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Recommendations for Research Policy Makers

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IMPART

**Improving the understanding of the impact of
nanoparticles on human health and the environment**

Integrating Activity

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Coordination Action

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Table of contents

1	Introduction	4
1.1	The IMPART project, goals and implementation	4
1.2	Added value	5
1.3	Structure of this report	5
2	Commented overview of current knowledge	6
2.1	Nanoparticle families treated	6
2.2	Health issues	8
2.2.1	Oxidative stress and lung inflammation	8
2.2.2	Thrombosis	9
2.2.3	Genotoxicity	9
2.3	Exposure	10
2.3.1	Health effect through inhalation exposure	10
2.3.2	Health effects through ingestion exposure	11
2.3.3	Health effects through dermal exposure	11
2.4	Environment	11
2.5	Legislation	12
2.5.1	Legislation concerning registration of the materials themselves	12
2.5.2	Legislation concerning protection of workers' and consumers health and safety, and environment	14
3	Assessment of existing data	15
3.1	Important factors in assessing the safety of nanomaterials	15
3.2	Legislation	17
3.3	Toxicology and hazards	17
3.3.1	TiO ₂ (and accordingly SiO ₂) particles	17
3.3.2	Carbon materials	18
3.3.3	Engineered quantum dots	18
3.3.4	Knowledge gaps concerning toxicology and internal dose	18
3.4	Environmental Risks	19
3.5	Best practices	21



4	Recommendations with regard to research policy	23
4.1	Introduction: current activities	23
4.2	Recommendations for immediate and long term measures with regard to research	24
4.2.1	Immediate measures	24
4.2.2	Long term measures	26
5	Summary and outlook	27
5.1	Summary.....	27
5.2	Outlook on future activities.....	27
6	References.....	29



1 Introduction

1.1 The IMPART project, goals and implementation

Nanotechnology is finding increased application in today's society and is being hailed as the next industrial revolution. Companies around the world are beginning to mass-produce nanoparticles (particles less than 100 nm in size) for use in everything from sunscreens to soil reclamation. The production of anthropogenically-derived nanoparticles will inevitably result in the introduction of these materials to the environment. However, despite rapid advances in nanotechnology, knowledge of the potential risks of nanoparticles to human health and the environment is limited. There is a concern that "size matters" with respect to toxicity, irrespective of the chemical composition. There are fears that materials that are biologically inert in bulk tend to become harmful in nanoparticle form. There is a need to encourage greater understanding of the short and long term implications of nanotechnology for health and the environment.

The primary aim of IMPART is to prevent knowledge of the health and environmental implications of nanoparticles from lagging behind the technological advances and incorporation of these materials in products widely distributed throughout society. In order to do this, IMPART has brought together experts involved in various regional, national and international initiatives in order to assess the state of the current understanding of health and environmental aspects of nanoparticles, collate this information into a manageable document, concluding with recommendations for the research and governmental bodies dealing with this issue, and communicate these recommendations to all groups concerned.

To accomplish this task, the IMPART Coordination Action was organized into the following work packages:

Collection of data: This work package provides an overview of ongoing projects concerning safety and risks of nanotechnology and brings together all of the existing reports on the health and safety aspects of nanoparticles including proceedings from conferences and meetings, as well as primary scientific literature on this topic. The information in this chapter serves as the input for the following three work packages.

Assessment of data: legislation, toxicology, risks, best practices: This work package reviews the collected data to provide an overview of the toxicology and risks of nanoparticles, and the existing legislation dealing both with nanoparticles and their macroscale counterparts. It then identifies gaps in knowledge concerning toxicology and risks as well as the gaps in legislation. Finally, if nanoparticles are toxic, they will be most dangerous to those working at the industrial scale either producing them in bulk or incorporating them into products, which also involves handling bulk quantities. Thus, as part of the risk assessment, this work package takes inventory of the attitudes in industries who work with nanoparticles, how they inform their own personnel and their customers and the practices which they have in-place to protect against exposure and treat in case of exposure.

Reports: recommendations and guidance booklet: The observations and assessments made in the previous two work packages are intended to lead to recommendations on

how to close the gaps in our knowledge of toxicology and safety issues related to exposure to, and handling of, nanoparticles. Recommendations will be formulated concerning the additional research necessary to close these gaps. If gaps have been sighted in legislation concerning the manufacture and application of nanoparticles, recommendations will be made in this regard as well. Recommendations will be also formulated in a guidance booklet for industry and all other target groups identified as potential recipients during the course of this Coordination Action.

Dissemination and Knowledge Transfer: Information exchange is the cornerstone of this Coordination Action; initially to collect information and identify knowledge gaps and subsequently to communicate the findings and the recommendations for further research and legislation. This work package is responsible for organizing effective mechanisms for this exchange.

1.2 Added value

The attention being paid to the health issues and environmental aspects of the production and use of nanoparticles is justified on the basis of what is already known from more than 50 years of research in particle toxicology (Donaldson and Borm, 2007). But the more recent realization that size and shape at the nano-scale can impart physical and chemical properties different from those of the same material in the micro or macro form has resulted in a growing body of new research data, some of which is of questionable value because of the lack of defined standards and the different assessment methods being employed.

The value of this report is that it not only reviews the conclusions being derived from these more recent studies but also, on the basis of critical evaluations by the IMPART experts participating in this Coordination Action, defines the gaps in our knowledge of the toxicity of nanoparticles as well as in matters dealing with methods to protect workers during production and handling and issues related to product life cycle, waste streams and diffusion in the environment.

1.3 Structure of this report

The goal of this report is to provide research policy makers and related target groups with a decision basis for taking further steps in the field of nano-safety issues such as defining research targets or funding opportunities. To this end, current information on health and environmental issues and existing legislation is reviewed, gaps are identified and, finally, recommendations are formulated.

2 Commented overview of current knowledge

2.1 Nanoparticle families treated

The nanoparticle families treated in this report include metals, metal oxides and ceramics, carbon and inorganic fullerenes, carbon and metallic nanotubes and quantum dots. Polymeric nanoparticles are not treated, since, for the most part, they are composed of organic building blocks generally used in everyday products, even for food and medical applications, and are seen as non-toxic and/or having a limited persistence in the environment.

Ceramics, Metals and Metal Oxides

Ceramics, metals, and metal oxide nanoparticles are assembled from nanometer-sized building blocks, mostly crystallites. They are microstructurally heterogeneous, consisting of grain boundaries in addition to the crystallites. It is this inherently heterogeneous structure on a nanometer scale that is crucial for many of their properties and distinguishes them from glasses, gels, etc. that are microstructurally homogeneous.

Fullerenes

C_{60} is a molecule that consists of 60 carbon atoms, arranged as 12 pentagons and 20 hexagons. The spherical molecules, 3.52 Å in diameter, do not exhibit "superaromaticity", the effect that the electrons in the hexagonal rings would be delocalized over the whole molecule. Therefore they have a high electron affinity at pentagon rings, which can lead to reaction with oxygen dissolved in water and the creation of oxygen free-radicals which are responsible for oxidative cell damage. Fullerenes are hydrophobic, very sparingly soluble in water and spontaneously form stable aggregates with nanoscale dimensions ($d=25-500$ nm), termed as "nano- C_{60} ", with a partially oxidized shell. The colour, hydrophobicity, and reactivity of individual C_{60} are substantially altered by its aggregation form.

