly designed enclosure or containment, although this was not universal. The situation is further amplified when considering what happens when containment is opened. Similarly, evidence indicates that worker exposure can be significantly reduced or prevented through the use of correctly designed and implemented extraction ventilation and filtration. Filtration theory indicates that filtration will be effective for particles in the nanometre size range. This also applies to personal protective equipment, where several studies clearly demonstrate the potential of respirator filters to capture nanoparticles. As for chemicals in general, further work is required to investigate human factors such as leakage around (rather than through) a face-piece filter. The situation is not as clear with protective suits and gloves, where much less work has been carried out.

Control Banding (CB) may have use in relation to the selection of control approaches. Attempts are being made to develop this approach for nanomaterials, but they are at an early stage. However, given the current level of development, CB cannot be used to demonstrate that the risks are adequately controlled. As an interim measure, users might consider CB approaches to provide an initial selection of control measures, while collecting further information about exposure, toxicity and risk. Although preliminary medical surveillance activities, such as documentation of the presence of engineered nanoparticles and identification of potentially exposed workers, are likely to be beneficial in the long term, no clear guidance can be given at this time as to which specific medical endpoints should be examined. For safety data sheets (SDS), it is important that information provided for a nanomaterial is representative, valid and provides the protection needed for the forms addressed by the SDS.

Other than in the case of filtration, no recommendations for risk management measures in REACH guidance relating to the environment can be made at this time, due to lack of evidence. Almost no work has been done on the effectiveness of consumer risk management measures. For OC, only limited information was found to be available in the public literature. Information is available on the risk management measures adopted, and in some cases the quantity of material produced and used on a daily or batch basis. Information concerning room sizes, ventilation rates, and temperature is almost entirely absent.

Regarding exposure estimation, the key issues identified included: discrimination from background nanoparticles; measurement of size distribution; maximum relevant particle size; effect of high spatial and temporal variability; assessment of high aspect ratio nanomaterials; application of exposure models and choice of metric; and instrument and measurement strategy. Alternative approaches in dealing with background particle measurements included a time series approach, near- and far-field paralleled measurements and off-line analysis to confirm whether peak concentrations observed correspond to an identified nanoparticle, either by composition, morphology or both.

Consideration of the use of size distribution data concluded that recommended methods should be able to account for complex distributions (e.g. bimodal distributions) and that the full size distribution curve should be reported. Particle size issues were concerned with aggregates and agglomerates and the need to identify and characterize these. Nanoparticles of interest may be present as primary particles, larger aggregates or agglomerates, and potentially background particles from which primary particles may subsequently be released. It was suggested that the respirable convention is the appropriate upper size limit. Given the effect of high spatial and temporal variability, measurements of workplace air concentrations are unlikely to represent

personal exposure. Therefore strategies which encourage comparison (even limited) between workplace air concentrations and personal exposure are recommended. At this time, it is not possible to make a definitive statement concerning which of the metrics are the most appropriate for nanoparticles. In relation to measuring exposure, the recommended practice at this time is that measurements should encompass assessment of at least mass, but where possible also number and/or surface area concentration.

For high aspect ratio nanomaterials, the application of WHO approach has not yet been validated. Given an absence of measurement methods or terminology to describe “bundles” or “clumps” of high aspect ratio nanomaterials, no specific guidance can be given at this time for quantitative assessment of these entities. However, their presence should be noted in any assessment. The limited evidence of validation for occupational exposure indicates that model estimates should not be relied on alone without further confirmation of their validity in individual cases. In any case, model estimates should be used with caution and with further scientific justification. Detailed implications for these issues in relation to the REACH guidance has been developed in Task B4 and refined through discussions with the Stakeholder Consultation Group into proposals for guidance amendments, which have been fully elaborated in the Final Project Report.

Consideration and evaluation of the REACH approach for deriving no-effect levels was made through the use of case studies for multiwalled carbon nanotubes (MWCNTs), nano-titanium dioxide and silver nanoparticles. In relation to the case studies, in all instances data gaps were observed that could hinder a full evaluation under REACH. Normally, the approach for dealing with deficiencies in data would be to look for other studies using similar materials, which may provide some knowledge of the likely effects of the materials (e.g. long-term effects, systemic effects, etc.). However, in relation to many nanomaterials, evidence is insufficient to apply such an approach.

Where data was available, and a case study was performed, it emerged that a major question relating to the REACH guidance was the applicability of the current assessment factors for nanomaterials, as these assessment factors have been derived from classical (soluble substance) toxicity in relation to both human and environmental health. Considerations have been made regarding their applicability to nanoparticles and the impact that alternative metrics and other issues, such as agglomeration and aggregation state, could have on the different assessment factors. However, it was considered that, for the most part, the current guidance on deriving exposure limits provides sufficient flexibility to address areas of uncertainty, data gaps and, if justified, deviations from the default approach and assessment factors. It was considered unclear whether the aggregation and agglomeration of nanoparticles will result in higher or lower toxicities found in standard tests. However, the aggregation or agglomeration state could affect various parameters, such as deposition zone in the lung or uptake by organisms, and thus characterization of particles both within test systems and the exposure environment is important.

Considerations were made of on-going hazard and risk characterization approaches, using the case study nanomaterials (MWCNTs, titanium dioxide, nanosilver). Evaluation of the identified alternative approaches for hazard and risk characterization under REACH revealed both merits and deficiencies in the derivation of exposure limits. This was very much the case in relation to extrapolating from experimental animals to humans for inhalation exposure (pertaining to both initial starting point modification and interspecies adjustments). Based on the information gathered and considered, in addition to the wider particle toxicology literature, an alternative approach for extrapolating from experimental animals to humans for inhalation exposure was suggested for possible future incorporation into guidance.

Lastly, jointly between the RIP-oN 2 and RIP-oN 3 projects, the critical items on exposure and dose descriptors were identified and used to outline needs for adequate metrics and parameters, as appropriate for exposure assessment and compatible with those used for hazard assessment. The metrics currently used in risk assessment (both regulatory and otherwise) across the three elements of exposure, toxicology and risk, are based on mass or number. The most prominent emerging alternative or additional metric identified for use

in relation to the risk assessment of nanomaterials is surface area. This is based primarily on toxicological evidence relating particle surface area to inflammation, an indicator of toxicity. There are currently no definitive conclusions on the best metric. However, there is consensus that there should be sufficient characterization of the forms of a substance tested to allow the dose response to be expressed in the different metrics discussed: number, surface area and mass. It is important to note that there are other parameters that can act as modifiers of the toxicity, including particle size, size distribution, density, surface modification, aggregation and agglomeration state, and shape, but these parameters would not generally be considered as scalable quantities. They do not appear to conform to the current use of the term "metric" under REACH, and were therefore not considered further in relation to the metric issue.

Nanomaterials in EU regulation

Andrej Kobe, Directorate-General for the Environment, European Commission

There are no specific EU regulations on nanomaterials. Two successful EC regulatory reviews, in 2008 and 2012, have concluded that the existing EU regulatory framework covers nanomaterials in principle; however, individual pieces of legislation may require adaptation to ensure adequate and effective implementation. In 2009 the European Parliament disagreed with the EC 2008 assessment and called for responsible use of the new technology and actions to ensure adequate information on safety and uses of materials on the market. It also prompted the introduction of some specific provisions in the enacting terms of legislation on biocides (EU, 2012), cosmetics (EU, 2009) and food labelling (EU, 2011) in co-decision. In a 2012 review, the EC affirmed the need to strengthen market surveillance and improve information and labelling requirements for nanomaterials in consumer product safety legislation. It also concluded that principle risk assessment paradigms applied in existing legislation were adequate, with the proviso that risks arising from specific properties, challenges in application of methods and tools, and variation between different materials must be adequately addressed. No generalizations are possible at present so a case-by-case approach to risk assessment is required. There is still a general lack of data, although development since 2008 has been significant. A review of environmental legislation (e.g. air, water, waste) emphasized the challenges in assessing nanomaterials in the environment, the ineffectiveness of end-of-pipe measures and the strong dependence on upstream chemicals legislation (e.g. REACH and CLP) to generate appropriate data and classify hazardous nanomaterials. An assessment on workplace legislation will only be concluded in 2014.

At present, cosmetics, biocides and food labelling regulations include nano-specific provisions in enacting terms, to better ensure their specificity is taken into account in the respective risk assessment and/or labelling of product ingredients. The first step in revising REACH was improving guidance for implementation and set-up of the specific working group at the ECHA, while further steps, such as revision of its annexes are still in progress. All changes have been informed by a series of studies, stakeholder consultations and the opinions prepared by the EU scientific committees. In order to support coherent regulatory introduction of nano-specific provisions, in 2011 the EC published a recommendation on the definition of "nanomaterial" and invited Member States and EU agencies to apply it in their work. Practical aspects of the implementation of the recommendation are continuously being addressed (e.g. the 2012 Joint Research Centre report on measurement methods), while the definition itself will be reviewed in 2014.

In 2012, France introduced a notification scheme to address the lack of knowledge regarding nanomaterials on the market. Several other Member States are considering similar schemes. In 2013, the EC is performing an impact assessment on establishing an inventory at EU level and will take into account national experiences. It also announced the third regulatory review in 2015.

References

EU (2009). Regulation (EC) No 1223 /2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. *Official Journal of the European Union*, L 342 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF, accessed 17 July 2013).

EU (2001). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. *Official Journal of the European Union*, L 304/18 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF, accessed 17 July 2013).

EU (2012). Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. *Official Journal of the European Union*, L167 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:167:0001:0123:EN:PDF, accessed 17 July 2013).

Pulmonary effects of exposure to nanoparticles

Ulla Vogel, Anne T Saber, Nicklas R Jacobsen, Petra Jackson, Karin S Hougaard, Ismo K Koponen, Keld A Jensen, Håkan Wallin

Danish Centre for Nanosafety, National Research Centre for the Working Environment

Nano-sized titanium dioxide and carbon black are both high-volume industrial chemicals. Airway exposure to nano-sized titanium dioxide and carbon black in the working environment is therefore likely. The potential risk of airway exposure to nanoparticles is of concern. Depending on primary and agglomerate particle size-distributions, a significant fraction of inhaled nanoparticles may deposit in the alveolar region of the lungs where clearance is slow. Prolonged lung retention is likely to result in long-lasting inflammation, which is linked to several adverse health effects. Inflammation has been shown to be proportional to the total surface area of the pulmonary deposited particles (Duffin et al., 2007; Saber et al., 2011)

As an example, we have studied the hazard of two industrially relevant engineered nanoparticles: titanium dioxide nanoparticle UV-Titan L181, which is a surface coated rutile with an average crystallite size of 17 nm; and Printex 90, which is a carbon black nanoparticle with an average primary particle size of 14 nm. When aerosolized, the titanium dioxide had an average aerodynamic size of 97 nm (Hougaard et al., 2010) and the carbon black had an average agglomerate size of 45 nm (Jackson et al., 2012). Inhalation of the titanium dioxide and carbon black dusts for 1 hour daily for 11 days at about 40 mg/m^3 increased influx of neutrophils in the lungs at both 5 and 25 days after exposure in mice (Hougaard et al., 2010; Jackson et al., 2012). Titanium dioxide retention in the lungs was assessed by measuring the titanium content in lung tissue 5 and 25 days after exposure. Five days after exposure, 24% of the estimated lung titanium deposition could still be detected in lung tissue; after 25 days, 21% could be detected, indicating slow clearance of the nano-titanium dioxide from the lungs (Hougaard et al., 2010). Increased levels of DNA strand breaks in bronchiolar lavage fluid were observed following inhalation exposure to carbon black but not titanium dioxide (Hougaard et al., 2010; Jackson et al., 2012). The doses correspond to half the daily exposure at the Danish OEL to titanium dioxide (9.75 mg/m^3 for 8 hours) or 50% more than the daily exposure at the Danish OEL to carbon black (3.5 mg/m^3/8 hours).

In another series of studies, UV-Titan and Printex 90 were deposited in lungs of mice by intratracheal

instillation. The doses corresponded to the total pulmonary deposition following 1, 3 or 9 days of exposure at the Danish OEL for the studied carbon black, based on the observed size distribution when aerosolized; and 1.5, 4.5 and 14 days at the Danish OEL for studied titanium dioxide. Exposure resulted in dose-dependent lung inflammation for both particles (Saber et al., 2012), and induction of DNA strand breaks in lung fluid cells, lung tissue and liver tissue for carbon black (Bourdon et al., 2012a). Thus, persistent inflammation and DNA damage was observed 28 days after instillation of doses corresponding to 3 and 1 day exposure to the Danish OEL of carbon black, respectively (Saber et al., 2012).

The Printex 90, but not the UV-Titan L181, was shown to induce reactive oxygen species in vitro (Jacobsen et al., 2008; Saber et al., 2011). The carbon black is mutagenic in vitro and the observed mutation spectrum is consistent with the interpretation that the mutations are caused by oxidative damage (Jacobsen et al., 2007, 2011). Global gene expression was assessed in lung tissue. For Printex 90, the most differentially expressed gene was the acute phase protein, serum amyloid A (SAA) (Bourdon et al., 2012b). Inhalation of UV-Titan L181 also increased pulmonary expression of acute phase proteins, with SAA3 as the most differentially expressed gene (Halappanavar et al., 2011). This links pulmonary exposure to cardiovascular risk, because it was recently shown that overexpression of SAA was sufficient to accelerate plaque progression in susceptible ApoE-/- mice (Dong et al., 2011).

CNTs represent another industrially relevant nanomaterial. As CNTs are high aspect ratio particles, that is long, thin, inhalable and insoluble fibres, they are suspected of having asbestos-like properties. Takagi et al. (2012) showed that intraperitoneal injection of the multiwalled carbon nanotube (MWCNT) Mitsui-7 resulted in mesothelioma in a susceptible mouse strain (p53 heterozygous mice) in a dose-dependent manner and at relatively low doses (1 microgram [µg]/mouse or 106 fibres/mouse). However, another noncommercially available MWCNT (11 nm wide and 0.7 micrometres [µm] long) did not induce cancer following intraperitoneal injection in rats (Muller et al., 2009).

Subchronic inhalation studies of two different MWCNTs, Baytubes and Nanocyl NC7000, both identified 0.1 mg/m3 as the lowest-observed-effect level or no-observed-effect levels (Ma-Hock et al., 2009; Pauluhn, 2010). The pulmonary retention time was estimated as 375 days (Pauluhn, 2010).

Concerns are also raised regarding nanomaterial-based products, for example paint and lacquers. The potential hazards of sanding dust from paint nanocomposites were assessed by generating dust particles by sanding wall paint with and without UV-Titan L181 (Koponen et al., 2011). Toxicity was assessed by deposition of the sanding dust into the lungs of mice (Saber et al., 2012). There was no difference in the inflammatory response to paint with and without UV-Titan, and the inflammatory response was much lower than that observed for the pure UV-Titan (Saber et al., 2012). No induction of DNA damage was observed.

In summary, toxicological effects after pulmonary exposure to pure titanium dioxide- and carbon-based nanoparticles and CNTs were observed at doses well below or close to the OELs. This indicates that the present OELs do not protect against adverse effects following exposure to the studied nanomaterials. NIOSH has proposed an OEL of 7 µg/m3 for CNTs in air, and an OEL of 0.25 µg/m3 for the general population. Others have proposed an OEL of 1 µg/m3 (Aschberger et al., 2010). NIOSH has furthermore proposed a specific exposure limit for ultrafine (and nano-sized) titanium dioxide of 0.3 mg/m^3.

References

Aschberger K et al. (2010). Review of carbon nanotubes toxicity and exposure-appraisal of human health risk assessment based on open literature. *Critical Reviews in Toxicology*, 40(9):759–790.

Bourdon JA et al. (2012a). Carbon black nanoparticle instillation induces sustained inflammation and genotoxicity in mouse lung and liver. *Particle and Fibre Toxicology*, 9(1):5.

Bourdon JA et al. (2012b). Hepatic and pulmonary toxicogenomic profiles in mice intratracheally instilled with carbon black nanoparticles reveal pulmonary inflammation, acute phase response and alterations in lipid homeostasis. *Toxicological Sciences*, 127(2):474-484.

Dong Z et al. (2011). Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Molecular Medicine*, 17(11–12):1357–1364.

Duffin R et al. (2007). Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: Highlighting the role of particle surface area and surface reactivity. *Inhalation Toxicology*, 19(10):849–856.

Halappanavar S et al. (2011). Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: A toxicogenomic study. *Environmental and Molecular Mutagenesis*, 52(6):425–439.

Hougaard KS et al. (2010). Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. *Particle and Fibre Toxicology*, 7(1):16.

Jackson P et al. (2012). Pulmonary exposure to carbon black by inhalation or instillation in pregnant mice: Effects on liver DNA strand breaks in dams and offspring. *Nanotoxicology*, 6(5):486–500.

Jacobsen NR et al. (2007). Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells. *Environmental and Molecular Mutagenesi*s, 48(6):451–461.

Jacobsen NR et al. (2008). Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C(60) fullerenes in the FE1-Mutatrade markMouse lung epithelial cells. *Environmental and Molecular Mutagenesis*, 49(6):476–487.

Jacobsen NR et al. (2011). Mutation spectrum in FE1-MUTA(TM) Mouse lung epithelial cells exposed to nanoparticulate carbon black. *Environmental and Molecular Mutagenesis*, 52(4):331–337.

Koponen IK, Jensen KA and Schneider T (2011). Comparison of dust released from sanding conventional and nanoparticle-doped wall and wood coatings. *Journal of Exposure Science and Environmental Epidemiology*, 21(4):408–418.

Ma-Hock L et al. (2009). Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. *Toxicological Sciences*, 112(2):468–481.

Muller J et al. (2009). Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicological Sciences*, 110(2):442–448.

Pauluhn J (2010). Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: Toxic effects are determined by density of agglomerate structures, not fibrillar structures. *Toxicological Sciences*, 113(1):226–242.

Saber AT et al. (2012). Nanotitanium dioxide toxicity in mouse lung is reduced in sanding dust from paint. *Particle and Fibre Toxicology*, 9(1):4.

Saber AT et al. (2011). Inflammatory and genotoxic effects of nanoparticles designed for inclusion in paints and lacquers. *Nanotoxicology*, 6(5):453–457.

Takagi A et al. (2012). Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. *Cancer Science*, 103(8):1440–1444.

The IRGC Risk Governance Framework: Applications in food and cosmetics

Dr Antje Grobe, DIALOG BASIS, University of Stuttgart, Germany and University of St. Gallen, Switzerland

Nanotechnologies have been identified as one of the key technologies for innovation in the fields of sustainable energy, transport, medical treatment and water purification, to name but a few. Many consumer products using nanotechnologies are under development or already on the market, such as smart textiles, intelligent packaging, cosmetics, paints and construction materials. However, in the light of the debate about GMOs in Europe, with millions of euro disinvestment due to negative public perceptions the development of nanotechnologies is accompanied by an extended societal debate. The idea of an inclusion of societal issues in the full process of technology assessment was developed by the IRGC in 2006. The IRGC report, *Appropriate risk governance strategies for nanotechnology applications in food and cosmetics* (IRGC, 2008) analysed the communicational patterns of the nano-debate and the changes of risk awareness among the broader public in these special fields of interest.

The authors recommended stakeholder dialogues as an early warning system in the pre-assessment phase, a systematic concern assessment in the risk appraisal phase, followed by a commonly shared tolerability judgement and adequate risk management measures. Each of the steps required the inclusion of stakeholders and a minimum of transparency and shared information. As discussed by Grobe and Rissanen (2012), we are still far from an application of the IRGC Risk Governance Framework for nanotechnologies in the food or cosmetics industries. The consequences are becoming more and more visible in public perception studies. For example, in Germany and Switzerland the quality of consumer knowledge about nanotechnologies decreased significantly between 2008 and 2011 (Grobe et al., 2012). Consumers know less about potential applications and benefits, but showed more detailed knowledge about risks. The data were interpreted as the result of two main factors: first the "non-communication" strategies of industry, and second, as the product of well connected and actively communicating environmental groups and consumer organizations. According to the first axiom of Paul Watzlawick: "One cannot not communicate" (Watzlawick et al., 1967), the absence of information about the benefits and the quality of nanotechnology products led to a transformation of concerns and uncertainties from former technology debates to the debate about nanotechnologies. The consumer's requirements for more information and transparency were exemplified: industry should provide easily accessible information about the functionality and the added value of nanomaterials in consumer products, about their safety for humans and the environment, and whether the products have been tested by an independent organization. Over all, consumer attitudes and expectations are still positive. But if industry fails to build trust among regulators, civil society organizations and consumers, nanotechnologies will share the fate of GMOs. Therefore, innovation for responsible communication strategies on nanotechnologies is needed, in the same way as responsible innovation in nanomaterials themselves.

References

Grobe A, Rissanen M (2012). Nanotechnologies in agriculture and food: An overview of different fields of application, risk assessment and public perception. *Recent patents on food, nutrition and agriculture*, 4(3):176–186. (http://www.benthamdirect.org/pages/article/1/3182099/nanotechnologies-in-agriculture-and-food-an-overview-of-different-fields-of-application-risk-assessment-and-public-perception.html, accessed 24 June 2013).

Grobe A et al. (2012). *Nanotechnologies from a consumer's point of view. What consumers know and what they would like to know*. Bern, Switzerland, Federal Office of Public Health; and St. Gallen, Switzerland, Risk Dialogue Foundation.

IRGC (2008). *Risk governance of nanotechnology applications in food and cosmetics*. Geneva, International Risk Governance Council.

Watzlawick P et al. (1967). *Pragmatics of human communication*. New York, WW Norton & Company.

Annex 2

Meeting documents

Nanotechnology and human health:
Scientific evidence and risk governance

EUDCE1206041/4.1/2

United Nations Premises, Bonn, Germany
10-11 December 2012

2 October 2012
Original: English

Scope and Purpose

The use of nanotechnology and nanomaterials stands to offer great societal benefits in areas such as clean water and energy, medicine and manufacturing of novel products. Nanomaterials and products based on nanotechnological applications are being commercialized at an increasing pace and human and environmental exposure can occur either during manufacturing of goods, use and disposal of products.

Given the increasing use of nanotechnology, concerns have emerged about the potential adverse health effects of nanomaterials and nanoparticles. At present, in fact, it is not fully known which nanomaterials can be hazardous to human and the environment, nor is it fully understood which properties make nanomaterials more or less toxic. Although significant progress is being made in regard to studying and mapping the environmental, health and safety aspects of nanomaterials, the field is pervaded by scientific uncertainty ("we know what we do not know") and ignorance ("we do not know what we do not know").

At the Fifth Ministerial Conference of Environment and Health (Parma, Italy, 10-12 March 2010)1, the health implications of nanotechnology and nanoparticles were listed among the key environment and health challenges that Ministers in the WHO European Region committed to act on. Along with a call for an increase of research into the use of nanomaterials, the Ministers, acknowledging the complex nature of the question, pledged to develop and use improved health risk and benefit assessment methods.

Preliminary work carried out by WHO indicates that a rigorous risk assessment is currently not feasible and that a less structured model of "risk governance" is a preferable way to proceed. Such an approach must be based on the best available science and evidence in regard to possible biological and health effects, but also on what is known about the present and future uses of nanomaterials, as well as regulation and risk management strategies.

This consultation organized by the WHO Regional Office for Europe aims at reviewing and discussing the current state of knowledge concerning the health risks and impacts of nanotechnology, established, suspected, and potential. Research into the environmental, health and safety aspects of nanomaterials is vast and growing rapidly, and so are the systematic literature reviews.

Specific issues for discussion include: present and future use and diffusion of nanomaterials; human health toxicity; risk assessment limitations and strategies, and alternative approaches; current nanomaterial regulatory frameworks; nanomaterial risk governance experiences and needs. The main objective of the consultation is thus to take stock of the recent insights from research and outcomes of assessment exercises in order to further characterize the complex picture of the health implications of nanotechnology and provide European Member States with some suggestions on appropriate risk and benefit assessment methods and, ultimately, for developing appropriate policies.

1 Parma Declaration on Environment and Health. Copenhagen, WHO Regional Office for Europe, 2010 (http://www.euro.who.int/__data/assets/pdf_file/0011/78608/E93618.pdf, accessed 2 October 2012).

