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SCIENTIFIC COMMITTEE ON EMERGING AND NEWLY IDENTIFIED HEALTH RISKS (SCENIHR)

modified Opinion (after public consultation) on

The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies

Adopted by the SCENIHR

during the 10th plenary meeting of 10 March 2006

after public consultation

Synthesis report : http://ec.europa.eu/health/ph_risk/documents/synth_report.pdf
Stakeholder comments : http://ec.europa.eu/health/ph_risk/documents/stakeholder_comments.zip

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EXECUTIVE SUMMARY

In view of the growing importance of nanotechnologies, and following from the conclusions of the Council of the European Union on the European strategy for nanotechnologies[1] highlighting the importance of the "assessment of potential risks throughout the life cycle of nanotechnology-based products" and the nanotechnologies action plan[2], the European Commission asked the independent experts of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)[3] for a scientific opinion on the appropriateness of existing methodologies to assess the potential risks of nanotechnologies. This report provides this Opinion and the relevant scientific background.

SCENIHR concludes that current risk assessment methodologies require some modification in order to deal with the hazards associated with nanotechnology and in particular that existing toxicological and ecotoxicological methods may not be sufficient to address all of the issues arising with nanoparticles. For exposure evaluation, dose requires information on the number of nanoparticles and/or their surface area in addition to traditional mass concentration characterization. Equipment for routine measurements in various media for representative exposure to free nanoparticles is inadequate. In addition, existing exposure assessment methods may not be appropriate to determine the environmental fate of nanoparticles.

Very little is known about the physiological responses to nanoparticles. Although some conventional toxicity and ecotoxicity tests have been shown to be useful in evaluating the hazards of nanoparticles, existing methodologies may require modification regarding hazard evaluation, including the assessment of whether nanoparticles can exacerbate pre-existing medical conditions, and the detection of nanoparticle distribution in the human body and in environmental compartments. The Committee points to major gaps in the knowledge necessary for risk assessment. These include nanoparticle characterisation, the detection and measurement of nanoparticles, the dose-response, fate, and persistence of nanoparticles in humans and in the environment, and all aspects of toxicology and environmental toxicology related to nanoparticles. Of special importance are the questions concerned with the transport of nanoparticles in the human body and the mechanisms of interaction at the sub-cellular and molecular levels. The monitoring of occupational exposure and the epidemiological data on the potential impact of nanoparticles on human health constitute priorities for further research.

This Report describes nanomaterials properties, identifies sources of free nanoparticles, discusses their detection and measurement and then examines interactions between nanoparticles and living systems. The report addresses the toxicology of nanoparticles and the potential exposure scenarios, and then addresses risk assessment methodologies, the core of the Scientific Opinion, through exposure assessment, hazard identification and characterization, risk characterization and an integrated assessment. The Report complements this scientific background and Opinion by an assessment of the gaps in knowledge required to address the risks of nanotechnologies and an examination of regulatory aspects related to risk assessment.

[1] Towards a European strategy for nanotechnology, COM(2004) 338 Final adopted on 12 May 2004 and approved by the Council of European Union on 24 September 2005

[2] Nanosciences and nanotechnologies: An action plan for Europe 2005-2009 (COM(2005) 243) adopted on 7 June 2005

[3] http://europa.eu.int/comm/health/ph_risk/committees/04_scenihr/04_scenihr_en.htm

1. BACKGROUND

General characteristics of nanotechnologies

The term "nanotechnologies" refers to technologies of the very small, with dimensions in the range of nanometers. Nanotechnologies exploit the specific properties that arise from structuring matter at a (meso-) scale characterized by the interplay of classical physics and quantum mechanics. Today, these properties are often difficult to predict a priori.

Nanotechnologies enable other technologies. Thus, they will mostly result in the production of intermediate goods. Because nanotechnologies connect disciplines as diverse as physics, chemistry, genetics, information and communication technologies (ICTs), and cognitive sciences amongst others, they offer the foundation of the so-called nano-bio-info-cogno (NBIC) "convergence".

Expected benefits from nanotechnologies

Technology analysts highlight nanotechnologies as benefiting today or likely to benefit in the foreseeable future:

- materials sciences (esp., ceramics; more generally, lighter and stronger materials);
- cosmetics (e.g., non-ghosting sunscreen, nano-liposome-based skin care products);
- house-cleaning products (e.g., window-washing sprays);
- paints, vanishes, and other coatings;
- chemistry (e.g., tailor-made catalysts);
- information and communication technology (e.g., nano-electronics);
- biomedical applications (e.g., "lab-on-a-chip", biosensors, medical imaging, prostheses and implants, drug delivery devices);
- environmental remediation technology;
- energy capture and storage technology (e.g., solar cells, batteries, fuel cells, fuels and catalysts);
- agriculture (e.g., sensors, seed improvement);
- food (ranging from non-permeable membranes and, national legislation permitting, antibacterial powders to pathogen and contaminant sensors, environmental monitors, and remote sensing and tracking devices);
- military technology.

  textiles, surface finishing and lubrication agents

The market for nanotechnologies is estimated at $700 billion by 2008 and more than $1 trillion by 2015 by the US National Science Foundation (NSF).

Community interest

The high expectations raised by nanotechnologies have led the Community to manifest its interest in boosting nanotechnologies in its Communication of 12 May 2004 entitled "Towards a European strategy for nanotechnology" [COM(2004) 338 final]. Council endorsed "the main message of this Communication, namely the need to develop an integrated and responsible strategy" for nanotechnologies [12487/04 (Presse 269), Point 4, p. 25]. Most recently, the Commission Communication 'Nanosciences and Nanotechnologies: an Action Programme for Europe 2005-2009' (from on 7 June 2005) requires a safe, integrated and responsible approach to the development of nanotechnologies.

Potential areas for concern

Some nanoparticles, nanospheres, nanotubes, and nanofibers produced via nanotechnologies including adventitious by-products have the potential to raise concerns for humans (public health, consumer safety, and the health and safety of workers) and the environment. The concerns that nanoparticles, nanotubes, and nanofibers raise constitute the most significant ones relating to nanotechnologies within the next 3–5 years. They require further studies. In this respect, more often than not, the toxicological, ecotoxicological, and exposure data needed to perform a complete risk analysis are lacking.

Experts are of the unanimous opinion that the adverse effects of nanoparticles cannot be predicted (or derived) from the known toxicity of material of macroscopic size, which obey the laws of classical physics. This has led the UK Royal Society and the Royal Academy of Engineering to recommend "that chemicals in the form of nanoparticles or nanotubes be treated as new substances under the existing Notification of New Substances (NONS) regulations and in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)" (Chapter 8 "Regulatory issues", Point 18, p. 71).

European Council recommendations

The European Council highlighted "the need to pay special attention to […] integrating societal, environmental and health considerations into the R&D process and assessment of potential risks throughout the life cycle of nanotechnology-based products" [12487/04 (Presse 269), Point 6, p. 26] and welcomed "the Commission's intention to […] to engage in a dialogue at international level, with a view to establishing a framework of shared principles for the safe, sustainable, responsible and socially acceptable development and use of nanotechnologies" [12487/04 (Presse 269), Point 8, p. 26; emphasis added].

In view of the Council's call for a "safe, sustainable, responsible and socially acceptable development and use of nanotechnologies", it is therefore timely to undertake a general reflection on the adequacy of existing risk assessment methodologies as concern nanotechnologies and their engineered and adventitious products and to identify any gaps in current knowledge which may be an obstruction to the demonstration of their safety.

2. TERMS OF REFERENCE

The Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) has been requested to answer the following questions, in each case, considering the different kinds of nanotechnologies separately:

1. *Are existing methodologies appropriate to assess potential and plausible risks associated with different kinds of nanotechnologies and processes associated with nanosized materials as well as the engineered and adventitious products of nanotechnologies?*

2. If existing methodologies are not appropriate to assess the hypothetical and potential risks associated with certain kinds of nanotechnologies and their engineered and adventitious products, how should existing methodologies be adapted and/or completed?

3. In general terms, what are the major gaps in knowledge necessary to underpin risk assessment in the areas of concern?

In making its report, the Committee is asked to include considerations of both engineered and adventitious products and potential risks related to public health, to consumer safety, to the health and safety of workers, and to the environment.

3. SCIENTIFIC RATIONALE

3.1 Introduction

Nanotechnology is the term given to those areas of science and engineering where phenomena that take place at dimensions in the nanometre scale are utilised in the design, characterisation, production and application of materials, structures, devices and systems. Although in the natural world there are many examples of structures that exist with nanometre dimensions (hereafter referred to as the nanoscale), including essential molecules within the human body and components of foods, and although many technologies have incidentally involved nanoscale structures for many years, it has only been in the last quarter of a century that it has been possible to actively and intentionally modify molecules and structures within this size range. It is this control at the nanometre scale that distinguishes nanotechnology from other areas of technology.

Clearly the various forms of nanotechnology have the potential to make a very significant impact on society. In general it may be assumed that the application of nanotechnology will be very beneficial to individuals and organisations. Many of these applications involve new materials which provide radically different properties through functioning at the nanoscale, where new phenomena are associated with the very large surface area to volume ratios experienced at these dimensions and with quantum effects that are not seen with larger sizes. These include materials in the form of very thin films used in catalysis and electronics, two-dimensional nanotubes and nanowires for optical and magnetic systems, and as nanoparticles used in cosmetics, pharmaceuticals and coatings. The industrial sectors most readily embracing nanotechnology are the information and communications sector, including electronic and optoelectronic fields, food technology, energy technology and the medical products sector, including many

different facets of pharmaceuticals and drug delivery systems, diagnostics and medical technology, where the terms nanomedicine and bionanotechnology are already commonplace. Nanotechnology products may also offer novel challengies for the reduction of environmental pollution.

However, just as phenomena taking place at the nanoscale may be quite different to those occurring at larger dimensions and may be exploitable for the benefit of mankind, so these newly identified processes and their products may expose the same humans, and the environment in general, to new health risks, possibly involving quite different mechanisms of interference with the physiology of human and environmental species. These possibilities may well be focussed on the fate of free nanoparticles generated in nanotechnology processes and either intentionally or unintentionally released into the environment, or actually delivered directly to individuals through the functioning of a nanotechnology based product. Of special concern would be those individuals whose work places them in regular and sustained contact with free nanoparticles. Central to these health risk concerns is the fact that evolution has determined that the human species has developed mechanisms of protection against environmental agents, either living or dead, this process being determined by the nature of the agents commonly encountered, within which size is an important factor. The exposure to nanoparticles having characteristics not previously encountered may well challenge the normal defence mechanisms associated with, for example, immune and inflammatory systems. It is also possible for there to be an environmental impact of the products of nanotechnology, related to the processes of dispersion and persistence of nanoparticles in the environment.

Wherever the potential for an entirely new risk is identified, it is necessary to carry out an extensive analysis of the nature of the risk, which can then, if necessary, be used in the processes of risk management. It is widely accepted that the risks associated with nanotechnology need to be analysed in this way. Many international organisations (e.g. Asia Pacific Nanotechnology Forum 2005), governmental bodies within the European Union (European Commission 2004,), National Institutions (e.g. De Jong et al 2005, Roszek et al 2005, US National Science and Technology Council 2004, IEEE 2004, US National Institute of Environmental Health Sciences 2004), non-governmental organisations (e.g.UN-NGLS 2005), learned institutions and societies (e.g. Institute of Nanotechnology 2005, Australian Academy of Sciences 2005, METI 2005, UK Royal Society and Royal Academy of Engineering 2004) and individuals (e.g. Oberdörster et al 2005, Donaldson and Stone 2003) have published reports on the current state of nanotechnology, and most draw attention to this need for a thorough risk analysis.

The European Council has highlighted the need to pay special attention to the potential risks throughout the life cycle of nanotechnology based products and the European Commission has signalled its intention to work on an international basis towards establishing a framework of shared principles for the safe, sustainable, responsible and socially acceptable use of nanotechnologies

3.2 Definitions and Scope

There are several definitions of nanotechnology and of the products of nanotechnology, often these been generated for specific purposes.

In this Opinion, the underlying scientific concepts of nanotechnology have been considered more important than the semantics of a definition, so these are considered first. The Committee considers that the scope of nanoscience and nanotechnology used by the UK Royal Society and Royal Academy of Engineering in their 2004 report (Royal Society and Royal Academy of Engineering 2004) adequately expresses these concepts. This suggests that the range of the nanoscale is from the atomic level, at around 0.2 nm up to around 100nm. It is within this range that materials can have substantially different properties compared to the same substances at larger sizes, both because of the substantially increased ratio of surface area to mass, and also because quantum effects begin to play a role at these dimensions, leading to significant changes in several types of physical property.

The present Opinion uses the various terms of nanotechnology in a manner consistent with the recently published Publicly Available Specification on the Vocabulary for Nanoparticles of the British Standards Institution (BSI 2005), in which the following definitions for the major general terms are proposed:

Nanoscale: *having one or more dimensions of the order of 100 nm or less*.

Nanoscience: *the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale*.

Nanotechnology: *the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanoscale*.

Nanomaterial: *material with one or more external dimensions, or an internal structure, which could exhibit novel characteristics compared to the same material without nanoscale features*.

Nanoparticle: *particle with one or more dimensions at the nanoscale*. (Note: In the present report, nanoparticles are considered to have two or more dimensions at the nanoscale).

Nanocomposite: *composite in which at least one of the phases has at least one dimension on the nanoscale.*

Nanostructured: *having a structure at the nanoscale*,

It should be noted that nanoscience and nanotechnology have been emerging rapidly during recent years, and that the vocabulary used within the contributing disciplines has not been consistent during this time. Also, as this report notes, there have been, and continue to be, serious difficulties with the precise measurement of the parameters of the nanoscale, such that it is not always possible to have complete confidence in the data and conclusions drawn about specific phenomena relating to specific features of nanostructures and nanomaterials. This Opinion recognises the inevitability of this situation and has drawn some general conclusions in the knowledge that the literature may contain inconsistencies and inaccuracies. Whilst, therefore, this Opinion uses the definition that nanoscale should now be considered to involve dimensions up to 100 nm, it recognises that some of the literature will have represented nanoscale as having larger dimensions than 100 nm. Much of the literature related to particles, especially that

concerned with aerosols, air pollution and inhalation toxicology, has referred to particles as either ultrafine, fine or conventional. This report has assumed that, unless otherwise stated, 'ultrafine particles' are essentially equivalent to nanoparticles.

Also, in relation to nanoparticles, it must be borne in mind that a sample of a substance that contains nanoparticles will not be monodisperse, but will normally contain a range of particle sizes. This makes it even more difficult to assess accurately the parameters of the nanoscale, especially when considering the doses for toxicological studies. In this Opinion reference is frequently made to studies of exposure and toxicology data concerned with particles and will quote the particle size given in the papers as either single figures (e.g. 40 nm) or ranges (e.g. 40 – 80 nm) recognising that these will be approximations.

Moreover, there will be a tendency in some situations for nanoparticles to aggregate. It might be assumed that an aggregate of nanoparticles, which may have dimensions measured in microns rather than nanometres, would behave differently to the individual nanoparticles, but at the same time there is no reason to expect the aggregate to behave like one large particle. Equally, it might be expected that the behaviour of nanoparticles will be dependent on their solubility and susceptibility to degradation and that neither the chemical composition nor particle size are guaranteed to remain constant over time.

With the above definitions and caveats in mind, it is clear that, as far as both intrinsic properties and health risks are concerned, there are two types of nanostructure to consider, those where the structure itself is a free particle and those where the nanostructure is an integral feature of a larger object.

In the latter group are nanocomposites, which are solid materials in which one or more dispersed phases are present as nanoscale particles, and nanocrystalline solids, in which individual crystals are of nanoscale dimensions. This group also includes objects which have been provided with a surface topography with features of nanoscale size, and functional components that have critical features of nanometre dimension, primarily including electronic components. . For medical purposes surface modifications can be obtained by using specific coatings composed of nanosized materials (Roszek et al 2005). This Opinion recognises the existence of such materials and products, and recognises that material features of nanoscale dimensions can influence interactions with living systems. However, although the science of interactions between biological systems and nanotopographical features is developing rapidly, very little is known of the potential of such interactions to induce adverse effects . The risk would be dependent on the strength of the adherence to the carrier material, and associated with the release during use or at the end of the life time of the product. As long as the nanomaterials are fixed on the surface of the carrier there is at the moment no reason to suppose that immobilized nanoparticles pose a greater risk for health or environment than the larger scale materials.

It is the former group, involving free nanoparticles, that provides the greater concern with respect to health risks, and which is the subject of the major part of this Opinion. The term 'free' should be qualified, since it implies that at some stage in production or use the substance in question consists of individual particles, of nanoscale dimensions. In the application of the substance, these individual particles may be incorporated into a quantity of another substance, which could be a gas, a liquid or a solid, typically to

produce a paste, a gel or a coating. These particles may still be considered to be free, although their bioavailability will vary with the nature of the phase in which they are dispersed. Ultrafine aerosols and colloids, and cream-based cosmetics and pharmaceutical preparations would be included in this category, and it is with these examples that much of the recent work on nanotechnology health risks has been concerned.

This opinion essentially discusses the potential risks associated with the manufacture and use of products incorporating engineered nanomaterials. Nanostructures of biological origin such as proteins, phospholipids, lipids etc. are not considered in this context.

3.3 Nanoscience and Nanotechnology

3.3.1 Introduction

Current knowledge of science at the nanometre scale is derived from many disciplines, originating with the atomic and molecular concepts in chemistry and physics, and then incorporating molecular life sciences, medicine and engineering. The observation and understanding of atomic and molecular behaviour from first principles was followed by the increasing ability to control and selectively modify properties of ever smaller pieces of matter in a functional way. Early examples here are the discoveries in self assembly (Bain et al 1989) which culminated in current synthetic and supra-molecular chemistry (Lehn 1988, Gomez –Lopez et al 1996), the increasing knowledge about life's replication processes and the co-evolution of physical (Perutz et al 1960, Aue et al 1976, Wuthrich 1995) and chemical methodologies. These have resulted in the portfolio of current molecular life sciences such as molecular motors and other functional entities (Mavroidis et al 2004, Clark et al 2004), including biomolecular and medical engineering and the emerging area of systems biology. On the other hand, man made micro and nanoscale sensing devices originate from other domains in microscopy and device engineering but relate to biomedical applications (Ziegler 2004, Emerich and Thanos 2003).