WS_2 and MoS_2 inorganic fullerenes (Tenne *et al.*, 1992) are onion-like nanoparticles with several molecular layers. They can be hollow or partially filled with MoO_x or WO_x compounds, which serve as precursor material during the synthesis. The particle diameters range from 20 nm to 150 nm for WS_2 and up to 80 nm for MoS_2 . The particles are frequently agglomerated and sintered into clusters composed of a few up to several tens of nanoparticles of different diameter. The surface of the particles is inert due to saturated sulphur bonds composing the top most atomic layer. The only reactive sites are surface defects in the form of screw dislocations, edges of broken molecular layers or exfoliated layers. These sites are saturated by oxygen forming transition metal oxides. The pure material is hydrophobic although it is possible to disaggregate the assemblies in solvents containing surfactants using ultrasound.

Nanotubes

Nanotubes are quasi one-dimensional objects with extremely large aspect ratios. Diameters range from 1 nm up to several microns, and lengths from a few microns up to several millimetres. They are hollow with wall thickness from 0.3 nm up to 100 nm. The tubes prepared from layered materials (graphite, MoS₂, WS₂, TiS₂, etc.) show a tendency for spontaneous formation of self-terminated cylinders, but the metal or metal oxide nanotubes need a template growth process to force the structure to form the cylindrical shape.

Carbon nanotubes These are unique, one dimensional macromolecules, composed entirely of carbon. They consist of extended tubes of rolled graphene sheets with an axial symmetry and a diameter in the nanometer range and can grow up to several centimetres long (Saito, 1998). There are two main types of CNT, differentiated by their structure, single wall carbon nanotubes (SWCNT) and multiwall carbon nanotubes (MWCNT). CNT exhibit a number of unique properties including strength, toughness, chemical robustness, thermal conductivity and electrical conductivity.

Inorganic nanotubes Since the first report on WS₂ and MoS₂ nanotubes in 1992, several compounds have been found in stable cylindrical geometry. Six families of inorganic nanotubes (NTs) have been synthesized up to now (Remskar, 2004):

1. Transition metal chalcogenide NTs: MoS₂, MoSe₂, WS₂, WSe₂, NbS₂, NbSe₂, TaS₂, ZrS₂, HfS₂, TiS₂, ZnS, NiS, CdSe, CdS;
2. Oxide NTs: transition metal oxides: TiO₂, ZnO, GaO/ZnO, VO_x, W₁₈O₄₉, V₂O₅, Al₂O₃, In₂O₃, Ga₂O₃, BaTiO₃, PbTiO₃; silicon oxide: SiO₂; MoO₃; RuO₂; rare earth oxides: (Er, Tm, Yb, Lu) oxide;
3. Transition metal halogenous NTs: NiCl₂;
4. Mixed phase and metal doped NTs: PbNb_nS_{2n+1}, Mo_{1-x}WS₂, W_xMo_yC_zS_z; Nb-WS₂, WS₂-carbon NTs, NbS₂-carbon NTs; Au-MoS₂, Ag-WS₂, Ag-MoS₂; Cu_{5.5}FeS_{6.5};
5. Boron and silicon based NTs: BN, BCN, Si;
6. Metal nanotubes: Au, Co, Fe, Cu, Ni, Te, Bi.

Inorganic NTs exist in different crystalline states. Semi-single crystal structures are typical for non-helical or mono-helical NTs while polycrystallinity appears either in the structure of the nanotube wall, composed of small thin crystal flakes or in the radial direction as multi-helicity. Many of the inorganic NTs prepared by the decomposition process appear as an assembly of nanocrystallites forming the nanotube wall (e.g. HfS₂, NbS₂).

Engineered Quantum Dots

Quantum dots are one of the most interesting classes of nanomaterials where physics, chemistry and biology have merged to yield a generic tool with a broad range of applications. A quantum dot (QD) may be defined as a semiconductor nanostructure which confines the motion of valence band electrons, conduction band holes or excitons (bound pairs of electrons and holes) in all 3 spatial directions. Quantum dots, per se, cannot be considered as an entirely novel classification of compounds. Biogenic and anthropogenic nanosized inorganic particles occur in water streams, silts, clays

and other natural sources. There is little evidence to indicate that such nanosized materials have had a more detrimental ecotoxicological or environmental impact than their corresponding bulk analogues.

Commercially manufactured quantum dots generally are based on CdSe spherical nanoparticle cores coated or capped by a thin layer of ZnS as a stabilizing agent (Alivisatos et al., 2005) although recently a number of non-heavy metal based InGaP/ZnS materials and gold and silver nanoparticles have also become commercially available. Their global production is relatively small in comparison to other nanomaterials and the concentrations at which such materials are deployed are quite low (on the order of μM). A recent comprehensive review by Hardman (2006) suggests strongly that one must take account of many specific factors when assessing the toxicity of QDs.

2.2 Health issues

Research has shown that exposure to environmental PM_{10} can cause cardiovascular morbidity and mortality as well as effects on the lungs. The mechanisms by which they cause these effects are not yet fully understood.

Ultrafine particles may be more toxic than larger particles of the same substance because of their larger surface area, enhanced chemical reactivity and easier penetration of cells (Monteiller et al., 2007). It has been demonstrated that pulmonary toxicity studies in rats with ultrafine particles induce enhanced inflammatory responses when compared to fine particles of identical chemical composition at equivalent mass concentrations (Donaldson et al., 2001; Oberdorster et al., 2005). However, other studies demonstrated that there was no difference between the effects of ultrafine and fine particles (Warheit et al., 2006). One aspect that may be of particular importance to the novel carbon-based materials, whose production involves the use of metal catalysts, is the issue of toxicity due to the residual metal contained within the final product.

2.2.1 Oxidative stress and lung inflammation

Oxidative stress is the condition wherein the physiological balance between oxidants and anti-oxidants is disturbed and can, for example, be quantified by measuring the balance between glutathione and oxidised glutathione. Ultrafine particles, even when they are composed of materials with a relative low toxicity, have been shown to cause oxidative stress and inflammatory effects in a number of *in vivo* and *in vitro* models (Ferin et al., 1992; Li et al., 1996). After inhalation of nanoparticles the natural antioxidant system may be overwhelmed by excessive production of reactive oxygen species (ROS). At high levels of oxidative stress, pro-inflammatory cascades will be initiated with inflammation and even cytotoxicity as a consequence (Donaldson and Stone, 2003). It should be noted that not only size, but also surface characteristics play an important role, therefore nanoparticles with a low oxidative potential should be designed (Warheit et al., 2007).

2.2.2 Thrombosis

Epidemiological studies have shown that there is a strong link between PM exposure and cardiovascular morbidity and mortality, which are mainly driven by thrombotic events in arteries (Hoek et al., 2001; Pope, III et al., 1999; Samet et al., 2000). Baccarelli et al. (2007) investigated the association between air pollution levels and changes in coagulation in 1218 normal subjects in Italy and reported that PM₁₀ is associated with changes in the global coagulation function (shortened prothrombin time), suggesting a tendency towards hypercoagulability after short-term exposure to PM₁₀. Whether these changes contribute to triggering cardiovascular effects remains to be established.

The formation of thrombi can be explained through the direct passage of ultrafine particles into the circulation and through the release into the systemic circulation of cytokines and other mediators produced in the lungs during lung inflammation caused by particle exposure (Nemmar et al., 2004).