Nanotechnology and human health:
Scientific evidence and risk governance

EUDCE1206041/4.1/4

United Nations Premises, Bonn, Germany
10-11 December 2012

Original: English

Programme

Day 1 - Monday 10 December
Meeting Room 2712

Chair: Michael Depledge
Rapporteur: Steffen F. Hansen

09:00 – 09:30	*Registration*
09:30 – 10:00	**Opening**
	Welcome and introduction, *Marco Martuzzi*, *Michael Depledge*
Session I	**Present and future uses of nanotechnology; exposure assessment**
10:00 – 10:30	Uses and future uses of nanomaterials, *Qasim Chaundry*
10:30 – 11:00	Exposure pathways, *Steffen F. Hansen*
11:00 – 11:30	*Coffee break*
11:30 – 12:00	Possible route of intake, *Craig Poland*
12:00 – 12:30	Discussion
12:30 – 13:30	*Lunch break*
Session II	**Human health toxicity**
13:30 – 14:00	General toxicity of NM, *Vyvyan Howard*
14:00 – 14:30	Pulmonary and reproductive effects of nanoparticles, *Ulla Vogel*
14:30 – 15:00	Cardiovascular and other systemic effects of nanoparticles, *Steffen Loft*
15:00 – 15:30	*Coffee break*
15:30 – 16:00	Discussion
Session III	**Risk assessment and alternatives**
16:00 – 16:30	Nanomaterials registration, Julia *Fabrega Climent*
16:30 – 17:00	Wrapping up and closure of the day
19:00 – 21:00	*Social dinner*

Day 2 – Tuesday 11 December
Meeting Room 2712

Chair: Michael Depledge
Rapporteur: Steffen F. Hansen

Session III	**Risk assessment and alternatives (continuation)**
09:30 – 10:00	The Swiss Precautionary Matrix, *Michael Riediker*
10:00 – 10:30	Control Banding nanotool, *Derk Brouwer*
10:30 – 11:00	Discussion
11:00 – 11:30	*Coffee break*
Session IV	**Current regulation of NM and future risk governance**
11:30 – 12:00	Current regulation of nanomaterials, *Andrej Kobe*
12:00 – 12:30	OECD programme on nanomaterials, *Peter Kearns*
12:30 – 13:30	*Lunch break*
13:30 – 14:00	REACH Implementation Projects on nanomaterials – Outcomes & Implementation – *Steve Hankin*
14:00 – 14:30	NANOSUPPORT - Assessment of nanomaterials in REACH registration dossiers, *Karin Aschberger*
14:30 – 15:00	IRGC framework, *Antje Grobe*
15:00 – 16:00	**Discussion and Closure of the meeting**
16:00 – 16:30	*Farewell coffee*

Nanotechnology and human health:
Scientific evidence and risk governance

EUDCE1206041/4.1/5

United Nations Premises, Bonn, Germany
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List of participants

Temporary advisers

Karin ASCHBERGER
Scientific Officer, Nanobiosciences Unit
Joint Research Centre Ispra
European Commission
Ispra (Varese), Italy

Alexandre D. AZOULAY
Managing Attorney
Center for International Environmental Law (CIEL)
Geneva, Switzerland

Derk BROUWER
Senior Scientist
TNO Research Group Quality & Safety
Zeist, Netherlands

Qasim CHAUDHRY
Principal Research Scientist
Food and Environment Safety
The Food and Environment Research Agency
York, United Kingdom

Michael DEPLEDGE (*Chair*)
Professor of Environment and Human Health
European Centre for Environment and Human Health
University of Exeter Medical School
Plymouth, United Kingdom

Julia FABREGA CLIMENT
Scientific Officer, Evaluation Directorate
European Chemicals Agency
Helsinki, Finland

Antje GROBE
Project Manager, Managing Director
DIALOG BASIS
Environmental and Technological Sociology Dept.
University of Stuttgart
Dettenhausen/Tübingen, Germany

Steve HANKIN
Senior Consultant in Risk Assessment & Section Head
SAFENANO
Institute of Occupational Medicine
Edinburgh, United Kingdom

Steffen F. HANSEN (*Rapporteur*)
Senior Researcher, DTU Environment
Technical University of Denmark
Lyngby, Denmark

Charles V. HOWARD
Centre for Molecular Biosciences
School of Biomedical Sciences
University of Ulster
Coleraine, United Kingdom

Peter KEARNS
Principal Administrator ENV-EHS
Organisation for Economic Co-operation and Development (OECD)
Paris, France

Hans-Jürgen KLOCKNER
Head of Section Science and Research,
Science, Technical and Environmental Affairs
German Chemical Industry Association
Frankfurt, Germany

Andrej KOBE
Policy Officer, European Commission DG Environment
Unit D3/ Chemicals, Biocides and Nanomaterials
Brussels, Belgium

Steffen H. LOFT
Professor, Department of Public Health
University of Copenhagen
Copenhagen, Denmark

Craig POLAND
Research Toxicologist
SAFENANO
Institute of Occupational Medicine
Research Avenue North, Riccarton
Edinburgh, United Kingdom

Jacques RAGOT
Head of Public Affairs
Communications and Public Affairs
Bayer MaterialScience AG
Leverkusen, Germany

Michael RIEDIKER
Leader of Group Particles and Health
Institute for Work and Health
Rue du Bugnon 21
Lausanne, Switzerland

Tatiana SANTOS OTERO
Senior Policy Officer
Chemicals and nanotechnology
The European Environmental Bureau (EEB)
Brussels, Belgium

Nathalie THIERIET
Scientific Manager
French agency for food, environmental and occupational health & safety (ANSES)
Maisons-Alfort, France

Ulla Birgitte VOGEL
Professor and Head of Nanosafety research
National Research Centre for the Working Environment
Copenhagen, Denmark

World Health Organization

Bianca BORTOT
Secretary, Environment and Health Intelligence and Forecasting
WHO European Centre for Environment and Health
Bonn, Germany

Andrey EGOROV
Manager, Environment and Health Intelligence and Forecasting
WHO European Centre for Environment and Health
Bonn, Germany

Angella KARAMAGI
Intern, Environment and Health Intelligence and Forecasting
WHO European Centre for Environment and Health
Bonn, Germany

Marco MARTUZZI
Programme Manager, Environment and Health Intelligence and Forecasting
WHO European Centre for Environment and Health
Bonn, Germany

Irina ZASTENSKAYA
Technical Officer, Environmental Exposures and Risk
WHO European Centre for Environment and Health
Bonn, Germany

Annex 3

Background Document

Charles V. Howard
Steffen F. Hansen
Khara D. Grieger
Michael H. Depledge
Marco Martuzzi

Contents

Abbreviations

µm	micrometre
CHA	comparative hazard assessment
CNS	central nervous system
CNT	carbon nanotube
MWCNT	multiwalled carbon nanotube
nm	nanometre
ROS	reactive oxygen species
SWCNT	single-walled carbon nanotube
UV	ultraviolet

Executive summary

Nanotechnology is the science and application of materials with a size below 100 nanometres (nm) (10-9 m). A number of substances, at this scale, acquire properties that are different both from those at an atomic or molecular level as well as at bulk scale. This has opened the way for a variety of applications in many different fields, from medicine to consumer products, from the creation of new materials to food additives. Research and commercial applications are already widespread, but nanotechnology is in its early days and the potential for developing and applying new generations of nanomaterials is huge. Harnessing the potential of current and future applications is the goal of nanotechnology, and the prospect of large benefits and returns has attracted considerable research and financial investment. At the same time, concerns have emerged with regard to the potential of nanomaterials, engineered nanoparticles in particular, to have unwanted or unexpected interactions with biological systems, resulting in adverse consequences for human and ecosystem health. The literature on these aspects is growing rapidly, but many areas of uncertainty remain, not least because nanotechnology is evolving fast and new research questions arise frequently. The issue will likely be surrounded by considerable uncertainty for some time.

This report aims to synthesize the current state of knowledge and the key evidence on possible health implications of nanomaterials, and identify options for risk governance and policy formulation. In this respect, it is important to underline that the term "nanotechnology" encompasses a very large and heterogeneous set of materials and applications which are difficult to classify, and impossible to discuss in their entirety. For the sake of discussing the health implications, it is convenient to consider the location of the nanoscale structure in the materials, i.e. whether nanostructures are in the bulk or on the surface, or if the material contains nanoparticles; in the latter case, most relevant for health effects, one can distinguish between the presence of nanoparticles bound to the surface, suspended in a liquid, suspended in a solid or airborne.

Humans have limited evolutionary experience of exogenous nanomaterials, with very few types of nanoparticles being present from prehistory, such as some inorganic nanoparticles from marine aerosols and viruses. As humans learnt to control fire, nanoparticles from combustion also became part of our evolutionary history. This may be a reason for the diminishing ability of cells to interact with particles (through the main mechanisms of dealing with them, phagocytosis and endocytosis), as their size decreases to nanoscale.

When they come into contact with humans, nanoparticles can enter the body relatively easily through inhalation, gastrointestinal assimilation and dermal absorption. They can also gain access to the central nervous system through the olfactory mucosa or through the blood–brain barrier, especially during the fetal stage and early life. These properties are of great interest for the targeted delivery of drugs.

Once inside the body, nanoparticles are highly mobile: they can access the circulation and be transported by the blood stream. How this happens in practice, the properties of nanoparticles as they travel the body and their dependence on particle size, however, is not well understood, as most of the evidence is based on animal models, with uncertain extrapolations to humans. Some degree of bioaccumulation due to incomplete excretion is known to occur, for example in the lung, gastrointestinal tract and the brain.

Evidence of the health effects of airborne particles, especially particulate matter (PM) of 2.5 micrometres (μm) and below PM2.5 (fine particles) is well established and includes severe and measurable impacts on mortality, morbidity, hospital admission and occurrence of symptoms. PM2.5 includes ultrafine and nanosized particles, whose role in determining the health effects is not well known. It is also clear that for nanoparticles, exposure metrics other than gravimetric concentrations (i.e. micrograms (μg) per cubic metre) are more relevant to assessing human exposure, for example the particle count or their total surface area.

Several mechanisms by which nanoparticles can induce cell damage have been reported, mainly of a chemical nature (e.g. oxidative stress, inflammation, protein misfolding), but also of a physical nature (e.g. direct physical damage, production of secondary photoelectrons), and involving the cell nucleus,

the membrane and cell organelles. Many of these mechanisms are known specifically for certain types of nanoparticles; they are often dependent on particle size, with a tendency to become more active as the particle size decreases.

The overall data and evidence on nanotechnology and health is far from conclusive. A cautionary approach is recommended by many evaluations, on the basis of the wide range of possible effects; the lack of effective defence mechanisms in the human body; the potential for damage; the possible extent of population exposure, for example through consumer products, cosmetics and food additives; the speed of technological development; and the projected proliferation of applications. The need for caution is reinforced by recent evidence of the asbestos-like action of carbon nanotubes in animal models and, to a lesser extent, by episodes of human health impacts following localized acute exposures.

Translating information about nanoparticle exposure and distribution in the human body, toxicological mechanisms and possible adverse health effects into assessment of the risks for human health is highly challenging. Traditional risk assessment methodology, for example as applied in chemical safety, has some important limitations. There is a need for the development of "nanotoxicology" as a specialized branch of toxicology that takes into account the property of materials at the nanoscale, similarly, approaches and methodology for describing the likelihood of human health impacts must take into account the unique complexities of the interaction between nanomaterials and the human body. Such complexities challenge all steps in traditional risk assessment: hazard identification is complicated by the diversity of nanomaterials and their properties, and by the variety of the possible relevant health endpoints. The few dose–response relationships known are based on experimental studies, especially in vitro, and interpretation and extrapolation of these data to human health is difficult; also some health effects may not depend on dose, but on other physical and chemical properties. As to exposure assessment, some attempts have been made in controlled circumstances in the occupational setting, but information is chronically lacking, due, among other things, to uncertain exposure metrics and technical difficulties in measuring concentrations.

Models and frameworks alternative to traditional risk assessment are thus being developed and applied to organize the available evidence on biological and health effects of nanomaterials in ways that can inform policy. Many of these approaches, developed by consortia of public and sometimes private agencies and expert groups, recognize the high degree of complexity and uncertainty. Thus, they are based on several steps (sequential or organized in flow charts), make provision for extended stakeholder consultation and some, rather than focusing on one agent, involve the consideration of alternatives, as in the case of comparative hazard assessment.

As no agency is currently in a position to formulate conclusive and reliable assessments of the health risks of nanomaterials, it is imperative that adequate mechanisms are put in place, at various levels, to ensure transparent and fair negotiation between stakeholders, and that evidence on health implications is used to inform the risk governance of nanotechnology. Profitable areas of discussion, in terms of policy action, include: the creation of regulatory bodies and their scope; the development of labelling schemes for consumer products (with a separate regime for pharmacological and medical applications); the most suitable means of providing information to the public for exercising informed choices; the relevance, at nanoscale, of licenses for production of materials at bulk scale; the criteria for identification of priority nanomaterials, for research and regulatory action; and the institution of monitoring and surveillance systems, possibly aimed with priority at vulnerable groups, for early detection of health consequences of nanomaterials.

1. Remit

The purpose of this document is to provide an overview of the potential impacts of nanotechnology on human health, and discuss if such evidence can be used to inform processes of risk governance of nanotechnology. The review first focuses on the potential health sequelae that could result from exposure to engineered nanoparticles or products containing them. Since 2003, a large number of detailed reviews have been published examining the impacts of nanotechnology (e.g. ETC Group, 2003a; ETC Group, 2003b; Bucher, 2004; EU, 2004; Royal Society, 2004; Swiss Re, 2004; Allianz & OECD, 2005; Oberdörster et al., 2005; Borm et al., 2006; DEFRA, 2006; FSA, 2006; Gwinn & Vallyathan, 2006; HSE, 2006; Maynard, 2006a; DEFRA, 2007; IRGC, 2007; CCA, 2008; NNI, 2008; DEFRA, 2009; Linkov & Steevens, 2009; Oberdörster, 2009; Seaton et al., 2009; Stone et al., 2010). Given this exhaustive background information, the aim is not to provide another extensive review, but rather to inform the policy debate in Member States of the WHO Regional Office for Europe by critically examining the current knowledge base from a toxico-pathological viewpoint and discussing the likely disease patterns that could result from exposure. On the basis of the available evidence, the commentary addresses questions of risk assessment and risk governance in nanotechnology, by discussing pros and cons of different risk assessment strategies and other frameworks for dealing with the risks of nanomaterials. The report concludes with a set of points meant to develop a suitable risk governance framework. This document was prepared following consultation work begun by WHO in 2008, in the framework of the EU co-funded PAVEL Project (Enhanced policy advice in environment and health Grant Agreement 2006WHO01). Draft versions were compiled and updated by the authors, taking into account the outcome of the expert consultations and on the basis of selected specialized literature.

2. What is nanotechnology?

2.1 Evolution

Broadly speaking, nanotechnology consists of the study and use of substances and materials at the scale 1–100 nm (10-9 m) in size. At this scale, substances and materials acquire properties that differ from the properties at both the atomic or molecular level and from those at bulk size.

Controlling and taking advantage of such novel physical, chemical and biological properties is the objective of nanotechnology. This holds the prospect of considerable benefits, through the development of countless applications: from new drugs and therapies to water purification technologies, from photovoltaic devices to food additives (see Figure 1 for some examples of existing applications). While many applications are already in use, including for health and the environment, and despite the substantial technological advances already achieved, nanotechnology is in its early stages, with an exceptionally rapid pace of innovation.

Four generations of nanotechnology development have been identified (Roco, 2003). The first generation, developed until the year 2000, consists of simple “passive” nanostructures. The second generation, undergoing development between 2000 and 2005, includes the development of “active (evolving function) nanostructures” such as targeted drugs and chemicals, light-driven molecular motors, nanoscale fluidics, laser-emitting devices and adaptive structures. The third generation consists of “systems of nanosystems”. This generation, projected to range from 2005 to 2010, includes the use of various syntheses and assembling techniques such as bioassembling, networking at the nanoscale and multiscale and hierarchical architectures, robotics on surfaces, modular nanosystems, chemomechanical processing of molecular assemblies, and quantum-based nanoscale systems. From 2010 to 2015/2020, a fourth generation was projected to involve the development of heterogeneous molecular nanosystems, where each molecule in the nanosystem has a specific structure and plays a different role (Roco & Renn, 2006).

2.2 Types of application

The word "nanotechnology" is probably of limited usefulness as it encompasses a wide range of enabling technologies that will facilitate progress in a number of fields (often unrelated to the initial development). According to the British Standards Institution (BSI, 2007) the number of nanotechnology methods, processes and techniques exceeds 30. The catchall nature of the term "nanotechnology", hence, may be confusing, as it can lead to a classification of a whole range of technologies as a single entity. In fact, the definition of "nanomaterial" or "nanoparticle" is still subject to debate

A great number of different nanomaterials can be manufactured (using various techniques) including buckminsterfullerene, carbon nanotubes, micelles, self-assembled monolayers, dendrimers, and aerogels in all kinds of sizes and shapes. The nature and the properties of nanomaterials differ substantially, and so do the potential biological effects and possible health hazards; however, information on a particular aspect of nanotechnologies has tended to be applied to the whole spectrum, which can be misleading.

In order to facilitate hazard identification and to focus risk assessment, Hansen et al. (2007) suggest categorizing nanomaterials depending on the location of the nanoscale structure in the system. This leads to a division of nanomaterials into three main categories (see Figure A3.1 for examples):

1. materials that are nanostructured in the bulk
2. materials that have nanostructure on the surface
3. materials that contain nanostructured particles.

A benefit of this categorization is that it provides a tool for dividing nanosystems into identifiable parts, thereby facilitating the evaluations and analysis ofexposure routes or effect studies. Others have proposed that nanomaterials are classified into different groups, depending on their chemical composition, for example the National Academy of Sciences (2008) grouped nanomaterials into metal oxides, nanoclays, nanotubes and quantum dots, while the United States Environmental Protection Agency (EPA) (2007) proposed groups of carbon-based materials (nanotubes, fullerenes), metal-based materials (metal oxides and quantum dots), dendrimers (nanosized polymers) and composites (including nanoclays).

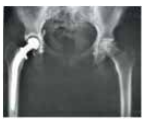
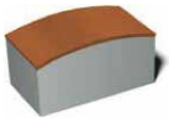
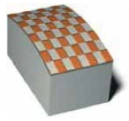
While the scientific literature on nanomaterials toxicity and ecotoxicity is growing rapidly, a number of aspects of nanotechnology do not appear to pose an appreciable immediate health threat, although extensive uncertainties remain. For example, the use of nanostructured surfaces to produce self-cleaning glass for use in buildings is a case of non-pervasive application, unlikely to have major health implications. However, this will not apply during manufacture or subsequent breakdown, and indeed the nanotechnology industry is currently engaged in the production of powders consisting of these nanoparticles, potentially hazardous and locally or widely pervasive.

Nanoparticles, an important subset of nanomaterials, are defined as discrete particles with at least one dimension less than 100 nm (which includes thin enough fibres); for a fuller definition see ISO (2008). Nanoparticles can have various forms and shapes, and include quantum dots, fullerenes, nanotubes and nanowires (Maynard & Aitken, 2007). In the third category of the classification of nanomaterials described by Hansen et al. (2007) – materials that contain nanostructured particles – four subcategories can be identified, depending on the environment around the nanoparticles:

- subcategory IIIa: nanoparticles bound to the surface of another solid structure
- subcategory IIIb: systems where nanoparticles are suspended in a liquid
- subcategory IIIc: nanoparticles suspended in solids
- subcategory IIId: of airborne nanoparticles.

This report will mainly focus on the health implications of free nanoparticles (subcategory III; b, c and d) of the first generation of nanotechnology; newer generations might pose profoundly different challenges. Carbon nanotubes and titanium dioxide are specifically considered in this report, given their widespread use and the available toxicity data.

Figure A3.1. Examples of nanomaterials and applications

Nanostructured materials		Bulk material structure at the nanoscale (e.g. nanocrystals with large breaking strength)	
		Bulk consisting of several materials (e.g. porous pills of magnesium chloride for the storage of ammonia)	
Surface-structured nanomaterials		Bulk material with structures at the nanoscale (e.g. cell-growth promoting plastics for implants)	
		Unpatterned nanofilm (e.g. dew free sunglasses)	
		Patterned nanofilm (e.g. magnetic film on hard disks)	
Nanoparticles		Surface bound platinum particles used in car catalysts	
		Carbon nanotubes incorporated into solid materials (e.g. ratchets, bikes)	
		Titanium dioxide suspended in liquids (e.g. sunscreens)	
		Airborne nanoparticles	

Source: Hansen SF (2012). Reproduced by permission of DTU Environment.

3. Nanotechnology and health: background

Despite the great potential of materials at the nanoscale, concerns also exist about their possible unintended adverse effects on human health and the environment. As substances acquire new, sometimes unexpected properties when engineered at nanoscale, and as the complexity of the nanomaterials' structures and functions increases, their ability to interact with biological systems, their known toxic characteristics, and their potential to cause adverse health effects, can also change.

Concerns over the possible health effects of exposure to nanomaterials were somewhat heightened by

two episodes, in different settings, suggesting the occurrence of acute respiratory conditions . In 2006, 80–100 people experienced respiratory problems and six were admitted to hospital with pulmonary conditions following use of "Nano Magic", a glass sealant home product, which was recalled. In 2008, seven workers in a printing factory in China were admitted to hospital with severe respiratory problems and two of them died, following exposure to nanoparticles over several months. Both episodes made international news and stimulated the debate over nanotechnology safety, in the general population as well as in the occupational setting. While these episodes provide some warning, neither established a clear causal link between exposure and health effects: in the first case, it turned out that the sealant contained no nanomaterials, but was designed to produce a nanoscale layer on the treated surfaces through deposition of an aerosol, which may have affected the users' lungs. In the second case, limited information on the nature and extent of the actual exposure to nanomaterials prevented the formulation of robust conclusions (see boxed text for further details).

3.1 Evolutionary perspective

To put in context the question of the possible adverse health effects of nanoparticles, it may be useful to examine the conditions that prevailed during human evolution. Seaton et al. (2009) provide a brief background on this topic in their review. It is likely that through evolutionary processes all species become adapted to prevailing conditions. However, if new agents or exposures are introduced, or marked changes in intensity of exposure occur, a lack of natural defences should cautiously be assumed, until it can be shown that there are no deleterious influences. An example can be made by considering environmental levels of heavy metals. The geochemical record indicates that traces of lead, cadmium, mercury etc. have always been present in the environment, albeit at very low levels. Thus, we have evolved mechanisms for their removal, which involve carrier proteins. However, these mechanisms evolved to cope with the pre-industrial levels to which we were exposed. When faced with higher levels of exposure, the performance of such systems is not optimal, and exposure to heavy metals is known to have deleterious health effects.

Humankind has always been exposed to some inorganic particles, possibly including some at nanoscale, mainly consisting of marine aerosols, minute crystals of soluble salts windblown from the action of the sea. However, the air contained relatively few other inorganic nanoparticles of less than 100 nm throughout our prehistory. The main biological objects at nanoscale were viruses and some other biological particles. This changed when humans harnessed fire about 150 000 years ago and combustion nanoparticles became a common feature of the human environment. Our defence mechanisms against nanoparticles probably evolved principally to cope with the biological threat posed by viruses.

The other evolutionary aspect that requires consideration is the propensity of cells to engulf particulate matter. Unicellular organisms, which evolved about 3.5 billion years ago, interact with their environment by internalizing matter through the cell wall and similarly externalizing waste materials. With the evolution of multicellular creatures some 450 million years ago, this basic ability was kept, and is essential for many aspects of the functioning of complex multicellular organisms.

Two cellular mechanisms for handling nano- and microparticles are of relevance, phagocytosis and endocytosis. Phagocytosis is typically triggered by larger particles (1–2 μm) such as apoptotic cell remnants, bacteria and viruses. A pseudopodial outgrowth of the cellular membrane surrounds and engulfs the particle before internalization into a phagosome. This appears to be an "active" process and to operate under a molecular recognition system. It is interesting to note that alveolar macrophages appear to have "difficulty" recognizing foreign particles of a size less than 65 nm (Donaldson et al., 1999). A number of studies support the hypothesis that alveolar macrophages are less efficient at engulfing nanoparticles than micron sized particles (Pratten & Lloyd, 1986; Oberdörster et al., 2002; Kreyling, 2002). This is probably indicative of the size range of particles that the body had to cope with through inhalation during our evolutionary history.

A second mechanism, endocytosis, is a basic property of cell membranes, which has evolved to transport much smaller objects, such as macromolecules, and is typified by an invagination of the cell membrane. It is divided

into: pinocytosis (cell drinking), caveolar endocytosis and receptor medicated endocytosis in clathrin coated pits. A review of nanoparticle mobility and endocytosis has been produced by Gumbleton (2001).

In parallel to the continuous process of endocytosis, the opposite mechanism of exocytosis is also operating. In the nervous system, this has become specialized in the telodendria for the regulation and release of neuron-transmitter substances into the synaptic cleft. In other cell membranes, exocytosis is a continuous process.

Engineered nanoparticles are in the size range of objects that can cross biological membranes, in common with many viruses, by endo- and exocytosis. Therefore, the expectation is that their mobility within the body will be high once they have gained entry, because the mechanisms that they would "highjack" are rapid, continuous and unstoppable. Likewise, it should be expected that nanoparticles will also be easily expelled from cells by exocytosis.

Two episodes of intoxication and acute health effects

1. Death of Chinese workers

During January–April 2008, seven young female workers in a Chinese print plant were diagnosed with shortness of breath and excess of liquid in the lungs, following exposure to a cocktail of dust, fumes and polyacrylate nanoparticles (~30 nm) for 5–13 months. All seven were admitted to hospital and eventually two of the women died. The incident was later investigated and described by Song et al. (2009). The women had been working 8–12-hour shifts in a 70 m^2 room with no windows and no ventilation, spraying a polyacrylic ester paste onto a polystyrene substrate that was subsequently heat-cured. Five months prior to the incidents, the local exhaust ventilation in the facility had broken down and had not been repaired (Maynard, 2009; Song et al., 2009).

The women underwent a number of tests, including immunologic tests, internal thoracoscopy and video-assisted thoracic surgery; a survey of the workplace was also conducted (Song et al., 2009). Pathological examinations of patients' lung tissue displayed nonspecific pulmonary inflammation, pulmonary fibrosis and foreign-body granulomas of the pleura. Using transmission electron microscopy, nanoparticles were found in the cytoplasm and caryoplasm of pulmonary epithelial and mesothelial cells, and located in the chest fluid. Particles of ~30 nm diameter were found in the fluid surrounding the patients' lungs and in the cell linings inside and outside the lungs. Similar sized nanoparticles were found in the polyacrylic ester paste, and in the workplace ventilation system, leading Song et al. (2009) to conclude that their study might be the "… first study on the clinical toxicity in humans due to long-term exposure to nanoparticles …"

As in the case of Nano Magic, the study by Song et al. (2009) made internationals news, but was also questioned by the scientific community. The main areas of criticism focused on the lack of exposure data and that no details were reported about the nature of exposure (i.e. the combination of fumes, dust and nanoparticles) and its magnitude and duration. Furthermore, it was not reported how the nanoparticles were produced and whether they were inhaled as single species or as large agglomerates or aggregates; chemical analysis of the nanoparticles identified in the women's lungs was limited (Maynard et al., 2009; Brain et al., 2010). Also, while nanoparticles were identified as the causative agent, no assessment was made of other plausible causes of the symptoms. Thus, one should be careful in extrapolating the conclusions of this study into recommendations on the handling of nanoparticles in general (Maynard et al., 2009; Brain et al., 2010). Some have argued that the study by Song et al. (2009) should never have been published due these deficiencies. Others have suggested that appropriate workplace hygiene needs to implemented in the nanotechnology industry, as in other industries, in order to avoid preventable tragedies (Maynard et al., 2009; Brain et al., 2010).