The deviation of surface and interface properties from the bulk properties of larger amounts of materials led to the sometimes unexpected significance of surface effects, including catalytic activity and wetting behaviour in material composed of nanosized entities, such as nanoparticles, composites and colloids (Kamat 2002, Schwerdtfeger 2003). Quantum mechanical principles manifest themselves in the properties of surfaces of clusters of very small particles, especially those of the order of 1000 atoms or molecules and less. Composite materials (Komarneni 1992, Schmidt 2000, Hadjipanayis 1999), with increasingly smaller characteristic sizes of the domains or phases, allowed for the design of materials with new and optimised physical and / or chemical properties. In electronic engineering, the miniaturization of devices has progressed well into the nanometre range with gate oxides in devices being routinely 25 nm thick. The recently increased public awareness of nanoscience is closely related to the availability of first real space images of atomic and molecular processes at surfaces through the invention of Scanning Probe Microscopies (Binnig and Rohrer 1985).

With the continuous development of nanotechnology, the possibility for the bottom-up production of nanoscale materials may result in some kind of self assembly of structures similar to the self assembly of phospholipid bilayers that resembles cellular membranes.

On the basis of current knowledge however, the spontaneous formation of artificial living systems through self assembly and related processes, suggested by some prominent commentators, is considered highly improbable. The combination of self replication with self perpetuation in an engineered nanosystem is extremely difficult to realize on the basis of current scientific knowledge.

3.3.2 Examples of Engineered Nanostructures and Materials and Their Applications

There are several areas of science and technology in which nanoscale structures are under active development or already in practical use.

In materials science, nanocomposites with nanoscale dispersed phases and nanocrystalline materials in which the very fine grain size affords quite different mechanical properties to conventional microstructures are already in use. In surface science and surface engineering, nanotopographies offer substantially different properties related to adhesion, tribology, optics and electronic behaviour. Supramolecular chemistry and catalysis have led to novel surface and size dependent chemistry, such as enantioselective catalysis at surfaces. In biological sciences, fundamental understanding of molecular motors and molecular functional entities on the nanometre scale has been responsible for advances in drug design and targeting. Nanoscale functionalised entities and devices are in development for analytical and instrumental applications in biology and medicine, including tissue engineering and imaging.

The application areas in which these advances in nanoscience are making their biggest impact include electronic, electro-optic and optical devices. The transition from semiconductor (conventional and organic) technology to nanoscale devices has anticipated improved properties and resolution, e.g. fluorescence labelling, scanning probe microscopy and confocal microscopy. Data storage devices based on nanostructures provide smaller, faster, and lower consumption systems.

In medicine, greater understanding of the origin of diseases on the nanometre scale is being derived, and drug delivery through functionalised nanostructures may result in improved pharmacokinetic and targeting properties.

A wide variety of functional nanoscale materials and functional nanoscale surfaces are in use in consumer products, including cosmetics and sunscreens, fibres and textiles, dyes, fillers, paints, emulsions and colloids.

3.3.3 The essentials of Nanostructure Generation: Top-Down vs. Bottom-Up Chemical and Physical Self Assembly

Nanotechnology is dependent on nanostructures that require creation and characterization. Two fundamentally different approaches for the controlled generation of nanostructures have evolved. On one hand there is growth and self assembly, from the bottom up, involving single atoms and molecules. On the other hand there is the top-down approach in which the powerful techniques of lithography and etching start with large uniform pieces of material and generate the required nanostructures from them. Both methods have inherent advantages. Top down assembly methods are currently superior for the possibility of interconnection and integration, as in electronic circuitry.

Bottom-up assembly is very powerful in creating identical structures with atomic precision, such as the supramolecular functional entities in living organisms. In many different fields of nanoscale science, e.g. the production of semiconductor quantum dots for lasers, the production of nanoparticles by self organization, and the generation of vesicles from lipids, self organization is used for the generation of functional nanometre sized objects. To date, man made self organised structures (Niemeyer 2001) remain much simpler than nature's complex self organised processes and structures.

3.3.4 Nanoscale materials properties

Material properties depend on structure and composition, and can typically be engineered or modified by changing the relative influence of interfacial or interphase properties and the macroscopic bulk properties through the characteristic size or dimension of components and domains. This approach had already emerged centuries ago with steel alloys and has been so powerful that many engineering materials today are composites with micro to nanoscale domain sizes. Depending on the physical or chemical character of each domain, there is a complex interrelation between the structure and the composition of the material, which may relate to the bulk and surface properties of each ingredient and newly emerging properties localized at the interface. Selective chemical reactivity is quite common with nanocomposites, which gives the potential for disintegration of the material into one or the other component. Complex processes govern this behaviour, which clearly relates to nanoparticle release into the environment.

3.3.5 Conclusions.

The exploitation of the properties associated with the nanoscale is based on a small number of discrete differences between features of the nanoscale and those of more conventional sizes, namely the markedly increased surface area of nanoparticles compared to larger particles of the same volume or mass, and also quantum effects. Questions naturally arise as to whether these features pose any inherent threats to humans and the environment. Bearing in mind that naturally occurring processes, such as volcanoes and fires, in the environment have been generating nanoparticles and other nanostructures for a very long time, it would appear that there is no intrinsic risk associated with the nanoscale per se for the population as a whole. As noted above, there is also no reason to believe that processes of self assembly, which are scientifically very important for the generation of nanoscale structures, could lead to uncontrolled self perpetuation. The real issues facing the assessment of risks associated with the nanoscale are largely concerned with the increased exposure levels, of both humans and environmental species, now that engineered nanostructures are being manufactured and generated in larger and larger amounts, in the new materials that are being so generated, and the potentially new routes by which exposure may occur with the current and anticipated applications.

3.4 Nanoparticles: Physical and Chemical Properties

The principal parameters of nanoparticles are their shape (including aspect ratios where appropriate), size, and the morphological sub-structure of the substance. Nanoparticles are presented as an aerosol (mostly solid or liquid phase in air), a suspension (mostly solid in liquids) or an emulsion (two liquid phases). In the presence of chemical agents (surfactants), the surface and interfacial properties may be modified. Indirectly such

agents can stabilise against coagulation or aggregation by conserving particle charge and by modifying the outmost layer of the particle. Depending on the growth history and the lifetime of a nanoparticle, very complex compositions, possibly with complex mixtures of adsorbates, have to be expected. In the typical history of a combustion nanoparticle, for example, many different agents are prone to condensation on the particle while it cools down and is exposed to different ambient atmospheres. Complex surface chemical processes are to be expected and have been identified only for a small number of particulate model systems. At the nanoparticle - liquid interface, polyelectrolytes have been utilised to modify surface properties and the interactions between particles and their environment. They have been used in a wide range of technologies, including adhesion, lubrication, stabilization, and controlled flocculation of colloidal dispersions (Liufu et al 2004).

At some point between the Angstrom level and the micrometre scale, the simple picture of a nanoparticle as a ball or droplet changes. Both physical and chemical properties are derived from atomic and molecular origin in a complex way. For example the electronic and optical properties and the chemical reactivity of small clusters are completely different from the better known property of each component in the bulk or at extended surfaces. Complex quantum mechanical models are required to predict the evolution of such properties with particle size, and typically very well defined conditions are needed to compare experiments and theoretical predictions.

3.4.1 Nanoparticle - Nanoparticle Interaction

At the nanoscale, particle-particle interactions are either dominated by weak Van der Waals forces, stronger polar and electrostatic interactions or covalent interactions. Depending on the viscosity and polarisability of the fluid, particle aggregation is determined by the interparticle interaction. By the modification of the surface layer, the tendency of a colloid to coagulate can be enhanced or hindered. For nanoparticles suspended in air, charges can be accumulated by physical processes such as glow discharge or photoemission. In liquids, particle charge can be stabilised by electrochemical processes at surfaces. The details of nanoparticle - nanoparticle interaction forces and nanoparticle – fluid interactions are of key importance to describe physical and chemical processes, and the temporal evolution of free nanoparticles. They remain difficult to characterise due to the small amount of molecules involved in the surface active layer. Both surface energy, charge and solvation are relevant parameters to be considered. Due to the crucial role of the nanoparticle – nanoparticle interaction and the nanoparticle – fluid interaction, the term free nanoparticle can be easily misunderstood. The interaction forces, either attractive or repulsive, crucially determine the fate of individual and collective nanoparticles. This interaction between nanoparticles resulting in aggregates and/or agglomerates may influence on their behaviour. In gas suspensions, aggregation is crucially determined by the size and diffusion, and coagulation typically occurs faster than in the liquid phase as the sticking coefficient is closer to unity than in liquids. 3.5 Sources of Free Nanoparticles

Nanoparticles are formed through the natural or human mediated disintegration of larger structures or by controlled assembly processes. The associated processes occur either in the gas phase, in a plasma, in a vacuum phase or in the liquid phase, eventually followed by the intentional or unintentional transfer into one or more relevant fluid media and then to an individual receptor in an exposure setting.

3.5.1 Formation of nanoparticles in the liquid phase

Defined bottom-up production of nanoparticles in the liquid phase with respect to particle size, chemical composition, surface and charge properties occurs mainly through controlled chemical reactions (Frens 1973), and self limiting self assembly processes have evolved by controlling growth conditions. In view of the ecological cycling of nanomaterials, some emphasis has to be given to the corrosion and disintegration of bulk materials, where little knowledge is currently available (Oberdörster G et al 2005]. Naturally occurring processes generating nanosized structures in the liquid phase include erosion and chemical disintegration of organic (plant or microorganism debris) or geological (e.g. clays) parent materials. In all these types of disintegration process, the surface properties and their change through chemical reaction are critical in determining whether individual nanoparticles will be formed in the respective medium (Boyle et al 2005).

3.5.2 Formation of nanoparticles suspended in the gas phase

The main route of bottom-up formation of nanoparticles in the gas-phase is by a chemical reaction leading to a non-volatile product, which undergoes homogeneous nucleation followed by condensation and growth. Recently, this has become an important pathway for the industrial production of nanoparticle powders, which may be of metals, oxides, semiconductors, polymers and various forms of carbon, and which may be in the form of spheres, wires, needles, tubes, platelets or other shapes. This is also the unintentional pathway by which nanoparticles are formed following the oxidation of gas-phase precursors in the atmosphere, in volcanic plumes, in natural and man-made combustion processes, or in fumes associated with any man-made process involving volatilizable material at elevated temperature, such as welding or smelting, polymer fabrication, or even cooking .

As with the liquid phase case, disintegration processes of parent materials provide a pathway which only leads to nanoparticles suspended in the gas phase under special conditions. While in the liquid phase the presence of emulsifying agents accompanying an erosion or chemical disintegration process could support the suspension process, the dispersion of nanoparticles into a gas from liquid emulsions or dry powders is severely limited by the strong adhesive forces between individual nanoparticles. Therefore, any mechanically induced stress on the parent material mostly leads to particles in the micrometer range and above. Only under accidental conditions, e.g. in the case of uncontrolled release of a powder or an emulsion from a highly pressurized vessel could strong shear forces overcome these adhesive forces (Reeks and Hall 2001). In contrast, the spraying of liquids containing nanoparticles or soluble material at very low concentrations, followed by drying of the solvent, can lead to the resuspension of nanoparticles or to the formation of new nanoparticles from the solutes. This can lead to redistribution of nanoparticles, biological material or toxic substances into nanoparticulate airborne form.

3.5.3 Environmental Sources of airborne Nanoparticles

The amount of nanoparticles in the air can be surprisingly similar in urban and rural areas, with as much as 10^6 to 10^8 nanoparticles per litre of air depending on conditions. In rural areas, nanoparticles mostly originate from the oxidation of volatile compounds of

biogenic or anthropogenic origin, including secondary organic aerosols. In urban areas, the primary sources of these particles are diesel engines (Schneider et al 2005) or cars with defective or cold catalytic converters (Zhiqiang et al 2000). Photo-oxidation processes also lead to significant numbers of nanoparticles in urban areas. Real-time measurements show that exhaust aerosol concentrations range between 10^4 to 10^6 particles.cm^{-3}, with the majority of the particles by number being less than 50 nm in diameter. The highest particle number concentrations and smallest particle size are associated with high-speed road traffic, presumably due to the subtle conditions during concomitant cooling and dilution of the exhaust gases. Emission factors for gasoline vehicles ranged from 1.9 to 9.9×10^{14} particles.km^{-1} and 2.2×10^{15} to 1.1×10^{16} particles.kg^{-1} fuel (Kittelson et al 2003 a,b). The awareness that combustion processes significantly contribute to the nanoparticle load by number has been rising recently and has provided a new motivation for airborne particle research (Donaldson et al 2001a).

Wieser and Gaegauf (2000) have evaluated different wood combustion systems with respect to emissions. Particle sizes were mainly in the range of 30 to 300 nm. Particles of less than 300 nm did not add much to the total particle count in flue gas. The particle distribution of manually operated appliances varied during a burn cycle, while continuous fed wood combustion systems show a fairly constant particle size distribution. Total particle numbers for automatically fed burners were smaller than with manual operation. Around 95% of the particles were smaller than 400 nm. The most frequent size of the particle number concentrations for batch operated appliances is approximately 110 nm, whereas the particle distribution changes significantly during the burn cycle.

3.5.4 Occupational Sources of airborne Nanoparticles

Inhaled nanoparticles may represent a potential health risk. Aerosols in workplace environments may be derived from a wide variety of sources, depending on the type of activity and processes taking place. Nanoparticle aerosols arising from mechanical processes (e.g. the breaking or fracture of solid or liquid material) are unlikely to be formed. Grinding and surface finishing typically releases micrometre and submicrometre particles, possibly down to 100 nm but rarely below this. Most plasma and laser deposition and aerosol processes are performed in evacuated or at least closed reaction chambers. Therefore exposure to nanoparticles is more likely to happen after the manufacturing process itself, except in those cases of failures during the processing (Luther 2004). In processes involving high pressure (e.g. supercritical fluid techniques), or with high energy mechanical forces, particle release could occur in the case of failure of sealing of the reactor or the mills. Nanoparticles exhibit increased diffusivity with decreasing size and therefore show delayed sedimentation in the earth's gravitational field, which translates into potentially increased lifetimes for nanoparticulate impurities at low concentration. In the presence of larger microparticles, as with the wide size distribution in aerosols such as smoke, the highly diffusive character of nanoparticles may lead to faster agglomeration or impaction on the larger particles. Furthermore, many particles, including metallic particles, are highly pyrophoric and there is a considerable risk of dust explosions.

3.5.5 Nanoparticles in and from Consumer Products

Nanoparticles are now being used in the manufacture of scratchproof eyeglasses, crack-resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells. Nanoparticles can contribute to stronger, lighter, cleaner and "smarter" surfaces and systems. At the nanoscale, the properties of particles may change in unpredictable ways. Nanoparticles of titanium oxide used in sunscreens, for example, have the same chemical composition as the larger white titanium oxide particles used in conventional products for decades, but nanoscale titanium oxide is transparent. Antimony - tin oxide provides another example since nanoparticles of this oxide are incorporated into a coating to provide scratch-resistance and offer transparent protection from ultra-violet radiation, not seen with larger size particles.

There are several safety concerns in the automotive sector relating to nanotechnology. Nanoparticles as fillers in tyres can improve adhesion to the road, reducing the stopping distance in wet conditions. The stiffness of the car body can be improved by use of nanoparticle-strengthened steels. New sol-gel deposition methods make it possible to apply, economically, nanometre thick antireflection layers of silicon dioxide or other materials onto displays or panes. Ultra-thin transparent layers on a silver base can be used for heatable, and therefore mist and ice-free, window panes. Transparent and light materials could substitute car body parts that reduce all-round vision at the moment. (Nanoforum Report 2004)

Nanotechnology can be applied in the production, processing, safety and packaging of food. It is possible that nanotechnology will allow the manipulation of the molecular forms of food to provide more capability, lower costs and greater sustainability than at present. A nanocomposite coating process should improve food packaging by placing anti-microbial agents directly on the surface of the coated film and could increase or decrease gas permeability as required for different products. They can also improve the mechanical and heat-resistance properties and lower the oxygen transmission rate. It should also be possible to apply nanotechnology to the detection of chemical and biological substances for sensing biochemical changes in foods, extending to the whole food chain in the future (Nanoforum Report 2004).

As reviewed by Salata (2004), nanomaterials are also being used in biology and medicine in a wide variety of ways, including the direct application of products into patients. Examples include products for drug delivery and gene therapy, the separation and purification of biological molecules and cells, fluorescent biological labels, imaging contrast agents, tissue engineering, DNA probes and nanoscale biochips, and microsurgical technology.

3.5.6 Conclusions

Nanoparticles are produced by natural phenomena, and many human industrial and domestic endeavours, such as cooking, material fabrication and transportation utilising internal combustion and jet engines, unintentionally release nanoparticles into the atmosphere. In recent years a new type of source of nanoparticle has been introduced, within the sphere of intentionally engineered nanoscale components of consumer products and advanced technologies. It is not yet clear just how significant is the

increase in exposure to nanoparticles associated with these new products, either in the workplace or in the context of consumers of nanotechnology-based products.

3.6 The Detection and Measurement of Nanoparticles

The detection of nanoparticles and the measurement of specific properties associated with them are necessary for two distinctly different reasons. First, methods are required that reliably detect nanoparticles and measure their physico-chemical properties in the media in which humans and ecosystems are exposed to them, such as air, water, soil, consumer products and nanocomposites. Methods must also be available that support the studies to assess the risk of nanoparticles, such as with toxicological and ecotoxicological studies. Additional tools are required in this case, which are able to detect nanoparticles in the relevant medium, including cells, fluids and plant tissue.

The second issue relates to the physical or chemical properties associated with nanoparticles that are the basis of the detection of nanoparticles in these media. The range of properties of nanoparticles of potential relevance to risk assessment highlights the principal needs for extremely sensitive methods. The typical dimensions of nanoparticles are below the diffraction limit of visible light, so that they are out of range for optical microscopy. In low concentration liquids and gases however, single chromophore detection is possible and in Scanning Near Field Optical Microscopy (SNOM) sub-wavelength feature can be analysed. While the chemical composition of nanoparticles might be accessible by classic analytic methods for macroscopic amounts of nanoparticulate material, chemical analysis of individual nanoparticles in a dilute environment was for a long time impossible due to their low mass, and only recently have methods become available for this purpose, so that even surface coatings may be detected.

3.6.1 In situ and on-line detection principles for nanoparticles in gas suspension

Driven by the recent developments in atmospheric chemistry and physics, which have highlighted the role of nanoparticles in areas such as climate and health research, the measurement technologies for atmospheric aerosols offers a suite of tools specialized to the nanometer size range. An HSE Report (2004) and Luther (2004) have summarized the various types of device which might be or have been used to provide measurement information on nanometre size aerosols. Due to the lack of significant scattering or absorption by particles in the nanometre size range, particles are counted in commercially available, condensation nucleus counters (CPC or CNC), in which the particles are activated to droplets in a supersaturated atmosphere of alcohol, which can then be detected optically. Currently available instruments can detect particles as small as 3 nm, while new developments may reach the 1 nm limit (Kim et al 2003). CPCs cover a large dynamic range from a few up to 10^6 particles.cm^{-3}.