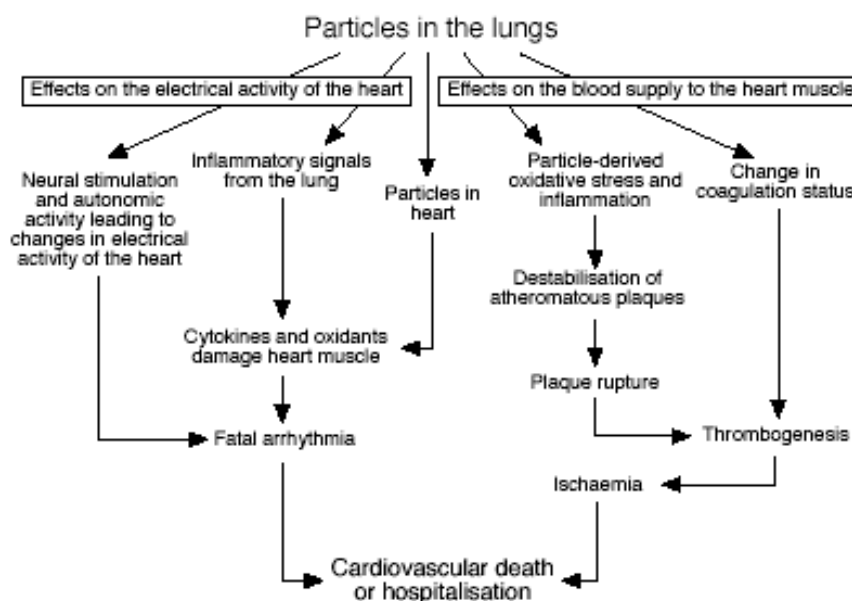


Fig. 1: Hypothetical events that link particles exposure via the lungs to adverse cardiovascular effects (Donaldson and Stone, 2003).

2.2.3 Genotoxicity

Genotoxicity is the degree to which a substance causes damage to DNA that can result in mutations or cancer. Two principle modes of genotoxic action can be considered for nanoparticles, referred to as primary and secondary genotoxicity. Primary genotoxicity is defined as genetic damage elicited by a substance directly (in the absence of inflammation), whereas secondary genotoxicity implies a pathway of genetic damage resulting from substance-elicited oxidative DNA attack by ROS in the presence of inflammation.

Currently available literature data indicates that tumorigenesis of poorly soluble particles involves a mechanism of secondary genotoxicity. However, further research is required since (1) causality between pulmonary inflammation and genotoxicity has not yet been established, and (2) effects of inflammation on fundamental DNA damage responses that orchestrate mutagenesis and carcinogenic outcome, that is, cell cycle arrest, DNA repair, proliferation and apoptosis are currently poorly understood (Schins and Knaapen, 2007).

2.3 Exposure

The primary routes of occupational exposure to nanoparticles include inhalation, transdermal desorption and ingestion (Borm *et al.*, 2006). The currently mostly investigated exposure route is via inhalation (Hoet *et al.*, 2004; Nemmar *et al.*, 2002). There are four main groups of nanoparticle production processes (gas-phase, vapour deposition, colloidal and attrition) all of which may potentially result in exposure by inhalation, dermal or ingestion routes. All processes may give rise to exposure to agglomerated nanoparticles during recovery, powder handling and product processing (Aitken *et al.*, 2004, IRSST, 2006). Information about production volume and occupational exposure potential of nano-sized material is urgently needed to determine potential risk to employees since the workplace represents a critical interface between people and nanotechnology, and an area where potential impact needs to be understood and managed. In the future, it is also likely that even wider distribution of these particles may have significant effects on organisms. For this reason the potential release of nanoparticles throughout the application life cycle including the recycling process and the potential effects of engineered nanoparticles on the ecosystem must be evaluated.

2.3.1 Health effect through inhalation exposure

The most common route of exposure to airborne particles in the workplace is by inhalation. The deposition of discrete nanoparticles in the respiratory tract is determined by the particle's aerodynamic or thermodynamic diameter (depending on particle size). Agglomerates of nanoparticles will deposit according to the diameter of the agglomerate.

The biological mechanisms of particle-related lung diseases (e.g., oxidative stress, inflammation, thrombotic effects and production of cytokines, chemokines, and cell growth factors) appear to be a consistent lung response for respirable particles including ultrafine or nanoparticles. Persistent inflammation is likely to lead to diseases such as fibrosis and cancer, therefore it is important to control inflammation.

Due to their small diameter, nanoparticles are capable of penetrating epithelial cells – or cell layer, entering the bloodstream from the lungs (Gilmour *et al.*, 2004), and even translocating to the brain via the olfactory nerves (Oberdorster *et al.*, 2004). Carbon nanoparticles (Oberdorster *et al.*, 2004), and manganese oxide (30 nm) (Elder *et al.*, 2006) can accumulate in the olfactory bulb following inhalation, and the manganese nanoparticles induce inflammatory changes in the brain.

2.3.2 Health effects through ingestion exposure

Nanoparticles can be ingested directly in food, water, cosmetics, or drugs. In general the kinetics of nanomaterials is not well understood yet; for instance, radioactive iridium nanoparticles do not show substantial GIT (Gastro Intestinal Tract) uptake, whereas ingestion of water-soluble radio-labeled C₆₀ fullerenes in rats show a 98% clearance in the faeces. In contrast, 90% of intravenously administered radio-labeled fullerenes are retained for at least a week, with 70% lodging in the liver (Nel et al., 2006).

There is also some evidence that smaller particles can be transferred more readily than their larger counterparts across the intestinal wall (Behrens et al., 2002). Although nanoparticles in food are infrequently taken up into gut lymphatic and distributed to other organs, most nanoparticles are rapidly eliminated via faeces. Little is currently known about the health effects of nanoparticles on the liver and kidneys after ingestion.

Another area which merits further research is the transfer of nanoparticles across the placenta barrier. Exposure to nanoparticles during the critical window of foetal development may lead to developmental damage in the offspring.

2.3.3 Health effects through dermal exposure

Some studies suggest that nanoparticles also could enter the body through the skin during occupational exposure or use of consumer products. Recent studies indicate that nanoparticles of titanium dioxide (TiO₂) used in sunscreens do not penetrate beyond the epidermis of healthy skin. However, Ryman-Rasmussen et al. (2006) reported that nanoparticles with varying physicochemical properties were able to penetrate the intact skin of pigs.

2.4 Environment

There are concerns about the negative impact that engineered nanoparticles (ENPs) may have on the environment. Most ENPs are fixed within materials (e.g. composites). However, these particles may become free in the environment due to damage, degradation and recycling following disposal. Currently, exposure to free nanoparticles is mostly limited to workplaces of manufacture and research and a number of cosmetic uses, including sunscreens. However, the central issue is that there has been very little research into the life cycles of products containing ENPs and any possible risks from their potential to release free nanoparticles. An assessment of the risks of ENPs in the environment requires an understanding of their mobility, reactivity, ecotoxicity and persistency (Nowack and Brucheli, 2007) and there is, therefore, a need for a life cycle analysis of products containing ENPs. A product life cycle analysis should consider the processes and materials used in manufacture, the likely interactions between the product, people and the environment during its manufacture and useful life, and the methods used in its eventual disposal (Royal Society Report, 2004).

Nanoparticles have different levels of interaction with biological systems and have different mobility based on their size, shape and chemical composition. It is not, therefore, possible to address the hazards and risks of nanoparticles in a general way,

as each type of nanoparticle needs to be evaluated. At this time there is not enough research on engineered nanoparticles to know whether or not they present a serious problem to human health and the environment. In order to prevent a backlash of negative public opinion and a political and regulatory backlash, the nanotechnology industry is keen to accumulate risk data to answer questions and address risks early. This will enable the nanotechnology industry to develop and flourish responsibly and with public support (Hood, 2004)

It is important to establish an environmental context for considering the potential impact of engineered nanoparticles (ENPs). Relatively recent, detailed observations of natural atmospheric nanoparticulates have shown that carbon nanotubes (CNTs) and other fullerene-related nanocrystals are ubiquitous in the Earth's atmosphere and have even been observed in a 10,000 year-old ice core (Murr et al. 2004). We are already exposed to ultrafine particles on a daily basis, for example, particulates from diesel exhausts. Indeed it has been suggested that we may have more to fear from the nanoparticles we encounter on a daily basis, than from newer ones arising from the potentially cleaner nanotechnologies of the future (Oberdorster, 2005).