2. Nano Magic

In late March 2006, a protective glass and bathroom tile sealant known as "Nano Magic" was recalled in Germany after 80–100 consumers experienced severe breathing problems after using it in confined spaces (Maynard, 2006b; Glaza, 2010). Six people had to be hospitalized with pulmonary oedema (water in the lungs). The recall made news headlines around the world. The product, made by Kleinmann GmbH, was sold in a pump spray container in supermarkets and discount stores, but was pulled from the market by the manufacturers after the German Federal Institute for Risk Assessment (BfR) issued a product warning. It later turned out that the product did not contain any nanoparticles. The manufacturer attributed the nanotechnological element of their product to the nanoscale layer of silicon dioxide that the product creates on surfaces (Bulls, 2006; Pescovitz, 2006). One major element of controversy was that neither the German government nor the manufacturer could provide clear information on what was in the product and whether appropriate safety testing had been performed. It took the BfR about 2 months to establish the absence of nanoparticles in the product (Elvin, 2006; von Bubnoff, 2010). It was later speculated that the effects observed where due to the tiny droplets produced via the aerosol liquid spray, which enabled the solvent to penetrate deep into the lungs. At the time, the incident was seen as an early warning on the risks of nanotechnology. In hindsight, it does seem to have led to greater attention on the potential health and environmental threats of this new technology (Maynard, 2006b).

3.2 Human exposure to nanomaterials

One of the key issues, from a public health viewpoint, is to establish the extent to which the population is exposed to nanomaterials. More precisely, it is important to know distributions of exposure to different types of nanomaterials in different population subgroups. This information seems to be quite elusive. Apart from airborne ultrafine and nanoparticles from combustion processes (e.g. from transport or industrial sources), some nanopowders, such as titanium dioxide, carbon black and fumed silicas, have been in industrial production for decades. Other newer nanopowder products are moving from research to industrial-scale production and may become more widespread, for example single-walled carbon nanotubes (SWCNT) (Aitken et al., 2006; Next Big Future, 2007; Thayer, 2007; NNI, 2008). However, the current exposure of the general population to the newer types of nanoparticle is largely unknown. Table A3.1 summarizes available data on exposure to nanoparticles from different sources. Some kinds of engineered nanoparticles have limited use and exposure to them is likely to be minimal. However, possible exceptions include cosmetics and personal care products containing titanium and zinc oxide nanoparticles (absorption through the skin), food additives (by ingestion) and fuel additives (such as ceria, by inhalation). Also, the workforce employed in nanoparticle production could well experience substantial exposure. In any case, given the paucity of data on exposure, it is currently not possible to attempt estimates of risk or health impacts of different types of nanomaterials for the general population.

Table A3.1. Available data on nanoparticle exposure

	Setting/scenario	Nanoparticle
Worker exposure		
Maynard et al. (2004)	Production (research)	SWCNT
Han et al. (2008)	Research lab	MWCNT
Bello et al. (2008a)	Research lab	MWCNT
Bello et al. (2008b)	Research lab	CNT carbon
		CNT alumina
Fujitani et al. (2008)	Production/bagging operation	Fullerene
Tsai et al. (2008a)	Research lab	Aluminium oxide
Tsai et al. (2008b)	Production/compounding	Aluminium
Demou et al. (2008)	Pilot-scale production	Metal-based
Hsu & Chein (2007)	Tile	Titanium dioxide
	Wood	Titanium dioxide
	Polymer	Titanium dioxide
Methner (2008)	Pilot-scale production	Manganese
		Silver
		Cobalt
Methner et al. (2007)	Production (research)	Caron nanofibers
Peters et al. (2009)	Production (commercial)	Lithium titanate metal oxide
Kuhlbusch et al. (2004)	Production (commercial)	Carbon black
Worker exposure continued		
Kuhlbusch & Fissan (2006)	Production (commercial)	Carbon black
Yeganeh et al. (2008)	Research lab	Fullerenes

Air	Water	Soil
< 53 µg/m^3		
172.9–193.6 MWCNT/cm^3		
ND		
≤2–5e6 (i)/380.7 (ii) particles/cm^3		
≤2–5e4 particles/cm^3 (i)		
1.0e4–2.0e4 particles/cm^3		
< 4.6e5		
50 000–150 000 particles/cm^3		
59 100 particles/cm^3 (+20%)a		
629.2 particles/cm^3 (iii)		
478.5 particles/cm^3 (iii)		
378.9 particles/cm^3 (iii)		
150 µg/m^3 after LEV (3 600 µg/m^3 without LEV)		
1 700 µg/m^3 after LEV (6 700 µg/m^3 without LEV)		
41 µg/m^3 after LEV (710 µg/m^3 without LEV)		
< 4.5E+04 p/cm^3		
0.118 +/- 0.023 mg/m^3		
0.035 +/- 0.006 mg/m^3		
0.026 +/- 0.007 mg/m^3		
0.039 +/- 0.016 mg/m^3		
0.036 +/- 0.015 mg/m^3		
0.028 +/- 0.012 mg/m^3		
PM1 Bag filling area, no bagging 14–24 µg/m^3 (Plant 1 & 2)		
PM1 Bag filling area 33–41 µg/m^3 (Plant 1–3)		
Bag filling fluffy 18–37 µg/m^3 (Plant 2)		
PM1 Bag filling (small bags) 46–52 µg/m^3 (Plant 3)		
PM1 Bag filling (large bags) 181–280 µg/m^3 (Plant 3)		
PM10 18 ± 20 µg/m^3 (Plant 1 Reactor 1)		
PM10 29 ± 25 µg/m^3 (Plant 1 Reactor 2)		
PM10 29 ± 25 µg/m^3 (Plant 1 Reactor 2)		
PM10 38 ± 35 µg/m^3 (Plant 1 Pelletizer 1)		
PM10 47 ± 39 µg/m^3 (Plant 1 Pelletizer 2)		
PM10 24 ± 7 µg/m^3 (Plant 2 Reactor)		
PM10 50 ± 50 µg/m^3 (Plant 2 Pelletizer)		
PM10 21 ± 12 µg/m^3 (Plant 3 Reactor)		
PM10 2080 ± 1 613 µg/m^3 (Plant 3 Pelletizer)		
4 Aug 05: Fume hood 150 ± 29 µg/m^3		
4 Aug 05: Work zone 123 ± 7 µg/m^3		
9 Aug 05: Fume hood 85 ± 122 µg/m^3		
9 Aug 05: Work zone 36 ± 3 µg/m^3		
21 Jun 06: Fume hood 52 ± 4 µg/m^3		
21 Jun 06: Work zone 52 ± 11 µg/m^3		
25 Jun 06: Fume hood 81 ± 116 µg/m^3		

	Setting/scenario	Nanoparticle
Bello et al. (2009b)	Research lab	CNT composites
Vorbau et al. (2009)		Mixed coating with zinc oxide
Human exposure		
Hansen et al. (2008)	Sunscreen lotion	Titanium dioxide
	Fluid product	Not specified
	Spray product	Not specified
Park et al. (2008)	Car emissions in the United Kingdom	Cerium oxide
	Car emissions in the EU by 2020	Cerium oxide
Environmental exposure		
Kaegi et al. (2008)	Exterior paint discharge	Titanium dioxide
Mueller & Nowack (2008)	Textiles, cosmetics, etc.	Silver
	Sporting good, cosmetics, etc.	Titanium dioxide
	Plastics and electronics	CNT
Luoma (2008)	Socks	Silver
	Washing machines	Silver
	Swimming pools	Silver
Blaser et al. (2008)	Biocidal plastics and textiles	Silver
Boxall et al. (2008)	Laundry detergents	Latex
	Paints, sunscreens, etc.	Zinc oxide
	Paints, sunscreens, etc.	Titanium dioxide
	Toothpaste	Hydroxyapatite
	Cosmetics	Buckminsterfullerene
		Organo-silica
		Silicon dioxide
	Fuel additive	Cerium oxide

CNT = carbon nanotube, LEV = local exhaust ventilation, MWCNT = multiwalled carbon nanotubes, ND = not determined, PM = particulate matter, SWCNT = single-walled carbon nanotubes.

[a] Particle number retention 97–100%
[b] Sunscreen contains 2% titanium dioxide
[c] Assuming that all cerium is emitted to atmosphere
[d] Assuming that 10% and 30% of the population in the United States use silver

Air	Water	Soil
25 Jun 06: Work zone 85 ± 48 µg/m^3		
Wet cutting (at source), arithmetic mean PM10 dust = 0.054 (mg/m^3)		
Dry cutting base-alumina (at source), AM PM10 dust = 1.19 (mg/m^3)		
Base-alumina (at personal breathing zone), AM PM10 dust = 0.73 mg/m^3		
Dry cutting CNT-alumina (at source), AM PM10 dust = 2.11 (mg/m^3)		
CNT-alumina (at personal breathing zone), AM PM10 dust = 0.80 mg/m^3		
Dry cutting base-carbon (at source), AM PM10 dust = 5.61 (mg/m^3)		
Base-carbon (at personal breathing zone), AM PM10 dust = 5.41 mg/m^3		
Dry cutting CNT-carbon (at source), AM PM10 dust = 8.38 (mg/m^3)		
CNT-carbon (at personal breathing zone), AM PM10 dust = 2.40 mg/m^3		
< 100 nm		
26 µg/kg bw/year[b]		
15 µg/kg bw/year		
44 µg/kg bw/year		
161 kg/year		< 0.01 mg/kg
< 1255 tonnes[c]		0.28—1.12 µg/g**
3.5e8 particles/L		
1.7e-3–4.4e-3 µg/m^3	0.03–0.08 µg/L	0.02–0.10 µg/kg
1.5e-3–4.2e-2 µg/m^3	0.70–16.00 µg/L	0.40–4.80 µg/kg
1.5e-3–42.3e-3 µg/m^3	0.5e-3–0.8e-3 µg/L	0.01–0.20 µg/kg
	< 2 790 kg [d]	
	2 850 kg [e]	
	30 tonnes[f]	
	320 ng/L[g]	14 mg/kg
	103–1 025 µg/L[h]	4.30–43.00 mg/kg
	76.00–760.00 µg/L[h]	3 194 µg/kg
7 mg/m^3[g]	24.50–245.00 µg/L[h]	1 030 µg/kg
	10.1µg/L	422 µg/kg
	0.000 5 µg/L	4 307 µg/kg
	0.000 7 µg/L	0.03 µg/kg
6,00E-07[i]	<0.000 1 [j]	< 0.01 [j]

[e)] Households in the United States that are wealthy enough will buy silver washing machines
[f)] 1 million pools in the United States use silver as a biocide
[g)] Removal in the STP was assumed to be 99–85% of wastewater
[h)] 10% and 100% market penetration
[i)] All diesel fuel doped with 10 ppm will lead to an emission rate of 10 ppm

4. Health effects of airborne particles

For other preceding novel technologies, such as genetically modified food crops, little toxicological research existed when they were introduced. In the case of nanomaterials and nanoparticles in particular, some literature already exists on exposure and adverse health effects of particulate matter. Therefore, before considering the possible health effects of engineered nanoparticles, it is important to consider what is known of the adverse effects of particulate pollution. The earliest understanding that particle exposure could harm health came from the "dust diseases" of silicosis (Agricola, 1556), pneumoconiosis, byssinosis and, more latterly, asbestosis. These were high-dose exposures associated with particular industrial activities.

They all resulted in lung destruction and fibrosis and were the result of high levels of exposure. The realization that poor air quality alone could cause acute morbidity and mortality became manifest during the Great Smog of London in December 1952, which caused 4000 acute deaths and a further 8000 deaths in the subsequent weeks, principally from acute (on top of pre-existing chronic) respiratory and cardiovascular disease. It led to the passing of the Clean Air Acts of 1956 and 1968 in the United Kingdom.

Particles in aerosols are defined with respect to their size: "coarse" particles, particulate matter (PM)10 (i.e. particles of 10 µm); "fine", PM2.5; and "ultrafine", PM0.1. In each case, the suffix refers to the upper size limit of the mean aerodynamic diameter of the particle population, in µm (Maynard and Howard, 1999). The ultrafine fraction has the same size range as defined for nanoparticles, i.e. 1–100 nm. The size distribution of particles in the atmosphere is not uniform, but tends to be trimodal (Harrison, 1999). This is associated with their mode of formation.

- **Nucleation mode particles** are generally < 100 nm diameter and are mainly the result of primary combustion particle production such as from traffic fumes. They are not particularly long-lived in the atmosphere in their initial form because of agglomeration and condensation mechanisms.
- **Accumulation mode particles** are typically found with sizes between 100 nm and 2.5 µm diameter and arise because of the growth of nucleation mode particles. They tend to consist of secondary particles, predominantly sulphate nitrates of ammonium. Their lifetime in the atmosphere is longer than for nucleation mode particles and can typically be over 1 week. They are thus subject to undergo long-range transport.
- **Coarse particles** are the result of grinding mechanical processes and resuspension processes from the surface of the land or sea. Their size is 2.5–10.0 µm.

The ultrafine range of atmospheric particles varies considerably from location to location. Urban ultrafine composition has been addressed by Cass et al. (2000). The average chemical composition of ultrafine particles in southern California, for example, was found to include:

- 50.0% organic compounds
- 14.0% trace metal oxides
- 8.7% elemental carbon
- 8.2% sulphate
- 6.8% nitrate
- 3.7% ammonium ion
- 0.6% sodium
- 0.5% chloride.

Mobile or stationary fuel combustion sources predominated, with an estimated consistency that includes:

- 65% organic compounds
- 7% elemental carbon
- 7% sulphate
- 4% trace elements.

The health hazard created by airborne particles is among the main concerns in environmental health, and the size of the particles is of key importance. Particles larger than 10 µm generally get caught in the nose and throat, and don't enter the lungs. Particles smaller than 10 µm can get into the large upper branches just below the throat, where they are caught and removed (by coughing and spitting or by swallowing). Particles smaller than 5 µm can get into the bronchial tubes,

at the top of the lungs. Particles smaller than 2.5 µm in diameter can get down to the deepest (alveolar) portions of the lungs, where gas exchange occurs between the air and the blood stream, oxygen moving in and carbon dioxide moving out.

Although the toxicological mechanisms are not completely clear, the epidemiological evidence on the association between exposure to fine particulate air pollution and adverse health effects is strong. Both WHO Air Quality Guidelines (WHO, 2006) and United States EPA air quality criteria for particulate matter (2004) point to a strong association between fine particulate matter (particle size less than 2.5 µm) and (i) chronic mortality due to long-term exposure (all-cause mortality, lung cancer and cardiovascular causes); (ii) acute mortality due to short-term exposure (respiratory and cardiovascular admissions, asthma, bronchitis); and (iii) a series of less severe but more frequent cardiovascular and respiratory morbidity effects or symptoms (restricted activity days, work days lost, etc.). For most of the health outcomes the relationship between pollutant exposure and outcome is linear and without threshold (WHO, 2006). This has been reinforced by more recent studies (Pope & Dockery, 2006; Brook, 2007; Chen et al., 2008).

Particles smaller than 0.1 µm in diameter are potentially the most hazardous, because the deepest (alveolar) portions of the lung have no efficient mechanisms for removing them. If these particles are soluble in water, they pass directly into the bloodstream within minutes. If they are not soluble in water, they are collected by macrophages (scavenging cells) and then transported to lymph nodes, where they are retained in the deep lung for long periods (months or years) (NRC, 1979).

About 60% of PM10 particles (by weight) have a diameter of 2.5 µm or less. Ultrafine particles are more penetrative than larger particles, and buildings do not offer complete protection from exposure (Rim et al., 2010). In a modern city or major town, on many days, the air will contain 100 billion (1011) particles of 1 nm diameter in each cubic meter of air, including indoors. By weight, these 100 billion particles will only amount to 0.00005 µg (one ten-thousandth of 1% of 50 µg/m^3, a limit value used in legislation), yet they may be implicated in the health damage created by fine particle pollution. For this reason, in 1979, the United States National Research Council suggested that measuring particles by weight, without regard to particle size, has "little utility for judging effects" (NRC, 1979). The continued validity of this statement, 30 years on, is supported by Maynard & Aitken (2007).

Research is underway to clarify the health effects of ultrafine particles, given their relevant chemical–physical and toxicological characteristics. However, the epidemiologic studies specifically investigating the health effects of exposure to ultrafine particles are still few as compared to those available for the coarser fractions of particulate matter. This is mainly due to the scarce availability of specific and valid environmental data and to technical difficulties in measuring them. The current epidemiologic evidence is, however, compatible with a role for ultrafine particles in the health effects of particulate matter, which is also corroborated by toxicological studies and biological plausibility. It is possible, in other words, that part of the health effects documented for fine particles is due to the ultrafine fraction. However, the lack of studies based on measurements of exposure to ultrafine- or nanoparticles for sizeable populations limits the evaluation of such hypothesis. Identification of the role of the ultrafine fraction in determining known health impacts is further complicated by the choice of metric for concentration and exposure.

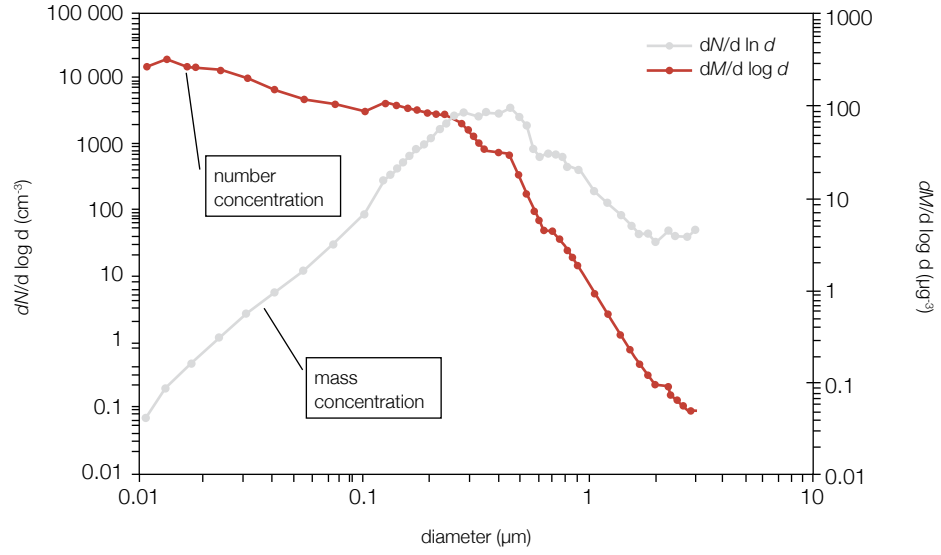
4.1 Relevant dose metric

There is some debate as to the most suitable metric to use for human exposure to ultrafine particles; this has been discussed by Seaton et al. (2009) and Oberdörster (2010). The classical dose metric for measurement and regulation of particle concentration has been gravimetric. For many decades, the accepted fraction for particles, used as standard for air quality, was PM10. More recently, some jurisdictions have adopted the standards for PM2.5, in consideration of its higher relevance for health effects. With regards to nanoparticles, consideration should be given to the question of the most relevant metric for PM0.1, in terms of concentrations, exposures and dose.

Particle number seems to be a more informative metric than particle mass, when the interest is in ultrafine particles, as is illustrated by figures A3.2

and A3.3. The majority of the particles that are of concern in the ultrafine and nanoscale range, weigh practically nothing. Figure A3.2 shows two frequency distributions made from measurements on one population of particles. The area (integral) under the black curve (number weighted) will result in the total number of particles within that size range.The area under the grey curve (mass weighted) will give the the total mass of all particles within that size range.

Figure A3.2. Typical particle number (N) and mass (M) distribution averages from approximately 10 000 single measurements, Erfurt.



Source: Wichmann et al. (2000:23).

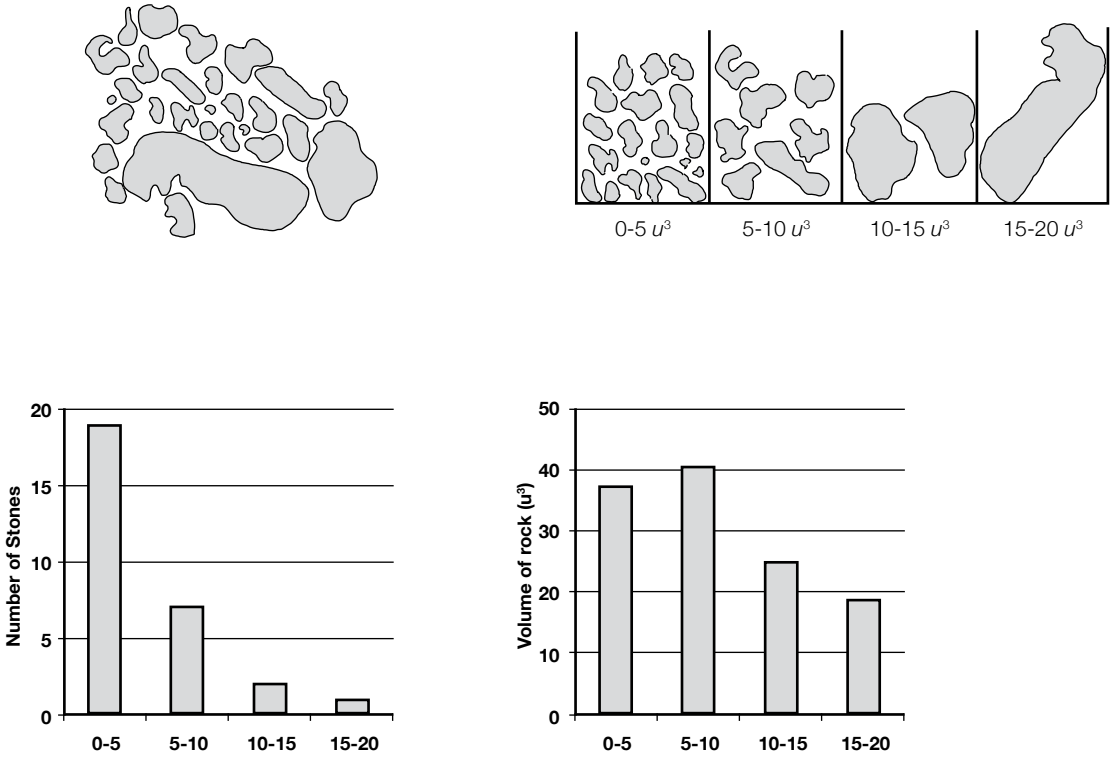
This distribution is also shown in Figure A3.3 in a simplified form. The upper-left image represents a set of arbitrarily selected particles. In the upper-right image, the particles have been binned according to their size in arbitrary units of volume (which is directly related to mass). In the bottom-left graph, the number of particles in each bin is represented on the y-ordinate, while in the bottom-right graph they are binned according to volume (mass). The influence of the larger particles is illustrated in this hypothetical example by the fact that nearly 20% of the total volume (mass) is represented by a single particle.

Despite its advantages, particle count may make it difficult to compare concentrations when different sizes and types of particle are present, and other metrics have been proposed. Total particle surface area appears to be a most promising metric. Other parameters of possible interest as dose metrics, but still to be elucidated, include biologically available surface area and surface reactivity (Oberdörster 2009). Cedervall et al. (2007) demonstrated that nanoparticles' surface characteristics and size determine the protein binding properties of plasma proteins. Maynard and Aitken (2007) have published a review of what is possible to measure with current technologies and what would be the most desirable metric to use when considering nanoparticles. They considered number-, length-, surface- and mass-weighted distributions of size. These are illustrated graphically for various classes of particle size. Clearly, the best option would be to have the capability to capture the multiple dimensions of concentration, exposure and dose, and monitor all weightings simultaneously. This would permit the collection of a large amount of additional information about particle shape, in addition to higher order extrapolations about particle size variability, convexity and surface roughness, etc.

The measurement of ultrafine particles has implications in terms of air pollution abatement. The ultrafine fraction of particulate aerosols is the most stubbornly resistant to abatement through regulation. This is illustrated in Figure A3.4, which shows a reduction in PM10 in Erfurt, Germany

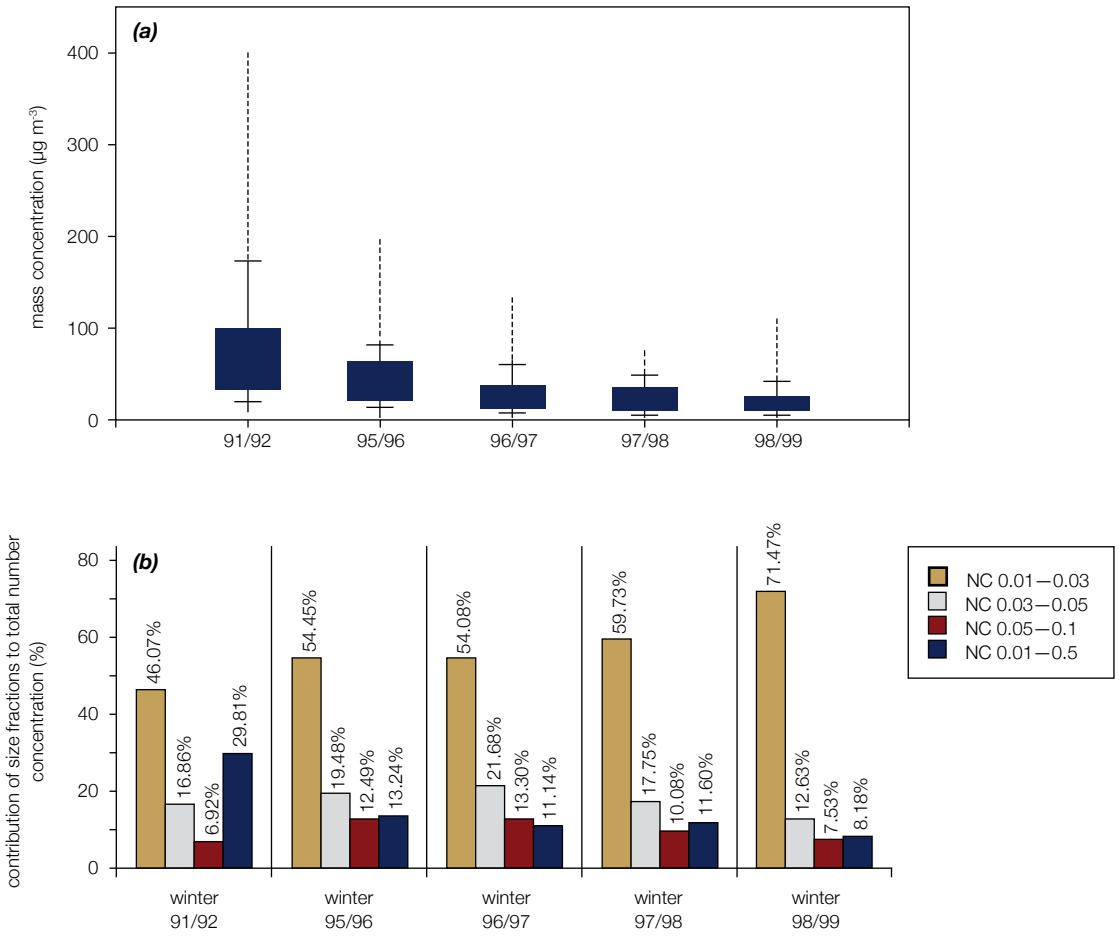
during 1991–1999. Inspection of the breakdown of the size fractions of the overall PM10 shows that while the larger particles were abated, the smallest fraction remained constant and therefore increased proportionally in its contribution to the total PM10. In urban areas, the major source of particulate emissions is motorized vehicles, with contributions from heating systems and from industrial sources. It is well known that reduction in particulate concentrations through appropriate transport policies leads to tangible health benefits, but the role of ultrafine particles is not clear. In particular, primary emissions from diesel engines and industrial processes tend to be monitored on the basis of coarse and fine particles, while ultrafine particle emissions are not well known.