A relatively simple technique involves charging particles by ion attachment and subsequent trapping of particles in a filter within a Faraday cup, which is connected to a sensitive electrometer. Although this method provides a signal which needs to be calibrated, it does give a sensitive proxy of aerosol surface area, under conditions of substantial aerosol load. This is similar to the epiphaniometer which relies on attachment and detection of a radioactive lead isotope rather than an ion, and is thus sensitive to rather low concentrations of particles.

While these instruments are not themselves size selective, they can be coupled to size selecting instruments, such as the commercially available differential mobility analyzer (DMA), which specifically covers the low nanometre size range. It discriminates charged particles with respect to their drift velocity under the action of an electric field. The combination of a DMA and CPC is often referred to as a scanning mobility particle sizer (SMPS). Low pressure impactors, where particles are separated by inertial impaction, can easily separate and count nanoparticles from larger particles, but commercially available systems do not provide a size resolution down to the nanometre range. An important advantage of the impactor systems is that aerosols with nanometre sizes can be collected for further analysis.

The recent developments of aerosol mass spectrometry, in which particles are vaporized and the resulting ions analyzed in a mass spectrometer have provided new alternative procedures. Depending on sampling inlet configuration, size separation method, vaporization method and type of mass spectrometer coupled to it, very specific, size resolved chemical composition of nanoparticles in gas suspension can be obtained.

3.6.2 In situ and on-line detection of particles in a liquid medium

Direct detection of nanoparticles in liquid media faces similar physical obstacles as in the gas phase. The most successful approach into the nanometre range involves measuring the size dependent Brownian motion of an ensemble of particles through the change of interference patterns with time. Commercially available instruments reach a lower detection limit of 3 nm. Other important techniques include optical chromophore counting, resonant light scattering and Raman scattering techniques, as well as the microscopic analysis of precipitates and cross section cuts. Highly sensitive techniques within electrochemistry and mass spectrometry and Rutherford backscattering have been used to identify compounds which have been brought into tissue in the form of particles (Penn et al 2003).

Most of these techniques are off-line and involve complicated sampling / sample preparation techniques. Using fluorescent molecules, quantum dots or magnetic nanoparticles as tracers, it is possible to count low concentrations of particles online. However, the choice of particles and chromophores puts further restrictions on the system studied.

Scanning Electron Microscopy (SEM) is the method of choice to investigate particle size shape and structure. When equipped with an Electron Dispersive Spectrometer (EDS) chemical composition can be determined, at least for larger particles and refractory components. The X-ray microanalysis system is not always suitable for chemical analysis because identification of substances can only be performed on the elemental level and cannot be quantified. Only solid, very high vapour pressure particles can be analysed due to the high vacuum of the system required for X-ray microanalysis.

The resolution of SEM and the related techniques has progressed below 10nm due to the implementation of cold electron sources in recent instruments. SEM resolution has been improved in Scanning Transmission Electron Microscopy (STEM) or High Resolution Transmission Electron Microscopy (HRTEM) techniques, which can again be combined to very powerful analytical techniques using electron probes and x-ray analysis.

3.7 The Potential for Interactions Between Nanoparticles and Living Systems

3.7.1 Introduction

The hierarchical self organization of life spans from single molecules around 1 nm in size to large animals and plants (~10 m) and to very large organized populations of a species (~100 m). Nanoparticles may be of the same dimensions as some biological molecules such as proteins and nucleic acids. Many of these biomolecules consist of long macromolecular chains which are folded and shaped by cooperative and weak interaction between side groups, H-bridges and salt bridges. Here, functionalized nanoparticles, such as colloidal gold (Hayatt 1989), may intrude into the complex folded structures (Cheng et al 1999, Hainfeld and Powell 2000). Evidence for such interactions is seen from the experience with immunolabelling (Romano and Romano 1977) and related surface functionalisation techniques to target nanoparticles to biomolecules as markers for high resolution Transmission Electron Microscopy and optical imaging systems. Other nanoparticle systems which are established for research purposes in cell systems include quantum dots (Chan and Nie 1998) and magnetic nanoparticles (Josephson et al 1999). For a recent review see (Penn et al 2003). Surface active agents have been shown to alter the path of nanoparticles (Schurch 1990).

3.7.2 Nanoparticles in Living Systems – The Surface Effects

All nanoparticles, on exposure to tissues and fluids of the body, will immediately adsorb onto their surface some of the macromolecules that they encounter at their portal of entry. The specific features of this adsorption process will depend on the surface characteristics of the particles, including surface chemistry and surface energy, and may be modulated by intentional modification or functionalisation of the surfaces (Schellenberger et al 2004). This is well demonstrated through the use of specific biomolecular linkers that are anchored on the surface of nanoparticles or within vesicles and liposomes (Nardin 2000). In this way the affinity of a nanoparticle can be shaped to fit to a particular protein, and thus target a specific biomolecular assembly on a membrane, or within a specific organelle or cell surface. The specificity of such surface layers is used for analytical purposes (Elghanian et al 1997), for optical labelling of biomolecules in molecular libraries (Han et al 2001) and for drug or gene delivery to cells (Hood et al 2002). Thus, both the existence of passive surface layers and surface active agents compromise the risk evaluation of nanoparticles by mere chemical composition. In agreement with bulk surface chemistry, metallic nanoparticles are of considerable chemical reactivity while ionic crystal nanoparticles have been observed to accumulate protein layers when exposed to the cytoplasm or in the lymphatic fluid. This protein layer is possibly involved in the interaction of the nanoparticle by the cellular system.

3.7.3 The Effects of Size, Shape, Surface and Bulk Composition

The interaction of nanoparticles with living systems is also affected by the characteristic dimensions. As noted above, nanoparticles, of a few nm in size, may reach well inside biomolecules, a situation not possible for larger particles. It has been reported that inhaled nanoparticles reach the blood and may reach other target sites such as the liver, heart or blood cells (Oberdörster G et al 2002, MacNee et al 2000, Kreyling et al 2002).

Nanoparticles may translocate through membranes. There is little evidence for an intact cellular or sub-cellular protection mechanism. For humans, inhalation is the most frequent route of access, and therefore the process of aggregation of the nanoparticles in the inhaled air has to be taken into account.

In order to understand and categorize the mechanisms for nanoparticle toxicity, information is needed on the response of living systems to the presence of nanoparticles of varying size, shape, surface and bulk chemical composition, as well as the temporal fate of the nanoparticles that are subject to translocation and degradation processes. The typical path within the organ and / or cell, which may be the result of either diffusion or active intracellular transportation, is also of relevance. Very little information on these aspects is presently available and this implies that there is an urgent need for toxicokinetic data for nanoparticles.

3.7.4 Solubility and Persistence

In view of the active functionalisation and the possible interaction of nanoparticles with bio-molecular structures, it is important to consider the dose and dose rate of the particulate agent, its ability to spread within the body and ecosystem, the decay of number concentration and the erosion of individual particles. Many nanoparticles will have considerable solubility. For these materials the interaction with living systems remains close enough to the bulk chemical agent to justify the use of well established toxicological testing procedures and approaches. For biodegradable particles, the particle composition and degradation products will influence their biological effects. On the other hand, materials with very low solubility or degradability, could accumulate within biological systems and persist there for long durations. It is with nanoparticles of this character that the greatest concerns must arise, and attention will have to be paid to the comparison of the persistence of the particles and the time constants of the metabolic and cellular activities within the target host.

It should be noted that solubility might be modified by surface active agents (surfactants), which could pose some new questions. Also of importance with soluble nanoparticles is the physics of exposure, where particle size plays a major role in aerodynamic and hydrodynamic or diffusive processes, which could affect the ability to reach different tissues. Examples are the size dependence of transport of airborne particles into the respiratory system, and the involvement of diffusive and trans membrane transport channels with smaller entities.

3.7.5 Conclusions

The major emerging issue to be discussed in the context of the biological interactions of nanoparticles is related to those particles with little or no solubility, or being non-degradable at the locality where accumulation is observed. There remain many unknown details about the interaction of nanoparticles and biological systems.

3.8 Toxicology of Nanoparticles

Studies specifically dealing with the toxicity of nanoparticles have only appeared recently and, although now emerging in the literature, are still rare. Data concerning the behaviour and toxicity of particles mainly comes from studies on inhaled nanoparticles (reviewed by Oberdörster G 1996, Oberdörster G et al 2005, Donaldson and Stone 2003, Borm 2002, Donaldson et al 2001a, 2004, Dreher 2004, Kreyling et al. 2004). Data on the behaviour of particles is also available from pharmaceutical studies in which formulations involving nanoscale components are used to solve problems dealing with insolubility of drug formulations and for drug delivery (Baran et al 2002, Cascone et al 2002, Duncan 2003, Kipp 2004).
Not all toxicological studies to date deal with nanoparticles as recently defined (size <100 nm) or have characterised the nanoparticles according to recent knowledge. However, this does not necessarily interfere with the conclusions reached in these studies.

3.8.1 The Mediators of the Toxicity of Particles

Size

Reduction in size to the nanoscale level results in an enormous increase of surface to volume ratio, so relatively more molecules of the chemical are present on the surface, thus enhancing the intrinsic toxicity (Donaldson et al 2004). This may be one of the reasons why nanoparticles are generally more toxic than larger particles of the same insoluble material when compared on a mass dose base. The expression of a dose response relationship on the basis of particle size resulted in a similar dose response relationship between low solubility - low toxicity, particles of different sizes (Oberdörster G et al 2000). In studies of low toxicity particles, TiO_2 induced a more severe lung inflammation and particle lymph node burden compared to $BaSO_4$ when dosed at mass burden in milligrams (Tran et al 2000). Surface area was therefore a driver for inflammation for these materials; the differences in severity of the response disappeared when the dose was expressed as surface area. These examples emphasize the importance of particle size, and by implication, the amount of surface area presented to the biological system for particle toxicity.

Chemical Composition

The chemical composition and the intrinsic toxicological properties of the chemical are of importance for the toxicity of particles (Donaldson et al 2004). The effect of carbon black has been shown to be more severe than that of titanium dioxide (Renwick et al 2004), while for both compounds the nanoparticles induced lung inflammation and epithelial damage in rats at greater extent than their larger counterparts. In addition, chemicals adsorbed on the surface may affect the reactivity of nanoparticles. Fractions isolated from particulate air pollutants (diesel exhaust particles) were demonstrated to exert toxic effects on cells in vitro (Xia et al 2004). Nanoparticles in ambient air can have a very complex composition, and these components, such as organics and metals, can interact. Metallic iron was able to potentiate the effect of carbon black nanoparticles, resulting in enhanced reactivity, including oxidative stress (Wilson et al 2002). In contrast, surface modification of nanoparticles can also result in a diminishing of

cytotoxicity. The *in vitro* cytotoxicity of superparamagnetic iron oxide nanoparticles could be abrogated by coating the nanoparticles with pullulan (Gupta and Gupta 2005). Also for dextran and albumin derivatised iron oxide nanoparticles, a reduction in *in vitro* cytotoxicity was noted (Berry et al 2003).

For several different nanoscale particles (polyvinyl chloride, TiO_2, SiO_2, Co, Ni), only Co induced toxicity in endothelial cells, which was accompanied by the production of the pro-inflammatory cytokine IL8 (Peters et al 2004). For other particles only TiO_2 and SiO_2 induced minor and profound IL8 releases, respectively. An explanation of the differences in cytotoxicity was not presented but might be due to both material differences and/or size difference at the nanoscale, as the particle size ranged from a mean diameter of 14nm to 120 nm and even clusters of 420 nm (Peters et al 2004).

For micron sized biomaterial particles, the *in vivo* distribution was dependent on the composition of the material. With two polymers, polymethylmethacrylate (PMMA) particles but not polystyrene (PS) particles could be recovered from the spleen after intraperitoneal administration (Tomazic-Jezic et al 2001). The PS particles regardless of size were accumulated primarily in the adipose tissue of the peritoneal cavity, with very few particles in the spleen.

Although nanoparticles in air can be used as an information source for particle toxicity, one has to be aware that particles in ambient air as part of pollution of combustion origin are coated with all kinds of reactive chemicals including biological compounds such as endotoxin (Carty et al 2003, Kreyling et al 2004, Schins et al 2004). Thus the information obtained from ambient air particles for nanoparticle toxicity should take into account the possible influence of particle composition and contamination.

Shape

Shape is also likely to be an important factor although there is little definitive evidence. Fibres provide a significant example of the debate about shape, especially in relation to inhalation, where the physical parameters of thinness and length appear to determine respirability and inflammatory potential. The biopersistence of fibres effectively determines their dose.

A special category of fibres are nanotubes, which may be of a few nanometres in diameter but with a length that could be several micrometers. Risks should be assessed bearing in mind the well known carcinogenic effects of certain asbestos fibres. In two recently published *in vivo* studies, single-wall carbon nanotubes (SWCNTs) were demonstrated to induce lung granulomas after intratracheal administration (Lam 2004, Warheit et al 2004), indicating that these nanotubes cannot be classified as a new form of graphite on material safety data sheets. On a dose per mass basis the nanotubes were more toxic than quartz particles, well known for their lung toxicity, although the mass dose was very high and mechanical blockage of some airways was noted. Carbon black, carbonyl iron and graphite produced no significant adverse effects. Multifocal granulomatous lesions were observed without accompanying inflammation, cell proliferation or cytotoxicity, which was suggested to indicate a potentially new mechanism of pulmonary toxicity and injury by the nanotubes, not following the normal paradigm of toxic dusts (Warheit et al 2004). *In vitro* studies using a human keratinocyte

cell line showed that carbon nanotube exposure resulted in accelerated oxidative stress and cellular toxicity, which may be interpreted as potential for dermal toxicity (Shvedova et al 2003).

3.8.2 Inhaled Particles

Epidemiological Evidence

The role of particulate matter as a component of air pollution with an influence on human health is well established, although the mechanisms of action are poorly understood (Englert 2004). Ambient particulate air pollution was found to be statistically associated with cardiovascular morbidity and mortality (Pope 2000, Samet et al 2000, Peters et al 2001). However, very little is known on the relationship between the specific exposure to nanoparticles and health effects, in contrast with the large number of epidemiological studies on larger particles.

Von Klot et al (2002) could not distinguish between ambient fine particles and nanoparticles with respect to the association with increased asthma medication use. In another study, fine particles were more strongly related to cardio-respiratory symptoms than were nanoparticles (de Hartog et al 2003, Pekkanen et al 2002). Peters et al (1997) demonstrated that the number of nanoparticles is more strongly associated with health effects than the mass. Epidemiological studies on ambient air pollution do not provide consistent evidence that nanoparticles are more hazardous than larger particles. It may well be that epidemiological studies are not well suited to demonstrating differences between the toxicity of the various components of particulate matter. The exposure-dose relationships depend so much on time and location, and epidemiological studies are hampered by the lack of appropriate measurement. There is some evidence that combustion-derived particles emanating from traffic are a key driver for adverse health effects.

Dosimetry

Estimating the dose of inhaled particles requires the knowledge of several mechanisms including regional deposition, retention, solubility, redistribution, translocation into the circulation, metabolism, accumulation in certain organs and the excretion pathways via urine and faeces. The factors that control or affect particle deposition include the particle characteristics themselves, the respiratory tract geometry and individual features of ventilation such as the mode of breathing.

Inhaled particulate matter can be deposited throughout the human respiratory system including pharyngeal, nasal, tracheobronchial and alveolar regions, depending on particle size as described in one model , shown in Figure 1, after Price et al (2002). A similar model has been proposed by ICRP (1994). The fractional deposition efficiency of particles with a size below 100 nm is between 30 and 70 % in pulmonary regions, although the predictability becomes less accurate at the nanoscale. With decreasing size there is a major increase in alveolar deposition. Cassee et al demonstrated that the toxicity of various size of soluble aerosolized cadmium chloride ($CdCl_2$) could be accurately predicted by calculating the dose rather than using the exposure concentration (Cassee et al. 2002).

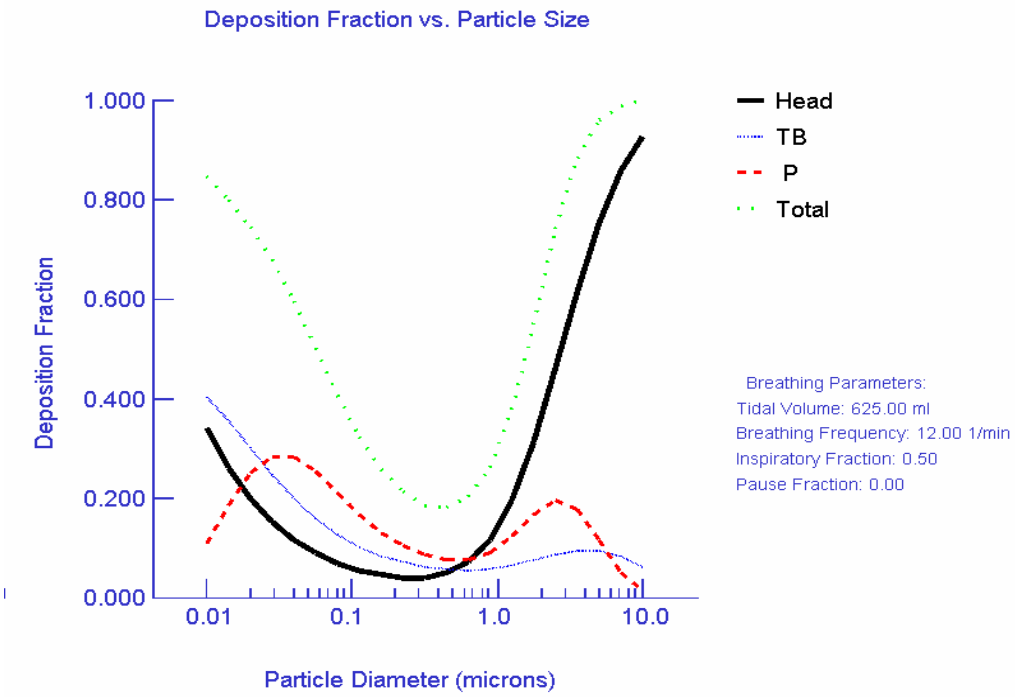


Figure 1 Non-monotonic relationships of particle deposition as function of diameter in a healthy adult (Price et al, 2002) TB= Tracheo-brochial region; P= pulmonary region.