It is also necessary to keep in mind the positive impact of the improved environmental technologies that are developing from new nanotechnologies. There is considerable promise not only for removing persistent pollutants from soils and water supplies, but also for improving the efficiency of energy production by using nanomaterials as catalysts and in energy storage, reducing waste to benefit the environment and increase sustainability (Masciaglioli and Zhang, 2003; Dror et al. 2005).

2.5 Legislation

Legislation potentially applicable to nanoparticles has been enacted at two levels, the materials themselves and the safety of workers, consumers and environment, but is focussed primarily on bulk quantities and industrial settings. There is no legislation specifically addressing nanoparticles in a research setting. Existing legislation is, however, considered applicable by legislation makers.

2.5.1 Legislation concerning registration of the materials themselves

Various legislations extending back to the 1960 have been assessed in the modern context, amended and incorporated into REACH, the Regulation concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals (Regulation No 1907, 2006). The provisions of REACH apply to the manufacture, import, placing on the market or use of substances on their own, in preparations or in articles, if so stated. REACH abolishes the distinction between «existing» and «new» substances and establishes a single legislative system for the marketing of chemical substances in Europe.

Uses of a substance for product and process oriented R&D are exempt for five years from the obligation to be included in the registration dossier of this substance. Nevertheless, other obligations, e.g. for risk assessment, classification and labelling,

and occupational health and safety apply. It means that the manufacturer or importer, or producer of nanomaterials for product and process oriented R&D with volumes of less than one tonne/yr after 5 year of uses, must notify the Agency of the following information: the identity of the manufacturer or importer or producer, the identity of the substance, the classification of the substance, the estimated quantity and the list of customers.

The European Chemicals Agency has stated on 3 December 2007 at the European NanOSH Conference in Helsinki, that REACH treats both, the bulk material and the nanosized material, as the same substance. The Agency added that this, however, does not prevent the registrant from identifying dangerous properties of this substance depending on its size and classify the different types accordingly.

In the context of nanotechnologies, the REACH Regulation defines a substance as “*a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process...*”. A key consideration in this regard is the fact that the nano-equivalent of a substance could have different physicochemical and ecotoxicological properties from the substance itself. If it is considered to be a different substance, then the registrant may submit a different registration dossier for the nano-substance (if produced in volumes greater than 1 ton/yr).

The OECD (2008) presented the list of endpoints to take into account, when testing specific nanomaterials for human health and environmental safety. Addressing this set should ensure consistency between the various tests to be carried out on specific nanomaterials. It should also lead to the development of dossiers for each nanomaterial describing basic characterization, fate, ecotoxicity and mammalian toxicity information.

In the opinion of IMPART, the REACH provisions apply in principle to nanomaterials, including placing them on the market and controlling of the risk.

The European Commission has adopted a so-called “incremental approach”, which focuses on adapting existing laws to regulate nanotechnologies. Franco et al. (2007) tested the effectiveness of the “incremental approach”. Their conclusion was that the “incremental approach” can only be applicable with the implementation of new amendments. Based on the findings, the authors concluded that the European “incremental approach” could work. In the short-term, they specifically have six recommendations for amending current rules and regulations:

1. Define standards for labs and other workplaces handling nanoparticles
2. Actively promote research and development of usable metrological tools
3. Establish a Technical Working Group within the EU Bureau of Integrated Pollution Prevention and Control to organize an exchange of information
4. Adapt the Chemical Abstract Service (CAS) to properly classify nanoparticles
5. Establish a specific regime for nanoparticles within REACH requiring industry to submit information on nanoparticles characteristics and health, safety and environmental information
6. Add free nanoparticles to the list of Annex II in Directive 1991/689 on hazardous wastes (Council Directive 91/689/EEC of 1991)

2.5.2 Legislation concerning protection of workers' and consumers health and safety, and environment

The employer is obliged to make an a priori overall risk assessment and to undertake measures to prevent occupational as well as consumers' and environmental risks. This is essential to combat risks at source either by eliminating/avoiding or, if not possible, by taking the appropriate control measures in order to reduce them.

As an example, applicable legislations are:

- Framework Council Directive 89/391/EEC (1989) on the introduction of measures to encourage improvements in the safety and health of workers
- Council Directive 98/24/EC (1998) on the protection of the health and safety of workers from the risks related to chemical agents at work
- Council Directive 2004/37/EC (2004) on the protection of workers from the risks related to exposure to carcinogens or mutagens at work
- Council Directive 91/414/EEC (1991) concerning the placing of plant protection products on the market
- Directive 98/8/EC (1998) concerning the placing of biocidal products on the market
- Council Directive 76/768/EEC (1976) on the approximation of the laws of the Member States relating to cosmetic products
- Council Directive 2008/1/EC (2008) concerning integrated pollution prevention and control
- Seveso II Directive 96/82 (1997)
- Water Framework Directive 2000/60/EC (2000)

In as much as nanoparticles have, potentially, mutagenic and carcinogenic properties as well as other health hazards and are comparable in nature to some of the substances defined in the 2004 directive, this directive provides an excellent framework for monitoring and protecting workers who handle nano-particulate material. It is clear that these regulations can be integrally applied to nano-particulate material along with the Article in the same directive on Information and Training of Workers and the article on Health Surveillance.

3 Assessment of existing data

3.1 Important factors in assessing the safety of nanomaterials

Risk assessment in general comprises several components including:

- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk calculation

On the basis of a reliable risk assessment, measures for risk management have to be undertaken comprising preventive measures, standardisation and regulation activities. The following figure (Fig. 2) gives an overview of different aspects and components, which have to be taken into account for the assessment and management of risks associated with industrial nanoparticle production and use.

<p>1. Hazard identification</p> <p>Particle characteristics</p> <ul style="list-style-type: none"> ◆ Aspect ratio ◆ Diameter ◆ Surface area ◆ Water solubility ◆ Chemical composition <p>Emission</p> <ul style="list-style-type: none"> ◆ Production volume ◆ Material flow ◆ Potential release <p>Health effects</p> <ul style="list-style-type: none"> ◆ Humans ◆ Experimental animals <p>Environmental effects</p> <ul style="list-style-type: none"> ◆ Persistence ◆ Biomagnification ◆ Long range transport 	<p>2. Hazard characterization</p> <p>Epidemiological Studies</p> <ul style="list-style-type: none"> ◆ Workers ◆ Consumers ◆ Exposed population <p>In vivo studies</p> <ul style="list-style-type: none"> ◆ Acute/chronic ◆ Different species <p>In vitro studies</p> <ul style="list-style-type: none"> ◆ Human/animal, different cell types ◆ Models (lung, skin, systemic effects) 	<p>4. Risk calculation</p> <p>Susceptibility extrapolation models</p> <ul style="list-style-type: none"> ◆ High dose – low dose ◆ Animal – human <p>Threshold value calculation</p> <ul style="list-style-type: none"> ◆ Intake emission concentration ◆ Maximum workplace concentration
<p>5. Risk Management</p> <p>Preventative measures</p> <ul style="list-style-type: none"> ◆ Personal protection equipment ◆ Modification of processes <p>Standardization</p> <ul style="list-style-type: none"> ◆ Measurement techniques ◆ Toxicological assessment <p>Regulation</p> <ul style="list-style-type: none"> ◆ Exposure/emission schedule ◆ Production standards/restrictions 		
<p>3. Exposure Assessment</p> <p>Exposure routes</p> <ul style="list-style-type: none"> ◆ Inhalation, dermal ingestion <p>Environmental monitoring</p> <ul style="list-style-type: none"> ◆ Biological uptake <p>Occupational monitoring</p> <ul style="list-style-type: none"> ◆ Personal exposure 		