Figure A3.3. Illustration of the difference between the number-weighted and volume (mass)- weighted distributions of size



Source: Howard & Reed (2010:141). Reproduced by permission of QTP Publications.

Figure A3.4. Mass concentration of fine particles in Erfurt, Germany (1991–1999)



Notes:

NC = Number Concentration

(a) Seven years trend of the mass concentration (MC 0.01–2.5 = $PM_{2.5}$ of FPs in Erfurt, winters 1991/2 to 1998/9)

(b) Seven years trend of the relative particle number concentration (in %); different size ranges (0.01–0.03, 0.03–0.05, 0.05–0.1, 0.1–0.5 µm diameter). The concentration of Ups is approximately constant and the fraction is the smallest size fraction increases steadily

Source: (2000:2764). Adapted from Wichmann et al. (2000:56–57). Reproduced by permission of the Royal Society.

5. Nanoparticle toxicity

When bulk materials are made into particles, the surface to volume ratio of the material increases. When this process reaches the nanoscale, the proportion of "surface" atoms or molecules in the material increases exponentially and the surface chemistry also changes, with the material tending to become more chemically reactive. This is the basis for the production of heterogeneous catalysts in the chemical industry. Platinum, for example, in the bulk state is particularly chemically unreactive. In the form of ultrafine particles, however, it can facilitate a number of chemical reactions. Jefferson & Tilley (1999) demonstrated that nanoscale platinum particles take on crystalline

forms with facets and isolated atoms at vertices, and discuss the implications of this morphology to the surface chemistry.

The special properties of nanomaterials must then be considered when studying their interactions with biological systems and when assessing their potential health effects. Because the surface chemistry of nanoparticles is different from that of the bulk materials that they were derived from, their toxicological properties differ, in many cases, from both the bulk and atomic or molecular forms of the materials. In fact, many aspects of nanoparticle interactions with biological systems are different, including transport, reactivity and excretion. These aspects are further elaborated and described in subsequent sections.

The term nanotoxicology, first used by Donaldson et al. (2004), indicates the fact that such differences require an ad hoc form of toxicology. The essential nature of the nanotoxicological problem was captured by Myllynen et al. (2008): "There are basically two questions to address: (1) what is the fate of nanoparticles? (i.e. where do they get to) and (2) if they get to a particular location, does it matter?".

The following sections aim to provide some key points in terms of the mechanisms of action of nanoparticle toxicity, as shown in the available literature (e.g. Oberdörster et al. 2005; Balbus et al., 2007; Helland et al. 2007; Singh & Nalwa, 2007; Elder et al., 2009; Genaidy et al., 2009; Isaacson et al., 2009; Oberdörster G., 2009; Seaton et al., 2009; Stone et al., 2010).

5.1 Mechanisms of toxicity

A number of mechanisms by which nanoparticles can induce cell damage have been reported, including, for example:

- oxidative damage through catalysis (Foley et al., 2002)
- lipid peroxidation (Kamat et al., 2000)
- surfactant properties (Cottingham et al., 2002; Cottingham et al., 2004)
- protein misfolding (Billsten et al., 1997)
- direct physical damage (Lam et al., 2004)
- enzyme poisoning (Nel et al., 2009).

These are known mechanisms mainly operating at a chemical level and have been reviewed in Nel et al. (2009). However, more data on the purely physical mechanisms of nanoparticle toxicity is needed. A recent article by Tickell (2008) has drawn attention to another possible route to harm, that of secondary photoelectron production, discussed in section 5.1.1.

Another source of vulnerability in an organ or tissue happens when it cannot repair itself by replacing damaged or dying cells, which makes it more susceptible to chronic, low-dose toxicity. Many tissues have the ability to repair through cellular proliferation. In mammals, for example, the liver is the organ with the highest regenerative capacity. Other functions, such as haemopoiesis and the renewal of the continually shedding gut lining, rely on lifelong cellular proliferation. However, in the central nervous system (CNS), the functional neurons that differentiate and mature during selective stabilization lose the ability to replicate and have to remain functional for the whole of life (Jontes & Phillips, 2008). Therefore in considering mechanisms of action, chronic effects on degenerative processes must also be considered.

5.1.1 Oxidative stress and inflammation

A common thread in nanoparticle research is the induction of oxidative stress and inflammation. This may happen via a number of mechanisms. The link between pro-inflammatory influences such as transcription factors, nuclear factor kappa beta (NF-kB) and activating protein 1 (AP-1), highlight the central role of oxidative stress in the inflammatory process (Meyer et al., 1993). Seaton et al. (1995) developed the biologically plausible hypothesis that the inflammation induced in the lung by ultrafine particles could increase blood coagulability, which in turn was associated with the increase in cardiovascular deaths during pollution episodes.

Also, catalytic activity can cause ionization events in what is, basically, an aqueous medium, leading to free radical production. This has been shown, for example, with certain nanoparticles, e.g. titania in sunlight (Barker & Branch, 2007). In vitro studies on living cells have confirmed the increased ability of ultrafine particles to produce free radicals, which then cause cellular damage (Rahman et al., 2002; Uchino et al., 2002; Li et al., 2003). This damage can be

manifested in different ways, including genotoxicity (Rahman et al., 2002) and altered rates of cell death, including apoptosis (Afaq et al., 1998; Kim et al., 1999; Rahman et al., 2002; Uchino et al., 2002).

A recent development in nanotechnology has been the application of buckminsterfullerene (C60), a 60+ atom form of carbon in the shape of a geodesic sphere, hence the nickname, "buckyball" (Johnston, 2010). These objects are very small, being about 1 nm in diameter and are therefore likely to be extremely mobile in biological systems. Kamat et al. (2000) demonstrated the ability of fullerenes to induce the production of reactive oxygen species and cause lipid peroxidation. However, Sayes et al. (2004) have demonstrated that the cytotoxic potential of fullerenes can be altered by over 7 orders of magnitude by changing their surface properties. Oberdörster E. (2004) has demonstrated the effects of Buckminsterfullerene in causing oxidative stress in fish, though more recently it has been suggested that the effect may have been due to residual tetrahydrofuran, used to solubilize the particles (Zhu et al., 2006).

Oxidative stress can induce inflammation. The cellular responses to nanoparticles have been reviewed by Unfried et al. (2007). The various pathways for free radical production leading to mitogen activated protein kinase (MAPK)-linked signalling are discussed in detail. There is increasing interest in the scientific literature in innate inflammosome interactions, see for example Dostert et al. (2008). It appears that a mechanism has evolved for coping with naturally occurring biological nanoparticles such as viruses. It is biologically plausible that engineered nanoparticles may be able to trigger such innate mechanisms and lead to the commonly observed inflammatory response of tissues to nanoparticle challenge. However, very little is known of the potential interactions with important immune system receptors, such as toll-like receptors and scavenger receptors. With the additional complexity, due to the protein "corona" that internalized nanoparticles garner on entry to the body, such interactions need to be further examined.

The toxicity and the ability of ultrafine particles to cause inflammation tend to increase as the particle size becomes smaller. This has been shown by a series of experiments with laboratory rodents; by Oberdörster G. (2000) using ultrafine particle inhalation; and by Donaldson et al. (1999, 2000) using nanoparticle instillation. For example, Donaldson et al. (1999) showed that 14 nm carbon black was roughly three times more toxic than 50 nm carbon black, and 10 times more toxic than 250 nm carbon black on a mass basis. Other experiments (Donaldson et al., 2000) showed that materials as dissimilar as titanium dioxide and latex demonstrated similar levels of toxicity, dependent on size and surface area rather than on composition. Oberdörster G.'s (2000) experiments with exposure to polytetrafluoroethylene fumes showed low-dose toxicity (50 $\mu g/m^3$ for 15 minutes led to very high mortality). However, the instillation experiments must be considered as having been conducted at high dose, compared to the levels of particles normally present in ambient air.

More recently, some in vivo investigations have been conducted into the effects of engineered nanoparticles. Lam et al. (2004) instilled nanotubes into the tracheas of mice and found resulting pathological changes persisting up to 90 days post-exposure.

Another potential mechanism gaining some attention, involves the toxicology of heavy metals, which is not yet fully explained. As mentioned above, a number of hypotheses based on chemical processes have been put forward, but less consideration has been given to physical rather than chemical processes. One possible explanation for genotoxic damage by induction of oxidative stress is through the production of secondary photoelectrons. When a material is irradiated with electromagnetic radiation, it will produce photoelectrons, the amount being a function of the fifth power of the atomic number (Einstein, 1905). The escape depth for photoelectrons from a bulk material is about 5 nm. Therefore, in bulk materials the majority of photoelectrons generated will be internally absorbed within the material. When an equivalent mass of bulk material is transformed into nanoparticles, the number of photoelectrons escaping into the surrounding environment of the particle increases greatly. This has been modelled by Elsaesser et al., (2008), who demonstrated a 25 000-fold amplification in photoelectron production, with a plateau at a particle size of 10 nm, which is consistent with the known escape depth. The population is

continuously exposed to background radiation and therefore, if high atomic number nanoparticles are internalized, they will give rise to photoelectrons that will enter surrounding tissues.

The main known mechanism for damage from photoelectrons is through the production of reactive oxygen species (ROS) in the main body constituent, water. On average, for every 60 electronvolts of energy lost by a photoelectron along its track there will be an ionization event, which will produce ROS. Counter-intuitively, therefore, the photoelectrons produced by low energy "soft" x-rays are predicted to be much more damaging to biological systems than those produced by high energy cosmic or gamma rays. High energy photoelectrons will leave the body with relatively few ionization events, while low energy photoelectrons will have a short intracorporeal course, which will give rise to thousands of ionization events. This could well be a significant mechanism for the induction of oxidative stress. It is interesting to note that the element with the highest atomic number occurring as part of natural biochemistry is iodine and that the thyroid gland, which contains almost all the body's iodine, is the most radiosensitive organ in the body.

5.1.2 Cellular toxicity

Possible targets for nanoparticle induction of cellular toxicity include the following:

Membranes

The integrity of cellular membranes is essential for many cellular processes involving fluid transport, maintenance of differential ionic concentrations and partitioning of various intracellular processes. Damage to phospholipid bilayer membranes through surfactant and lipid peroxidation effects will lead to cellular dysfunction and, if severe enough, to cell death. In particular, the vulnerability of cells that display electrical activity, such as neurons and cardiac muscle, requires further investigation.

Macromolecules

Fully functional proteins are essential for normal activity. When proteins misfold, they can become non-functional or produce oligomers, which themselves may be toxic. For a fuller discussion of nanoparticles and protein misfolding, see section 6.1.

Mitochondria

It appears that mitochondria may be a major target organelle for nanoparticle toxicity. The process of oxidative phosphorylation in the mitochondria is vital for cell function. Damage to the permeability of the outer mitochondrial membrane can precipitate apoptosis through the release of cytochrome c. Fullerenes have been shown to preferentially localize in mitochondria (Foley et al., 2002) as have MWCNT (Zhu et al., 2006). Salnikov et al. (2007) have shown that 3 nm gold particles can enter mitochondria via voltage dependent channels, while 6 nm particles cannot.

Air pollution can also interfere with mitochondrial activity: Li et al. (2003) showed that ultrafine air pollution particles, as opposed to coarse, are commonly found within mitochondria and cause vacuolar changes. Xia et al. (2006) have found mitochondrial mass loss and apoptosis in RAW264.7 macrophages. They have also shown that ambient ultrafine particles and cationic polystyrene nanoparticles cause mitochondrial damage with increased calcium uptake and ROS production (Xia et al., 2006). Hussain et al. (2005) have compared the mitochondrial impact of a number of different types of metallic nanoparticles. They found silver nanoparticles to be the most potent in disrupting mitochondrial function. A comparison of the mitochondrial toxicity of CNTs and fullerenes was made by Jia et al. (2005). They found SWCNTs to be the most toxic on a mass basis.

The nucleus

Damage to DNA within the nucleus can result in mutation and transmissible disease. The genotoxicity of silica particles has recently been studied in a multicentre in vitro investigation using the "comet assay" (Barnes et al., 2008), and no genotoxic activity was detected. However, there are questions about the sensitivity of the comet assay in detecting clastogens and therefore its suitability in this context.

Unfried et al. (2007) report on the work of a number of studies that have examined intranuclear nanoparticles. One portal of entry could be from nanoparticles coated in phospholipids interacting with the nuclear membrane (Godbey, 1999) or nanoparticles diffusing through the nuclear pores. Panté & Kann (2002) reported the passage of 24–39 nm gold particles coated in cargo receptors, and

Hoshino et al. (2004) detected quantum dots in cell nuclei within 15 minutes of incubation.

5.2 Nanoparticle assimilation, absorption, fate and mobility within the body

Nanoparticles can gain entry to the body by a number of routes, including inhalation, ingestion and across the skin, and can then travel around the body into various organs, including across the blood–brain barrier (Gumbleton, 2001; Elder et al., 2009; Oberdörster G., 2010). These properties are being positively harnessed by the pharmaceutical industry to improve the efficiency of drug delivery; however, there is growing recognition that the same properties could apply to ambient nanoparticles resulting from pollution or from manufactured products.

5.2.1 Inhalation

The airways of the respiratory system are lined with a pseudostratified columnar ciliated epithelium from the nose downwards to the respiratory bronchioles, except for a part of the lower pharynx and upper larynx, which have a stratified squamous epithelium. The surfaces of the epithelia are kept moist by secretions of mucus from goblet cells and, more importantly, submucosal glands. The lining of the major airways of the trachea and bronchi are covered with a "mucociliary escalator". The cilia move the mucus slowly upwards to the larynx, where it is swallowed and ingested. Beyond the respiratory bronchioles is the alveolar air space, which has a simple epithelium with no cilia, onto which a surfactant is secreted. The alveolar surface is patrolled by alveolar macrophages, living within the layer of surfactant, which engulf foreign matter arriving at the alveolar surface. Particle deposition may occur within the airways via three different mechanisms: sedimentation, inertial impaction and diffusion.

- **Sedimentation** occurs under the influence of gravity and tends to increase with increasing particle size.

- **Inertial impaction** occurs when a particle is being carried in air and the direction of the air changes, the momentum of the particle carrying it forward on its initial path. Particles have a tendency to impact at bifurcations in the bronchial tree. Deposition is usually determined by the momentum (weight and speed) of the particle. Increased flow tends to increase impaction, especially of larger particles. This turbulent impaction is more common in the upper, larger airways and predominantly affects particles greater than 1 µm in diameter.

- **Diffusion** (or Brownian motion) occurs with very small particles, as a result of being bombarded by other molecules, similar to the behaviour of gas molecules. Movement of these particles is completely random. Therefore, if they are close to a wet mucosa they are likely to deposit. Re-suspension does not happen subsequently. Diffusion is the method of deposition for the smaller ultrafine particles with a diameter less than 10 nm and happens predominantly in the nasal and upper pharyngeal parts of the respiratory tract.

The relative deposition of particles according to their size is shown in Figure A3.5 (Wichmann & Peters, 2000). Note that the very smallest particles tend to deposit in the upper airways by diffusion, while the bulk of the ultrafine particles deposit predominantly in the alveolar region of the lung by impaction. Other particles which deposit on the lining of the trachea and bronchi are usually ingested.

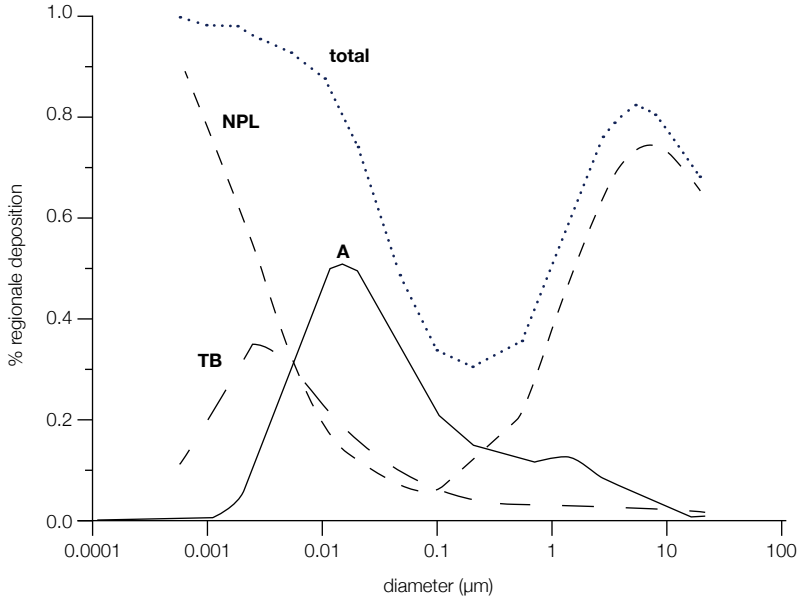
The animal literature on translocation of nanoparticles to the pulmonary circulation is not univocal.

Evidence has been found of accumulation of 13C nanoparticles (20–29 nm) in the rat liver within 30 minutes of inhalational exposure, with a further five-fold increase in the following 24 hours (Oberdörster et al., 2002). Earlier work also indicated nanoparticle uptake from the lung (Patrick and Stirling, 1992). This suggests a rapid translocation, although questions concerning confounding by ingestional exposure have been addressed in Stern and McNeil (2008). Kreyling et al. (2002) showed a very low (< 1%) rate of translocation from rat lung to liver, spleen, heart and brain when dosing with 80 and 15 nm iridium nanoparticles by instillation. The study did, however, report that the smaller particles showed a higher rate of translocation.

The results for human studies are also mixed. While Brown et al. (2002) and Mills et al. (2006) did not demonstrate any significant translocation of radio labelled carbon nanoparticles, Nemmar et al. (2001) showed 3–5% uptake within a few minutes of exposure in the blood, and subsequent deposition in the liver. The latter paper attracted some comment that the possibility of label removal from nanoparticles was not controlled for.

On the evidence available at the time, Hoet et al. (2004) concluded that endocytosis by epithelial and endothelial cells and phagocytosis by alveolar macrophages were the most important routes for nanoparticle translocation.

Figure A3.5. Predicted deposition of inhaled particles of different sizes of unit density in the human respiratory tract during nose breathing, light exercise



NPL = nose, pharynx, larynx, TB = trachea, bronchi, A = alveolus.

Source: Wichmann & Peters (2000:2755). Reproduced by permission of the Royal Society.

5.2.2 Dermal absorption

Another possible portal of entry into the body is via the skin. The findings in scientific papers are mixed, some showing dermal nanoparticle assimilation, others not. The question is a key one, because the skin is the largest organ in the body and the quantity of nanoparticles that crosses per unit area of skin would not have to be large to achieve a substantial internalized dose. In addition, because a number of commercial skincare products containing nanoparticles are already on the market, including sunscreens, their application to a large proportion of the skin surface area may contribute to high levels of exposure.

A number of sunscreen preparations are now available which incorporate nanoparticle titanium dioxide. Tan et al. (1996) showed the presence of titanium in the epidermis and dermis after the use of sunscreen. Schulz et al. (2002) and Gamer et al. (2006) could not reproduce Tan's results; however, they did not address confounders such as movement of the skin or the charge of the particle.

Tinkle et al. (2003) have shown in an animal model that beryllium particles of 0.5 and 1.0 µm diameter (i.e. within the category of "fine" particles) can get deep enough into the skin to be taken up into the lymphatic system, while larger particles did not. The

lymphatics are a system of blind capillary vessels which drain the spaces between cells of interstitial fluid and then drain into bigger vessels, eventually returning the lymph to the general circulation. The implication is that nanoparticles can be assimilated into the body through the skin. The exact proportion of deposited particles which will be absorbed remains unknown. Tinkle et al. (2003) have studied the penetration of dextran beads into postmortem human skin and demonstrated that 0.5 µm and 1.0 µm beads can penetrate the stratum corneum of skin being flexed. This affected over 50% of samples if the process continued for 1 hour. In a small proportion of cases, the beads got as far as the dermis. More recently, Rouse et al. (2007) demonstrated that buckminsterfullerene amino acid penetrated the skin after mechanical flexing, thus supporting the findings of Tinkle et al. (2003). Lademann et al. (1999) demonstrated that microparticles of titanium dioxide contained in sunscreen penetrated into the stratum corneum and hair follicle orifices.

Particle charge appears to be a significant factor. Kohli & Alpar (2004) found that positively charged or neutral latex particles of 50 and 500 nm could not penetrate the dermis, while negatively charged particles could. The size and shape of quantum dots was shown by Ryman-Rasmussen et al. (2006) to be significant in the penetration of intact skin.

A comprehensive, industry-funded review of skin absorption of nanomaterials has recently been published (Nohynek et al., 2007). This indicated that most studies on the skin absorption of metal oxide nanoparticles (both in vitro and in vivo) showed no significant absorption into the systemic circulation. However, the relevance of some in vitro models has been questioned, as the role of flexing may be more important with nanomaterials (EC, 2007). The effect of abrasion of rat skin causing a small increase in the penetration of quantum dots is reported in Zhang & Monteiro-Riviere (2008). However, the effect on skin damaged by other mechanisms (e.g. through sunburn), or on infant skin, remains unknown.

5.2.3 Gastrointestinal assimilation

Particles enter the gut through ingestion. Nanotechnology applications in the food industry include the use of ingredients processed or formulated to form nanostructures, and nanosized or nano-encapsulated additives. Also, food can be a source of nanoparticle ingestion owing to nanotechnology applications in coating, packaging and filtering. These involve organic and inorganic nanomaterials (WHO/FAO 2010).

An additional source is the constant swallowing of mucus produced by the mucociliary escalator of the respiratory system, which will contain impacted particles. Lomer et al. (2002) have estimated that 1012–1014 particles of up to 3 µm are ingested each day, per person in developed countries. These are predominantly in the form of titanium dioxide, food colorants and silica.

Persorption is a process in the gut whereby quite large particles, of up to 20 µm, can be assimilated into the lymphatics and portal capillaries across Peyer's patches in the ileum (Volkheimer, 1974; Hodges et al., 1995). In contrast, Kreyling et al. (2002) found that no 192IrCl 18 nm particles were absorbed across the gut wall in rats. This was a similar result to that obtained with the same material in their respiratory exposure studies.

Another mechanism that has been demonstrated is via intestinal enterocytes (Jani et al., 1990; Florence, 2005). Positive charge of particles appears to enhance their uptake in the gut (Florence, 2005). Jani et al. (1990), using polystyrene particles (50 nm, 100 nm, 500 nm, 1 µm and 10 µm) found a size effect: the smaller the particle, the higher the uptake.

5.2.4 Translocation to and assimilation by the central nervous system

Oberdörster et al. (2005) reviewed three ways in which nanoparticles can potentially gain access to the CNS: absorption by the olfactory mucosa, assimilation via the peripheral nervous system and traversing of the blood–brain barrier. Virtually the first thing that vertebrates do during ontogeny is "internalize" their CNS during gastrulation (i.e., they turn a piece of the skin on the dorsum "inside-out" and sink it under the integument), which brings it under the influence of the homeostatic robustness of the internal milieu. However, this system has an "Achilles heel" – the olfactory mucosa. The olfactory mucosa is the only part of the CNS to remain in direct

contact with the external milieu. It is an absorptive "sampling" mucosa, operating by endocytosis. It is widely recognized that this is a portal of entry to the CNS for some viruses, as demonstrated by Bodian & Howe (1941a, 1941b) for the 30 nm polio virus in a primate model. They showed that the virus was moved by anterograde axonal transport at 2.4 mm/hr. de Lorenzo (1970) used 50 nm silver-coated colloidal gold nanoparticles in squirrel monkeys and showed uptake in the olfactory bulb. He also observed trans-synaptic propagation to reach mitral cell dendrites. Oberdörster et al. (2004) reported on the translocation of inhaled ultrafine particles to the brain. They exposed rats to 13C nanoparticles (35 nm) by inhalation. They observed a significant increase in 13C in the olfactory bulb on day 1 with a further continued increase up to day 7 post exposure.

The second route of entry, assimilation via the peripheral nervous system, is again achieved by retrograde axonal transport. A number of viruses are known to be able to travel via the trigeminal nerve to the semilunar ganglion in the middle cranial fossa. Herpes viruses can use both anterograde and retrograde axonal transport via branches of the trigeminal nerve (Kennedy & Chaudhuri, 2002). Non-biological nanoparticles have also been observed to undergo retrograde axonal transport from the peripheral nervous system. Hunter & Dey (1998) demonstrated the uptake of rhodamine labelled microspheres of 20–200 nm in rats and Hunter & Undem (1999) used the same method in guinea pigs.