The general pathways for the mechanical clearance of insoluble particles in the pulmonary region, after McClelland (1998) is given in Figure 2. After deposition in the respiratory tract, translocation of nanoparticles may potentially occur to the lung interstitium, the brain, liver, spleen and possibly to the foetus in pregnant females (MacNee et al 2000, Oberdörster G et al 2000, 2002). It is emphasised that there is extremely limited data available on these pathways. With insoluble iridium nanoparticles in the size range of about 15 and 80 nm, clearance was found to be primarily via the airways into the gastrointestinal tract. Only a small fraction (<1%) of the particles was translocated into secondary organs like liver, spleen, heart and brain, of which the 80 nm sized particles were translocated an order of magnitude less that the 15 nm sized particles, indicating the importance of size even within the nanometre range (Kreyling et al 2002). The presence of low amounts of particles in the liver and spleen could be attributed to translocation from the lung to the blood and sequestration by the sinusoidal macrophages of these organs. Particles were not dissolved nor absorbed from the gut (Kreyling et al 2002). However, depending on the exposure time, the actual amount of translocated particles could be considerable. Circulating particles (after intravenous administration) were accumulated in liver and spleen and retained there. Passage of inhaled nanoparticles into the bloodstream was demonstrated in one human study (Nemmar et al 2002), but two other similar studies have failed to show such a translocation. Another potential route of translocation of inhaled nanoparticles is the olfactory nerve in the nose leading to the olfactory bulb of the brain. ^{13}C nanoparticles with a size about 35 nm were detected in the brain olfactory bulb after inhalation exposure. The route of brain entry was suggested to be by migration along the olfactory

nerve into the olfactory bulb of the brain after deposition on the olfactory mucosa in the nasal region (Oberdörster E et al 2004).

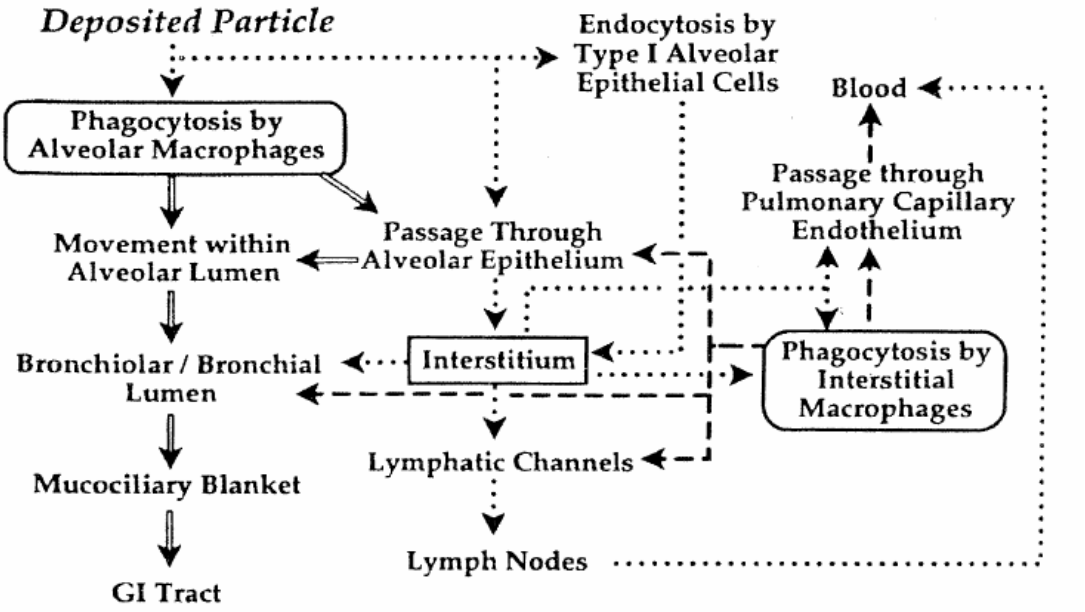


Figure 2 Diagram of known and suspended mechanical clearance pathways for insoluble particles depositing in the pulmonary region (McClellan et al 1998)

Experimental Evidence for Respiratory Toxicity

As noted above, several model nanoparticulate materials have been used for the evaluation of air particulate toxicity, These include, polystyrene, titanium dioxide, carbon black, cobalt, nickel and latex (Oberdörster G et al 2000, Donaldson et al 2000, Dick et al 2003, Nygaard et al 2005). Nanoparticles of titanium dioxide induced more bronchoalveolar inflammation than fine TiO_2 when rats were exposed to an equal concentration (Ferin et al 1992, Oberdörster G et al 1994, 2000). Similar results were obtained for nanoparticulate polystyrene (Brown et al 2001). These studies indicate that materials which by themselves are low in toxicity could be toxic when administered in formulations involving nanoparticles. If these particles are of low solubility, this effect could be solely due to the increased surface area of the inhaled dose. Similar results were obtained for low doses of nanoscale and fine carbon black and latex particles, (Li et al 1999, Donaldson et al 2000, 2001a, Wilson et al 2002, Renwick et al 2004). For nickel nanoparticles, an enhanced lung inflammation and toxicity was observed compared to larger sized nickel (Zhang et al 2003). Thus, for inhalation exposure it can be concluded that nanoparticles may show an increased toxicity compared to larger particles of the same chemical composition. Besides size, the chemical nature itself has an impact on the induced lung inflammation after intratracheal instillation, as nanoparticulate Ni was more toxic than nanoparticulate Co, with nanoparticulate TiO_2 being the least toxic (Zhang et al 1998). The ranking of toxicity was reflected in the capability of the materials to induce free radical damage to plasmid DNA, indicating that free radical generation may underlie these observed differences in toxicity.

Systemic Toxicity

As mentioned above nanoparticles may be able to translocate from the lung into the blood resulting in systemic exposure of internal organs, although the extent of this may vary. Another route of translocation from the airways may be by neuronal uptake. Inflammatory biomarkers such as Interleukin 1α (IL1α) and Tumour Necrosis Factor α (TNFα) were increased in the brain of mice exposed to ambient air particulate matter compared to controls (Campbell et al 2005). It is unknown whether this leads to potentially adverse consequences, but certainly warrants further studies. In view of the induction of inflammatory cytokines, a relation with a variety of neurological diseases might be considered.

Vascular effects in terms of thrombosis were observed for intratracheally administered 60 nm amine modified polystyrene particles but not for 400 nm sized particles (Nemmar et al 2003). Both 60 nm and 400 nm sized polystyrene particles induced pulmonary inflammation, so inflammation and thrombogenesis are not necessarily coupled. In a recent study in rats carbon nanoparticles (about 38 nm) were found to induce a mild but consistent increase in heart rate (Harder et al 2005) but only induced a low grade pulmonary inflammation. The effect on the heart rate could not be related to blood hypercoagulability, which is in discrepancy with other reports (Donaldson et al 2001b, Nemmar et al 2003). Studies with inhalation of diesel soot in human subjects indicate that the function of the endothelial cells in the forearm is impaired following inhalation, which was evident from impaired vasomotor and secretory responses to pharmacological stimulation. The available data are consistent with the occurrence of a systemic inflammatory response and an alteration of autonomic cardiac control, but there is little evidence of endothelial dysfunction, pro-coagulatory states or nanoparticle-related myocardial malfunction.

Mechanisms of Particulate Toxicity

Several possible mechanisms of action for the toxicity of particles in general have been postulated (see figure 7), including injury of epithelial tissue (Pagan et al 2003), inflammation, oxidative stress response (Nel et al 2001, Donaldson et al 2001, Donaldson and Stone 2003), and allergy (Dybing et al 2004). At the cellular level oxidative stress is considered to be of importance (Donaldson et al 2001a,b, Oberdörster G et al 2005). Nanoparticle induced oxidative stress responses in keratinocytes, macrophages and blood monocytes after *in vitro* exposure (Shvedova et al 2003, Brown et al 2004). In a recent study gene expression profiles indicated that pulmonary injury and inflammation are likely due to increased expression of an oxidative stress response and subsequent contributions from cytokines and chemokines after exposure to urban particulate matter (Kooter et al 2005). Enhancement of antioxidant enzymes of alveolar macrophages was demonstrated after *in vivo* exposure to TiO_2 nanoparticles, but this was not sufficient to counteract the lipid peroxidation and hydrogen peroxide generation that occurred (Afaq et al 1998). Thus, the overall resultant effect appears to be induction of oxidative stress in the cells, although the extrapolation of this mechanism to all types of nanoparticles is not possible.

3.8.3 Particles for Drug Delivery

Carriers for Drug Delivery

Nanostructures and nanoparticles can be used for drug delivery purposes, either as the drug formulation itself or as the drug delivery carrier (Cascone et al 2002, Baran et al 2002, Duncan 2003, Kipp 2004). Current research focuses on cancer therapy, diagnostics and imaging, although many challenges still need to be solved (Ferrari 2005). In addition, nanostructures are being investigated for gene delivery purposes (Kneuer et al 2000, Salem et al 2003, Ravi Kumar et al 2004, Gemeinhart et al 2005, Yoo et al 2005, Roy et al 2005).

Many different formulations involving nanoparticles have been used for drug delivery purposes, including albumin (Damascelli et al 2003), poly(D,L-lactic-co-glycolide)acid (PLGA) (Panyam et al 2002, Weissenbock et al 2004), solid lipid formulations (Muller et al 1997, 2000, Wissing et al 2004), cetyl alcohol/polysorbate nanoparticles (Koziara et al 2004), hydrogels (Gupta and Gupta 2004), gold (Hainfeld et al 2004, Paciotti et al 2004), polyalkylcyanoacrylate composites (Cruz et al 1997, Olivier et al 1999, Kreuter et al 2003), magnetic iron oxide (Gupta and Gupta 2005), methoxy poly(ethylene glycol)/poly(ε-caprolactone) (Kim et al 2003), and gelatin (Cascone et al 2002). Albumin nanoparticles are already the subject of clinical studies for anticancer drug delivery purposes (Damascelli et al 2003).

Not all of the 'nanoparticle formulations' mentioned are strictly or solely nanoparticulate in the sense that their size is not always below 100 nm and some drug delivery systems include particles up to several hundreds of nanometres. In many cases, the technology to produce very small particles did not exist in the early stages of development, but now there is an increasing refinement in their size and it is relevant to this Opinion to discuss this group of products together. The route of administration may be oral, parental (subcutaneous, intramuscular, intra-arterial, intravenous) and via the skin.

Distribution

The aims for nanoparticle entrapment of drugs are either enhanced delivery and uptake by cells and/or the reduction in toxicity of the free drug to non target organs. For intravenous administration, long circulating and target- specific nanoparticles are needed. One of the problems is evasion of the entrapment of nanoparticles in the mononuclear phagocytic system, as present in liver and spleen (Gibaud et al 1996, Moghimi et al 2001). Surface modification with poly(ethyleneglycol), (PEG) resulted in a prolonged presence in the circulation by avoiding recognition and phagocytosis by the mononuclear phagocytic system (Bazile et al 1995). Besides reduction of therapeutic efficacy, liver entrapment also may have an effect on liver function. For cyanoacrylate and polystyrene nanoparticles (around 214 nm and 128 nm, respectively) transient liver alterations were observed after a single and chronic intravenous administration (Fernandez-Urrusuno et al 1995, 1997). Inflammatory responses were characterized by secretion of acute phase protein α1-acid glycoprotein by hepatocytes. In addition, antioxidant defences of hepatocytes were depleted, probably as a result of local release of oxidative species. Although nanoscale formulation is aimed at enhancing drug delivery without loss of drug

activity, a study comparing insulin-chitosan nanoparticles to chitosan solution and chitosan powder formulations showed that the insulin-chitosan nanoparticles were less effective in terms of bioavailability and lowering blood glucose level in both a rat and sheep model (Dyer et al 2002).

After oral administration, only 10% of 60 nm polystyrene particles were recovered from the tissue of the gastrointestinal tract. Most of these particles were present in lymphoid tissue such as Peyer's Patches and lymphoid aggregates in the large intestine (Hillery et al 1994).

After dermal administration, negatively charged nanoparticles of about 50 and 500 nm were found to permeate the skin, while positively charged and neutral particles of all sizes did not. It was suggested that particle size was less important than the total charge, explaining why both 50 and 500 nm sized latex particles showed permeation and 100 or 200 nm negatively charged particles did not (Kohli and Alpar 2004). A greater concentration of charge was suggested to be responsible for overcoming the skin barrier, explained for the 50 nm particles as being the small size and large surface area, and for the 500 nm explained by the high number of charged groups. It can be expected that penetration of damaged skin will be easier. Thus, when nanoparticles are used in ointments there should be particular consideration of skin permeation.

Intracellular Uptake

Encapsulation in sub 130 nm size poly(lactic acid – glycolic acid), PLGA, particles increased cellular uptake of a photosensitizer, resulting in enhanced cytotoxicity *in vitro* (Konan et al 2003). Toxicity of free nanoparticles was not determined in this study. Chemical characteristics such as surface charge may determine the fate of nanoparticles in cells. PLGA nanoparticles were found to be ingested by cells by endocytosis (Panyam et al 2002, Konan et al 2003). The escape from these endosomes into the cellular cytoplasm was suggested to be caused by a change in surface charge from negative to positive, resulting in cytoplasmic delivery of the incorporated drug. The hypothesis concerning the influence of the positive surface charge for escaping the endosomes was supported by data obtained with negatively charged polystyrene nanoparticles which remained in the endosomal compartment of the smooth muscle cells used in this study.

Nanoparticles may be used for gene delivery, applications including plasmid DNA administration for vaccination (Salem et al 2003, Cui and Mumper 2002, 2003, Zhang et al 2005) and cancer therapy (Ramesh et al 2004, Gordon and Hall 2005). Gene transfer was accomplished *in vitro* and *in vivo* using various types of nanoparticles. With silica nanoparticles of about 42 nm, gene transfer was obtained with very low cell toxicity (Ravi Kumar et al 2004). A clinical trial with gene therapy aimed at determining safety and tolerability was performed in cystic fibrosis patients (Konstan et al 2004).

Cellular Targeting

Specific targeting to retinal epithelium cells in the eye is possible (Bourges et al 2003). For very small quantum dots (<10 nm) specific targeting of peptide coated quantum dots to the vasculature of lungs and tumours has been reported (Åkerman et al 2002). PEG coating abrogated uptake by the reticuloendothelial system of liver and spleen. In

contrast, about 40-50 nm magnetic nanoparticles coated with PEG were quite well taken up by endocytosis (Gupta and Curtis 2004)

For indomethacin loaded nanospheres (size below 200 nm) composed of methoxy poly(ethylene glycol)/poly(ε-caprolactone) polymers, the *in vitro* cytotoxicity was reduced when compared to free indomethacin, although some minor toxicity of 15-20% growth reduction was still present (Kim et al 2003). *In vivo* acute toxicity studies found a LD_{50} value of 1.47 $g.kg^{-1}$, and 50% of this LD_{50} value administered for 7 days did not induce acute toxicity in heart, lung, liver and kidney. It was concluded that these methoxy poly(ethylene glycol)/poly(ε-caprolactone) polymer nanospheres were non-toxic.

Surface modifications of nanoparticles offer possibilities for medical applications such as drug targeting in terms of cellular adhesion and invasion and transcellular transport. Carbohydrate binding ligands on the surface of biodegradable PLGA nanospheres were found to associate at higher rates with cell membranes (Weissenböck et al 2004). Such increased adherence may lead to an enhanced activity of the drug presented as or incorporated in nanoparticles. For solid lipid nanoparticles, (SLN), *in vitro* cytotoxicity was dependent on the surfactant used for stabilization of the nanoparticles with one of the investigated surfactants inducing cytotoxicity (Muller et al 1997, Olbrich et al 2004). The stabilizing surfactants showed the largest differences in toxicity, although toxicity could be markedly reduced by binding to the nanoparticles. SLNs of various composition were investigated for their use in skin application (Santos Maia et al 2002). In vitro studies showed an increased drug (glucocorticoid) penetration of skin and epidermal localization. For nanoparticles in the size of 200 – 400 nm, the composition of the lipid matrix was shown to have an impact on the cytotoxicity of SLN (Schöler et al 2002). Coupling specific proteins such as antibodies to the nanoparticle surface may enable a more specific immune directed targeting of the particles to certain cells or organs (Nobs et al 2004).

Organ Specific Targeting

One of the advantages of the use of nanoparticles for pharmaceutical formulations is the potential to cross the blood brain barrier (BBB). However, this also may be the major drawback for systemic administration of nanoparticles in terms of potential brain toxicity. Such passage was suggested to be possible by the toxic effect of nanoparticles (about 200nm) on cerebral endothelial cells (Olivier et al 1999), although for similar nanoparticles (about 300nm) this was contradicted and not found for a different type of nanoparticle (Lockman et al 2003). Physical association of the drug to the nanoparticles was necessary for drug delivery to occur into the brain (Kreuter et al 2003). When nanoparticles with different surface characteristics were evaluated, neutral nanoparticles and low concentrations of anionic nanoparticles were found to have no effect on BBB integrity, whereas high concentrations of anionic nanoparticles and cationic nanoparticles were toxic for the BBB. The brain uptake rates of anionic nanoparticles at lower concentrations were superior to neutral or cationic formulations at the same concentrations. Therefore, nanoparticle surface charge must be considered for toxicity and brain distribution profiles (Lockman et al 2004).

Specific migration into draining lymph node is of importance for both treatment and diagnostic purposes. Nanoparticle formulations of polyisobutylcyanoacrylate (Nishioka and Yoshino 2001) and fluorescent quantum dots (Kim et al 2004, Soltesz et al 2005) were shown to localize into such draining lymph nodes. Also uptake by endothelial cells can be used for diagnostic as well as therapeutic (prevention of cardiovascular restenosis) purposes (Davda and Labhasetwa 2002, Uwatoka et al 2003, Westedt et al 2004).

Toxicity

The use of nanoparticles as drug carriers may reduce the toxicity of the incorporated drug (Kim et al 2003), although discrimination between the drug and the nanoparticle toxicity cannot always be made. The structure and properties of gold nanoparticles make them useful for a wide array of biological applications. Toxicity, however, has been observed at high concentrations using these systems. Goodman et al (2004) demonstrated that for 2 nm gold particles cationic particles were moderately toxic, whereas anionic particles were relatively non-toxic. Such very small sized gold nanoparticles were found to be non toxic when administered to mice for tumour therapy (Hainfeld et al 2004).

For thiol derivatized PEG - colloidal gold nanoparticles with tumour necrosis factor (TNF) an enhanced anti-tumour activity was reported when compared to free TNF (Paciotti et al 2004). Topoisomerase inhibitors when formulated in lipid containing nanoparticles showed increased anti-tumour activity in an *in vivo* nude mouse xenograft human tumour model (Williams et al 2003). Although phagocytosis by macrophages does not seem to be necessary for the uptake of nanoparticles, the immune system is not totally inactive when dealing with nanoparticles. For 100 nm polystyrene particles, an IgE adjuvant activity was observed in an animal model system of ovalbumin allergy (Nygaard et al 2005). Antibodies against fullerenes could be induced after the intraperitoneal injection of C60 conjugated with serum proteins.