Fig. 2: Components and aspects of risk assessment and management (adapted from "Industrial application of nanomaterials - chances and risks, Technology analysis, VDI-TZ, W. Luther (ed.), 2004)

In view of the fact that data on exposure assessment are lacking, a full risk assessment of nanoparticulate materials in most cases is not feasible at present. However a ranking of potential risks can be achieved by applying hazard trigger algorithms. Relevant factors which can give a first estimation of potential risks of nanoparticles are:

- Production volume
- Potential exposure to customers, workers, environment
- Potential aerosol release during production, handling, processing

- Solubility
- Aspect ratio (to distinguish between fibers and particles)
- Particle diameter (taking into account a potential deagglomeration in body liquids e.g. in the lungs)
- Toxicological and ecotoxicological parameters

A scheme for assessing the risks of nanomaterials is depicted in the following figure. This scheme is to be regarded separately from registration processes of new chemical substances in the frame of existing chemical regulations. Until now producers are not obliged to declare particle size of the substances in the frame of registration processes. Therefore, the proposed scheme should be applied also for already registered substances which are manufactured as nanoparticulate materials and therefore might differ significantly from bulk materials in their physical and toxicological properties. Further investigations could lead to more suitable parameters for risk assessing, e.g. surface properties (area and bioavailability). It can be assumed that many parameters of nanoparticulate materials with regard to toxicological and ecotoxicological properties will be unknown. Here, standardised screening tests would be of great use for giving a first assessment of potential risks. Nanoparticulate materials which are assigned a high priority by such an assessment should be subject to further investigations and/or regulatory measures.

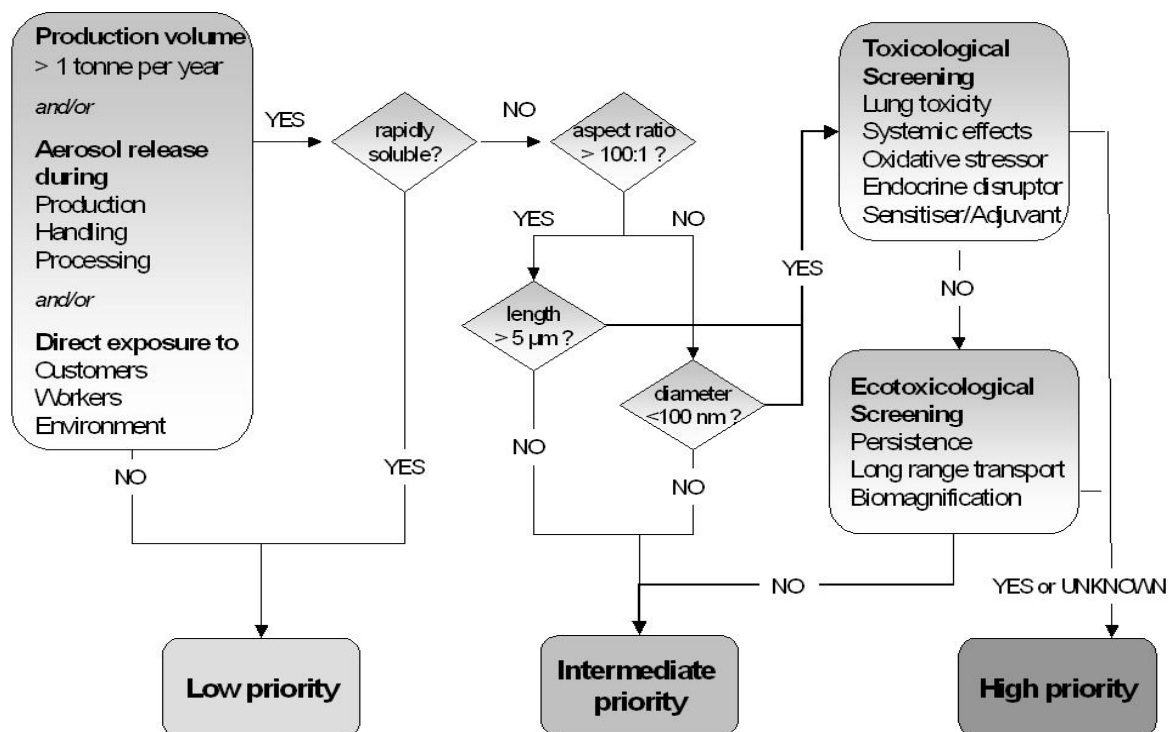


Fig. 3: Hazard trigger algorithm (source: VDI-TZ, modified from Howard and de Jong, 2004)

Knowledge gaps in the assessment of existing data:

Knowledge gaps, some of which have been identified by the NIOSH Nanotechnology Research Center, include:

- The application of risk assessment methods in using existing data to provide a framework for developing preliminary risk management strategies
- Biomathematical modelling approaches to fill data gaps concerning occupational health risks of exposure to non-spherical nanoparticles
- Standardized high-throughput methods of *in vitro* screening which reliably reflect *in vivo* toxicological potential

3.2 Legislation

Legislation as reviewed in section 2.5 makes it clear that there is no mandatory distinction required for registration of material on the basis of size. If produced or imported in volumes > 1 ton per year, the registrant may submit a different registration dossier for the nano-substance, but the producer/applicant could also consider the hazard information for the nano-equivalent to be the same as the registered macro-form and avoid any separate registration. From the point of view of substances whose toxic properties may directly relate to their nanometer dimensions, this is an obvious gap in legislation which needs to be addressed.

3.3 Toxicology and hazards

3.3.1 TiO₂ (and accordingly SiO₂) particles

- We have to examine to which concentrations of TiO₂ humans are exposed and these doses have to be used in toxicity studies. *In vivo* studies with TiO₂ doses causing an overload are not relevant for assessing their health effects on humans
- Further investigations are also required to establish the role of the crystal form and surface chemistry and the mechanisms of photoactivity of nanoscale TiO₂
- The effects of coatings on TiO₂ particles have to be further clarified because surface treatments can influence its toxicity. Impurities linked to the synthesis of the TiO₂ particles seem to be very important
- From an environmental viewpoint, the long term stability of these coatings needs to be characterized
- Ultrafine TiO₂ is frequently used in sunscreens. However, the fate of ultrafine-TiO₂ applied to skin is uncertain. Whether nanoparticles can penetrate through the skin has to be investigated further, because TiO₂ has the ability to form ROS such as hydroxyl radicals that can cause damage to cells and DNA
- Further research is needed to clarify the genotoxic effects of nanoparticles. It is not clear whether they can cause only single-strand breaks or both single- and double-strand breaks

3.3.2 Carbon materials

- Non purified, iron-containing CNT can produce cellular oxidative stress, whereas purified CNT don't to such an extent
- DNA damage (probably linked to oxidative stress) is linked to internalized CNT
- Cytotoxicity of CNT is dependent on the presence of metal impurities, surface reactivity and formation of agglomerates. Purified and water soluble CNT seem to be less cytotoxic than non-purified, water-insoluble materials
- For nanomaterials (CNT and non-fibre metallic and non-metallic materials) oxidative stress appears to be the common mechanism involved in cytotoxicity
- Role of internalisation on the cytotoxicity and the biocompatibility of surfaces containing CNT
- The comparison with asbestos is an important issue, certainly since a recent publication showing that MWCNT induced length-dependent pathology in the mesothelial lining of the mouse body cavity (as a model of the chest mesothelial lining), (Poland et al., 2008). The *in vitro* study by Wick et al. (2007) also reported similar effects of SWCNT agglomerates and asbestos on cytotoxicity and cell proliferation in a human mesothelioma cell line

3.3.3 Engineered quantum dots

In addition to their potential toxicological properties arising from their nano-particulate nature, some of these quantum dots are composed of elements such as Cd, which are toxic. To prevent toxicity, they are coated with a protective layer, however, the stability of the coating over the long term is a matter of concern, especially if the materials diffuse into the environment.