The third route is for nanoparticles to traverse the blood–brain barrier. The blood–brain barrier does not become fully competent until about 6 months after birth. Therefore, a window of vulnerability exists for the ingress of nanoparticles into the CNS during fetal and early postnatal life. The blood–brain barrier has specialized tight junctions which help minimize paracellular transport. It stops most small molecules and nearly all macromolecules from entering the CNS (de Boer & Galliard, 2007). The pharmaceutical industry is conducting a large amount of active research into nanoparticle preparations to increase drug delivery to the CNS by crossing the blood–brain barrier. This has been extensively reviewed by Kabanov & Gendelman (2007). Candidate nanoparticles under consideration include liposomes, polymeric micelles, nanogels, dendrimers and insoluble nanoparticles. Kreuter et al. (2001, 2002) have shown that poly(butyl cyanoacrylate) nanoparticles pre-coated with polysorbate 80 can be used to enhance the delivery of apolipoproteins to the brain. Alyaudtin et al. (2001) have demonstrated that similar ultrafine particle mediate delivery of [3H]-dalargin to the brain. Furthermore, Foley et al. (2002) have established that fullerenes can cross the membranes of living cells and accumulate preferentially in the mitochondria.

5.2.5 Nanoparticle distribution in the body

The kinetics of nanoparticles in the body has been reviewed by Hagens et al. (2007). Once nanoparticles have been assimilated, if they can gain access to the circulation they can be transported throughout the body. Several authors have demonstrated the presence of nanoparticles in the blood stream (Hillyer and Albrecht, 2001; Gatti et al., 2004), and a number of studies have shown distribution of nanoparticles to the liver, spleen, heart and brain (Hillyer & Albrecht, 2001; Nemmar et al., 2002; Oberdörster et al., 2002; Ji et al., 2006). When nanoparticles enter the circulation, they can interact with elements of the blood, plasma proteins, cells and factors affecting coagulation; however, the exact mechanism of this interaction is unknown. Evidence suggests that nanoparticle interactions with plasma proteins can reduce their toxicity. Lovric et al. (2005) showed that bovine serum albumin reduced the toxicity of quantum dots in vitro. Apolipoprotein-A1 has been shown to bind to silica (Barrett et al., 1999); although, little appears to be known about the stability of such binding or the proportion of nanoparticles likely to be so bound. Human studies are not conclusive, as they may suffer a lower level of sensitivity, compared to animal experiments.

After oral dosing with colloidal gold in mice, Hillyer & Albrecht (2001) demonstrated that 4 nm nanoparticles spread to distant organs, including the brain. However, 58 nm nanoparticles remained in the gut, suggesting a size dependent phenomenon.

Oberdörster et al. (2002) showed in animals that after inhalation there was a small but measurable redistribution of radio-labelled nanoparticles to distant organs, suggesting haematogenous spread.

5.2.6 Nanoparticle excretion

The kidney forms urine as an ultrafiltrate of blood, through the glomerular wall, whose pores are ~8 nm in size. Small positively charged ions such as sodium and potassium can pass easily through the kidneys, while larger molecules, particularly those with a negative charge, such as proteins, hardly pass at all. If nanoparticles cannot be completely removed from the body then, however slowly they enter, some degree of bioaccumulation will occur. It is evident from the literature that non-soluble particulate matter can sequester for years in the lungs, gastrointestinal tract or brain.

There is evidence of translocation of nanoparticles from the lung and gut to the vascular system. Nigavekar et al. (2004) report that poly(amidoamine) dendrimers 5 nm in diameter have been shown in a mouse model to be excreted in urine, with accumulation in the kidney observed. Borm et al. (2006) describe unpublished observations (Curtis) that suggested it is conceivable that nanoparticles of an appropriate size could block the renal fenestrae and precipitate acute renal failure. There is clearly a large data gap in this topic.

Nanoparticles can also be excreted in the bile (Nefzger et al., 1984); the process of transcytosis is involved. Lowe et al. (1985) studied the transport of horse radish peroxidise, a high molecular weight protein into the bile. Their studies suggested that in the presence of liver damage paracellular routes of excretion into the bile became more significant. Sweat and cell shedding are other possible routes of nanoparticle excretion.

This topic is of critical importance to the pharmaceutical industry, which needs to know the pharmacokinetics of nanomedicine products. A review of nanoparticle clearance has been published by Longmire et al. (2008).

5.3 Internally produced nanoparticles

Internally produced nanoparticles cause a number of "co-pathologies", which should be considered because they can inform hazard identification from engineered nanoparticles. In addition, these pathologies could be affected by interactions between nanoparticles produced by the human body and engineered ones.

Examples include:

- Acute glomerulonephritis. In the post-beta streptococcal infection variety the pathology is caused by the deposition of nanoscale protein immune complexes which obstruct the pores in the glomerular basement membrane.
- Disseminated intravascular coagulopathy is an acute pathological activation of coagulation mechanisms which can be activated by the appearance in the blood of nanoscale lipopolysaccharide complexes. The latter can be a consequence of a gram negative bacterial infection.
- Amyloid precursor protein oligomer toxicity. The presence of this oligomer is associated with Alzheimer's disease.

6. Target organ susceptibility

6.1 The brain and protein misfolding

Over 40 human protein misfolding diseases are known. The majority of these result in damage to the CNS as shown in Table A3.2. The spectrum of sequelae associated with protein misfolding include non-functioning proteins, insoluble proteins or protein fractions and/or the production of toxic oligomers (Cottingham et al., 2002). However, through evolution the CNS has developed a system for coping with a basal level of such misfolding by internalizing such products in specialized endosomes. The occurrence of lipofuscin granules, for example, increases with age. Some specific proteins have their own distinctive endosomes, for example the protein associated with Parkinson's disease, alpha-synuclein accumulates in Lewy bodies.

The mechanisms that have evolved throughout pre-industrial history to deal with misfolded protein fragments can be expected to have the capacity to cope over a lifetime without major adverse effects. However, if the rate of misfolding is increased, it may result in adverse consequences. The possible sequelae might be an increase in the incidence of protein misfolding disease in the general population and an earlier average age onset.

Some literature exists on the ability of nanoparticles

to disrupt protein folding, termed “chaperone effects”. Billsten et al. (1997) showed that 9 nm silica particles can alter the configuration of the enzyme human carbonic anhydrase II. Akiyoshi et al. (1999) have demonstrated the chaperone-like activity of nanoparticles with the beneficial effect of facilitating the thermal stabilization with refolding of carbonic anhydrase B. Ishii et al. (2003) have shown that semiconductor nanoparticles can be stabilized by chaperoning molecules, and then their release can be mediated by Adenosine triphosphate (ATP). This is presented as a possible basis for bio-mediated devices in the future. Protein misfolding mechanisms and cellular recognition systems have been reviewed by Goldberg (2003).

In vitro work by Linse et al. (2007) has demonstrated that the rate of fibrillation of amyloid protein can be perturbed by nanoparticles. Chen & von Mikecz (2005) have reported that silicon dioxide nanoparticle induced protein aggregates have a similar composition to intracellular inclusions that are diagnostic markers for protein misfolding diseases such as Huntington’s chorea and muscular dystrophy.

It is clear that more data are needed about the degree of translocation of nanoparticles into the brain and the interactions of nanoparticles with critical proteins such as amyloid precursor protein, Parkin, alpha synuclein and tau.

Table A3.2. List of proteopathies

Proteopathy	Major aggregating protein
Alzheimer's disease	Amyloid β peptide (Aβ); Tau protein (see tauopathies)
Cerebral β-amyloid angiopathy	Amyloid β peptide (Aβ)
Retinal ganglion cell degeneration in glaucoma[38]	Amyloid β peptide (Aβ)
Prion diseases (multiple)	Prion protein
Parkinson's disease and other synucleinopathies (multiple)	α-Synuclein
Tauopathies (multiple)	Microtubule-associated protein tau (Tau protein)
Frontotemporal lobar degeneration (FTLD) (Ubi+, Tau-)	TDP-43
FTLD–FUS	Fused in sarcoma (FUS) protein
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase, TDP-43, FUS
Huntington's disease and other triplet repeat disorders (multiple)	Proteins with tandem glutamine expansions
Familial British dementia	ABri
Familial Danish dementia	ADan
Hereditary cerebral hemorrhage with amyloidosis (Icelandic) (HCHWA-I)	Cystatin C
CADASIL	Notch3
Alexander disease[39]	Glial fibrillary acidic protein (GFAP)
Seipinopathies[40]	Seipin
Familial amyloidotic neuropathy, Senile systemic amyloidosis	Transthyretin
Serpinopathies (multiple)	Serpins
AL (light chain) amyloidosis (primary systemic amyloidosis)	Monoclonal immunoglobulin light chains
AH (heavy chain) amyloidosis	Immunoglobulin heavy chains
AA (secondary) amyloidosis	Amyloid A protein
Type II diabetes	Islet amyloid polypeptide (IAPP; amylin)
Aortic medial amyloidosis	Medin (lactadherin)

Proteopathy	Major aggregating protein
ApoAI amyloidosis	Apolipoprotein AI
ApoAII amyloidosis	Apolipoprotein AII
ApoAIV amyloidosis	Apolipoprotein AIV
Familial amyloidosis of the Finnish type (FAF)	Gelsolin
Lysozyme amyloidosis	Lysozyme
Fibrinogen amyloidosis	Fibrinogen
Dialysis amyloidosis	Beta-2 microglobulin
Inclusion body myositis/myopathy	Amyloid β peptide (Aβ)
Cataracts	Crystallins
Retinitis pigmentosa with rhodopsin mutations[41]	rhodopsin
Medullary thyroid carcinoma	Calcitonin
Cardiac atrial amyloidosis	Atrial natriuretic factor
Pituitary prolactinoma	Prolactin
Hereditary lattice corneal dystrophy	Keratoepithelin
Cutaneous lichen amyloidosis	Keratins
Mallory bodies	Keratin intermediate filament proteins
Corneal lactoferrin amyloidosis	Lactoferrin
Pulmonary alveolar proteinosis	Surfactant protein C (SP-C)
Odontogenic (Pindborg) tumor amyloid	Odontogenic ameloblast-associated protein
Seminal vesicle amyloid	Semenogelin I
Cystic Fibrosis	cystic fibrosis transmembrane conductance regulator (CFTR) protein
Sickle cell disease[42]	Hemoglobin
Critical illness myopathy (CIM)	Hyperproteolytic state of myosin ubiquitination

Source: Proteopathy [web page]. San Francisco, Wikimedia Foundation Inc. (http://en.wikipedia.org/wiki/Proteopathy, accessed 8 October 2013).

6.2 Vascular and respiratory systems

As discussed earlier, epidemiological studies demonstrate the links between air pollution and cardiovascular and respiratory mortality and morbidity (Schwartz & Dockery, 1992; Dockery et al., 1993; Peters et al., 2001; Clancy et al., 2002). Particulate aerosols have also been associated with changes in heart physiology (Brook et al., 2002; Devlin et al., 2003). The fact that tobacco smoke damages the arterial endothelium, and increases incidence of atherosclerosis, myocardial infarction and stroke is widely known (Auerbach et al., 1965). Particle research is uncovering the mechanisms for these observations; Donaldson et al. (2005) have reviewed this topic. The current hypothesis is that combustion derived nanoparticles (i) on gaining the blood stream, disrupt platelet and endothelial cell function, destabilizing plaque, leading to plaque rupture and thrombogenesis; and (ii) in the lung interstitium, nanoparticles cause lung inflammation, which produces cytokines and induces systemic inflammation, leading to destabilization of atheromatous plaques, plaque rupture and thrombogenesis.

The lung is also a major target for direct consequences of nanoparticle aerosol exposure. Donaldson et al. (1996) showed that, for titanium dioxide instilled into rat lungs, the smaller the size of particle the more intense the inflammation. Stone et al. (1998) have shown that

installation of carbon black induced a similar particle size related inflammation. Furthermore, it appeared that the material used was of less significance than the particle size. Oberdörster et al. (2005) showed acute lung inflammation in rats following inhalation of freshly generated polytetrafluoroethylene fumes. In all cases, the most intense inflammation, assessed by the loading of bronchoalveolar lavage fluid with neutrophils, was associated with exposure to particles in the ultrafine fraction. The common denominator was inflammation and the implications for systemic disease have been discussed by Seaton (1995).

6.3 The fetus

A suitable model for the study of nanoparticles on rapidly dividing cells is provided by the fetus, where at certain stages cells around the notochord are dividing every 3.5 hours, many times faster than in the most malignant tumours. In addition, epigenetic phenomena involving apoptosis in the selective stabilization of a number of systems makes the intrauterine part of life very vulnerable to damage.

Myllynen et al. (2008) have recently shown, on a human placenta perfusion model, that PEGylated gold nanoparticles of 5, 10 and 15 nm did not cross the materno–fetal barrier into the fetal circulation after a 6 hour perfusion. However, a dispersion of nanoparticles took place and these were found in the syncytiotrophoblast. The result of a more prolonged perfusion in vivo in human placenta remains unknown.

Tsuchiya et al. (1996) exposed pregnant mice to buckminsterfullerene. At high dose (137 mg/kg) all embryos died. At a lower dose, (25–50 mg/kg) teratogenesis was observed, particularly in the head region. Although the nanoparticles may have crossed the placenta, an intraperitoneal route of exposure could not be ruled out. The dosages used were extremely high. An in vitro study on mouse embryos (Bosman, 2005) showed that polystyrene nanoparticles were internalized by the embryo, but no hindrance of development was detected.

Developmental toxicity of nanoparticles would be dependent on their potential to have effects either on placental function or by direct migration to the fetus. Very little data is available on this.

6.4 The anticipated spectrum of pathology from nanoparticle exposure

Some general conclusions can be drawn from the previous sections, concerning known pathogenic mechanisms, which can inform the formulation of policy. The likely unwanted consequences of human exposure to engineered nanoparticles will fall into two main categories, depending on whether or not particles can get inside cells. For nanoparticles that can be fully internalized by cells, the predominant hazard will involve cytotoxicological effects at the cellular level and/or inflammatory effects at the tissue level. Fibres, when they reach a length at which they become “indigestible” by the cell (generally accepted as being in the region of 20 µm), tend to give rise to a different spectrum of disease, associated with chronic inflammation and oxidative stress of the interstitial regions between cells. The demonstration of mesothelioma-like activity in certain varieties of carbon nanotubes suggests that human exposure may have important consequences. For those particles that cannot be fully internalized, usually high aspect ratio fibres, the additional complication of extracellular “foreign body” type of reactions exists. This is particularly the case for those associated with “frustrated phagocytosis”, where the cell tries to engulf the particle but because of its dimensions only partially achieves this.

The acute response of tissues to nanoparticles has been more widely studied than chronic long-term endpoints. The common factor for the acute response is oxidative stress leading to inflammation (Unfried et al., 2007), which is detectable and the sequelae of which are well understood. However, the possibility of longer-term phenomena, not necessarily involving inflammation, has to be considered. The growing literature on the ability of nanoparticles to interfere with normal protein folding raises questions about the possibility that chronic low-dose exposure of particles over a lifetime may contribute to degenerative diseases, particularly of the nervous system. While acute effects such as inflammation are more likely to be detected, particularly with modern nitric oxide measurement equipment, and associated with nanoparticles exposure, chronic low-dose toxicity leading to degenerative disease is much less likely to be identified.

Thus, the health consequences of human exposure to nanomaterials might include diseases that: are aspecific (i.e. admit several risk factors of a different nature, for example linked to lifestyle); are possibly more powerful; have long-term latency; and are not easily predictable given the complex causal chain, or possible synergistic effects with other exposures. The combination of these characteristics would make some of the health effects particularly difficult to detect and may produce some "unknown unknowns".

7. Consideration of two nanomaterials produced in industrial quantities

7.1 Titanium dioxide

Titanium dioxide has been widely used for decades, for instance as an ultraviolet (UV) filter in sunscreens. Originally the particle size for use in sunscreens was ~200 nm and sunscreen creams where opaque and white in appearance. More recently the particle size has been reduced to the nanoscale of ~30–60 nm, making creams transparent. Due to these applications, nanosized titanium dioxide may be among the most widespread nanoparticles to be encountered in the daily human environment. Various effects have been observed in different strains of rodents after exposure to titanium dioxide, and several studies report differences in the responses for particles smaller than 100 nm compared to those for larger particles (Ferin et al., 1992; Baggs, et al. 1997; Oberdörster et al., 2005). Difference in the toxicological profile in rodents has also been observed to be dependent of the surface area of titanium dioxide particles rather than mass dose. When comparing anatase (one of the three mineral forms of titanium dioxide) particles of 20 and 250 nm, Oberdörster G. (2000) found that the former generated a much greater pulmonary-inflammatory response in rats after exposure to equal-mass doses. With dose expressed as a function of mass and surface particle number, marked differences in the dose-response curve of the two materials were found; however, when dose was expressed in terms of particle surface area, both forms of titanium dioxide followed a similar dose-response curve. Donaldson et al. (2002) reported observing a similar correlation for carbon black, when studying the ability of nano- and microparticles to cause inflammatory effect in rats (Donaldson et al., 2002). In contrast, Warheit et al., (2006) and Sayes et al. (2007) reported no association with surface area when evaluating biological response in rats after exposure to nanosized titanium dioxide, silicon dioxide and other particles.

A number of factors have been hypothesized to influence the toxicity of titanium dioxide besides surface area. These include crystalline structure, shape, coated versus non-coated type, as well as exposure routes (e.g. intratracheal, oral, inhalation) (Oberdörster et al., 1992; Osier & Oberdörster, 1997; Höhr et al., 2002; Warheit et al., 2007). The cytotoxicity of titanium dioxide has also been found to be dependent on crystalline structure, size and purity. For instance Sayes et al. (2006) observed a time-dependent decrease in human dermal fibroblasts for anatase titanium dioxide (30 µg/mL/1–48 h) and found a 50% lethal concentration (LC50 = 3.6 µg/mL). However, for rutile titanium dioxide (another mineral form), the LC50 was substantially higher and equal to 550 µg/mL, with LC50 of anatase/rutile titanium dioxide falling between the two. Limbach et al. (2007) found promoted increase of ROS in human lung epithelial A459 cells that increased with the purity of the particles (30 µg/mL/4 h). This observation was supported by Long et al. (2006) who observed a rapid (< 5 minute) and sustained (120 minute) release of ROS at non-cytotoxic concentrations (2.5–120 ppm/1–18 h) in BV2 brain microglia. This work has also been reviewed by Stone et al. (2010).

The UV reactivity of titanium dioxide has been the subject of several studies. Uchino et al. (2002) and Gurr et al. (2005) found no additional effect of photoactivation against human bronchial epithelial BEAS-2B cells and Chinese hamster ovary cells; however, evidence indicates that the cytotoxicity of titanium dioxide is influenced by UV irradiation (Sayes et al., 2006). Recent graphical evidence concerning titanium dioxide and ROS production can be found in Oberdörster (2010).

7.2 Carbon nanotubes

CNTs are fibrous nanoparticles, already being

produced in industrial quantities, with annual production rising sharply since 2005. Current applications include materials and components for a variety of industries (sports, construction, textiles, scientific laboratories); the remarkable characteristics in terms of strength, lightness, flexibility, conductivity and thermal properties make CNTs likely to become very widely used in the near future. Their health effects have been thoroughly reviewed by Genaidy et al. (2009). In addition, their toxicology is discussed in Stone et al. (2010) and Oberdörster (2010). As with titanium dioxide, the widespread use of CNTs creates a greater likelihood of generalized exposure than with other nanoparticle types. Thus, the available knowledge on CNTs' nanotoxicology is of special importance.

The publication of two papers (Poland et al., 2008; Takagi et al., 2008) indicating that CNTs have mesothelioma-like activity in animal models, acts as a serious warning in the risk assessment of these novel materials. The paper by Poland et al. examined the effect of long MWCNT on the peritoneal cavity of mice; the results indicated an asbestos-like, length-dependent, pathogenic behaviour. Takagi et al. (2008), reported that MWCNT with a fibrous or rod-shaped length around 10–20 µm, with an aspect ratio of more than three, induced mesothelioma when administered intraperitoneally to p53-heterozygous mice that had been reported to be sensitive to asbestos – although the doses used were high. A positive control, crocidolite (blue asbestos) was used for comparison. The results point out the possibility that carbon-made, fibrous or rod-shaped micrometre particles may share the carcinogenic mechanisms postulated for blue asbestos. Thus, CNTs of similar dimensions and durability as asbestos fibres may be of concern.

This result was predictable from in vitro studies by Kaiser et al. (2008) investigating bundles of SWCNT. Findings are also consistent with the review conducted by Tomatis et al. (2007) on the association between asbestos fibre size and mesothelioma. This review concluded that stiffer, high aspect ratio fibres are also positively associated, and rejected suggestions that only short, low aspect ratio asbestos fibres could traverse the pleura and cause mesothelioma (Chiappino, 2005).

8. Risk assessment of nanomaterials

Risk assessment (mainly of chemical or physical agents) traditionally consists of the following four steps:

1. hazard identification
2. hazard characterization
3. exposure assessment
4. risk quantification.

The quality of the final step, the risk quantification, is entirely dependent on the quality of the first three steps. Current risk assessment procedures with corresponding regulations for nanomaterials have been based on procedures extrapolated from chemical risk assessment, (Rocks et al., 2008). This section briefly describes the basic risk assessment procedures used for nanomaterials, and gaps in knowledge, relevance and usefulness. The limitations of chemical risk assessment when applied to nanomaterials are also briefly described in later sections.

8.1 Hazard identification and characterization and exposure assessment in nanomaterial risk assessment

The diversity of nanomaterials and their properties makes it hugely challenging to conduct in vitro and in vivo evaluations of their biological effects (CCA, 2008). End-points usually considered include acute toxicity, repeated dose toxicity, irritation, sensitization potential, mutagenicity, clastogenicity (i.e. propensity to cause a point mutation as compared to disrupting a chromosome), carcinogenicity and reproductive toxicity (Rocks et al., 2008). However, as discussed in this report, there is not enough knowledge to exclude the possibility of other, potentially unknown, endpoints. Preliminary results suggest that in vitro testing may not always accurately predict hazards, and large in vivo studies are few and difficult to reproduce.

Hazard identification of chemical or physical agents is traditionally based on inherent physical, chemical, biological and toxicological properties, whereas

hazard characterization involves the establishment of a dose (concentration)–response (effects) assessment.

Several studies – for example on buckminsterfullerene, single- and multiwalled CNTs, and various forms of metal nanoparticles – have reported dose–response relationships. Based on these, some predicted "no effect concentrations" have even been estimated (Mueller & Nowack, 2008; Park et al., 2008).

Interpreting these results and extrapolating to the wide variety of nanomaterials is difficult, since the nanomaterials that have been tested differ substantially from other nanomaterials with regard to: (i) physical–chemical properties such as chemical composition, shape, etc. and (ii) endpoints tested for, duration of exposure, methods (e.g. assays) and standards used (Hansen et al., 2007). In addition, based on the knowledge from studies on nanoparticles detailed in sections 5 and 6 of this report, it has been suggested that the biological activity of nanoparticles might not always be dose-dependent, but rather dependent on physical and chemical properties not routinely considered in toxicity studies (Oberdörster et al., 2005). This creates serious challenges for all steps of risk assessment of nanomaterials.

Exposure assessment is another key step in risk assessment, invariably challenging, and the case of nanomaterials is no exception. Unfortunately, exposure data are lacking and no full exposure assessment has so far been published for any type of nanomaterial or group of nanomaterials. This is partly due to technical difficulties in measuring nanomaterial exposure in the workplace and/or the environment, and partly because the biological and environmental pathways of nanomaterials are still largely unexplored in detail (NIOSH, 2006; Owen & Handy, 2007; CCA, 2008). However, some efforts have been made to estimate, predict and assess occupational, consumer and environmental exposure levels (e.g. Boxall et al., 2008; Mueller & Nowack, 2008; Wijnhoven et al., 2009; Gottschalk et al., 2010). The applicability of current exposure assessment methods and guidelines has also been discussed (OECD, 2009; SCENIHR, 2009). These efforts have been hampered by the lack of information (or access to it), e.g. about manufacturing conditions, levels of production, industrial applications and uses, consumer products, environmental behaviour and environmental fate and distribution (Maynard et al. 2004; Boxall et al. 2008, Hansen et al., 2008).

8.2 Current status of risk assessment of nanomaterials

In 2007, the EC Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) concluded that the current chemical risk assessment framework was most likely to be sufficient for nanomaterials, but may need to be modified in some cases (SCENIHR 2007, 2009; Grieger et al., 2010). However, since then only a few attempts have been made to complete risk assessments of nanomaterials, which are described below.

In 2009, Shinohara et al. published a number of interim reports on risk assessments of manufactured nanomaterials, i.e. buckminsterfullerene, CNTs and titanium dioxide. Based on a limited number of inhalation exposure studies, the authors present several procedures to establish a provisional value for an acceptable exposure concentration in the occupational environment, as well as the general environment. When deriving the provisional no-observed-adverse-effect level (NOAEL) for buckminsterfullerene, inflammatory responses in the lung was selected as the critical endpoint, based on the assumption that inhalation is the primary route of human exposure. The occupational NOAEL was estimated to be 116 μg/m^3 and the general NOAEL 49.6 μg/m^3. In the case of buckminsterfullerene, the uncertainty factor was reported to be 6 in the occupational environment and 60 in the general environment. Shinohara et al. (2009) found that the acceptable and estimated exposures to CNTs were quite close to each other, but concluded that the risk cannot be determined since the estimated exposure values have a wide range, and vary greatly with different work processes at individual sites. Using lung inflammation as the critical endpoint, the acceptable amount of deposited titanium dioxide on the alveoli per day, per body weight was found to be 18 μg/kg/day. Worst-case exposure was estimated at 5.9 μg/kg/day, and following that, a hazard quotient was calculated as 0.3 (i.e. < 1), indicating that titanium dioxide is safe to use. Shinohara et al. (2009) subsequently concluded that the provisional values presented in the interim reports must be treated as preliminary

due to a number of challenges and uncertainties in the estimated values, including: whether the amount deposited on the alveoli should be based on mass concentration; the influence of surface area, primary particle size, and aggregation/agglomeration size; limited information from toxicity tests; and diversity of types of titanium dioxide nanomaterials.