Ecotoxicity

Colvin's (2003) discussion on the potential impact of engineered materials demonstrates the lack of data on the exposure and effects of nanoparticles. To date, only a few studies have been carried out with species used for ecotoxicological testing. Oberdörster (2004b) showed the 48 hours LC_{50} in *Daphnia magna* for uncoated water soluble fullerenes nC_{60} is 800 ppb. E. Oberdörster (2004a) demonstrated a significant increase of lipid peroxidation in the brain and glutathione depletion in the gill of juvenile largemouth bass *(Micropterus salmoides*) after exposure for 48 hours to 0.5 ppm of fullerenes $nC_{60.}$, but the increase was not significant at 1 ppm.

In their follow-up studies, Oberdörster G et al. (2005) report the possible molecular mechanism of these observations. The bactericidal properties of fullerenes have been reported by Yamakoshi et al (2003). However, considering that a large number of the above cited human toxicology studies have examined the uptake and effects of nanoparticles at a cellular level, it can be hypothesized that these observations may also hold for species other than humans. As such the reports may be useful for the assessment of the effects on environmental species. Work to support this hypothesis is needed. Careful examination and interpretation of existing data and careful planning of new research is, however, required if we are to establish the true ecotoxicity of nanoparticles, and the differences with conventional forms of the substances.

3.8.4 Toxicological Testing

The consideration of dose response relationships in the toxicology of nanoparticles poses a significant problem. In toxicology the paradigm exists that health effects are correlated to the mass of the agent to which the individual is exposed, resulting in an accumulated mass as internal or organ dose/exposure. For nanoparticles the concentration number and the resulting total surface area determine the interactions with biological systems. Therefore the surface area and number concentration appear to be more reasonable parameters for doses in terms of exposure (Brown et al 2001, Oberdörster G et al 2000, Höhr et al 2002). The increase in lung inflammation for nanoparticles compared to fine particles was noted when doses were expressed as mass. In contrast when doses were expressed as surface area similar responses were observed for both fine and nanoparticles (Oberdörster G et al 2000). Also when comparing toxicity differences between TiO_2 and $BaSO_4$, dose response relationships were similar when compared at a dose expressed as surface area burden (Tran et al 2000). For TiO_2 nanoparticles, significant species differences were noted after inhalation exposure with rats, mice and hamsters, the rat being the most sensitive (Bermudez et al 2004). Pulmonary responses and dosimetry (particle retention and overload) were considered to be responsible for these differences.

Several hypotheses were proposed for the adverse health effects of nanoparticles as part of ambient air pollution (reviewed by Kreyling et al 2004). These hypotheses for adverse health effects of nanoparticles include:

Particle characteristics:

- Importance of large surface area for interactions with cells and tissues
- Complex formation with biomolecules
- Formation of increased level of radical species compared to larger particles
- Increased induction of oxidative stress
- Induction of cellular DNA damage
- Induction of oxidative stress by lipid peroxidation

Distribution

- Deposition characteristics dependent on size
- Uptake by cells of respiratory epithelium
- Increased access to interstitial spaces
- Access to systemic circulation

Organ system effects, including effects on immune and inflammatory systems

- Reduced function of macrophages, reduced phagocytosis of particles themselves, reduced macrophage mobility and cytoskeletal dysfunction
- Increased pro-inflammatory activity and induction of cytokines and other mediators
- Adverse effects on cardiac functions and vascular homeostasis

Some hypotheses raised for ambient air nanoparticles may be of limited or no relevance for engineered nanoparticles, such as adsorbance of toxic substances. Although such adsorbance cannot be ruled out, it is probably of less importance for production and handling facilities of large volumes of engineered nanoparticles compared to the particles

in ambient air. Limitations of the studies cited may be the relatively high doses used, the short periods of time investigated, and/or artefacts occurring during sampling of the particles on filters (Wittmaack et al 2002).

In addition, the use of healthy animal models may hamper the interpretation of the results as some of the effects listed and may only be a risk for susceptible organisms and predisposed individuals, but not to healthy people (Kreyling et al 2004). Age, co-pollutants and a compromised respiratory tract can modify the pulmonary inflammation and oxidative stress induced by carbonaceous nanoparticles (Elder et al 2000). For chronic obstructive pulmonary disease, the generation of free radicals on the surface due to high reactivity of nanoparticles, and the induction of oxidative stress, might contribute to the induction of inflammation (MacNee et al 2003).

In vitro observations with keratinocytes, macrophages and blood monocytes revealed the induction of oxidative stress in these cells after exposure to nanoparticles (Shvedova et al 2003, Brown et al 2004). A role for free radicals and reactive oxygen species was also suggested by *in vitro* studies in which antioxidants were able to block the particle induced release of TNFα from alveolar macrophages (Dick et al 2003). Also macrophage phagocytosis was impaired by nanoparticles (Renwick et al 2001). For TiO_2 and ZnO nanoparticles, oxidative damage to DNA was demonstrated (Dunford et al 1997, Rahman et al 2002), resulting in micronucleus formation and apoptosis.

The type of cell under investigation may also be of importance for the ultimate effect of the particles investigated. For epithelial lung cells, either as cell line or primary rat type 2 cells, the coarse fraction of urban ambient air showed similar or higher potency to induce cytokine release and cytotoxicity compared to the finer fractions (Hetland et al 2004). Human macrophages and osteoblasts showed a different behaviour towards nanotopography surfaces, macrophages showing preference for the nanosurface and being activated, while the osteoblasts moved away from the nanosurfaces (Rice et al 2003).

In view of the specific characteristics demonstrated for nanoparticles and nanoparticle formulations, the assays usually performed for determining toxicity of products may not be sufficient to detect all possible adverse effects of nanoparticles. However, this may not be the case for assessing potential environmental effects given the nature and the simplicity of standard, regulatory tests. Nanoparticles may differ in reactivity and solubility and may interact with all kinds of endogenous proteins, lipids, polysaccharides and cells. Based on experiences in inhalation toxicology, a series of tests was proposed for evaluation of the toxicity of nanoparticles used in drug delivery systems (Borm and Kreyling 2004). These included tests for blood cell damage after intravenous administration, acute phase responses of hepatocytes or lung cells, permeability tests of endothelial cells, for destabilization of atheromatous plaques in animal models for atherosclerosis, for effects on the autonomic nervous system, for adjuvant activity in an immunization model, for immune activation by measuring T cell activity and cytokine induction in lymph nodes, for the determination of surface activity and induction of oxidative stress in cell lines, for toxicity on various cell lines in vitro, and for biopersistance.

3.8.5 Conclusions

Reduction in size to the nanoscale changes the characteristics of particles, primarily due to the increased surface to volume ratio. There are as yet no paradigms to anticipate the significance of any of these changes in characteristics, so the safety evaluation of nanoparticles and nanostructures cannot rely on the toxicological and ecotoxicological profile of the bulk material that has been historically determined. The biological behaviour of nanoparticles is determined by the chemical composition, including coatings on the surface, the decrease in size and corresponding shifts in chemical and physical properties, the associated increase in surface to volume ratio, and the shape. In addition, aggregations of nanoparticles may have an effect on their biological behaviour as well. The dose expressed as surface area or number of particles administered shows a better relationship with biological and/or toxic effects than dose expressed as mass. The biological evaluation of nanoparticles and/or products incorporating nanoparticles should be performed on a case by case basis.

Epidemiological studies on ambient air pollution demonstrate the general adverse effects of particulate matter on humans. However, chemical absorbents on the particulates themselves can be partly responsible for modifying the toxic effects, which limits extrapolation of these results to nanoparticle toxicity.

One mechanism of toxicity of nanoparticles is likely to be induction of reactive oxygen species and the consequential oxidative stress in cells and organs. Testing for interaction of nanoparticles with proteins and various cell types should be considered as part of the toxicological evaluation. Nanoparticle translocation and uptake by the body occurs after inhalation exposure (neuronal uptake, translocation across lung epithelium, and ingestion), oral exposure (ingestion), and dermal exposure depending on the characteristics of the nanoparticle under investigation. With the exception of airborne particles delivered to the lung, information on the biological fate of nanoparticles including distribution, accumulation, metabolism, and organ specific toxicity is still minimal.

3.9 Exposure Scenarios

Most human individuals are routinely exposed to particles in the ambient atmosphere, primarily from diesel fumes. Any combustion process produces nanoparticles in vast numbers from condensation of gases. Initially only about 10 nm in diameter, these rapidly coalesce to produce somewhat larger aggregates of up to about 100 nm, which may remain in the air for days or weeks. The air in a normal room can contain 10,000 to 20,000 nanoparticles.cm^{-3}, whilst these figures can reach 50,000 nanoparticles.cm^{-3} in a wood and 100,000 nanoparticles.cm^{-3} in urban streets. Although the mass concentration of nanoparticles is low, it still amounts to substantial numbers. These concentrations imply that every hour, individuals breath millions of nanoparticles, and it is estimated that at least half of these reach the alveoli. At present it is not known to what extent the engineered nanoparticles contribute to these numbers.

The current best practice for measuring the exposure of an individual to a material present as an aerosol is to use a personal sampling device. Samples collected are subsequently assessed either gravimetrically or via chemical analysis to determine the mass and provide an estimate of time weighted mass concentration. Currently pollution

standards are mass based; the dose by number or area will increase as size decreases. (Kittelson 1998). However for nanometre size aerosols, measurement of mass is not sufficient. Oberdörster et al (2005) showed the tremendous differences in number concentrations and surface areas for particles. (Table 1). The extraordinarily high number concentrations of nanoparticles per given mass may be of toxicological significance when these particles interact with cells and subcellular components. Likewise, their increased surface area per unit mass can be toxicologically important if other characteristics, such as surface chemistry and bulk chemistry, are the same.

Table 1 Particle Number and Particle Surface Area per 10 µgm^{-3} Airborne Particles. (Oberdörster G et al., 2005)

Particle Diameter Nm	Particle Number cm^{-3}	Particle Surface Area $\mu m^2.cm^{-3}$
5	153,000,000	12,000
20	2,400,000	3,016
250	1,200	240
5,000	0.15	12

In general, the use of mass concentration data alone is insufficient for the expression of dose, and the number concentration and / or surface area need to be included. Serita et al (1999) exposed rats to metallic nickel nanoparticles at around the Japanese occupational exposure level (OEL). This OEL was based on particles larger than the nanoscale, but exposure to concentrations around the OEL (1.4 mg.m^{-3}) in the form of nanoparticles caused severe lung injury after a single exposure. This finding supports the concept that surface area is the dose measure that predicts pulmonary response, rather than mass, and this has far reaching potential consequences for occupational standards that are based on mass. Therefore the ideal sampler to measure biologically relevant exposure to nanoparticle aerosols would be a personal sampling device which collects the relevant size fraction and provides either an instantaneous measure of a sample surface area or which facilitates the off-line analysis of the sample to provide a measure of surface area.

3.9.1 Sampling

Sampling of nanoparticles is a challenging task for several reasons. First, the sampling strategy should ensure that the particle collection methods, including location, represent as accurately as possible the real exposure at the site in question and methods should be developed and chosen according to the size and nature of the particles under investigation. Secondly, because of their small mass, separation of nanoparticles from larger particles by inertial impaction can only be achieved at a relatively high pressure drop. Thirdly, considering that typical ambient atmosphere nanoparticle concentrations are less than 1 µg.m^{-3}, collection of filter samples for gravimetric analysis and chemical characterization is only feasible with certain high volume sampling techniques (Sarnat et al 2003). In addition, the discrimination between existing ambient particles and engineered nanoparticles is an important factor in the sampling strategy.

Analysis of relatively large particles in a scanning electron microscope requires relatively little preparation of the samples. At the opposite end of the spectrum, nanometre-diameter particles to be analysed in the transmission electron microscope or scanning transmission electron microscope must be presented without contaminants on a suitably thin electron-transparent support. Inertial collection methods such as gravitational settling and centrifugal collection, are suitable only for particles greater than 1-10 μm in diameter, and are impractical to implement for nanoparticles.

Inertial deposition in impactors is achieved by increasing particle momentum in a high velocity air flow, enabling deposition onto a substrate by rapidly changing the flow direction. Use of low pressure stages in cascade impactors allows the collection of particles as small as 50 nm in devices such as the electrical low pressure impactor. Recent developments in nozzle design have led to hypersonic impactors capable of collecting particles down to 50 nm, and focusing impactors capable in principle of operating below 10 nm. However, deposition forces are necessarily high, leading to the possibility of particle damage. Aerosol samples collected by impaction are generally restricted to a small region of the substrate, thus increasing the probability of particle coincidence, and may be non-uniform with respect to particle size.

Electrostatic deposition allows relatively high deposition velocities, especially at high particle charge-to-mass ratios. Where particles are unlikely to be damaged by the charging mechanism used or the electric fields encountered, relatively gentle and uniform deposition is possible. If particles are charged to their theoretical limit, electrostatic deposition velocities are relatively independent of particle size. However, this limit is difficult to achieve under practical sampling conditions. Under conditions where positive and negative ions may freely attach to aerosol particles, a charge equilibrium is reached that is highly size dependent. The fraction of nanoparticles having a minimum of one charge drops off rapidly with decreasing size, leading to a dramatic fall in deposition velocity. Diffusional or photoelectric charging can be used to increase the average particle charge at small diameters, and electrostatic precipitation can be used effectively for particles larger than 20 nm in diameter.

Below 10-20 nm, diffusion begins to dominate other deposition mechanisms. For particles smaller than 10 nm diffusion is ideally suited to obtaining uniform particle deposits on a range of sampler substrates, although samples may be highly biased towards smaller particles, and are unlikely to contain a significant fraction of particles larger than 20-30 nm. Thermophoresis, the movement of aerosol particles in the presence of a temperature gradient, has the advantage that for a given particle composition, deposition velocity is constant below a size of around 100 nm. Achievable deposition velocities are relatively low, but deposition is gentle and unlikely to influence the physical nature of the particles. Implementation of thermophoresis in a uniform temperature gradient between two horizontal surfaces has enabled uniform deposits of discrete particles from below 5 nm to nearly 1 μm directly on to transmission electron microscope support grids (Maynard 2000).

3.9.2 Exposure Assessment Approaches

The following section is based on human health experiments and observations although some observations may also be relevant for other species.

Inhalation

The respiratory tract acts as a serial filter system and in each of its compartments (nose, larynx, airways, and alveoli) the predominance of characteristic physical mechanisms of particle deposition may change. In addition, these mechanisms significantly change with particle size. Nanoparticles are primarily displaced by Brownian motion and therefore underlie diffusive transport and deposition mechanisms. In practice it means that the smaller the particle the higher is the probability of a particle to reach the epithelium of the lung.

Motor vehicle emissions usually constitute the most significant source of nanoparticles in an urban environment. The relationship to traffic volumes indicates that the accumulation mode particles are associated with emissions from heavy-duty traffic (mainly diesel vehicles) whilst particles in the range 30–60nm show a stronger association with light duty traffic. Both of these size fractions show the anticipated dilution effect with increasing wind speed (Charron and Harrison 2003).

Combustion of fossil fuels, especially in diesel engines, produces waste by-products, including nanoparticles. Today, these combustion waste nanosized particles constitute the most important source of anthropogenic nanoparticles.

Environmental Exposure

Exposure, uptake, distribution and degradation of nanoparticles from the environment have been recently discussed by Oberdörster G et al. (2005). They believe that nanomaterials are likely to enter the environment for several reasons. With nanomaterials now being manufactured in large quantities, it is argued that manufacturing effluent and spillage, use and disposal through landfill, will inevitably result in environmental exposure. Moreover these materials are being used in personal-care products such as cosmetics and sunscreens, which can enter the environment on a continual basis from washing off of consumer products. However, it should be said that currently very little is known about the behaviour of nanoparticles in the environment. One study has shown that iron nanoparticles can travel within ground water over a distance of 20 m and remain reactive for 4-8 weeks (Zhang 2003).

Occupational Exposure

Based on the systematic study by the Institute of Occupational Medicine for the UK Health and Safety Executive it may be assumed that there are a few main industrial activities in which exposure to nanoparticles may occur (HSE 2004):

1. Nanotechnology sector, primary research & development (universities and other research groups and spin-offs);

2. Existing chemical and pharmaceutical companies;

3. Powder handling processes including paints, pigments and cement manufacture;

4. Welding

5. Other processes where the nanoparticles are by-products.

The potential risks following occupational exposure were also discussed in the same report, summarised in Table 2 (HSE 2004)

Table 2 The potential risks following occupational exposure to nanoparticles (HSE 2004)

Synthesis process	Particle formation	Potential inhalation risks	Potential dermal/ingestion risks
Gas phase	In air	Direct leakage from reactor Product recovery Post recovery processing and packaging	Airborne contamination of workplace Handling of product Cleaning/maintenance of plant
Vapour phase	On substrate	Product recovery Post recovery processing and packaging	Dry contamination of workplace Handling of product Cleaning/maintenance of plant
Colloidal	Liquid suspension	Drying of product (processing and spillage)	Spillage/contamination of workplace Handling of product Cleaning/maintenance of plant
Attrition	Liquid suspension	Drying of product (processing and spillage)	Spillage/contamination of workplace Handling of product Cleaning/maintenance of plant

Also in the same report (HSE, 2004) it was estimated that the number of workers in the UK who may be exposed to manufactures nanoparticles in the work environment in the university sector and in emerging nanoparticle companies may be as high as 2,000. Around 100,000 individuals may potentially be exposed to fine powders through various powder handling processes. It is not possible to say what proportion of these may be exposed to nanoparticles. More than 1,000,000 workers in the UK may be exposed to nanoparticles via incidental production in processes such as welding and refining (Aitken

et al 2004). In the U.S., an estimated 2 million people work with nanometre-diameter particles on a regular basis in the development, production, and use of nanomaterials or products, this being based on national industry-specific occupational employment estimates by the U.S. Department of Labor's Bureau of Labor Statistics for the year 2000. If growth in nanotechnology-related industries meets expectations, a similar number of additional workers will be required globally (NIOSH 2005). It should be emphasized that although some industrial processes have involved nanoscale compounds (e.g. carbon black, welding, etc) for decades, occupational exposure data, including size and mass of the particles, is very scarce.

The aim of one major workplace study (BIA 2003) was to gather and catalogue technical measurement information on nanoparticles occurring at different work processes, where those nanoparticles had been released occasionally as by-products of technical processes. Typical examples include welding fumes, metal fumes, soldering fumes, plasma cutting fumes, plasma spraying emissions, polymer fumes, vulcanisation fumes, amorphous silicic acids, powder coating emissions, oil mists, aircraft engine emissions, bakery oven emissions, meat smokery fumes, and particulate diesel motor emissions. The particles were for the most part the products of condensation in thermal and chemical reactions, the primary particles created having a size of only a few nanometres. The most frequently-occurring particle size was between 160 and 300 nm. The total concentration of all particles in the measurement range 14 to 673 nm was between 500,000 and 2,500,000 particles per cm^3. A comparison of the occurrence of nanoparticles in different workplace atmospheres is given Table 3 after Möhlmann (2004).