3.3.4 Knowledge gaps concerning toxicology and internal dose

Uncertainty remains about many toxicological aspects of nanoparticles in general, including:

- The nature and severity of effects on the lung from inhaled nanoparticles
- Whether exposure to nanoparticles has any effect on the immune system
- Which unique physical and chemical properties of nanoparticles affect the body's response when exposed to these particles
- The relative importance of particle size as a cause of observed health effects
- Whether nanoparticles move throughout the body and affect organs other than the one through which they entered the body
- Persistence in the body
- Long-term stability of coatings

3.4 Environmental Risks

A number of recent, comprehensive reports outlining the risk associated with the manufacture, modification, formulation and employment of nanomaterials have been placed in the public domain (Singh 2007, Aitken 2006 and NIOSH 2006). Other reports have examined the environmental risks associated with such materials (Guzman 2006) or examined occupational exposure (Aitken 2004), potential health concerns (Powell 2006), risk assessment approaches (Kuempel 2007) and precautionary measures (NIOSH 2006), including suggestions of best practice in this field. Many practitioners, including respiratory experts, toxicologists, environmentalists and meteorologists have argued strongly for the adoption of a uniform set of references and standards (Aitken 2006). Furthermore by considering risk at an early stage in the development of a technology, the potential (detrimental) impact on the environment and public health can be minimized. An account of EU-funded initiatives in research and development in this area of nanotechnology has recently been published by Aguar (2008).

Health and environmental hazards may occur from the production, use and disposal of nanoparticles. There is still a significant amount of uncertainty as to how nanomaterials differ in properties from their bulk counterparts and the corresponding risks. Little is also known about the environmental persistence or impact of engineered nanoparticles. It is difficult to predict which of these new materials may bioaccumulate and persist, as there have been no long-term studies observing the unique physiochemical characteristics of these new materials. Given the complexity of aquatic systems with their suspended sediment particles and natural colloids like humic acids and the water microlayer, predicting the behaviour of nanoparticles is likely to be much more difficult than predicting the behaviour of conventional chemical pollutants, which is still often a major challenge. We may be able to use the known behaviour of natural nanoscale or microscale particles, such as colloids, viruses and bacteria; and how these adsorb to or associate with larger biotic and non-biotic particles in the suspended and deposited sediment.

There is a serious lack of life-cycle analyses that look at the possibilities for environmental release of ENPs from production through to disposal of nanomaterials. There may be direct inputs into the aquatic, marine and terrestrial ecosystems and into the atmosphere from initial and downstream manufacturers. There may also be non-industrial inputs, e.g. consumer products including sunscreens and cosmetics from direct and indirect sources, leaching from landfill or soil-applied sewage sludge, and atmospheric sources of nanomaterials from waste combustion. It is certain that further use of nanomaterials will result in their introduction into environmental systems and ecosystems in greater quantities (Helland et al., 2008). Understanding what happens to nanomaterials during their journey from manufacture to waste disposal will help to focus studies that can tell us about transport pathways, biogeochemical cycling and environmental fate. Such work will help us to identify which, if any, environmental compartments are at risk of contamination by nanomaterials (Owen and Depledge, 2005).

Fortner et al. (2005) are currently conducting studies on how C₆₀ (fullerene) affects bacteria and simple organisms like worms. They are also exploring whether these fullerenes tend to move up the food chain. Initial results show that NPs accumulate in living cells over time, with ever-increasing concentrations in microbes, in the worms that

eat those microbes, and in animals higher up the food chain. It is possible that these NPs reach humans. NPs have been shown to inhibit the motility and phagocytosis of macrophages in the lungs, which suggests that similar effects might be expected in simple soil organisms (Lam et al., 2004). Lovorn and Klaper (2006) recently found that exposure of *Daphnia magna* to filtered C_{60} and filtered TiO_2 caused an increase in mortality with increasing concentration.

There are several ongoing studies evaluating fullerene toxicity in aqueous systems. Due to the possibility of C_{60} solubilisation through colloid formation under environmental conditions, many studies have focused on the effects of n- C_{60} (highly stable colloidal aggregates of C_{60}). The possibility of n- C_{60} formation following extended contact with water suggests that n- C_{60} could be a significant form of C_{60} if these fullerenes were introduced to aquatic systems. The first interest attracting published work on n- C_{60} toxicity to organisms concluded that n- C_{60} produced oxidative damage in the brains of exposed largemouth bass (Oberdörster, 2004). Nevertheless, the same group has to correct these results as the tetrahydrofuran (THF) solubilised fullerenes are toxic through the peroxides coming from the solvent THF (Oberdörster et al., 2006). The tendency of n- C_{60} to aggregate and deposit will play a key role in determining its longevity in aquatic systems and, therefore, provide key information on the exposure risk presented by these colloids. In one case, it was shown that hydrophobic contaminants can irreversibly interact with fullerene aggregates in water, and these species showed a high capacity for concentrating a model aromatic hydrocarbon (Cheng et al., 2004).

The mobility of eight particulate products of nanochemistry in a well-defined porous medium were evaluated by Lecoanet et al. (2004) to assess their potential for migration in porous media, such as groundwater aquifers and water treatment plant filters. Their results showed that nanomaterials exhibit widely differing transport behaviours, and consequently, they suggest that the potential for exposure to n- C_{60} through groundwater transport may be less than that of other fullerenes. Observations made by Brant et al. (2005) suggest that, under some conditions present in natural aquatic systems, these materials have limited mobility as they form large aggregates that may settle out of suspension or deposit on surfaces. These phenomena may, at least partially, offset any risk presented by n- C_{60} toxicity due to a reduced potential for exposure (Brant et al., 2005).

More recent data have shown for the first time how multiwalled carbon nanotubes might behave in natural aquatic environments (Hyung et al., 2007). This research suggests that natural organic matter present in river water could aid the dispersion of carbon nanotubes by stabilizing the nanotubes and increasing their potential for dispersal dramatically. In fact, their experiments showed that natural organic matter stabilizes the model carbon nanotube in the aqueous phase more efficiently than a surfactant. They also found that the nanotubes remain as discrete units. However, the toxicity of the new materials in natural environments remains relatively unknown. The paper on the occurrence, behaviour and effects of NPs in the environment by Nowack and Bucheli (2007) is a comprehensive and useful review of this area.

There is still currently very little evidence on which to determine the risks posed by engineered nanoscale materials. It is, therefore, difficult to assess the additional

measures that may be necessary to control potential risks. To address this, the UK Government has developed a comprehensive programme of research on potential risks and a Voluntary Reporting Scheme for engineered nanoscale materials. This scheme aims to contribute to the process of gathering evidence on the potential health and environmental impacts of nanomaterials. There are uncertainties about the risks of nanoparticles currently in production that need to be addressed immediately to safeguard workers, consumers, and the environment, and to support regulatory decisions that may be necessary. The report produced by the Royal Society and Royal Academy of Engineering in 2004 recommends that, until more is known about the environmental impact of NPs, their release into the environment should be avoided as far as possible. They also recommend that NPs should be treated as hazardous and be reduced in waste streams and that the use of free NPs in environmental applications such as remediation of groundwater be prohibited (Royal Society Report, 2004).