In 2010, Stone et al. published a scientific report including an exploration of key questions associated with risk assessment of nanomaterials. They carried out qualitative risk characterizations for buckminsterfullerene, CNTs, nanosilver and titanium dioxide, and concluded that a quantitative risk assessment was not possible due to "a significant lack of measured and modelled exposure data of nanoparticles, for humans (occupational and consumer exposure) and for the environment". In terms of human health, Stone et al. (2010) sought to establish "exposure values for the various routes of exposure (inhalation, dermal and oral) for consumers and workers and the establishment of a Derived-No-Effect Level (DNEL), typically based on extrapolation of animal data to the human situation by using appropriate assessment factors". In relation to environmental risk assessment, the authors developed the predicted exposure concentration (PEC) and the predicted no effect concentration (PNEC) for each environmental compartment (e.g. water, air, soil) and derived a risk quotient. If the risk quotient is greater than one, this is interpreted as an indicator of risk. Based on the available data, Stone et al. (2010) found that risk does not seem to be adequately controlled for fullerenes and CNTs in the workplace, as there may be a potential risk after prolonged inhalation exposure to nanosilver, as well as a chronic risk associated with occupational nano-titanium dioxide exposure. However, they note that this risk characterization is based on sparse information and includes many uncertainties. For instance, with regard to the buckminsterfullerene, there are information gaps and areas of uncertainty concerning:

- the availability of reliable exposure measurements of fullerenes in mass/volume, identification of fullerenes or fullerene types, distinction from background levels in the workplace, and also as released from consumer products and possibly in the environment;
- toxicokinetics (with consideration of detection methods and their influence on the results):
  - absorption of fullerenes via the different exposure routes (inhalation, dermal and oral);
  - metabolism/elimination via alveolar macrophages, Kupffer cells or other pathways;
- repeated dose toxicity studies via inhalation at concentrations relevant for the workplace to detect local and possible systemic effects; discussion is required to decide whether a 28-day or 90-day study would be more appropriate to see early/prolonged effects, but also not to be affected by recovery; dependent on the results from these studies (e.g. low toxicity confirmed) no guideline acute inhalation study might be necessary;
- dermal studies:
  - dermal uptake with a skin model;
  - local/systemic dermal effects (depending on results of dermal uptake);
- irritation (skin/eye);
- sensitization (skin, respiratory tract);
- genotoxicity: further in vitro and in vivo investigations to decide on primary and/or secondary genotoxic effects;
- carcinogenicity: depending on results from genotoxicity tests and subacute/subchronic studies, a testing strategy for carcinogenicity might be developed;
- reproductive toxicity: depending on results from absorption studies (systemic availability) and indications of effects on reproductive organs/hormones from a repeat dose toxicity study.

With regard to environmental risk assessment, Stone et al. (2010) were not able to perform a quantitative risk assessment of buckminsterfullerene and CNTs due to the lack of data. For instance, in order to "estimate the risk of C60 [buckminsterfullerene] for the aquatic environment, it would be necessary to measure/estimate fullerenes concentrations in water, and to study any toxic effect under realistic exposure conditions, considering the use of well characterized natural waters. Moreover, the effect of different functionalizations in relation to both biotic and abiotic factors should be studied case by case."

Additional gaps identified in regard to environment risk assessment are:

- lack of information on production volumes of buckminsterfullerene, in its many forms;

- lack of information on the number of buckminsterfullerene products, market penetration and amounts of the nanoparticles in these products;
- lack of information on behaviour of buckminsterfullerene during wastewater treatment;
- lack of exposure and monitoring data on buckminsterfullerene in environmental compartments;
- very little information about environmental fate, limited to aggregation in natural water;
- general lack of toxicity data, but especially for algae, sediment organisms and terrestrial organisms;
- lack of long-term toxicity studies for the aquatic compartment, i.e. long-term studies on fish and Daphnia;
- lack of information concerning interaction between organisms and buckminsterfullerene (adsorption, uptake, bioaccumulation, etc.).

Interaction of fullerenes with chemicals should also be considered in a full risk assessment, since it can increase the bioavailability and accumulation of organic and inorganic chemicals, increasing their toxicity.

For nanosilver and nano-titanium dioxide, a quantitative risk assessment was carried out for the aquatic compartment, but due to limited data it considered freshwater only. Normally, sediment and the food chain would be included as well. The PEC/PNEC ratio for the aquatic compartment of nanosilver was found to be 0.25–2.00, and for nano-titanium dioxide 0.10–4.00. As these ranges include 1, Stone et al. (2010) state that refinements are needed.

Some of the limitations summarized above are specific to the application of nanomaterials, while others may also be problematic in other areas, such as chemical safety. Such limitations have been identified over the past 20–30 years, through the use of risk assessment. These issues raise a number of challenging questions in the practice of human health risk assessment:

- To obtain complete risk assessments (of chemicals as well as nanomaterials), simplifications like lab-to-real world extrapolations and worst-case assumptions are made and some uncertainties are dealt with by using prudential correction factors. Even with these simplifications, chemical risk assessments have turned out to be very time- and resource intensive and their real influence on risk management decisions can be questioned (Tickner, 2007). Although not specific to nanomaterials, these limitations are important to consider when doing risk assessment of nanomaterials.

- There are no established standards for risk assessment of nanomaterials and there are no standard procedures or widely accepted methods for assessing nanomaterials safety hazards. When looking at the individual steps in the risk assessment procedure, it becomes apparent that even at the starting point – hazard identification – applying internationally consolidated practices from other fields is not straightforward. For example, a set of hazard-relevant physical and chemical parameters still needs to be identified by the scientific community and no standardized toxicity test guidelines are in use. For exposure assessments, no standards exist on how to measure nanoparticle dose in the body, or exposure in the workplace or the environment, which present additional complications in monitoring, detection and procedures. Assessment of health effects is hampered by the lack of basic toxicological test methods, and a lack of established health endpoints to investigate. Although standardization work in the field of risk assessment of nanomaterials is currently underway on an international scale, it is important to remember that standardization of chemical risk assessment procedures (including toxicity tests and related exposure/effect assessment protocols) took more than 20 years (Halffman, 1998).

- The current risk assessment paradigm is not ideal for taking into consideration the many sources and types of uncertainty involved in determining exposures and health effects of nanomaterials. Many different data or research gaps need to be addressed in order to analyse more accurately the risks of nanomaterials, and traditional means of dealing with these uncertainties may not adequately reflect the true degree of uncertainty. The United Kingdom Department for Environment, Food and Rural Affairs (DEFRA) has recommended that these uncertainties should be further clarified. Furthermore, Grieger et al., (2009)

concluded that recognized knowledge gaps exist in nearly all aspects of the environmental and health risks of nanomaterials. Further research will likely reduce these uncertainties, although the process will be time- and resource expensive.

- It is often stated that the risk of nanoparticles needs to be assessed on a case-by-case basis in order to take the unique properties of any nanomaterials into consideration (e.g. SCENIHR, 2007, 2009; Stone et al., 2010). Even with well defined data demands, the experience from chemical risk assessments tell us that this will be very time- and resources intensive. For nanomaterials, this situation is further complicated by the fact that hazard characteristics will not only be linked to chemical identity but also to a large number of other characteristics, and their combinations. For instance, there are 20 different structural types of SWCNT alone, and their length can vary from 5 to 300 nm. According to Schmidt (2007) four different processes exist for manufacturing SWCNTs, five methods for purifying them, and 10 surface coatings are typically applied. Hence, up to 50 000 potential combinations of SWCNT exist, and each version may have different chemical, physical and biological properties that determine their overall hazard. Although not all of them are expected to be of commercial relevance, the numerous kinds of nanoparticles – such as fullerenes, quantum dots, metal and metal-oxide nanoparticles – imply a great complexity in performing case-by-case risk assessments.

8.3 Other frameworks for dealing with nanomaterial risk

In addition to the traditional risk assessment paradigm, and in consideration of its limitations, other frameworks have been proposed which attempt to analyse the human and environmental risks associated with nanomaterials (Grieger et al., 2010). There has also been interest in alternative tools such as multicriteria decision analysis (Linkov et al., 2007) or life-cycle analysis (Shatkin, 2008), to help assess some of the potential health risks. Although none of the tools or frameworks listed below are currently used on a systematic basis, they may provide some important insights into developing alternative ways to address the potential risks of nanomaterials given the complexity and degrees of uncertainty involved.

8.3.1 Nano Risk Framework

Environmental Defense and DuPont Corporation have published a "Nano Risk Framework" describing a process for ensuring the responsible development of nanoscale materials (Environmental Defense and DuPont, 2007). The intent of the framework is to define a systematic process for identifying, managing and reducing potential environmental, health and safety risks of engineered nanomaterials across all stages of a product's life-cycle. The objective is to offer a voluntary scheme to facilitate the responsible development of nanomaterials by companies and private and public research institutions. The framework informs DuPont's decision-making over nanotechnology and on how to direct research and development of various applications of nanomaterials (e.g. surface-treated, high-rutile phase titanium dioxide, and the use of nano-iron(II) oxide to destroy contaminants in groundwater). The framework is designed to be used iteratively at different stages of development advancement (i.e. basic research and development, prototyping, pilot testing, test marketing and full commercial launch) and as new information becomes available. The framework consists of six distinct steps:

1. Develop a general description of the nanomaterial in question and its intended uses, based on information already available, and identify analogous materials and applications that may help fill data gaps in this and other steps.

2. Develop profiles of the nanomaterial's properties, inherent hazards and associated exposures, considering all the elements of the nanomaterial's full life-cycle and considering that a material's properties, hazards and exposures may change during its life-cycle.

3. Evaluate all the information generated in the profiles and identify and characterize the nature, magnitude and probability of risks of the nanomaterial and its application. Gaps in the life-cycle profiles should be prioritized and

a decision should be made on how to address them.

4. Evaluate the available risk management options and recommend a course of action including engineering controls, protective equipment, risk communication strategies and product or process modifications.

5. Decide alongside key stakeholders, experts and decision-makers whether or in what capacity to continue development and production, and document these decisions and their rationale and share appropriate information with the relevant stakeholders.

6. Update and re-execute the risk evaluation regularly or as necessary to ensure that risk management systems are working as expected, and adapt in the face of new information or conditions, and document and share appropriate information with relevant stakeholders (Environmental Defense and DuPont, 2007).

Environmental Defense and DuPont have developed a system to help guide information generation and update assumptions, decisions and practices as new information becomes available (Figure A3.6). At various stages in the product development process a worksheet is provided to help participants:

- organize, document and communicate the information they have about their material
- recognize where and how information is incomplete
- explain how information gaps were addressed
- explain the rationale behind the user's risk management decisions and actions.

The amount of information required in the framework is directly related to the potential extent and degree of exposure of the specified application. Environmental Defense and DuPont recommend that a broad range of stakeholders have access to such information as products move into commercialization in order to facilitate understanding (Environmental Defense and DuPont, 2007).

8.3.2 SCENIHR's staged approach

SCENIHR has recommended a tiered approach to assessing the potential risks from engineered nanoparticles (Figure A3.7). In order to put in place a process that is both scientifically valid and cost effective, and minimizes the use of animals, SCENIHR suggests a four stage process:

1. identify whether the manufacture, use and/or end of use disposal or recycling could result in exposure of humans or environmental species and ecosystems;
2. characterize the nature, level and duration of any exposure;
3. identify the hazardous properties of any forms of the nanomaterial to which significant exposure is likely;
4. characterize the hazard and make the final risk assessment.

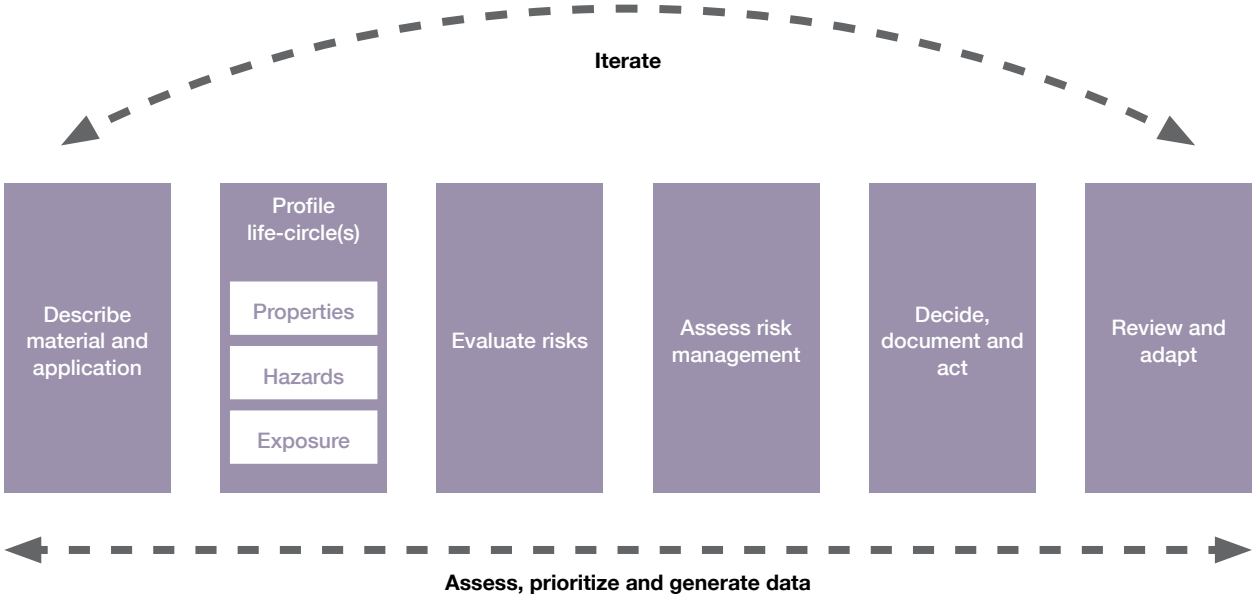
Each stage involves specific steps, illustrated in Figure A3.7. The staged approach could also be used as a guide for the safe and sustainable handling of nanoparticles at various stages of their life-cycle. However, SCENIHR notes that a full implementation of this framework will require substantial methodological developments, for instance in regard to validated in vitro tests and portable equipment for exposure monitoring for nanoparticles (SCENIHR, 2007).

8.3.3 Weight-of-evidence appraisal

In a DEFRA project (2009), 19 research objectives were identified, including 6 on human health effects of nanomaterials. The scientific literature was assessed, through expert judgement, against these objectives. Each of 293 studies included in a database and selected for evaluation was rated on its relevance, quality and contribution using a weight-of-evidence framework, considering the nature of the study, the quality of characterization of nanomaterials, peer review and publication procedures, specificity, relevance and reliability. After studies were ranked, a threshold was identified, for each research objective, over which studies were considered to be of the most scientific value. Among the research objectives, one was "human health hazard and risk assessment", which was based on data on the

toxicity (hazard) of nanoparticles and on nanoparticle physical-chemical characteristics and exposure (occupational, environmental, consumer). Eighteen studies on toxicology (including toxicokinetics), hazard identification (i.e. particle characterization), exposure and risk assessment were identified as relevant, and 17 reached a weight-of-evidence score high enough to be considered to have made (or be making) a contribution towards the objective. However, none of the studies involved humans, and data was lacking on occupational, environmental and consumer exposure. The authors conclude that "we are still some way from meeting the … objective".

Figure A3.6. Environmental Defense and DuPont framework for assessment of risks from nanomaterials



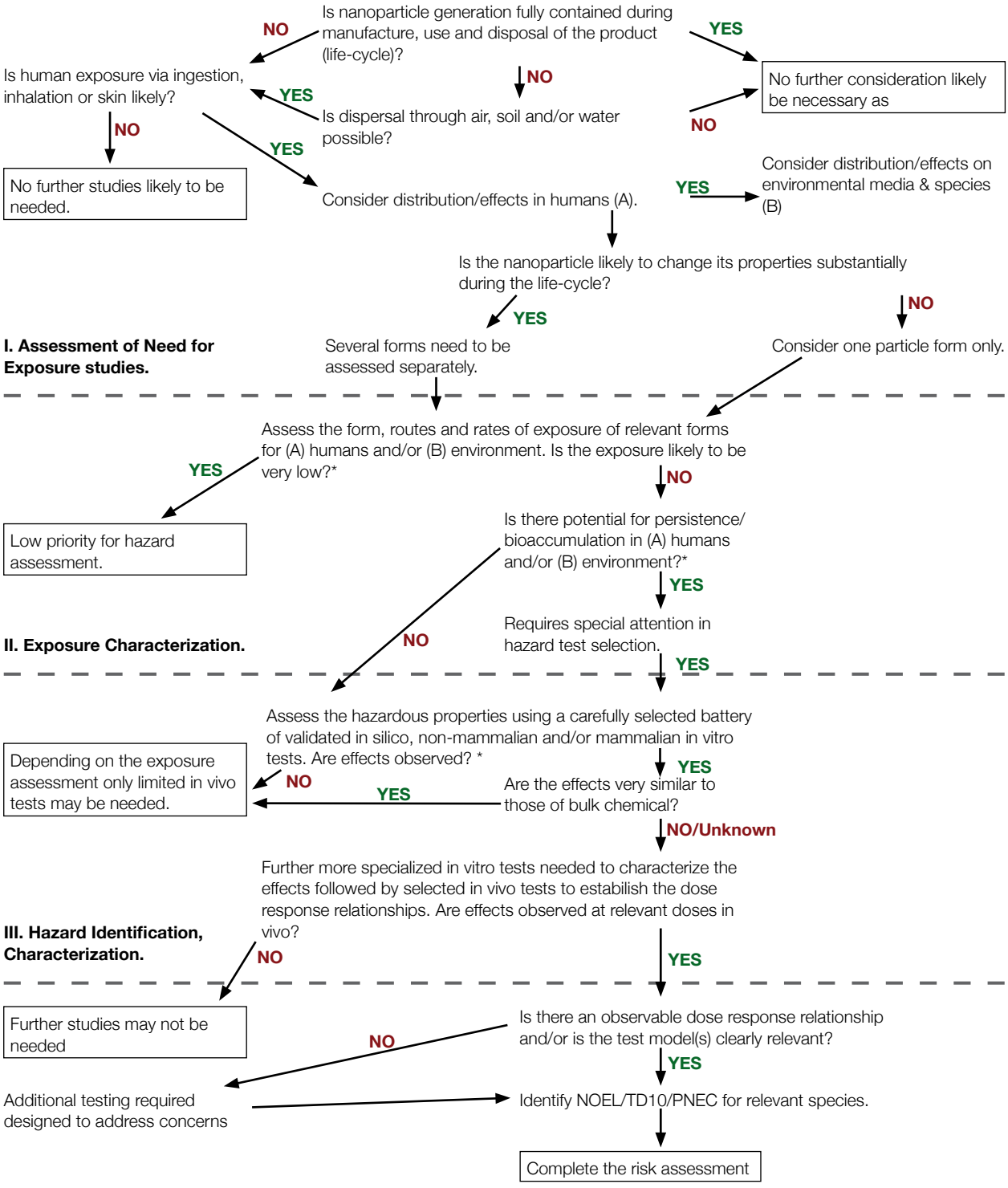
Source: Environmental Defense and DuPont (2007). Reproduced with permission.

8.3.4 Comparative hazard assessment

Comparative hazard assessment (CHA) is another means of prioritizing chemical substances for regulation. As outlined above, exposure assessment is complicated, expensive and often uncertain, and requires many model-based assumptions. Since hazard identification and characterization has many research needs and knowledge gaps, the deployment of ambitious exposure assessment exercises may be premature or not very cost effective, and CHA may be an alternative option. When using CHA, the step of exposure assessment is omitted. Substances or agents are ranked by hazard, and less hazardous materials are then substituted for more hazardous ones. This strategy has been successfully applied to reducing the number of hazardous pesticides in use. Pesticides lend themselves well to this approach, because some are relatively well characterized toxicologically. However, CHA can be applied in other situations.

The use of CHA for nanoparticles has been proposed by Howard & de Jong (2004). They developed a hazard trigger algorithm (EU, 2004). The majority of the hazard trigger values were unknown at that time, but progress has been made since. As a result, despite large knowledge gaps, this tool may be pragmatically used in regulatory work, as it allows the prioritization of nanoparticles to undergo regulatory scrutiny. Figure A3.8 illustrates such a CHA approach.

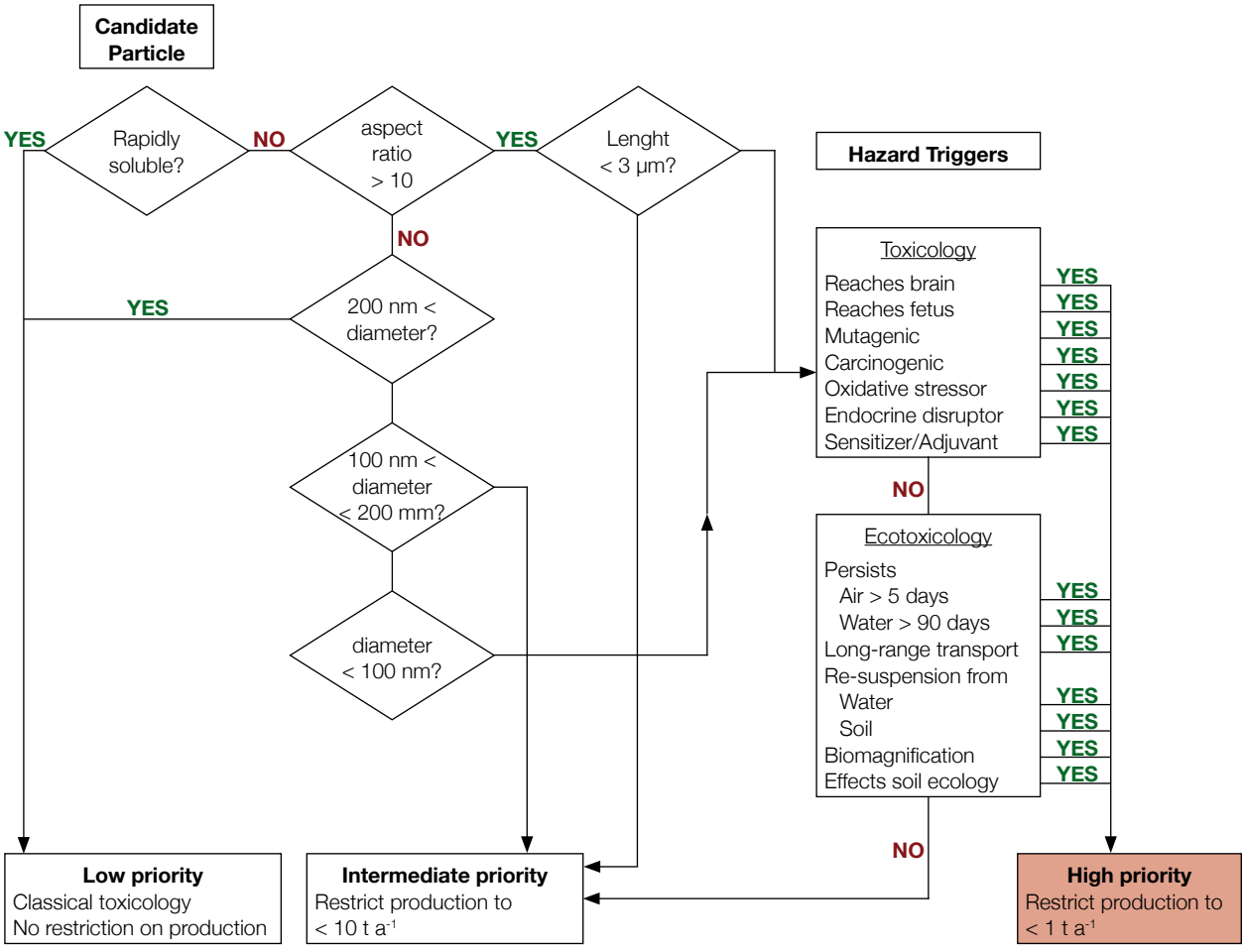
Figure A3.7. A staged approach to identifying the human and environmental risks from nanoparticles



*Compare against the risk assessment for appropriate well studied nanomaterials. Appropriate for benchmarking purposes.

Source: adapted from SCENHIR (2007:54–55). Reproduced by permission of the European Commission.

Figure A3.8. Proposed hazard trigger algorithm for nanoparticles



Note: At decision points, **unknown values** should be taken to — **yes**.

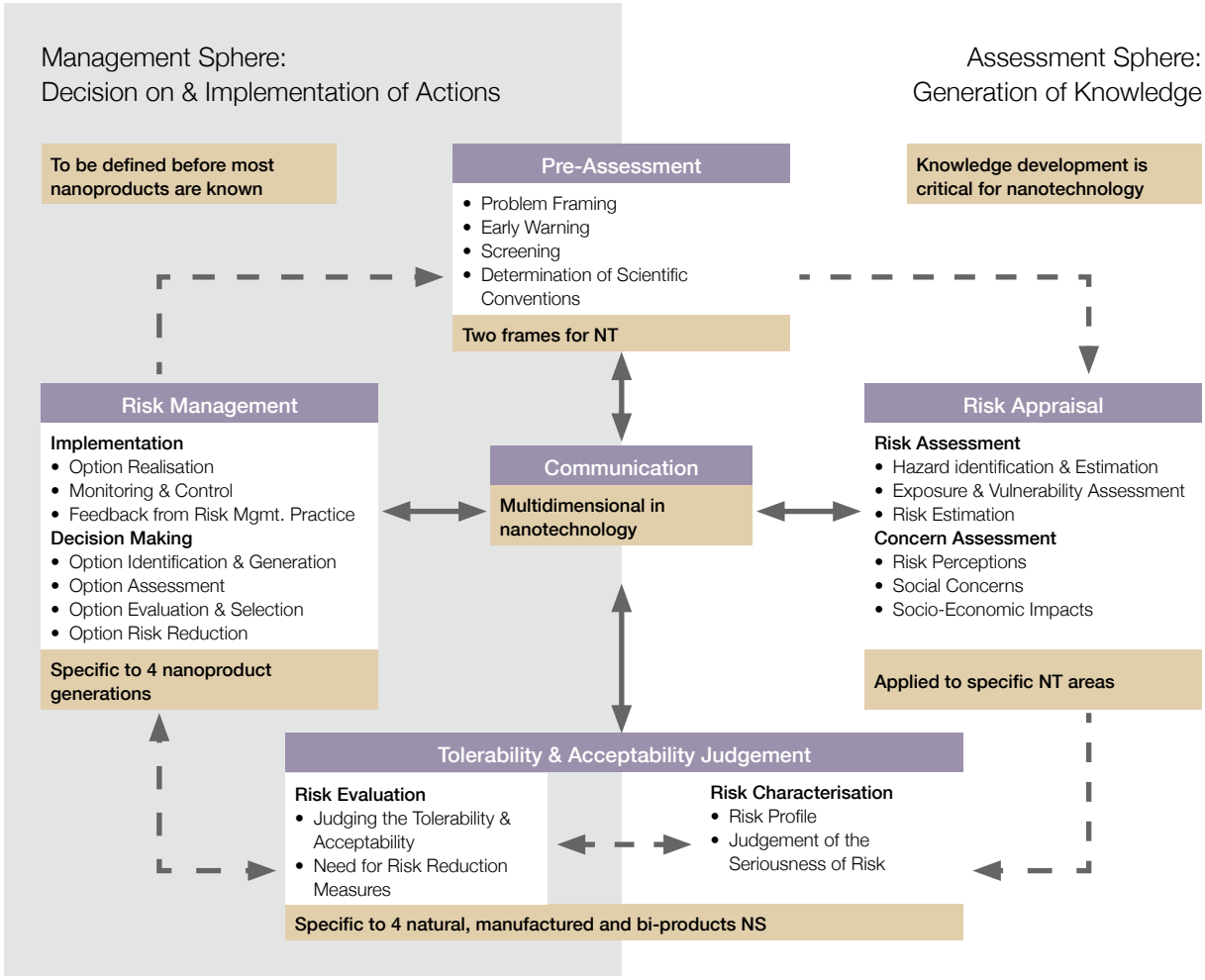
Source: adapted from Howard & de Jong (2004:36). Reproduced by permission of the European Commission.