Table 3 Comparison of nanoparticles in workplace air (Möhlmann, 2004)

Process	Total concentration in measurement range 14-673 nm, (particles.cm-3)	Maximum of number concentration (nm)
Outdoor, office	up to 10 000	
Silicon melt	100 000	280-520
Metal grinding	up to 130 000	17-170
Soldering	up to 400 000	36-64
Plasma cutting	up to 500 000	120-180
Bakery	up to 640 000	32-109
Airport field	up to 700 000	<45
Welding	100 000 up to 40 000 000	40-600

With respect to carbon nanotubes, Maynard et al (2004) carried out a laboratory based study, then complemented by a field study, in which airborne and dermal exposure to single-walled carbon nanotube material (SWCNT) was measured in 4 sampling sites where workers handled unrefined material. Estimates of nanotube concentrations ranged from 0.7 to 53 $\mu g.m^{-3}$. Filter samples indicated that many of the particles may have been compact, rather than having an open, low density structure more generally associated with unprocessed SWCNT. Glove deposits were estimated at between 0.2 and 6 mg per hand.

Exposure to carbon black has been a major concern for decades. Furnace black account for 98% of the worldwide production and has an average aggregate diameter of 80-500

nm and an average primary particle diameter of 17-70 nm. (IARC 1996). The exposure to carbon black dust has been measured in two phases of a large multi-national study (Gardiner et al 1996). The highest mean exposure was experienced by the warehouse packers and they are also most likely to exceed the OES 3.5 $mg.m^{-3}$ The range of means for 14 job titles varied from 0.3 to 10.4 $mg.m^{-3}$. In another study, exposure to inhalable dust in carbon black manufacturing industry was measured during three sampling periods (from 1987 to 1995) in several European countries. Prior to the exposure measurements, all workers were categorized into 14 job titles, which were amalgamated into eight job categories. Average inhalable dust exposure (directly calculated using the exposure data, dropped from 1.3 $mg.m^{-3}$ in Phase I (1987-89) to 0.8 $mg.m^{-3}$ in Phase II (1991-1992) and 0.7 $mg.m^{-3}$ in Phase III, 1994-95, (van Tongeren et al 2000).

Dermal exposure

There is very little data in the literature on potential exposure through the skin, even though nanomaterials have been used in cosmetics and pharmaceuticals for many years. Currently, most of the dermal exposure concerns skin preparations that use nanoparticles. A recent review of dermal exposure issues concluded that there was no evidence to indicate specific health problems are currently arising form dermal penetration of nanoparticles (HSE 2004).

In theory, harmful effects arising from skin exposure may either occur locally within the skin or alternatively the substance may be absorbed through the skin and disseminate via the bloodstream, possibly causing systemic effects, although there is no evidence of this as yet. Most studies concerning penetration of nanoparticles into the skin have focused on whether or not drugs penetrate through the skin using different formulations containing chemicals and/or particulate material as a vehicle. The main types of particulate materials commonly used are liposomes, poorly soluble solid materials such as TiO_2, ZnO_2 and polymer particulates and submicron emulsion particles such as solid lipid nanoparticles.

There is only limited data on the fate of nanoparticles of titanium dioxide when used in sunscreens and other products on the skin and it appears unlikely that this does not penetrate beyond the dermis. The investigations of Schulz et al. using optical and electron microscopy proved that neither surfacecharacteristics, particle size nor shape of the micronised pigments result in any dermal absorption of this substance. Micronised titanium dioxide is solely deposited on the outermost surface of the stratum corneum and has not been detected by light and electron microscopy in deeper stratum corneum layers, the human epidermis and dermis (Schulz et al 2002).

Ingestion exposure

It was already recognized in 1926 (by Kumagai cited by Salata 2004) that particles could translocate from the lumen of the intestinal tract via aggregations of intestinal lymphatic tissue (Peyer's patches), containing M cells. It is now known that uptake of inert particles can occur not only through immune cells present in Peyers' patches but also through enterocytes, and to lesser extent across para-cellular pathways (Aprahamian et al 1987). However, once again data in the literature on potential exposure through the GI tract are very scarce.

Szenkuti (1997) observed that cationic nanometre-sized latex particles became entrapped in the negatively charged mucus, whereas repulsive carboxylated fluorescent latex nanoparticles were able to diffuse across this layer. The smaller the particle diameter the faster they could permeate the mucus to reach the colonic enterocytes; 14 nm diameter permeated within 2 min, 415 nm particles took 30 min, while 1000 nm particles were unable to cross this barrier.

After oral gavage for several days, a sparse accumulation of charged latex particulates in the lamina propria was found compared to uncharged latex nanoparticles in the same size range (Jani et al 1989). The same authors (Jani at el 1990) investigated the body distribution after translocation of polystyrene particles ranging from 50 nm to 3000 nm. Rats were fed by gavage daily for 10 days at a dose of 1,25 $mg.kg^{-1}$. It was found that as much as 34% and 26% of the 50 nm and 100 nm particles were absorbed, respectively. Those larger than 300 nm were absent from the blood. No particles were detected in heart or lung tissue.

3.9.3 Conclusions

There is no clear opinion on which parameter(s) should be measured as a most appropriate measure of assessing exposure (mass/number/surface area). There is inadequate portable instrumentation for nanoparticles exposure available. New sampling techniques and strategies for exposure assessment at workplace and environment should be elaborated. The possibility of establishing of Occupational Exposure Limits for chemicals in the form of nanoparticles should be considered.

3.10 Risk Assessment Methodologies

3.10.1 Introduction

Nanoparticle forms of various chemicals (metals, carbon, other inorganic and organic chemicals) are being developed to produce new products that have properties that are qualitatively or quantitatively different from their other physical forms. It would not be surprising, therefore, if their interactions with and in biological systems are also altered. This section addresses the methodologies that may be used to assess the risks to man and the environment arising from the normal manufacture, use and disposal of nanotechnology products. It is recognised that the release of nanoparticles may be associated with abnormal events such as an explosion, spillage or equipment malfunction, but these are not considered further in this Opinion since these were not included in the questions asked of SCENIHR.

The first issue to consider in any discussion of the methodology required to assess the risks from nanoparticles to man or to the environment is the size range, shape and composition of the nanoparticles. For the purposes of this discussion a nanoparticle is considered to be a particle of 100 nm or less (in either a solid or liquid form) to which humans or the environment may be directly or indirectly exposed. In principle, nanoparticles can be manufactured from almost any chemical. However, the majority of the current limited evidence on the behaviour in biological systems is mainly limited to transition metals, silicon, carbon (nanotubes, fullerenes) metal oxides and a few agents that have been selected as potential delivery systems for pharmaceutical agents. Before addressing the possible risk to humans and the environment from nanoparticles it is

necessary to summarise how their biological properties could differ qualitatively or quantitatively from those of chemical and biological substances in other physical forms. Three situations may be distinguished. The hazard is due a) solely to the substance being in nanoparticle form, b) principally to the chemical composition of the particle and c) combination of a) and b) It should be noted that because of the restricted range of nanoparticle types whose biological properties have been studied to date, it is uncertain whether or not the findings are representative of nanoparticles in general. The possible stages in the fate of a nanoparticle once released are set out in Figure 3.

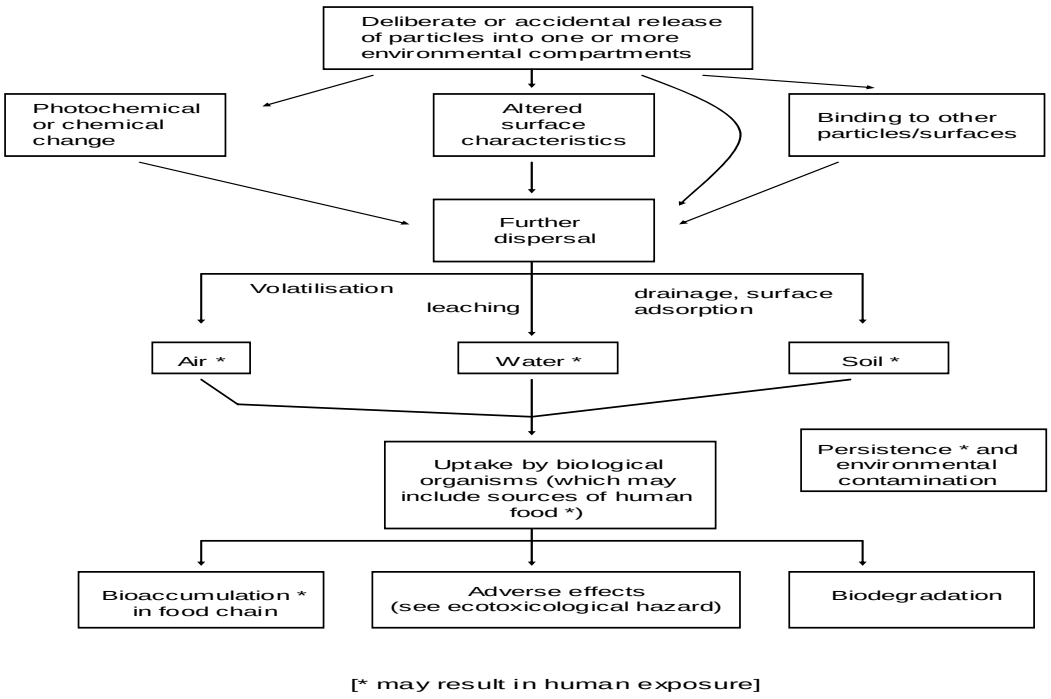


Figure 3 Fate of nanoparticles in the environment

3.10.2 General Exposure Considerations

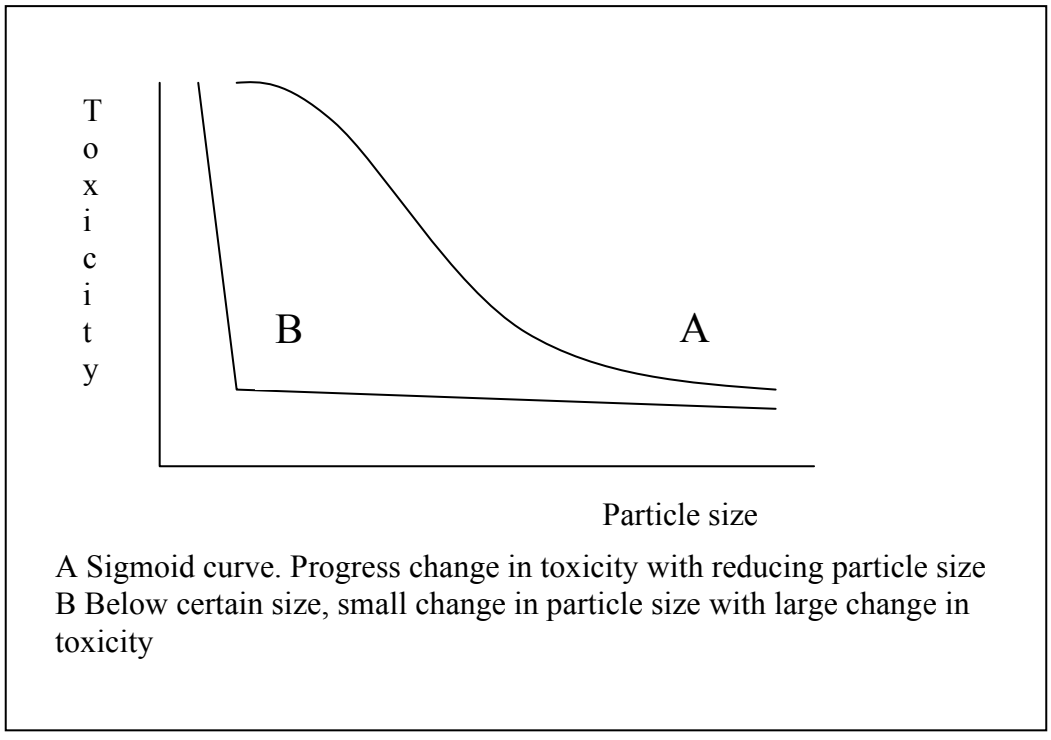
Route of exposure is the first consideration in developing the methodology to be used . Much of the published human toxicological and epidemiology data relates to airborne exposure. However there is a variety of additional routes by which man can be exposed to nanoparticles that may need to be considered including:

- Ingestion (foods, food additives and contaminants, various medicinal agents)
- Topical contact (surface finishing products, contaminants, cosmetics)
- Injection or implantation (some medicinal products)

Based on the information provided in the previous sections, it is evident that particle size may influence the biological properties of a substance in a number of different ways. With respect of exposure there is evidence that nanoparticles may be able to penetrate

cell membranes and thereby enter various cell types, whereas larger particles may be excluded. If a nanoparticle can penetrate cell membranes, it may be assumed that nanoparticles have the potential to reach other organs in addition to those which are the portals of entry.

Most of the work on this topic is being conducted by the pharmaceutical industry because of the potential to improve drug delivery to target tissues. There is very little information in the published literature on how nanoparticles may be distributed within cells once absorbed. There is some evidence that titanium dioxide nanoparticles are widely distributed in cells and are not necessarily membrane bound. In another study on endothelial cells using nanoparticles of poly DL lactide-co-glycolide polymer containing serum albumin, concentration of the nanoparticles was shown in the cytoplasm (Davda and Labhasetwar 2002). The extent to which the distribution is substance-specific is unclear. There is evidence that airborne nanoparticles, in contrast to larger particles, are able, via the nose, to pass along the olfactory nerve and enter the brain (Oberdörster G et al, 2004b). Following their ingestion, nanoparticles may be taken up by the Peyers patches in the intestine. It is not known how well absorbed nanoparticles can penetrate the fenestrated capillaries.



A Sigmoid curve. Progress change in toxicity with reducing particle size
B Below certain size, small change in particle size with large change in toxicity

Figure 4 Possible relationship between particle size and toxicity

To date there is no good evidence of a specific particle size, shape and surface charge at which altered penetration of cell membranes occurs. It is very important from a risk assessment viewpoint to understand whether the relationship between particle size and effect, indicated in Figure 4, is best represented by:

- a sigmoid curve, or

- involves a sharp change. It is biologically plausible that as the particle size reduces, a sudden increase in absorption and/or toxicity arises. It is important to establish whether this is the typical situation or specific to certain nanoparticles. In the absence of adequate data to the contrary, this particle size response model should be used as the default position.

It has also been found that for certain nanoparticles the clearance mechanisms may be less effective than that for larger particles. For example, impaired phagocytosis has been observed in a macrophage cell line containing nanoparticles (Renwick et al 2001). If this finding reflects a more general phenomenon, nanoparticles would need to be considered to have the potential for bioaccumulation in humans and possibly in other species and in the environment.

There have been even fewer studies on the behaviour of nanoparticles in the environment. It is probable that nanoparticles in ambient air will be widely dispersed unless they react with other components in the air. It needs to be established whether nanoparticles of a chemical, by virtue of their size and surface properties, may partition and distribute differently in the environment compared to other physical and chemical forms, for example (see Figure 3). It is also vital to establish whether or not there is a tendency for substances in nanoparticle form to be more or less persistent in the environment than larger sized counterparts.

What is clear from the published literature on human toxicology is that the expression of the exposure dose in terms of unit weight, which is the established practice in toxicology, is often not appropriate when studying the toxicity of nanoparticles. Instead either total surface area, or number of particles, or a combination of surface area and number of particles, should be used.

3.10.3 Hazard Considerations

In view of the potential for nanoparticles to penetrate proteins, nucleic acids and other biological molecules, it is possible that unique adverse effects never previously observed for chemicals in other physical forms could occur. There is no evidence that this is the case in practice. However, the methodology to evaluate the hazards needs to incorporate the possibility.

The main source of information on the potential for adverse human health effects with nanoparticles are the epidemiological studies of airborne particles in ambient air. These have shown that smaller particles of low solubility (less than 1μm) are substantially more toxic than larger particles. In part this is due to the fact that the dose in particle number terms is much higher per unit weight for small particles. There is evidence that these particles also penetrate the alveolar cell barrier more effectively than larger particles. It should be noted that the majority of studies to date have been confined to particle sizes from 0.1 to 10 μm. The size of airborne particles has also been reported to influence perceptions of adverse effects. For example Keady and Halvorsen (2000) have shown that the airborne level of nanoparticles in offices correlates directly with complaints of sick building syndrome. It has been found that as far as ambient air pollution with fine particles is concerned, there is a population subgroup that is much more sensitive to the adverse effects than the public as a whole. This subgroup includes individuals with severe chronic respiratory and heart disease. Whether the same population would be

much more sensitive to other forms of airborne nanoparticle is uncertain but this must be considered to be a real possibility.

There is little information on trends in species differences in either the toxicokinetics or toxicodynamics of nanoparticles and a cautious approach must be adopted in the extrapolation of findings in animals to man. There is some evidence from published animal studies that nanoparticles can have a greater toxicity or, perhaps, a different toxicity compared to larger particles of the same substance. Lam et al (2004) and Warheit et al (2004) have published the results of studies of the toxicity of single-wall carbon nanotubes in mice and rats respectively. Using an intratracheal route of administration they compared different means of nanotube production with effects of carbon black and quartz particles. In the Lam et al study the nanotubes were found to produce dose dependent lung lesions. The effects of carbon black were distinctively different. The study by Warheit et al was more comprehensive. It showed multifocal pulmonary granuloma but without evidence of ongoing pulmonary inflammation or cellular proliferation. These effects are different from those of quartz, carbon black and graphite. The conclusion from these two studies is that carbon nanotubes have different toxicological properties from other forms of carbon (Dreher 2004). This supports the case for a separate/ additional risk assessment of substances that are in nanoparticle form.

These differences may be attributable to the fact that they have a much greater surface area to weight ratio than larger particles and, as a consequence, they tend to be more chemically reactive and bind other substances to their surface more effectively.

Other Environmental Species

There is almost no published literature on the effects of nanoparticles on environmental species. Colvin's (2003) discussion on the potential impact of engineered materials demonstrates the lack of data on the exposure and effects of nanoparticles. To date, only a few studies have been carried out with species used for ecotoxicological testing. Oberdörster (2004b) showed the 48 hours LC_{50} in *Daphnia magna* for uncoated water soluble fullerenes nC_{60} is 800 ppb. Oberdörster G (2004a) demonstrated a significant increase of lipid peroxidation in the brain and glutathione depletion in the gill of juvenile largemouth bass *(Micropterus salmoides*) after exposure for 48 hours to 0.5 ppm of fullerenes $nC_{60.}$, but the increase was not significant at 1 ppm. In their follow-up studies, Oberdörster E et al. (2005) report the possible molecular mechanism of these observations. The bactericidal properties of fullerenes have been reported by Kai et al (2003). However, a number of the above cited human toxicology studies have examined the uptake and effects of nanoparticles at a cellular level, it is reasonable to assume that these observations can be extrapolated to environmental species. Work to support this hypothesis is needed. Careful examination and interpretation of existing data and careful planning of new research is required to establish the true ecotoxicity of nanoparticles and the differences with conventional forms of the substances.