More research into the hazards and exposure pathways of NPs and nanotubes is required to reduce the many uncertainties and knowledge gaps related to their potential impact on health, safety and the environment. Current funding and hence research is inadequate. Zhang (2003) suggests that more attention should be directed to the fundamentals of nanochemistry in the environment, such as the process of contaminant transformation at the nanoparticle-water interface. An interdisciplinary approach is necessary for an appropriate risk assessment. There are many opportunities for collaboration between the different centres of expertise in nanotechnology, environmental science, pharmaceutical science and toxicology within the European Community. It is important to appreciate that environmental scientists and engineers already investigate nanostructures and nanoscale systems, as in studies of the natural weathering of minerals or the production of nanoscale colloids by microorganisms that are important in the fate, transport and transformation of potentially toxic substances (Masciangioli and Zhang, 2003).

Knowledge gaps in environmental risks:

- Environmental persistence, mobility through the food chain and bio-accumulation of ENPs
- Environmental release profiles through the entire life cycle of ENP-containing products

3.5 Best practices

Best practices in dealing with potentially dangerous materials of any type involve the following steps:

- Hazard identification – is there reason to believe the material could be harmful?
- Hazard characterization – how and under what conditions could it be harmful?
- Exposure assessment – will there be exposure in real world conditions?
- Risk characterization – is the substance hazardous and will there be exposure?
- Risk management – develop procedures to minimize exposure

Best practice protocols specifically dealing with nanoparticle production and handling have been drawn up by various companies and government organizations and address the issue in varying degrees of detail. (See “Best practices” in the reference list). Once the hazard posed by the nanomaterial has been evaluated, based on available, physical and chemical property data and toxicology or health effects data, and the potential worker exposure has been assessed to determine the degree of risk, the issue of “best practice” becomes one of risk management. It is generally accepted that risk management involves:

- The education and training of workers in the proper handling of nanomaterials (e.g., good work practices). This activity is not restricted to those producing nanoparticles but applies also to customers handling the materials in downstream applications. Part of the education includes instructions and safety data sheets to be provided, along with the raw materials, to the customers
- The establishment of criteria and procedures for installing and evaluating engineering controls (e.g., exhaust ventilation) at locations where exposure to nanoparticles might occur
- The development of procedures for determining the need for- and selection of personal protective equipment (PPE) (e.g., clothing, gloves, respirators)
- The systematic evaluation of exposures to ensure that control measures are working properly and that workers are being provided the appropriate PPE
- Routine health surveillance programs to ensure that any work-related change in health status is quickly determined and steps taken to identify and address the causes

Knowledge gaps in exposure and personnel protection:

Much more information is still needed in several areas related to the execution of “best practice” protocols, including:

- Nanoparticle sampling procedures and measurements
- The development of personal-sized sampling instruments which are able to measure particle size, shape, surface area, concentration, electrical charge and other characteristics
- Potential for nanoparticles to penetrate respirators and other personal protective equipment
- Understanding the chemical, physical and reactive properties of nanoparticles
- Determining the potential for airborne nanomaterials to cause fire or explosion
- Understanding the extent to which combustible nanomaterials pose a higher risk of fire and explosion than coarser material of similar composition and quantity
- Determining any chemical and/or physical characteristics of nanomaterials that may initiate catalytic reactions and increase the potential of fire and explosion

4 Recommendations with regard to research policy

4.1 Introduction: current activities

The Royal Society and Royal Academy of Engineering addressed potential risks to the environment and human health in their report entitled 'Nanoscience and nanotechnologies: opportunities and uncertainties'. The report sets out a number of recommendations for the UK Government to ensure an appropriate control framework for nanotechnologies. The UK Government's response to these recommendations was published in February 2005, and its implementation is being co-ordinated through the Nanotechnology Issues Dialogue Group (NIDG).

The European Commission have published a Nanotechnology Action Plan (<http://cordis.europa.eu/nanotechnology/actionplan.htm>) which includes a section on risks to the environment and human health. The EU Framework 7 (FP7) research program has a 3.5 billion Euro (\$ 5.1 billion) funding for nanotechnology for the period 2007 through to 2013. The UK Department for the Environment, Food and Rural Affairs (DEFRA) is working with the Commission to promote awareness of the Action Plan, and the need for harmonised and consistent action across the EU. A European Commission document, called 'Towards a European strategy for nanotechnology' (12 May 2004), addresses not only the R&D and innovation aspects of nanotechnology, but also possible environmental, health, safety and societal concerns.

Internationally, DEFRA is working through the Organisation for Economic Co-operation and Development (OECD) to a similar purpose, focusing in particular on research priorities, and the harmonisation of test methods for assessing risks. A Nanotechnologies Research Co-ordination Group (NRCG), chaired by DEFRA, is working to ensure that the research effort is effectively coordinated across governments. The NRCG is becoming more directly involved in the co-ordination of funding strategies for research and has recently expanded its membership to include industry representatives and more independent scientists and social scientists in its taskforces.

Five task forces have been set up by the NRCG under its terms of reference in response to the concerns noted above:

- Task Force 1 (metrology, characterization and standards)
- Task Force 2 (exposure issues, occupational and environmental)
- Task Force 3 (human health hazard and risk assessment)
- Task Force 4 (environmental hazard and risk assessment)
- Task Force 5 (social and economic dimensions of nanotechnologies)

The OECD has a Working Party on Manufactured Nanomaterials (WPMN) with six projects, each overseen by a Steering Group (SG). These groups are:

- SG 1: Database on Environment Health and Safety (EHS) research (to be launched in February 2008)
- SG 2: EHS Research strategies on manufactured nanomaterials

- SG 3: Safety testing of a representative set of manufactured nanomaterials (14 materials provisionally agreed) with a sub-group to pursue *in vitro* methods
- SG 4: Manufactured nanomaterials and test guidelines (closely linked with SG3)
- SG 5: Co-operation on voluntary schemes and regulatory programs providing details on exposure measurement and mitigation
- SG 6: Co-operation on risk assessment for manufactured nanomaterials (A research project related to this objective has recently been funded by DEFRA at Cranfield University)
- SG 7: The role of alternative methods in nanotoxicology
- SG 8: Cooperation on exposure measurement and exposure mitigation

4.2 Recommendations for immediate and long term measures with regard to research

Immediate measures relating to research on nanoparticles will have to deal with characterization of the human and environmental toxicological effects of nanoparticles. Work has already started but, as is clear from the summaries provided above, the field is very much in its infancy; it is still learning how to study these effects, deciding the relevant parameters, developing the proper measuring equipment, etc. The major work of definitive characterization, classification and regulation has yet to begin.

4.2.1 Immediate measures

Use of reference materials

The use of Reference materials for manufactured nanoparticle toxicology studies will be necessary for the scientific world to come to a more homogeneous testing. One example of such efforts is the project REFNANO (www.iom-world.org/pubs/REFNANOReport.pdf & Aitken et al., 2007). The project aimed for consensus between toxicology and metrology. More effort is needed in this area.

Complete and accurate particle characterization is essential for understanding their potential toxicological properties. Furthermore, characterization of nanomaterials is fundamental to ensure consistency and reproducibility of any tests (Aitken et al., 2007; Powers et al., 2006; HSE, 2006; NIOSH, 2006).