8.3.5 IRGC's risk Governance Framework

The Risk Governance Framework developed by the International Risk Governance Council (IRGC), provides another approach to nanomaterials risk analysis. Unlike the previously described frameworks the IRGC's framework is a broad framework for risk governance, which combines some aspects of the traditional risk assessment framework with societal and ethical aspects (Figure A3.9). This framework was considered particularly relevant for nanotechnology due to the large and extensive data gaps and uncertainties surrounding the potential health and environmental risks, and hence the need to involve a wide range of stakeholders when making decisions regarding nanotechnology's use and implementation. For example, the IRGC's framework includes an important step early in the process, which frames the problem according to societal values, establishing the risk "question" to be addressed by the subsequent risk appraisal step. Factors within the traditional risk assessment paradigm (hazard identification, exposure assessment, etc.) are then included within the risk appraisal section of the framework. Risks are evaluated during the tolerability and acceptability judgement phase, which is placed in a societal context. Finally, the identified risks are managed and decisions are made from the data collected in the process. Communication between the different steps plays an

integral part of the framework. The Risk Governance Framework has been applied to nanomaterials used in foods and cosmetics (IRGC, 2007).

Figure A3.9. The IRGC Risk Governance Framework



Source: IRGC (2007). Reproduced by permission.

9. Risk governance of nanotechnology

Assessing the health effects of nanotechnology poses significant challenges. As discussed throughout this report, many distinctions must be made between types of nanomaterials, the possible nature of biological and health endpoints, and the route and likelihood of exposure. The extent to which humans come into contact with nanomaterials is only vaguely known, and so are the mechanisms of biological interaction. Many knowledge gaps exist, and the technology is in rapid evolution. Against this background, the role of scientific knowledge on nanotechnology and health effects, and the kind of advice that the research community can offer (in particular on health implications), must be realistic: it is virtually impossible that univocally determined, evidence-based recommendations on safe and unsafe types of nanomaterials and no-dose effects can be produced. The lack of such clear cut statements should not be misinterpreted as indicating

lack of effects, or conversely, suggestive of the need to ban all sorts of applications. Rather, it is important that health considerations, based on available scientific knowledge, are formulated as clearly as possible, and taken into full consideration through mechanisms of risk governance that ensure that an open, accountable, fair debate takes place. In essence, health advocates should contribute to ensuring that a fair process of negotiation occurs, between all relevant stakeholders, and results in reflective, legitimate deliberation, as mindful of health as possible.

Why is it unlikely that clear-cut, evidence-based standards and univocal risk assessments are produced? Nanotechnology and health presents a scenario that is rather typical of our era: high-stakes, systemic uncertainty, great complexity and conflicting values and cultural positions. The stakes are high in several respects, as many benefits are already being gained and many more likely to accrue from developments in the field of nanoscience. This has attracted large industrial investment, financial (and human) capital and expertise, involving not only the private sector but also academia, research and development. Several such investors stand to reap substantial benefits, financially and otherwise, so "positive" stakes are high. "Negative" stakes are also high, however, though perhaps more uncertain: while adverse effects on human health and ecosystems have not been clearly established, some factors are of concern and suggest that a cautionary approach should be followed.

Use of the precautionary principle has, in general, been endorsed in consultations around science and policy in environment and health (WHO, 2008), where complex risk factors are involved. Some specific factors related to nanomaterials also support this view:

1. the human evolutionary experience of nanomaterials is essentially limited to combustion nanoparticles and viruses. The very reason for the great technological potential of substances aggregating at sizes between molecular and bulk level (i.e. the appearance of completely different properties) has a mirroring potential for creating unwanted biological interactions for which we may have no defence mechanisms in place.

2. Similarly, nanomaterials have unfamiliar biokinetic properties within the human body. Nanoparticles have easy access to virtually all tissues and compartments of a biological system, including highly "protected" ones like the human central nervous system, at all stages of human development, beginning from preconception.

3. Some still sporadic observations should be interpreted as possible warning signals, notably the asbestos-like properties of carbon nanotubes, which have produced mesothelioma in animal models.

4. Such an observation, together with the variety of possible mechanisms of action outlined in this report, suggests that nanomaterials could result in adverse health effects of very different nature: some could be specific, like mesothelioma, and more easily detectable; other effects, occurring for example through inflammatory responses or oxidative stress, could be aspecific, and have other strong competing determinants. If a certain nanoparticle increased cardiovascular or lung cancer risks even modestly, for example, widespread exposure would produce a sizable impact, but would be extremely difficult to detect given the influence of other powerful risk factors, and against background trends.

The time dimension could be problematic: asbestos-like properties of nanofibres, for example, could produce health effects that require a long latency time to become manifest; exposures accrued over decades by a large enough population could then create the condition for irreversible epidemics. Thus, stakes around nanotechnology are high, both in terms of what we stand to lose by relinquishing certain benefits, or delaying them, and in terms of the potential detrimental consequences. Wrong decisions would have costly corollaries.

A second, related element, besides the high stakes, is that uncertainty and complexity are clearly of very high order. For a start, "nanotechnology" is a term that is too broad to be of use, in virtually all phases, from hazard identification to risk communication. It needs to be focussed and segmented for the

purpose of research and regulation, and for allowing a meaningful and accessible public debate. This would be in the interests of both producers and consumers. Uncertainty around the health implications of nanomaterials involves both recognized uncertainties, such as incomplete knowledge on the biological interaction of a given material, or on the choice of an appropriate exposure metric, but also ignorance, i.e. not knowing what we don't know. Complex systems interactions can produce unexpected responses, which cannot be ruled out given the largely unknown properties of newly developed nanomaterials. Given the multiplicity of the nanomaterial types, variants, applications, target organs and mechanisms of action, such uncertainty is likely to be irreducible and be a permanent feature of research in this domain. Uncertainty will also be fuelled by the speed of technological development, which in many cases outpaces advancement of scientific evidence. Thus, it is necessary to apply modern methodology to characterizing and dealing with systematic uncertainty and complexity, and take policy decisions under such uncertainty. The popular demand on science to reduce uncertainty may be problematic and result in delaying the formulation of the most urgent questions. The problem may be compounded by a misconception, consisting of confusing accuracy, or abundance of information, with relevance. Given the magnitude of the scientific efforts, the number of dedicated programmes and published scientific papers, one may be lead to overestimate the available knowledge, and overlook the possibility that crucial questions are not addressed, or that substantial knowledge gaps exist.

Thirdly, underlying ethical questions, involving values, are entwined with scientific considerations and their policy implications. Apart from a possible ideological divide between "technophiles" and "technophobes", it is very likely that some controversy may arise from issues of procedural as well as distributional justice. Procedurally unjust steps may occur, or be perceived to occur, if processes of governance are not fully transparent; if information is withheld from public circulation; if participatory decisions are not attended by a balanced and representative set of stakeholders; if vested interests unduly influence decisions; and ultimately, if the knowledge base and the policy course of action does not carry the necessary legitimacy. Distributional justice may be a subtle but important issue, at least on two accounts: (i) overall exposure to a certain type of nanomaterials may be low, but unevenly distributed among the population. If there are established or suspected health impacts, albeit small, they will be concentrated on a subgroup (perhaps a minority) receiving the majority of the overall exposure (such a subgroup may also happen to coincide with other vulnerabilities, adding to the problem). As seen in many other cases of environmental risk factors, such skewed distribution will substantially complicate decision-making and requires carefully managed negotiations. (ii) It may be of limited value to compare overall costs and benefits (monetary or otherwise) of a given nanotechnology or application. The most relevant issue may be who bears the costs and who reaps the benefits. If the discrepancy between the beneficiaries and those carrying the disbenefits (for example in terms of exposure) is marked, controversy will remain even if overall benefits outweigh overall costs. The aggregate, society-wide level of analysis, often used in economic assessments, may thus not be the most appropriate one.

9.1 Conclusions

Developing a prescriptive course of action for the risk management of nanotechnology is not feasible for any health agency. Rather, arrangements should be made to ensure that health considerations are fully appraised and given a high prominence in all deliberations. This is likely to be a continuous process, which needs to be set up and managed in consultation with a variety of stakeholders, and must address the most relevant questions. In particular:

- Consultative bodies and mechanisms on the risk governance of nanotechnology should be established, or strengthened where they exist, at the level where strategic decisions are made. Such bodies should be set up and/or run in a fair and transparent way, with the involvement of all legitimate stakeholders, to the extent possible. Consultation should be periodic, and held early enough in the process to meaningfully influence decisions. Leeway should be given to the phase of framing and formulating questions, i.e. avoiding situations where past decisions are presented as

fait accompli. Outcomes from such consultations should also inform the research agenda, towards the most policy-relevant objectives. Health agencies and health advocates should always be part of such bodies. Agencies in the United Nations system such as WHO should, in addition: facilitate the dialogue between stakeholders; ensure an appropriate use of available scientific evidence; and prevent the occurrence of unfair commercial or industrial practices between different regions of the world. Mechanisms should be identified to finance such efforts in a sustainable way.

- Given the multiplicity of different stakeholders, facilitating the necessary dialogue between commercial developers and regulators will be challenging, because technological progress is usually in advance of regulatory control. Yet, such dialogue should be continuous and accessible to other interested parties.

- Regulatory bodies have a role to play in exercising some level of control on the manufacture and commerce of some classes of nanoproducts. Regulatory schemes for products containing nanoparticles, possibly different from ordinary products, should be considered.

- Specific options to be considered include the following:
  - A full declaration of nanoscale ingredients should be made available in any consumer product (with the exception of medical products and devices, which will likely require a separate regime), with priority given to products with high exposure and/or intake potential, such as food, cosmetics and household chemicals. Arrangements should be made that ensure that commercial confidentiality does not become a reason for non-compliance. This requirement obviously demands that manufacturers be fully aware of the nanomaterials present in their products;
  - Pre-existing licences, granted to manufacture or use materials at larger than nanoscale, should not automatically allow the manufacture or use of the same material at nanoscale, without requiring further assessment.
  - Nanoscale materials should have a Chemical Abstracts Service Registry Number (CAS RN) which is distinct from the one for the bulk material.

- Identifying the priority nanomaterials for regulatory control and the priorities for research is critical:
  - This goal would be facilitated by the development of public databases bringing together available information on types and properties of nanomaterials. For example, the collation of data from all countries, using large volume air samplers to monitor trends of engineered nanoparticles in ambient air samples and assess changing patterns in population exposure is likely to be informative.
  - Such information would in turn enable the development and refinement of schemes of classification and taxonomy of nanomaterials, manufacturing processes, applications and products. It might be useful to include multiple criteria for classification, including criteria for classifying nanomaterials against their human exposure potential, for example as highly pervasive, pervasive or non-pervasive. The different categories could be subject to differing levels of regulation, hence improving cost–effectiveness of regulatory action.

- Nanomaterials that show the highest potential for health damage, or that can be expected to do so, on the basis of their chemical–physical properties, or for which existing toxicological evidence is a reason for concern, should be the highest priority candidates for regulation and research. However, because of the large scientific uncertainty, priorities should be based on other factors:
  - the extent of the current or anticipated population exposure;
  - its distribution, notably the possible concentration of exposure on vulnerable subgroups, such as children or people with existing health conditions;
  - a possible escalation of production and diffusion due to anticipated commercial driving forces, such as wider mass applications;
  - the degree of availability of alternative materials, of lesser damaging potential (e.g. as established by comparative hazard assessment);

  - the expected nature of possible adverse health effects (e.g. how easily detectable it might be, over what time effects might become manifest);
  - the nature of the technological application (e.g. a purely commercial application on recreational equipment will invite a more restrictive attitude than a medical application meant to increase the effectiveness of a drug).

- Setting the research agenda to include key questions on the health implications of nanotechnology is crucial. Encouragement and incentives should be given to the development of suitable methodology to complement risk assessment, such as nanotoxicology and comparative hazard assessment and other methods developed ad hoc. This will require the sponsoring of targeted research programmes to fill in missing data needed for the setting of standards and norms. International collaboration to this effect should be encouraged. Generally speaking, public funding of research and development efforts should be balanced and always include a component dedicated to investigating possible health risks and impact. During 2004–2009, the proportion of funding for environment, health and safety research in the United States of America and the EU was 0.9–5.0% (Balbus et al., 2005; EC, 2005, 2008; Hullmann, 2007; NNI, 2008, 2009), the latter figure being a better reflection of the current knowledge gaps.

- Governments and international agencies should consider instituting mandatory monitoring schemes and health surveillance systems, perhaps aimed with priority at vulnerable groups. Attention should be paid also to workers, possibly following the model of the nuclear industry in many countries. These populations, or some representative subgroups, should be monitored for diseases relevant to their potential exposures. For example, long-term longitudinal studies into the incidence of inflammatory diseases, cardiovascular disease and early-onset protein misfolding diseases.

10. References

Afaq F et al. (1998). Cytotoxicity, pro-oxidant effects and antioxidant depletion in rat lung alveolar macrophages exposed to ultrafine titanium dioxide. *Journal of Applied Toxicology*, 18(5):307–312.

Agricola (1556). *De Re Metallica*. Basel, Froben.

Aitken RJ et al. (2006). Manufacture and use of nanomaterials: current status in the United Kingdom and global trends. *Occupational Medicine*, 56(5):300–306.

Akiyoshi K, Sasaki Y, Sunamoto J (1999). Molecular chaperone-like activity of hydrogel nanoparticles of hydrophobized pullulan: thermal stabilization with refolding of carbonic anhydrase B. *Bioconjugate Chemistry*, 10:321–324.

Allianz, OECD (2005). *Small sizes that matter: opportunities and risks of nanotechnologies*. Munich, Allianz Group, and Paris, Organisation for Economic Co-operation and Development (http://www.oecd.org/science/nanosafety/44108334.pdf, accessed 15 April 2013).

Alyaudtin RN et al. (2001). Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo and in vitro. *Journal of Drug Targeting*, 9(3):209–221.

Anderson HR et al. (2004). *Meta-analysis of time-series studies and panel studies of particulate matter (PM) and ozone (O3): report of a WHO task group*. Copenhagen, WHO Regional Office for Europe (http://www.euro.who.int/__data/assets/pdf_file/0004/74731/e82792.pdf, accessed 16 April 2013).

Auerbach O, Cuyler Hammond E, Garfinkel L (1965). Smoking in relation to atherosclerosis of the coronary arteries. *New England Journal of Medicine*, 273:775–779.

Baggs RB, Ferin J, Oberdörster G (1997). Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. *Veterinary Pathology*, 34(6):592–597.

Balbus JM et al. (2005). Getting nanotechnology right the first time. *Issues in Science and Technology*, Summer 2005:5–71.

Balbus JM et al. (2007). Meeting report: hazard assessment for nanoparticles – Report from an interdisciplinary workshop. *Environmental Health Perspectives*, 115(11):1654–1659.

Barker PJ, Branch A (2007). The interaction of modern sunscreen formulations with surface coatings. *Progress in Organic Coatings*, 62:313–320.

Barnes CA et al. (2008). Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity. *Nano Letters*, 8(9):3069–3074.

Barrett EG et al. (1999). Silica binds serum proteins resulting in a shift of the dose–response for silica-induced chemokine expression in an alveolar type II cell line. *Toxicology and Applied Pharmacology*, (2):111–122.

Bello D et al. (2008a). Particle exposure levels during CVD growth and subsequent handling of vertically-aligned carbon nanotube films. *Carbon*, 46:974–981.

Bello D et al. (2008b). Exposure to nanoscale particles and fibres during machining of hybrid advanced composites containing carbon nanotubes. *Journal of Nanoparticle Research*, 11:231–249.

Billsten P et al. (1997). Adsorption to silica nanoparticles of human carbonic anhydrase II and truncated forms induce a molten-globule-like structure. *FEBS Letters*, 402:67–72.

Blaser S et al. (2008). Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano functionalized plastics and textiles. *Science of the Total Environment*, 390:396–409.

Bodian D, Howe HA (1941a). Experimental studies on intraneural spread of poliomyelitis virus. *Bulletin of the Johns Hopkins Hospital*, 69:248–267.

Bodian D, Howe HA (1941b). The rate of progression of poliomyelitis virus in nerves. *Bulletin of the Johns Hopkins Hospital*, 69:79–85.

de Boer AG, Gaillard PJ (2007). Drug targeting to the brain. *Annual Review of Pharmacology and Toxicology*, 47:323–355.

Borm PJ et al. (2006). The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and Fibre Toxicology*, 14(3):11.

Bosman SJ et al. (2005). Development of mammalian embryos exposed to mixed-size nanoparticles. *Clinical and Experimental Obstetrics and Gynecology*. 32(4):222–224.

Boxall ABA et al. (2008). *Current and future predicted environmental exposure to engineered nanoparticles*. York, Central Science Laboratory.

Brain JD, Kreyling W, Gehr P (2010). To the Editor. *European Respiratory Journal*, 35(1):226–227.

BSI (2007). *Nanotechnologies – Part 2: Guide to safe handling and disposal of manufactured nanomaterials*. British Standards Institution (PD 6699-2).

Brook RD (2007). Is air pollution a cause of cardiovascular disease? Updated review and controversies. *Reviews on Environmental Health*, 22(2):115–137.

Brook RD et al. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation*, 105:1534–1536.

Brown JS, Zeman KL, Bennett WD (2002) Ultrafine particle deposition and clearance in the healthy and obstructed lung. *American Journal of Respiratory and Critical Care Medicine*, 166:1240–1247.

Bucher J et al. (2004). *Developing experimental approaches for the evaluation of toxicological interactions of nanoscale materials*. Gainesville, University of Florida.

Bulls K (2006). "Nano" safety recall [web site]. Cambridge, MA, MIT Technology Review (http://www.technologyreview.com/NanoTech/wtr_16681,318,p1.html, accessed 16 April 2013).

Cass GR et al. (2000). The chemical composition of atmospheric ultrafine particles. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical & Engineering Sciences.* 358(1775):2581–2592.

CCA (2008). *Small is different: a science perspective on the regulatory challenges of the nanoscale. Report of the Expert Panel on Nanotechnology*. Ottawa, Council of Canadian Academies.

Cedervall T et al. (2007). Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proceedings of the National Academy of Sciences*, 104(7):2050–2055.

Chen H, Goldberg MS, Villeneuve PJ (2008). A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases. *Reviews on Environmental Health*, 23(4):243–297.

Chen M, von Mikecz A (2005). Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles. *Experimental Cell Research*, 305(1):51–62.

Chiappino G (2005). Mesotelioma: il ruolo delle fibre ultrafini e conseguenti riflessi in campo preventivo e medico legale [Mesothelioma: the role of ultrafine fibers and consequences for prevention and legal medicine]. *La Medicina del Lavoro*, 96:3–23.

Clancy L et al. (2002). Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. *Lancet*, 360:1210–1214.

Cottingham MG, Bain CD, Vaux DJT (2004). Rapid method for measurement of surface tension in multiwell plates. *Laboratory Investigation*, 84:523–529.

Cottingham MG, Hollinshead MS, Vaux DJ (2002). Amyloid fibril formation by a synthetic peptide from a region of human acetylcholinesterase that is homologous to the Alzheimer's amyloid-beta peptide. *Biochemistry*, 41(46): 13539–13547.

DEFRA (2006). *Characterising the potential risks posed by engineered nanoparticles. UK Government research – a progress report*. London, Department for Environment, Food and Rural Affairs.

DEFRA (2007). *Characterising the potential risks posed by engineered nanoparticles – A second UK Government Research Report*. London, Department for Environment, Food and Rural Affairs.

DEFRA (2009). *A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology.* London, Department for Environment, Food and Rural Affairs.

Demou E, Peter P, Hellweg S (2008). Exposure to manufactured nanostructured particles in an industrial pilot plant. *Annals of Occupational Hygiene*, 52(8):695–706.

Devlin RB et al. (2003). Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability. *European Respiratory Journal*, (Suppl.) 40:76–80.

Dockery DW et al. (1993). An association between air-pollution and mortality in 6 United-States cities. *New England Journal of Medicine*, 329:1753–1759.

Donaldson K, Beswick PH, Gilmour PS (1996). Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? *Toxicology Letters*, 88(1–3):293–298.

Donaldson J, Stone V, MacNee W (1999). The toxicology of ultrafine particles. In: Maynard RL, Howard CV, eds. *Particulate matter: properties and effects upon health*. Oxford, BIOS Scientific Publishers Ltd:115–129.

Donaldson K et al. (2000). Ultrafine particles: mechanisms of lung injury. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical & Engineering Sciences.* 358(1775):2741–2749.

Donaldson K et al. (2002). The pulmonary toxicology of ultrafine particles. *Journal of Aerosol Medicine*, 15:213–220.

Donaldson K et al. (2004). Nanotoxicology. *Occupational and Environmental Medicine*, 61:727–728.

Donaldson K et al. (2005). Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Particle and Fibre Toxicology*, 2:10.

Dostert C et al. (2008). Innate immune activation through NALP3 inflammasome sensing of asbestos and silica. *Science*, 320(5876):674–677.

EC (2005). *Some figures about nanotechnology R&D in Europe and beyond*. Brussels, European Commission (ftp://ftp.cordis.europa.eu/pub/nanotechnology/docs/nano_funding_data_08122005.pdf, accessed 16 April 2013).

EC (2007). *Draft commission recommendation on the definition of the term "nanomaterial"*. Brussels, European Commission (http://ec.europa.eu/environment/consultations/pdf/recommendation_nano.pdf, accessed 16 April 2013).

EC (2008). *EU nanotechnology R&D in the field of health and environmental impact of nanoparticles*. Brussels, European Commission (ftp://ftp.cordis.europa.eu/pub/nanotechnology/docs/final-version.pdf, accessed 16 April 2013).

Einstein A (1905). Ueber einen die Erzeugung und Verwandlung des Lichtes betreffenden heuristischen Gesichtspunkt. *Annalen der Physik*, 6:132–148.

Elder A et al. (2009). Human health risks of engineered nanomaterials: critical knowledge gaps in nanomaterials risk assessment. In: Linkov I, Steevens J, eds. *Nanotechnology. Risks and benefits*. Dordrecht, Springer, 3–29.

Elsaesser A et al. (2008). Personal communication.

Elvin G (2006). *No nano in Magic Nano*. nanotechbuzz [web site], 27 May (http://www.nanotechbuzz.com/50226711/no_nano_in_magic_nano.php, accessed 16 April 2013).

Environmental Defense, DuPont (2007). *Nano Risk Framework* (http://www.nanoriskframework.com/files/2011/11/6496_Nano-Risk-Framework.pdf, accessed 16 April 2013).

ETC Group (2003a). *The big down: atomtech: technologies converging at the nanoscale*. Winnipeg, ETC Group (http://www.etcgroup.org/content/big-down, accessed 16 April 2013).

ETC Group (2003b). *No small matter II: the case for a global moratorium. Size matters!* Occasional Paper Series, 7:1, Winnipeg, ETC Group (http://www.etcgroup.org/content/size-matters, accessed 16 April 2013).

EU (2004). *Nanotechnologies: a preliminary risk analysis on the basis of a workshop organized in Brussels on 1–2 March 2004 by the Health and Consumer Protection Directorate General of the European Commission*. Brussels, European Union (http://ec.europa.eu/health/ph_risk/documents/ev_20040301_en.pdf, accessed 16 April 2013).

Ferin J et al. (1992). Pulmonary retention of ultrafine and fine particles in rats. *American Journal of Respiratory Cell and Molecular Biology*, 6:535–542.

Florence AT (2005). Nanoparticle uptake by the oral route: fulfilling its potential? *Drug Discovery Today: Technologies*, 2(1):75–81.

Foley S et al. (2002). Cellular localization of a water-soluble fullerene derivative. *Biochemical and Biophysical Research Communications*, 294:116–119.

FSA (2006). *Draft report of FSA regulatory review. A*

review of potential implications of nanotechnologies for regulations and risk assessment in relation to food. United Kingdom Food Standards Agency.

Fujitani et al. (2008). Measurement of the physical properties of aerosols in a fullerene factory for inhalation exposure assessment. *Journal of Occupational and Environmental Hygiene*, 5(6):380–389.

Gamer AO, Leibold E, van Ravenzwaay B (2006). The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin. *Toxicology In Vitro*, 20(3):301–307.

Gatti AM et al (2004). Detection of micro- and nano-sized biocompatible particles in the blood. *Journal of Materials Science: Materials in Medicine*, 15(4):469–472.

Genaidy A et al. (2009). Health effects of exposure to carbon nanofibers: systematic review, critical appraisal, meta-analysis and research to practice perspectives. *Science of the Total Environment*, 407(12):3686–3701.