Because of the inverse relationship between particle size and surface area, it is imperative that, for various environmental (model) species, (1) dose (or concentration) – effect relationships are established as a function of total surface area and/or number of particles (and surface charge) rather than mass units and (2) a comparison is made between the effects of the conventional and the nanoparticle form(s) of the substance. It should also be recognized that the potential problems associated with persistent insoluble

nanoparticles in the environment may be considerably greater than with human health assessment. The protection goals and endpoints (i.e. protection of individuals vs. protection of populations) of an environmental effect assessment are clearly different than those used of the human health evaluation. As such, next to *in vitro* studies which may help to establish potential differences in the toxic action of nanoparticles and conventional forms of the substance, *in vivo* assays will have to be performed using model species representative of each of environmental compartment (terrestrial, aquatic etc.) and reflecting different exposure routes (water, food-borne, etc.).

3.10.4 Scope of Nanoparticle Risk Assessment

If there is a potential for exposure of humans, other species or the environment to free nanoparticles from a product or process, including disposal processes, some form of risk assessment is required. This is necessary regardless of whether or not the toxicology of the chemical(s) comprising the nanoparticle is well established.

The factors that need to be considered in the risk assessment of a new form of nanoparticle associated with a product or process are set out in Figure 5.

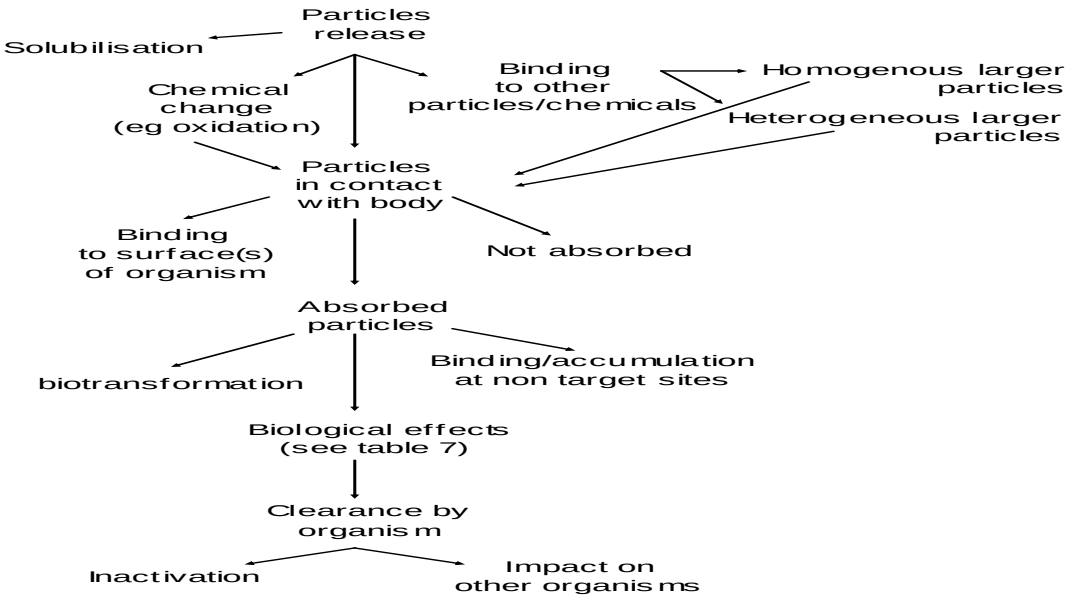


Figure 5 Toxicokinetics of nanoparticles

Depending on the conditions of manufacture, formulation, use and final disposal, a risk assessment of nanoparticles may need to address:

- Worker safety during the manufacture of nanoparticles. It is noted that typically workers are exposed to higher levels of chemicals and for more prolonged periods of time compared to the general population and this will probably be the case for nanoparticles production.

- Safety of consumers using products that contain nanoparticles.

- Safety of local human populations due to chronic or acute release of nanoparticles from manufacturing and /or processing facilities.

- The impact on the environment per se resulting from production, formulation and use, and on the potential for human re-exposure through the environment. Particular attention is required for products that are deliberately used in nanoparticle form in the environment, e.g. biocides, environment improving agents.

- The environmental and human health risks involved in the disposal or recycling of nanoparticle dependant products. This includes the potential for nanoparticles to escape from 'contained' waste disposal sites as well as their impact on sewage treatment plants.

One or more of these risk assessments may be omitted if there are valid reasons to conclude that that no exposure will occur. In principle, the traditional risk assessment procedure is an appropriate tool for assessing the risks from exposure to nanoparticles under specified exposure conditions. However it has to be recognized that the public expectation of new or emerging technologies is that higher requirements for safety are needed than for tried and tested technologies. Failure to meet the expectations may result in public fear or even rejection of nanotechnology based products.

The traditional risk assessment methodology comprises the following stages:

i) Exposure assessment

ii) Hazard identification

iii) Hazard characterization

iv) Risk characterization

This framework has not yet been applied to nanoparticles generally either in terms of their potential human or environmental impacts for a number of related reasons. There is an unclear situation in regard to regulatory requirements for risk assessment. As a consequence there are no official guidelines on what constitutes an appropriate testing regimen. The manufacture of nanoparticles commercially is relatively new and there is very limited relevant epidemiology or environmental monitoring data available. The focus has been on production expressed as mass (COM 67/548, see REACH for example) rather than particle size; this may severely underestimate the potential contribution of nanoparticles to overall risk posed by the substance.

It is evident from the foregoing discussion that there is already sufficient data to conclude that, from a risk assessment point of view and for some types of nanoparticle at least, it is not valid to rely entirely on toxicological findings from testing the component of a nanoparticle of interest in another physical form.

Three situations may be distinguished:

i) A substantial amount of good quality human and environmental hazard and exposure information on the substance (in its 'conventional' form) that comprises the nanoparticle of interest already exists. In this case the question is what further information is required to supplement this in order to provide the necessary confidence in the safety of the nanoparticle product. It must be reiterated that it is not scientifically valid to rely exclusively on the properties of the chemical in other physical forms for risk assessment purposes.

ii) The substance has already been produced in a one form of nanoparticle. A new form of nanoparticle is then produced. Is it necessary in this case to repeat all the studies required by i) for this new particle form?

iii) Limited or no information is available on the biological properties of the substance that comprise the nanoparticle. The question that needs to be addressed in this case is what is the full package of tests that needs to be conducted assuming that human and environmental exposure will only be to its nanoparticle form.

In the following text the emphasis is on the first situation, namely where there is accessible suitable data on the human and environmental hazards of the substance that comprises the nanoparticle.

3.10.5 Exposure Assessment Methodology

An algorithm is provided in Figure 6 setting out the key steps in the exposure assessment. Methodology is required to identify how nanoparticles distribute in environmental compartments and in human tissues.

Risk assessment may be applied either to a chemical (or a mixture of chemicals) in nanoparticle form and/or to a product in which this nanoparticle form is incorporated. The former approach has the benefit that if the identified risk is deemed to be acceptable, the risk assessment of the products in which it is incorporated can be restricted, unless there are reasons to assume that the exposure or the toxicology may be significantly different due the other components of the product. In the discussion below the term product is used for both the chemical itself and any item it may be incorporated in.

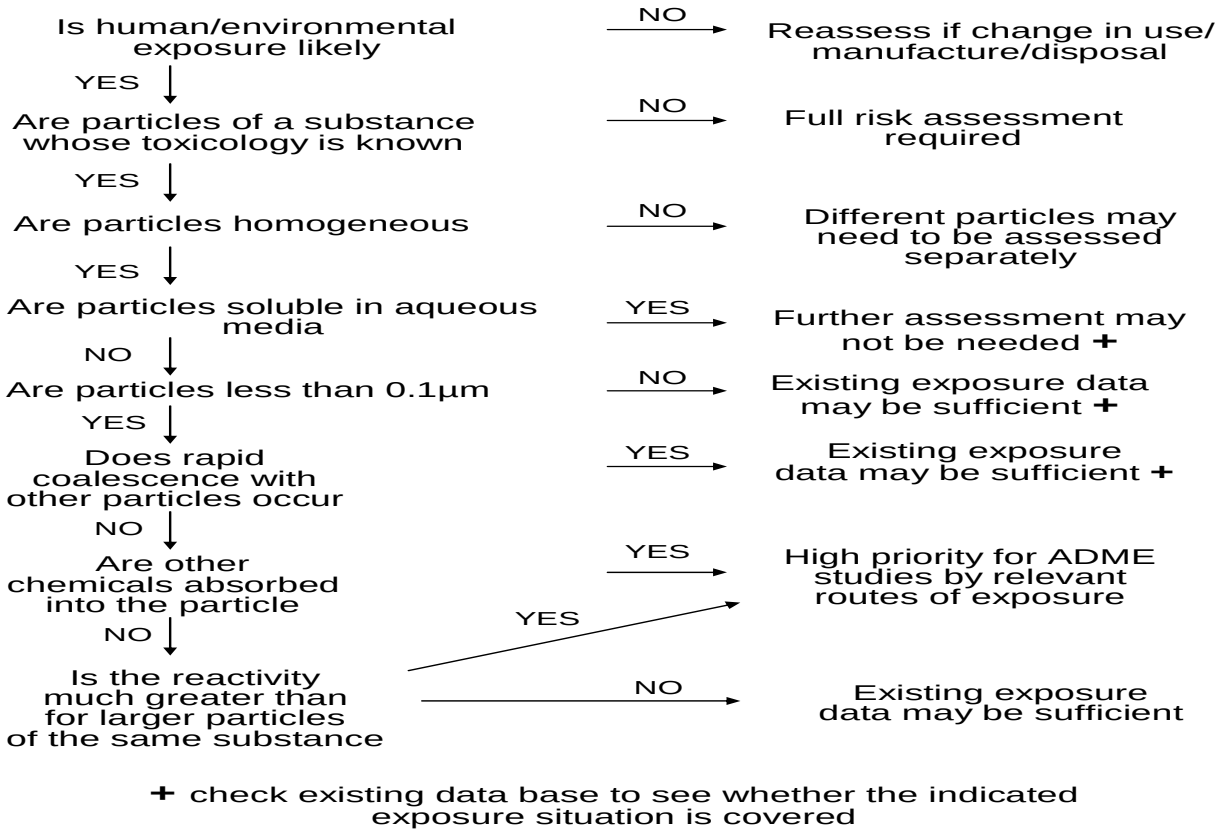


Figure 6 Exposure assessment algorithm

It is important for risk assessment purposes to specify clearly at the outset the following factors.

Product specification (physical chemical properties)

The amount that is expected to be produced along with the anticipated uses and proposed routes for disposal/recycling of the product(s) at the end of its useful life. Is probable that different manufacturers will produce nanoparticles of rather similar chemical composition that are not identical in all their properties. It is therefore vital that the specification of the nanoparticle form is thorough and comprehensive. The description should include:

- The chemical composition of the nanoparticle including formulation components and impurities, surface chemistry, acidity/basicity, redox potential, reactivity (redox, photoreactivity etc.) and the nature of any surface coating or adsorbed species.

- The particle size range (and distribution) to which humans and/or the environment will be exposed, along with information on other physical characteristics, e.g. shape, density, surface area and charge, solubility, porosity, roughness morphology, crystallinity and magnetic properties. Note that the nature of the nanoparticle to which organisms or individuals are exposed may differ, for example between workers, consumers and the environment and might also differ from the particle size distribution

in the product itself (NB This size range must also have been used in hazard assessment tests).

- The extent to which the released particles are soluble in aqueous media, and/or biodegradable. This is likely to be a major factor in limiting their accumulation and persistence in man and the environment.

- Their chemical and physical stability under relevant environmental conditions including potential for coalescence and/or degradation (along with the identification of the degradation products).

Intended use along with the identification of each of the likely exposure scenarios (including potential for accidental exposure)

- Both normal and high level use situations need to be identified in order to assess emission routes, levels and duration of human exposure and the release and distribution in the various environmental compartments. This may need to include possible misuses and accidents that could result in substantial human and /or environmental exposure although this is beyond the scope of this Opinion.

- Potential methods of disposal of the product at the end of its use and the exposure consequences for the environment should be considered.

Examination of human exposure

- Identification and quantification of relevant exposure.

- Determination of the absorption of the nanoparticle by the appropriate route(s) of exposure at relevant doses and dose rates, including all possible translocation routes.

- Identification of the metabolic fate. This includes the characterisation and quantification of nanoparticles in body tissues.

- Examination of the potential for bioaccumulation following repeated exposure to the nanoparticles.

If there is good data on the uptake, metabolism, distribution and excretion of the substance in other physical forms it may be sufficient to demonstrate that uptake and clearance is comparable for the nanoparticle form using the appropriate route of exposure. If this is demonstrated, then only limited hazard identification and characterisation may be needed.

Examination of environmental exposure

- Identification and quantification of relevant exposure

- Determination of the environmental release pattern (and quantities), the distribution (including the mobility and specific ‘sinks’) and fate (including persistence) of the nanoparticle in the various environmental compartments. For metal and metal oxide nanoparticles assessment of the dissolution rates and

speciation in the environmental compartment will be key to understanding the fate and ultimately the bioavailability of the substance. Attention should be given to those nanoparticles that are designed to be deliberately released into the environment (for example agents used to clean up chemical spillages) and the waste products of nanotechnology.

- Establishment of concentrations (calculated and/or measured), in terms of particle surface and or number, in the different environmental compartments.

- Examination of the potential for bioaccumulation in different aquatic and terrestrial species and possibly the potential for biomagnification in the different environmental compartments.

Data demonstrating that the above processes and characteristics of the nanoparticle are similar to that of the conventional substance may lead to a reduction of data needs for the risk assessment.

Summary

It is unclear at the present time the extent to which the toxicokinetics, the environmental distribution and fate of nanoparticles can be predicted from knowledge of their physicochemical properties. In view of the limited range of substances as yet produced in nanoparticle form and the potential for most chemical substances or mixtures to be produced in this form, caution needs to be used in extrapolation from published data.

A particular concern is the potential for persistence of nanoparticles in humans and the potential for bioaccumulation in the environment. In humans, and other species, there is concern as to whether the clearance mechanisms for larger particles are as efficient in dealing with nanoparticles. In the environment there is concern of the possible differences in distribution of nanoparticles both in air, aquatic and terrestrial compartments.

3.10.6 Hazard Identification and Hazard Characterization Methodology

It is assumed that the range and type of adverse effects that could arise from exposure to nanoparticles is likely to be similar to that identified for chemicals in other physical forms. If this assumption is correct, there would be no reason to change existing well established toxicity testing protocols. If it is not, additional endpoints may need to be considered for the toxicological assessment of nanoparticles.

A number of mechanisms by which nanoparticles may exert toxicity have been proposed and these are summarised in Figure 7. The critical issue to be resolved is whether the hazard is due principally to;

a) the toxicological properties of the chemical(s) that comprise the core of the nanoparticle,

b) the much greater relative surface area of the nanoparticle form and, consequently, the greater potential reactivity, or

c) the potential, due to the enhanced surface area and possible surface reactivity, for other chemicals of concern to be absorbed onto the nanoparticles.

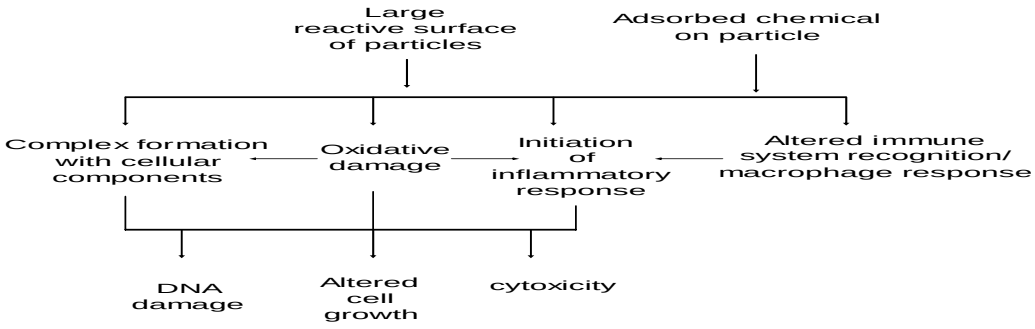


Figure 7 Algorithm of toxicodynamics of nanoparticles

In reality, a combination of these factors may be expressed. In the case of a), similar properties to the chemical in other physical forms could be anticipated. However, if the distribution of the nanoparticles in the human body or in environmental species is very different, this may result in altered toxicological profile. In the case of b) and c) the nanoparticle form would be anticipated to have altered toxicological properties. If chemicals of toxicological concern are likely to be adsorbed onto nanoparticles, the potential for the release of these chemicals will need to be addressed.

With chemicals for which the toxicology is well documented and where the toxicokinetics of the nanoparticle form are similar to those of the chemical in other physical forms, a screening test battery could be introduced to establish whether b) or c) are significant to the hazard profile.

This test battery could comprise mainly in vitro and chemical tests. However, no appropriate tests are currently available.

With those chemicals for which the toxicological properties of other physical forms are well established, a testing strategy (screening) is needed that will identify whether or not the nanoparticle form will or will not cause significantly different adverse effects. The proposed approach is set out in Figure.8. The selection of this test battery should be informed by knowledge of the chemical, physical and biological properties, along with data on the same chemical in other physical forms. *In vitro* tests could in principle play an important role in this screening process. If there are substantial differences between the nanoparticle form and other physical forms of the chemical, then the regulatory guidelines for testing of a new chemical/ particular type of product for its effects on human health and on the environment should be followed. (EU Technical Guidance Document, European Commission 2003).