Glaza P (2010). *Industry should take lead with media when an event like a recall occurs*. Electroiq [web site], 5 January (http://www.electroiq.com/articles/stm/print/volume-6/issue-3/opinion/guest-column/industry-should-take-lead-with-media-when-an-event-like-a-recall-occurs.html, accessed 16 April 2013).

Godbey WT, Wu KK, Mikos AG (1999). Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery. *Proceedings of the National Academy of Sciences*, 96(9):5177–5181.

Goldberg AL (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature*, 426:895–899.

Gottschalk F et al. (2010). Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. *Environmental Toxicology and Chemistry,* 29:1036–1048.

Grieger K, Hansen SF, Baun A (2009). The known unknowns of nanomaterials: describing and characterizing uncertainty within environmental, health and safety risks. *Nanotoxicology*, 3(3):1–12.

Grieger K, Baun A, Owen R (2010). Redefining risk research priorities for nanomaterials. *Journal of Nanoparticle Research*, 2(2):383–392.

Gumbleton M (2001). Caveolae as potential macromolecule trafficking compartments within alveolar epithelium. *Advanced Drug Delivery Reviews*, 49:281–300.

Gurr JR et al. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology*, 213(1–2):66–73.

Gwinn MR, Vallyathan V (2006). Nanoparticles: health effects – pros and cons, *Environmental Health Perspectives*, 114:1818–1825.

Hagens et al. (2007). What do we (need to) know about the kinetic properties of nanoparticles in the body? *Regulatory Toxicology and Pharmacology*, 49:217–229.

Halffman W (1998). Standardization as a trust device. In: Bal R, Halffman W, eds. *The politics of chemical risk. Scenarios for a regulatory future*. Dordrecht, Kluwer Academic Publishers, 265–292.

Han JH et al. (2008). Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. *Inhalation Toxicology*, 20:741–749.

Hansen SF et al. (2007). Categorization framework to aid Hazard Identification of Nanomaterials. *Nanotoxicology*, 1:243–250.

Hansen SF et al. (2008). Categorization framework to aid exposure assessment of nanomaterials in consumer products. *Ecotoxicology*, 17(5):438–447.

Harrison RM (1999). Sources and behaviour of atmospheric particulate matter. In: Maynard RL, Howard CV, eds. *Particulate matter: properties and effects upon health*. Oxford, BIOS Scientific Publishers Ltd, 63–84.

Helland A et al. (2007). Reviewing the environmental and human health knowledge base of carbon nanotubes. *Environmental Health Perspectives*, 115(8):1125–1131.

Hillyer JF, Albrecht RM (2001). Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. *Journal of Pharmaceutical Sciences*, 90(12):1927–1936.

Hodges GM et al. (1995). Uptake and translocation of microparticles in small intestine. Morphology and quantification of particle distribution. *Digestive Diseases and Sciences*, 40:967–975.

Hoet PH, Bruske-Hohlfeld I, Salata OV (2004). Nanoparticles – known and unknown health risks. *Journal of Nanobiotechnology*, 2(1):12.

Hohr D et al. (2002). The surface area rather than the surface coating determines the acute inflammatory response after instillation of fine and ultrafine TiO2 in the rat. *International Journal of Hygiene and Environmental Health*, 205(3):239–244.

Hoshino A et al. (2004). Quantum dots targeted to the assigned organelle in living cells. *Microbiology and Immunology*, 48(12):985–994.

Howard CV, de Jong W (2004). Concept note on a hazard trigger algorithm as a potential prioritization tool for use by regulators. In: *Nanotechnologies: a preliminary risk analysis on the basis of a workshop organized in Brussels on 1–2 March 2004 by the Health and Consumer Protection*

Directorate General of the European Commission. Brussels, European Commission, 35-36 (http://ec.europa.eu/health/ph_risk/documents/ev_20040301_en.pdf, accessed 16 April 2013).

Howard CV, Reed MG (2010). *Unbiased Stereology. 2nd edition*. Liverpool, QTP Publications.

HSE (2006). *The assessment of different metrics of the concentration of nano (ultrafine) particles in existing and new industries*. Health and Safety Executive report RR513 (http://www.hse.gov.uk/research/rrpdf/rr513.pdf, accessed 16 April 2013).

Hsu LY, Chein HM (2007). Evaluation of nanoparticle emission for TiO2 nanopowder coating materials. *Journal of Nanoparticle Research*, 89:157–163.

Hullmann A (2007). Measuring and assessing the development of nanotechnology. *Scientometrics*, 70(3):739–758.

Hunter DD, Dey RD (1998). Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium. *Neuroscience*, 83:591–599.

Hunter DD, Undem BJ (1999). Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea. *American Journal of Respiratory and Critical Care Medicine*, 159:1943–1948.

Hussain SM et al. (2005). In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in Vitro*, 19(7):975–983.

IRGC (2007). *Nanotechnology risk governance: recommendations for a global, coordinated approach to the governance of potential risks*. Geneva, International Risk Governance Council.

Isaacson CW, Kleber M, Field JA (2009). Quantitative analysis of fullerene nanomaterials in environmental systems: a critical review. *Environmental Science & Technology*, 43(17):6463–6474.

Ishii D et al. (2003). Chaperonin-mediated stabilization and ATP-triggered release of semiconductor nanoparticles. *Nature*, 423:628–632.

ISO (2008). *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate. ISO/TS 27687:2008*. Geneva, International Organization for Standardization.

Jani P et al. (1990). Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *Journal of Pharmacy and Pharmacology*, 42(12):821–826.

Jefferson DA, Tilley EEM (1999). The structural and physical chemistry of nanoparticles. In: Maynard RL, Howard CV, eds. *Particulate matter: properties and effects upon health*. Oxford, BIOS Scientific Publishers Ltd, 63–84.

Ji ZQ et al. (2006). Biodistribution and tumor uptake of C60(OH)x in mice. *Journal of Nanoparticle Research*, 8:53–63.

Jia G et al. (2005). Cytotoxicity of carbon nanomaterials: single-wall nanotube, multiwall nanotube, and fullerene. *Environmental Science & Technology*, 39(5):1378–1383.

Johnston HJ et al. (2010). The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. *Toxicological Sciences*, 114 (2):162–182.

Jontes JD, Phillips GR (2008). Selective stabilization and synaptic specificity: a new cell biological model. *Trends in Neuroscience*, 29:185–191.

Kabanov AV, Gendelman HE (2007). Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. *Progress in Polymer Science*, 32:1054–1082.

Kaegi R et al. (2008). Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. *Environmental Pollution*, 156(2):233–239.

Kaiser JP et al. (2008). Single walled carbon nanotubes (SWCNT) affect cell physiology and cell architecture. *Journal of Materials Science: Materials in Medicine*, 19(4):1523–1527.

Kamat JP et al. (2000). Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. *Toxicology*, 155:55–61.

Kennedy P, Chaudhuri A (2002). Herpes simplex encephalitis. *Journal of Neurology, Neurosurgery and Psychiatry*, 73:237–238.

Kim et al. (1999). Mechanism of silica- and titanium dioxide-induced cytotoxicity in alveolar macrophages. *Journal of Toxicology and Environmental Health, Part A*, 58(7):437–450.

Kohli AK, Alpar HO (2004). Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *International Journal of Pharmaceutics*, 275(1–2):13–7.

Kreuter J (2001). Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews*, 47:65–81.

Kreuter J et al. (2002) Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *Journal of Drug Targeting*, 10(4):317–325.

Kreyling WG et al. (2002). Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *Journal of Toxicology and Environmental Health, Part A*, 65(20):1513–1530.

Kuhlbusch TAJ, Fissan H (2006). Particle characteristics in the reactor and pelletizing areas of carbon black production. *Journal of Occupational and Environmental Hygiene*, 3:558–567.

Kuhlbusch TAJ, Neumann S, Fissan H (2004). Number size distribution, mass concentration, and particle composition of PM1, PM2.5, and PM10 in bag filling areas of carbon black production. *Journal of Occupational and Environmental Hygiene*, 1:660–671.

Lademann J et al. (1999). Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. *Skin Pharmacology and Physiology*, 12(5):247–256.

Lam CW et al. (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological Sciences*, 77:126–134.

Li N et al. (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, *Environmental Health Perspectives*, 111(4):455–460.

Limbach LK et al. (2007). Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. *Environmental Science & Technology*, 41(11):4158–4163.

Linkov I, Steevens J, eds. (2009). *Nanotechnology. Risks and benefits*. Dordrecht, Springer, 3–29.

Linkov I et al. (2007) Multicriteria decision analysis and environmental risk assessment for nanomaterials. *Journal of Nanoparticle Research*, 9(4):543–554.

Linse S et al. (2007). Nucleation of protein fibrillation by nanoparticles. *Proceedings of the National Academy of Sciences*, 104(21):8691–8696.

Lomer MC, Thompson RP, Powell JJ (2002). Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. *Proceedings of the Nutrition Society*, 61:123–130.

Long TC. (2006). Titanium Dioxide (P25) Produces Reactive Oxygen Species in Immortalized Brain Microglia (BV2): Implications for Nanoparticle Neurotoxicity. *Environmental Science & Technology* 40(14): 4346-4352.

Longmire M, Choyke PL, Kobayashi H (2008). Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomedicine*, 3:703–717.

de Lorenzo AJ (1970). The olfactory neuron and the blood-brain barrier. In: Wolstenholme G, Knight J, eds. *Taste and smell in vertebrates*. London, Churchill, 151–176.

Lovric J (2005). Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *Journal of Molecular Medicine*, 83(5):377–385.

Lowe PJ et al. (1985). Transcytosis and paracellular movements of horseradish peroxidase across liver parenchymal tissue from blood to bile. Effects of alpha-naphthylisothiocyanate and colchicine. *Biochemical Journal*, 229:529–537.

Luoma SN (2008). *Silver nanotechnologies and the environment: old problems or new challenges?* Washington, DC, Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

Maynard AD (2006a). *Nanotechnology: a research strategy for addressing risk*. Washington, DC, Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

Maynard AD (2006b). "*Magic nano" product recall update*. Nanotechwire [web site], 13 April (http://www.nanotechwire.com/news.asp?nid=3178, accessed 17 April 2013)

Maynard AD (2009). *New study seeks to link seven cases of occupational lung disease with nanoparticles and nanotechnology*. 2020 Science [web site], 18 August (http://2020science.org/2009/08/18/new-study-seeks-to-link-seven-cases-of-ocupational-lung-disease-with-nanoparticles-and-nanotechnology/, accessed 17 April 2013).

Maynard AD, Aitken RJ (2007). Assessing exposure to airborne nanomaterials: current abilities and future requirements. *Nanotoxicology*, 1:26–41.

Maynard RL, Howard CV (1999). *Particulate matter: properties and effects upon health*. Oxford, BIOS Scientific Publishers.

Maynard AD et al. (2004). Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. *Journal of Toxicology and Environmental Health, Part A*, 67:87–107.

Maynard AD et al. (2009). *Nanoparticle exposure and occupational lung disease – six expert perspectives on a new clinical study*. 2020 Science [web site], 18 August (http://2020science.org/?s= Nanoparticle+exposure+and+occupational+lung+disease+%E2%80%93+six+expert+perspectives+on +a+ new+clinical+study, accessed 17 April 2013).

Methner MM (2008). Effectiveness of local exhaust ventilation (LEV) in controlling engineered nanomaterial emissions during reactor cleanout operations. *Journal of Occupational and Environmental Hygiene*, 5(6):D63–D69.

Methner MM et al. (2007). Identification and characterization of potential sources of worker exposure to carbon nanofibers during polymer composite laboratory operations. *Journal of Occupational and Environmental Hygiene*, 4(12):D125–D130.

Meyer M, Schreck R, Baeuerle PA (1993). H2O2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary

antioxidant-responsive factor. *EMBO Journal*, 12:2005–2015.

Mills NL et al. (2006). Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *American Journal of Respiratory and Critical Care Medicine,* 173(4):426–431.

Mueller N, Nowack B (2008). Exposure modeling of engineered nanoparticles in the environment. *Environmental Science & Technology*, 42:4447–4453.

Myllynen PK et al. (2008). Kinetics of gold nanoparticles in the human placenta. *Reproductive Toxicology*, 26(2):130–137.

National Academy of Sciences (2008). *Review of the federal strategy for nanotechnology-related environmental, health, and safety research*. Washington, DC, National Academies Press, 1–191.

Nefzger M et al. (1984). Distribution and elimination of polymethyl ethacrylate nanoparticles after peroral administration to rats. *Journal of Pharmaceutical Sciences*, 73:1309–1311.

Nel AE et al. (2009). Understanding biophysicochemical interactions at the nano–bio interface. *Nature Materials*, 8:543–557.

Nemmar A et al. (2001). Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *American Journal of Respiratory and Critical Care Medicine*, 164:1665–1668.

Nemmar A et al. (2002). Passage of inhaled particles into the blood circulation in humans. *Circulation*, 105(4): 411–414.

Next Big Future (2007). *Carbon nanotubes production in 2007 and projected* (http://nextbigfuture.com/2007/07/carbon-nanotubes-production-in-2007-and.html, accessed 17 April 2013).

Nigavekar SS et al. (2004). 3H dendrimer nanoparticle organ/tumor distribution. *Pharmaceutical Research*, 21:476–483.

NIOSH (2006). *Approaches to safe nanotechnology: managing the health and safety concerns associated with engineered nanomaterials*. Cincinnati, National Institute for Occupational Safety and Health.

NNI (2008). *Strategy for nano*technology-related environmental, health and safety research. Washington, DC, United States National Nanotechnology Initiative.

NNI (2009). *FY 2009 budget and highlights*. Washington, DC, United States National Nanotechnology Initiative.

Nohynek GJ et al. (2007). Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Critical Reviews in Toxicology*, 37(3):251–277.

NRC (1979). *Airborne Particles*. Baltimore, National Research Council, National Academy of Sciences, University Park Press, 108–110.

Oberdörster G et al. (1992). Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environmetnal Health Perspectives*, 97:193-199.

Oberdörster E (2004). Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environmental Health Perspectives*, 112:1058–1062.

Oberdörster G (2000). Toxicology of ultrafine particles: in vivo studies. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical & Engineering Sciences.* 358(1775):2719–2740.

Oberdörster G (2009). Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. *Journal of Internal Medicine*, 267:89–105.

Oberdörster G (2010). Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. *Journal of Internal Medicine*, 267(1):89–105.

Oberdörster G, Oberdörster E, Oberdörster J (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, *Environmental Health Perspectives*, 113:823–839.

Oberdörster G et al. (2002) Extrapulmonary translocation of ultrafine carbon particles following wholebody inhalation exposure of rats. *Journal of Toxicology and Environmental Health*, Part A, 65(20):1531–1543.

Oberdörster G et al. (2004). Translocation of inhaled ultrafine particles to the brain. *Inhalation Toxicology*, 16(6/7):437–445.

OECD (2009). *Preliminary analysis of exposure measurement and exposure mitigation in occupational settings: manufactured nanomaterials*. Paris, Organisation for Economic Cooperation and Development.

Osier M, Oberdörster G (1997). Intratracheal inhalation vs intratracheal instillation: differences in particle effects. *Fundamental and Applied Toxicology*, 40(2):220–227.

Owen R, Handy R (2007). Formulating the problems for environmental risk assessment of nanomaterials. *Environmental Science & Technology*, 41(16):5582–5588.

Panté N, Kann M (2002). Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm. *Molecular Biology of the Cell*, 13(2):425–434.

Park B. et al. (2008). Hazard and Risk Assessment of a Nanoparticulate Cerium Oxide-Based Diesel Fuel Additive—A Case Study. *Inhalation Toxicology*, 20:547-566.

Patrick G, Stirling C (1992). Transport of particles of colloidal gold within and from rat lung after local deposition by alveolar microinjection. *Environmental Health Perspectives*, 97:47–51.

Pescovitz D (2006). *Magic Nano recall*. Boing boing [web site], April 10, (http://boingboing.net/2006/04/10/magic-nano-recall.html, accessed 17 April 2013).

Peters A et al. (2001). Increased particulate air pollution and the triggering of myocardial infarction. *Circulation*, 103:2810–2815.

Peters TM et al. (2009). Airborne monitoring to distinguish engineered nanomaterials from incidental particles for environmental health and safety. *Journal of Occupational and Environmental Hygiene*, 6:73–81.

Poland CA et al. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, 3(7):423–428.

Pope CA 3rd, Dockery DW (2006). Health effects of fine particulate air pollution: lines that connect. *Journal of the Air and Waste Management Association*, 56(6):709–742.

Pratten MK, Lloyd JB (1986). Pinocytosis and phagocytosis: the effect of size of a particulate substrate on its mode of capture by rat peritoneal macrophages cultured in vitro. *Biochimica et Biophysica Acta*, 881:307–313.

Rahman Q et al. (2002). Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. *Environmental Health Perspectives*, 110(8):797–800.

Rim D, Wallace L, Persily A (2010). Infiltration of outdoor ultrafine particles into a test house. *Environmental Science & Technology*, 44(15):5908–5913.

Rocks S et al. (2008). *Comparison of risk assessment approaches for manufactured nanomaterials*. United Kingdom Department for Environment, Food and Rural Affairs.

Roco MC (2003). Broader societal issues of nanotechnology. *Journal of Nanoparticle Research*, 5:181–189.

Roco M, Renn O (2006). Nanotechnology and the need for risk governance. *Journal of Nanoparticle Research*, 8(2):153–191.

Rouse JG et al. (2007). Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. *Nano Letters*, 7(1):155–160.

Royal Society (2004). *Nanoscience and nanotechnologies: opportunities and uncertainties*. London, Royal Society and Royal Academy of Engineering.

Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2006). Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicological Sciences*, 91(1):159–165.

Salnikov V et al. (2007). Probing the outer mitochondrial membrane in cardiac mitochondria with nanoparticles. *Biophysical Journal*, 92(3):1058–1071.

Sayes SM et al. (2004). The differential cytotoxicity of water-soluble fullerenes. *Nano Letters*, 4:1881–1887.

Sayes CM et al. (2006). Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. *Toxicological Sciences*, 92(1):174–185.

Sayes CM et al. (2007). Comparative pulmonary toxicity assessments of C60 water suspensions in rats: few differences in fullerene toxicity in vivo in contrast to in vitro profiles. *Nano Letters*, 7:2399–2406.

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, 21–22 June 2007*. Brussels, Scientific Committee on Emerging and Newly Identified Health Risks, European Commission (http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf, accessed 17 April 2013).

SCENIHR (2009). *Risk assessment of products of nanotechnologies*. Brussels, Scientific Committee on Emerging and Newly Identified Health Risks, European Commission (http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf, accessed 17 April 2013).

Schmidt K (2007). *NanoFrontiers: visions for the future of nanotechnology*. Washington, DC, Woodrow Wilson Center for International Scholars.

Schulz J et al. (2002). Distribution of sunscreens on skin. *Advanced Drug Delivery Reviews*, 54(1):S157–S163.

Schwartz J, Dockery DW (1992). Increased mortality in Philadelphia associated with daily air pollution concentrations. *American Review of Respiratory Disease*, 145:600–604.

Seaton A et al. (1995). Particulate air pollution and acute health effects. *Lancet* 345:176–178.

Seaton A et al. (2009). Nanoparticles, human health hazard and regulation. *Journal of The Royal Society Interface* (doi:10.1098/rsif.2009.0252.focus).

Shatkin JA (2008). Informing environmental decision-making by combining life cycle assessment and risk analysis. *Journal of Industrial Ecology*, 12(3):278–281.

Shinohara N, Gamo M, Nakanishi J (2009). *Risk assessment of manufactured nanomaterials*. Kawasaki, National Institute of Advanced Industrial Science and

Technology, Research Institute of Science for Safety and Sustainability.

Singh S, Nalwa HS (2007). Nanotechnology and health safety—toxicity and risk assessments of nanostructured materials on human health. *Journal of Nanoscience and Nanotechnology*, 7:3048–3070.

Song Y, Xue L, Du X (2009). Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *European Respiratory Journal*, 34(3):559–567.

Stern ST, McNeil SE (2008). Nanotechnology safety concerns revisited. *Toxicological Sciences*, 101(1):4–21.

Stone V et al. (1998). The role of oxidative stress in the prolonged inhibitory effect of ultrafine carbon black on epithelial cell function. *Toxicology in Vitro*, 12(6):649–659.

Stone V et al. (2010). *Engineered nanoparticles: review of health and environmental safety*. Edinburgh Napier University, Institute of Occupational Medicine, Technical University of Denmark, Institute for Health and Consumer Protection of the European Commission's Joint Research Centre and Institute of Nanotechnology.

Swiss Re (2004). *Nanotechnology: small matter, many unknowns*. Zurich, Swiss Re.

Takagi A et al. (2008). Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multiwall carbon nanotube. *Journal of Toxicological Sciences*, 33(1):105–116.

Tan MH et al. (1996). A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. *Australasian Journal of Dermatology*, 37(4):185–187.

Thayer AM (2007). Carbon nanotubes by the metric ton. Anticipating new commercial applications, producers increase capacity. *Chemical & Engineering News*, 85(46):29–35.

Tickell O (2008). How war debris could cause cancer. *New Scientist*, 7 September.

Tickner J (2007). Why risk assessment is not enough to protect public health: rationale for a precautionary approach to science and policy. In: Robinson M, Toscano W, eds. *Risk assessment for environmental health*. The Association of Schools of Public Health, Jossey-Bass Publisher, 431.

Tinkle SS et al. (2003). Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental Health Perspectives*, 111(9):1202–1208.

Tomatis L et al. (2007). The role of asbestos fiber dimensions in the prevention of mesothelioma. *International Journal of Occupational and Environmental Health*, 13(1):64–69.

Tsai S et al. (2008a). Airborne nanoparticle exposures associated with the manual handling of nanoalumina and nanosilver in fume hoods. *Journal of Nanoparticle Research*, 11(1):147.

Tsai S et al. (2008b). Airborne nanoparticle release associated with the compounding of nanocomposites using nanoalumina as fillers. *Journal of Aerosol and Air Quality Research*, 8:160–177.

Tsuchiya T et al. (1996). Novel harmful effects of [60] fullerene on mouse embryos in vitro and in vivo. *FEBS Letters.* 393(1):139–145.

Uchino T et al. (2002). Quantitative determination of OH radical generation and its cytotoxicity induced by TiO2–UVA treatment. *Toxicology in Vitro*, 16:629–635.

Unfried K et al. (2007). Cellular responses to nanoparticles: target structures and mechanisms. *Nanotoxicology*, 1:52–71.

Volkheimer G (1974). Passage of particles through the wall of the gastrointestinal tract. *Environmental Health Perspectives*, 9:215–225.

von Bubnoff A (2010). What's in a nano name? Electroiq [web site], 1 July (http://www.electroiq.com/articles/stm/print/volume-6/issue-4/features/global-watch/whatrsquos-in-a-nano-name.html, accessed 17 April 2013).

Vorbau M, Hillemann L, Stintz M (2009). Method for the characterization of the abrasion induced nanoparticle release into air from surface coatings. J*ournal of Aerosol Science*, 40:209–217.

Warheit DB et al. (2006). Pulmonary instillation studies with nanoscale TiO2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. *Toxicological Sciences*, 91:227–236.

Warheit DB et al. (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: differential responses related to surface properties. *Toxicology*, 230(1):90–104.

Wichmann HE et al. (2000). *Daily Mortality and Fine and Ultrafine Particles in Erfurt, Germany. Part I: Role of Particle Number and Particle Mass.* Cambridhe MA, Health Effects Institute (HEI Research Report No. 98).

Wichmann HE, Peters A (2000). Epidemiological evidence of the effects of ultrafine particle exposure. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical & Engineering Sciences.* 358(1775):2751–2769.

WHO Regional Office for Europe (2006) *Air Quality Guidelines. Global Update 2005*. Copenhagen, WHO Regional Office for Europe (http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf, accessed 17 April 2013)

WHO Regional Office for Europe (2008). *Fifth Ministerial Conference on Environment and Health. Report of the Second High-level Preparatory Meeting*, *Madrid, Spain, 22–24 October 2008*. Copenhagen, WHO Regional Office for Europe (http://www.euro.who.int/__data/assets/

pdf_file/0020/104555/26th_EEHC_Madrid_ereport.pdf, accessed 17 April 2013).

WHO/FAO (2010). *Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications*. Geneva, World Health Organization/Rome, Food and Agriculture Organization of the United Nations.

Wijnhoven SWP et al. (2009). Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*, 3:2,109–138.

Xia T et al. (2006). Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Letters*, 6(8):1794–1807.

Yeganeh B et al. (2008). Characterization of airborne particles during production of carbonaceous nanomaterials. *Environmental Science & Technology*, 42:4600–4606.

Zhang LW, Monteiro-Riviere NA (2008). Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin Pharmacology and Physiology* 21(3):166-180.

Zhu Y et al. (2006). The interaction and toxicity of multiwalled carbon nanotubes with Stylonychia mytilus. *Journal of Nanoscience and Nanotechnology*, 6(5):1357–1364.

The WHO Regional Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

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Nanotechnology, the science and application of objects smaller that 100 nanometres, is evolving rapidly in many fields. Besides the countless beneficial applications, including in health and medicine, concerns exist on adverse health consequences of unintended human exposure to nanomaterials.

In the 2010 Parma Declaration on Environment and Health, ministers of health and of environment of the 53 Member States of the WHO Regional Office for Europe listed the health implications of nanotechnology and nanoparticles among the key environment and health challenges.

The WHO Regional Office for Europe undertook a critical assessment of the current state of knowledge and the key evidence on the possible health implications of nanomaterials, with a view to identify options for risk assessment and policy formulation, and convened an expert meeting to address the issue.

Current evidence is not conclusive. As complexity and uncertainty are large, risk assessment is challenging, and formulation of evidence-based policies and regulations elusive.

Innovative models and frameworks for risk assessment and risk governance are being developed and applied to organize the available evidence on biological and health effects of nanomaterials in ways to inform policy.

World Health Organization
Regional Office for Europe

UN City, Marmorvej 51, DK-2100 Copenhagen Ø, Denmark
Tel.: +45 45 33 70 00 Fax: +45 45 33 70 01
Email: contact@euro.who.int
Website: www.euro.who.int

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