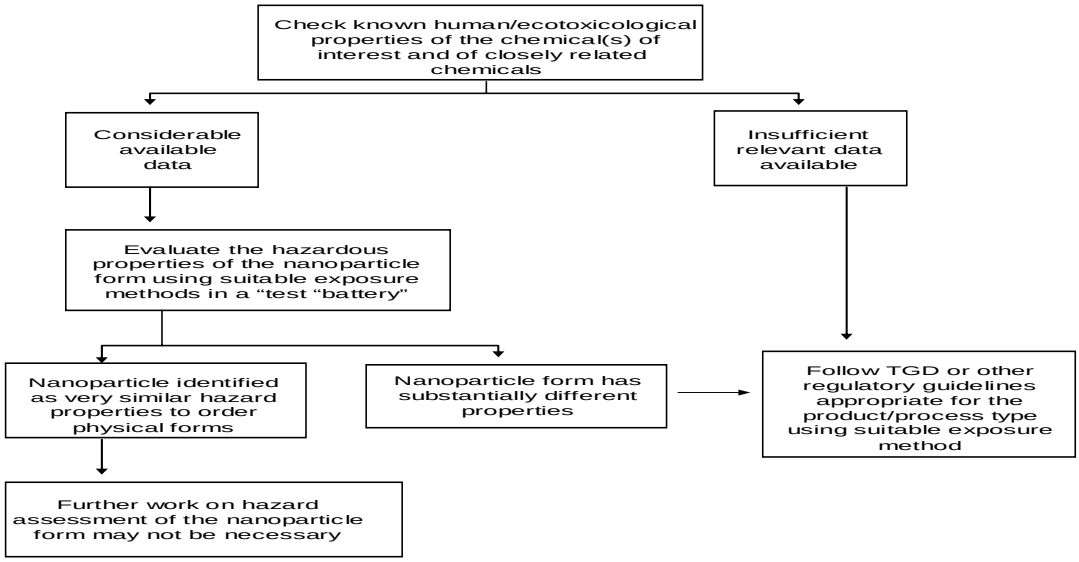


Figure 8 Hazard identification

At this stage in establishing the knowledge base for nanoparticles, it would be helpful to bench mark studies against substances with very well understood toxicology in man, such as quartz and asbestos.

In principle *in vitro* studies combined with information on the surface chemistry could provide an important early indicator of the differences or similarities in potential hazard between the nanoparticle form of a substance and other physicochemical forms. However, characterisation of the uptake, distribution, deposition and retention of nanoparticles and the comparison with their larger size counterparts may require an in vivo approach.

3.10.7 Risk Characterization and Integrated Risk Assessment

As discussed above the presentation of a chemical(s) in nanoparticle form may result in changes in both exposure (including environmental fate and persistence, uptake, metabolism, clearance and bioaccumulation) and the nature and magnitude of the adverse effects. Due to the lack of available data on the risk characterisation of different nanoparticle-based products, no generic conclusions are possible at this stage. Consequently, each product and process that involves nanoparticles must be considered separately in terms of:

- Worker safety during the manufacture of nanoparticles.
- Safety of consumers using products that contain nanoparticles.
- Safety of local populations due to chronic or acute release of nanoparticles from manufacturing and /or processing facilities.

- The impact on the various environmental compartments per se resulting from production, formulation and use, and on the potential for human re-exposure through the environment.
- The environmental and human health risks involved in the disposal or recycling of nanoparticle dependant products.

In the absence of suitable hazard data a precautionary approach may need to be adopted for nanoparticles which are likely to be highly biopersistent in humans and/or in environmental species. It should also be noted that there is no reliable information on the effect of the simultaneous exposure to multiple forms of nanoparticles, where it would be appropriate to assume the effects are additive, or on the interaction between nanoparticles and other stressors (either physical, chemical or biological), which should be considered on a case-by-case basis.

3.10.8 Critical gaps in knowledge required for risk assessment purposes

There is a paucity of information in a number of areas that are fundamental to the development of detailed guidelines on the risk assessment of nanoparticles. These include:

i) Protocols need to be established that enable the release of nanoparticles from a very wide range of production processes, formulation and use of products to be assessed

ii) Whether it is possible to extrapolate from the toxicology of non-nano sized fibres, particles and other physical forms of the same substance to nanosized materials, and between nanoparticles of different size ranges.

iii) The actual measured range of exposure levels (to man and the environment) experienced during use of nanoparticle based products. This will require the development of new measurement techniques for routine use.

iv) Information on the health of workers involved in the manufacture and processing of nanoparticles, since this group may receive the greatest exposure to engineered nanoparticles.

v) Information and measurement of environmental fate, distribution and, persistence (including bioaccumulation) of nanoparticles

vi) Effects of nanoparticles on various environmental species of each of the environmental compartments and representative of different trophic levels and different exposure (uptake) routes.

vii) There is a lack of background data on the current and historic exposure of humans and environmental species to nanoparticles. Such information is important to the assessment of a possible additional risk from exposure to nanoparticles arising from the development of nanotechnologies.

viii) Information on the possibility that simultaneous exposure to different particles could result in additive effects.

3.10.9 Regulatory and Other Aspects Related to Risk Assessment

The regulation of products containing nanoparticles based on tonnage, as proposed for existing chemicals under REACH, needs to be considered further because there are many more nanoparticles to the tonne than is the case for larger particles, and their behaviour in the body and in the environment may be different. If the nanoparticle form of a chemical does have distinctly different properties in biological systems from other physical forms of the same chemical, it will be necessary to readily identify the nanoparticle form of each chemical for the purposes of hazard warning labels etc. One approach to ensure that the effects of the nanoparticle form of a chemical is properly assessed would be to have a unique identification for it, either assigning different CAS numbers to the nanoparticle form, or adding a code (CAS-NP50) to existing CAS numbers leaving the CAS number for identifying similar chemical compounds .

It is also inappropriate to assume that current workplace exposure standards for dusts can be applied directly to the nanoparticle form of the dust component. New standards will therefore need to be considered. Similarly, classification and labelling for human health and the environment may need to be reconsidered.

3.10.10 Other Needed Developments

In order that nanotechnology and nanomaterials can be developed responsibly, with optimization of benefits and minimization of risks, international cooperation on identifying and resolving gaps in knowledge is required. It is recognized that a major barrier to progress in this area is the confidential nature of much of the research on nanoparticles. Means of facilitating co-operation with industry to fill some of the critical knowledge gaps for risk assessment purposes need to be found to avoid the experience of the biotechnology industry of public perception of the risks.

There is an urgent need for a harmonized terminology/ nomenclature for defining the physical characteristics of nanoparticles and their general properties. For the further development of risk assessment, standardisation of testing methodologies is needed to identify exposure scenarios and potential hazards of nanoparticles. In addition, the availability of reference materials would be of high importance to function as benchmark for adverse effects.

A transparent framework for risk benefit analysis should also be developed that is able to achieve wide acceptability

3.10.11 Conclusions

There is insufficient data available to identify any generic rules governing the likely toxicology and ecotoxicology of nanoparticles in general.

In the absence of data to the contrary it cannot be assumed, for risk assessment purposes, that the nanoparticle form of a chemical(s) has similar effects on biological systems to those of the same chemical in other physical forms. To maintain a high level of public health, occupational health and environmental protection in the European Union, it is essential that a specific risk assessment is conducted along the lines proposed above if

there is any potential for humans and the environment to be exposed to particular forms of nanoparticles.

Exposure dose needs to be defined in terms of number of particles and/or total surface area rather than the conventional use of mass. Change in the size/shape and other physicochemical properties of a nanoparticle could result in changes in the adverse effects. Therefore it is essential to specify the precise size and other characteristics of each nanoparticle product in order to conduct a reliable risk assessment.

A framework is proposed that enables both human and environmental risk assessment to be targeted and avoids unnecessary testing.

A number of the conventional toxicity tests may require some modification for the assessment of nanoparticles in order to ensure that the exposure conditions simulate realistic exposure scenarios and that endpoints are directly associated with the nanoparticles to be assessed.

These conclusions have very important regulatory implications.

3.11 Prioritisation of Needs in Knowledge

In general, and in spite of a rapidly increasing number of scientific publications dealing with nanoscience and nanotechnology, there is insufficient knowledge and data concerning nanoparticle characterisation, their detection and measurement, the fate (and especially the persistence) of nanoparticles in humans and in the environment, and all aspects of toxicology and environmental toxicology related to nanoparticles, to allow for satisfactory risk assessments for populations and ecosystems to be performed.

The major gaps in knowledge that need to be filled in relation to improved risk assessment for the products of nanotoxicology include the following. These are cited in a logical order starting with nanoproduct manufacture through human and environmental exposure to the toxicology and fate of nanoparticles. All of these areas require urgent attention. However, it is emphasised that the area in most need of attention is that concerned with the identification of exposure levels, both to man and the environment, which will require new and modified measurement techniques.

- The characterisation of the mechanisms and kinetics of the release of nanoparticles from a very wide range of production processes, formulations and uses of the products of nanotechnology.
- The actual range of exposure levels, both to man and to the environment, experienced during use of nanoparticle based products.
- The extent to which it is possible to extrapolate from the toxicology of non-nano sized fibres, particles and other physical forms of the same substance to the toxicology of nanosized materials.
- Toxicokinetic data following exposure at various portals of entry, so that target organs can be identified and doses for hazard assessment determined. This includes dose response data for the target organs, and knowledge of the

subcellular location of nanoparticles and their mechanistic effects at the cellular level.

- Information on the health of workers involved in the manufacture and processing of nanoparticles.

- The fate, distribution and, persistence (including bioaccumulation) of nanoparticles in the environment.

- The effects of nanoparticles on various environmental species, in each of the environmental compartments and representative of different trophic levels and exposure routes.

4. COMMITTEE OPINION

The following conclusions may be drawn from this analysis of the appropriateness of existing methodologies to assess the potential human health and environmental risks associated with engineered and adventitious products of nanotechnology.

It should be recognised that there is insufficient data available at the present time to allow the identification of any systematic rules that govern the toxicological characteristics of all products of nanotechnology. It follows therefore that risk assessment will need to be made on a case by case basis. In order to perform a risk assessment for the application of nanotechnologies, identification of the methodological issues requires consideration of both the exposure and the hazard.

In considering the potential of adverse health risks associated with nanotechnology products, two separate types of nanostructure may be identified, those where the structure itself is a free particle, and those where the nanostructure is an integral feature of a larger object. Although all nanostructures may interact with living systems in ways that may be influenced by the nanoscale characteristics, it is not considered that these nanoscale features of larger objects (for example nanotopographical features on medical devices) pose any additional human health and environmental risks. The situation with free nanoparticles, including agglomerates, is quite different. It is the generation, application, distribution, persistence and toxicological characteristics of free nanoparticles that give rise to concerns over possible human health and environmental risks. These concerns include the physical, chemical or biological degradation of nanocomposites, which potentially releases nanoparticles. For environmental risk analysis, these concerns imply the necessity for life cycle evaluation of these products.

Free nanoparticles may occur naturally, or be the unintentional products of an industrial or domestic process, or they may be specifically engineered for applications which depend on their unique properties. These properties will primarily be influenced by the high surface to volume ratio associated with nanoparticles and the quantum effects that occur in the nanometre range. Careful characterisation of the physico-chemical properties is essential, for which appropriate methodologies must become available for routine use.

Several different exposure scenarios can be identified with respect to nanoparticles. Nanoparticles of natural origin and those generated unintentionally by human activity ensure that all individuals are routinely exposed to nanoparticles throughout life. The principal route of human exposure is by inhalation in view of the presence of nanoparticles in air. The rapidly increasing use of manufactured nanoparticles in consumer products such as cosmetics, and pharmaceutical preparations and food technology implies that dermal, gastrointestinal, and parenteral routes of exposure are becoming more significant. For the environment, release and distribution of nanoparticles may occur through air, water and soil. As such, species living in all environmental compartments may be exposed to these particles. There is an urgent need for exposure data on humans (consumers and workers) and environmental species including micro-organisms.

The evaluation of exposure of individuals and the environment in general to nanoparticles, and therefore of the associated health risks, has been impeded by the difficulty of routine sampling, and of counting and measuring particles that are below the limit of detection by visible light. The use of mass concentration data alone for the expression of dose is insufficient, and number concentrations and surface area metrics are generally more relevant in exposure and risk assessment. This is not incorporated in current regulations. The development of methodologies and equipment that enables routine measurements in various media for representative exposure to free nanoparticles is an important priority.

In considering the hazards associated with nanoparticles, the size, shape and composition, including surface charge and adsorbed species, of the nanoparticles are important. The phenomena of surface modification, aggregation and dissolution or degradation are also significant. Since nanoparticles that are readily soluble in the physiological environment lose their particle specific effects, they only remain of concern if they dissolve into harmful molecules . For particles that are essentially insoluble, there is the possibility of biopersistence, resulting in long term exposure and associated nanoparticle-specific effects. So the characterisation of nanoparticles used in biological evaluations is essential.

There is little published data on the biological behaviour of nanoparticles, including the distribution, accumulation, metabolism and organ specific toxicity. Much of the data that is available concerns the respiratory system where there are experimental data to show that nanoparticles often exert greater toxic effects than larger particles of the same substance at the same mass concentration. Interactions of nanoparticles with biomolecules such as DNA, RNA or proteins are also more likely with decreasing particle size. Although no mechanisms unique to nanoparticles have yet been identified, a mechanism of toxicity for some nanoparticles is the induction of reactive oxygen species and the consequential oxidative stress experienced by cells.

Nanoparticle translocation can occur to a greater extent and to different sites than occurs with larger particles. There can therefore be a systemic distribution and accumulation of such particles. There is evidence that nanoparticles can translocate from their portal of entry and can reach other parts of the body, including the blood and the brain, although again very few studies have been performed and the extent and significance of this translocation is unclear. It is uncertain whether nanoparticles can reach the foetus. Obviously, in medical applications involving parenteral administration of nanoparticles, systemic distribution is probable. At this stage, the evidence of toxicity in man arising from such systemic exposure to manufactured nanoparticles is sparse. Current testing guidelines for the hazard identification and characterisation of chemicals and products do not yet require the identification of the systemic distribution of nanoparticles, although some potentially suitable methods do exist.

The safety evaluation of nanoparticles and nanostructures cannot rely solely on the toxicological profile of the equivalent bulk material. Nanomaterials need to be evaluated for their risk on a case by case basis for each preparartion including the intended use of the material. In carrying out the risk assessment for products of nanotechnology, new testing strategies will be required that will address the product specification, the intended use and the identification of potential exposure scenarios, both human and environmental. Conventional toxicity and ecotoxicity tests have already been shown to

be useful in evaluating the hazards of nanoparticles. However, some methods may require modification and some new testing methods may also be needed. It appears that nanoparticles can exacerbate certain pre-existing medical conditions and may increase susceptibility to some diseases, which may require modification of testing strategies.

There are regulatory and risk management implications of the above analysis, for example, in toxicological testing guidelines, the setting of occupational and environmental quality standards, and in the classification and labelling of products.

International cooperation is needed to address the multiple issues in nanotechnology. Standardisation including the avalability of reference materials/particles is the key issue to come to a mutual understanding in what we are dealing with in terms of risk assessment for the use of nanotechnology.

NOTE The standardisation process for nanotechnology has already been initiated by organisations like ISO and CEN.

In relation to the specific questions asked of SCENIHR:

Question 1

Are existing methodologies appropriate to assess potential and plausible risks associated with different kinds of nanotechnologies and processes associated with nanosized materials as well as the engineered and adventitious products of nanotechnologies?

Although the existing toxicological and ecotoxicological methods are appropriate to assess many of the hazards associated with the products and processes involving nanoparticles, they may not be sufficient to address all the hazards. Specifically, particular attention needs to be given to the mode of delivery of the nanoparticle to the test system to ensure that it reflects the relevant exposure scenarios. The assays may need to be supplemented by additional tests, or replaced by modified tests, as it cannot be assumed that current scientific knowledge has elucidated all the potential adverse effects of nanoparticles.

For exposure, the use of mass concentration data alone for the expression of dose is insufficient, and the number concentration and/or surface area need to be included. Equipment that enables routine measurements in various media for representative exposure to free nanoparticles is not yet available. The existing methods used for environmental exposure assessment are not necessarily appropriate for determing the distribution, partitioning and persistence of nanoparticles in the various environmental compartments.

Given the above uncertainties, the current risk assessment procedures require modification for nanoparticles.

Question 2

If existing methodologies are not appropriate to assess the hypothetical and potential risks associated with certain kinds of nanotechnologies and their engineered and adventitious products, how should existing methodologies be adapted and/or completed?

Three different situations can be identified where existing methodologies are considered unsuitable:

- Routine methodologies have not yet been made available and / or have not been included in the testing guidance and/or achieved regulatory acceptance.

- Scientific research has identified a phenomenon to be evaluated and existing methodologies need to be adapted.

- Advances in nanotechnology may require additional methodological principles and developments.

Included in the areas of requirements for new or modified methodologies are:

- Appropriate methodologies must be made available for the routine and careful characterisation of the physico-chemical properties of nanoparticles.

- Methodologies and equipment need to be developed that enable routine measurements, in various media, of representative exposure to free nanoparticles.

- Although conventional toxicity and ecotoxicity tests have been shown to be useful in evaluating the hazards of nanoparticles, some methods may require modification and some new testing methods may also be needed in order to optimise this process of hazard evaluation, including the assessment of whether nanoparticles can exacerbate pre-existing medical conditions.

- In this context, although again some potentially suitable methods exist for the detection of nanoparticle translocation, these need to be developed further and incorporated into new testing strategies and guidelines for the assessment of the systemic distribution of nanoparticles.

More specifically the above mentioned methodologies need to provide information on how nanoparticles distribute in human tissues and in environmental compartments. This information can then be used in the exposure assessment algorithm provided in figure 6 in section 3.10.5 of this opinion.

Question 3

In general terms, what are the major gaps in knowledge necessary to underpin risk assessment in the areas of concern?

In general, and in spite of a rapidly increasing number of scientific publications dealing with nanoscience and nanotechnology, there is insufficient knowledge and data concerning nanoparticle characterisation, their detection and measurement, the fate (and especially the persistence) of nanoparticles in humans and in the environment, and all aspects of toxicology and environmental toxicology related to nanoparticles, to allow for satisfactory risk assessments for humans and ecosystems to be performed.

The major gaps in knowledge that need to be filled in relation to improved risk assessment for the products of nanotechnology include:

- The characterisation of the mechanisms and kinetics of the release of nanoparticles from a very wide range of production processes, formulations and uses of the products of nanotechnology.

- The actual range of exposure levels to nanoparticles, both to man and to the environment.

- The extent to which it is possible to extrapolate from the toxicology of non-nano sized particles and other physical forms e.g. fibres of the same substance to the toxicology of nanosized materials, and between nanoparticles of different size ranges and shape

- Toxicokinetic data following exposure, so that target organs can be identified and doses for hazard assessment determined. This includes dose response data for the target organs, and knowledge of the subcellular location of nanoparticles and their mechanistic effects at the cellular level.

- Information from the occupational exposure and associated health effects on workers involved in the manufacture and processing of nanoparticles

- The fate, distribution and, persistence and bioaccumulation of nanoparticles in the environment and environmental species including micro-organisms

- The effects of nanoparticles on various environmental species, in each of the environmental compartments and representative of different trophic levels and exposure routes.

In addition, there are several aspects of the fundamental properties of nanoparticles that require elucidation, including the ability of nanoparticles to act as vectors of chemicals, micro-organisms and interactions with other stressors.

5. MINORITY OPINION

Not applicable.

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7. ACKNOWLEDGEMENTS

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