Use of more standardized and validated test protocols

In the effort to standardize the material, the property of interest in health and safety of nanomaterials remains their toxicity – which is at the moment somewhat secondary to the characterization. Therefore, more effort is needed to answer the question: “What toxicity parameter should be used as reference and how should it be measured?” For example:

- Warheit et al. (2007) recommend: substantial particle characterization, pulmonary toxicity studies, acute dermal toxicity and sensitization studies, acute oral and ocular studies, along with screening for genotoxicity, and, for environmental safety, aquatic studies
- Despite major efforts of several separate research groups, these questions have never been tackled in a large-scale exercise to verify the different opinions in the field. A strategy is needed to come to an “evidence based” approach. In other words: existing protocols should be validated for engineered nanoparticles, and new protocols should be set up
- All efforts in that area should be synchronised with ongoing OECD "Working Party on Manufactured Nanomaterials" efforts, especially with projects of SG 3 - Safety testing of a representative set of manufactured nanomaterials, SG 4 - Manufactured nanomaterials and test guidelines; and SG7 - The role of alternative methods in nanotoxicology
- A few recent observations emphasize the need for validated protocols:
 - Interference of nanomaterials with tests designed to examine their toxicity and inflammatory or oxidative capacities
 - Interference of SWCNT with MTT and neutral red assays and LDH activity in culture media. (Worle-Knirsch et al., 2006; Davoren et al., 2006)
 - Interference of CNT with different inflammatory metabolites in culture media (Pulskamp et al., 2007)

Specific point of action

Utilisation of measurement of oxidative stress as a marker of the potential toxicity of a nanomaterial

This possibility is proposed in a general review on toxicity of nanomaterials (Nel et al., 2006). However, the issue is complex because oxidative stress is a complex phenomenon, affecting different systems and cellular components. Many questions are unsolved yet: Which parameter should be measure? At what time? In which cellular type?

Specific targets to study

Since nanoparticles are able to translocate to the blood stream and pass the blood–brain barrier (Kreuter, 2001; Oberdorster and Utell, 2002; Oberdorster et al., 2002; Oberdorster et al., 2005), future research on NP cytotoxicity should include neuronal cells and neural function in addition to airway epithelia.

Development of nanoparticles whose surface characteristics impart a low oxidative potential (Warheit et al., 2007).

Dosimetrics to use?

The ‘ultrafine’ hypothesis stating that nanoparticles may be more cytotoxic than larger particles is almost generally accepted but some consideration has to be taken because it has been suggested that particle surface area and/or particle number both play a role and that maybe a combined threshold can be found. Therefore, it is possible that

particle aggregation could significantly modify dosimetrics and therefore should be considered in evaluations (Brown et al., 2007). The availability of particles to the cells may be a dominant dosimetric factor in particle toxicity, which suggests that the dynamics of agglomeration and spatial distributions of particles, as has also been emphasized in Section 3.4, will need to be considered for comparisons between particulate materials.

Registration

Expand the REACH protocol to require separate registration of nano-particulate material and chemical safety assessments (CSA) for each identified use once the hazard potential has been established.

Three challenges in risk research in nanotechnologies (Maynard et al., 2006).

- Develop and validate methods to evaluate the toxicity of engineered nanomaterials. “It seems clear that *in vitro* cellular systems will need to be further developed, standardized, and validated (relative to *in vivo* effects) in order to provide useful screening data on the relative toxicity of inhaled particle types (Sayes et al., 2007)”
- Develop models for predicting the potential impact of engineered nanomaterials on the environment and human health
- Develop robust systems for evaluating the health and environmental impact of engineered nanomaterials over their entire life

4.2.2 Long term measures

Even without the validated protocols and standards mentioned above, it is already clear that some nanomaterials are toxic under some conditions and yet, even without a thorough long-term testing, some nanoparticles and nanofibers are being incorporated into consumer products. The scientific community and governmental regulatory agencies are just not able to move at the speed of the consumer product industries or, better still, stay ahead of them, as should be the case in a perfectly ordered world. Therefore, on the long-term, the emphasis will need to be on following the impact of nano-particles on human health. As in the case of asbestos fibers, the best method to assess the health effect of something which gets distributed widely in society is to monitor the health of those who deal with the material during the production phase.

- Institute pan-European mandatory registration and health-screening of all workers involved in industrial large-scale nanoparticle production and handling
- Establish long-term cohort studies to follow the health status of these workers collecting not only health data, but also biological materials from these individuals to be stored in a Bio-bank for further characterization and correlation
- Establish and fund a separate department/organization to coordinate and manage the funding of the above activities

5 Summary and outlook

5.1 Summary

The purpose of this Coordination Action was to develop guidelines for the production and application of nanoparticles, starting from the premise that at least some of these materials may be toxic to human health and the environment. For this purpose, the current report first assesses what is currently known about these materials and what legislation is in place to regulate them. Four classes of engineered nanoparticles have been examined; ceramics, metals and metal oxides, carbon and inorganic fullerenes, carbon and metallic nanotubes and quantum dots. The various routes of exposure have been considered and a summary is provided of the information which is available on the toxicity and health impact of these materials, derived from experimental studies. On the basis of this information an analysis has been made of the gaps which still exist in our knowledge of the impact of these materials and the procedures to minimize exposure. This has resulted in a series of recommendations for further research on these topics which are summarized in Chapter 4, Recommendations with regard to research policy.

5.2 Outlook on future activities

The health, safety and environmental impact of nanoparticles will need to be addressed and monitored continuously for many years. On the short term, much of the focus will be on fundamental issues such as unravelling physiological responses to nanoparticles and establishing and understanding causal relationships between their presence and pathologies in cell culture and animal models. The credibility and broad acceptance of the results will depend on the establishment and use of standardized models, metrics, dosing and monitoring equipment. Legislation regulating the production, work-pace hygiene and application of these materials will be derived from these results. Nevertheless, mirroring the genetic engineering / food controversies of the past 20 years, one can expect a constant pressure from citizens' organizations, on the one hand, to prevent the application of nanoparticles, and from industries, on the other, to increase their application in consumer products and processes as a result of uncertainties in the extensibility of *in vitro* or animal model study results to humans.

Long-term cohort studies monitoring the health status of workers exposed to elevated levels of nanoparticles over extended periods will provide an upper limit of the threat which nanoparticles pose to human health. But over the same period, these materials will continue to diffuse in the environment and into the food chain in undefined ways and at virtually undetectable levels. One can expect, therefore, that if there are health hazards associated with certain classes of broadly used nanoparticles, they could well be untraceable and still have major impacts on our society. One needs only to look at the increasing incidence of breast cancer in women in the US and Western Europe or cancers which occur more selectively in other regions of the world to get a feeling for the intricacy of the link between human health, environment, customs, genetics and diet.



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This IMPART study is a snapshot, in time, of the nanoparticle issue. It has identified areas where more research is needed and made recommendations which, when implemented will bring more clarity to the discussions and controversies surrounding a technology that poses so many threats and, at the same time, hold so much promise.

6 References

- Aguar P, Nicolás JJ EU (2008) Nanotechnology R&D in the field of health and environmental impact of nanoparticles, OECD.
- Aitken RJ, Tran CL, Donaldson K, Stone V, Johnstone J, Chaudry Q, Cash S (2007) REFNANO Reference materials for engineered nanoparticle toxicology and metrology ACHS/07/09A 5 June 2007.
- Aitken RJ, Chaudhry MQ, Boxall ABA (2006) Manufacture and use of nanomaterials: current status in the UK and global trends. *Occupational Medicine* 56(5) 300-306.
- Aitken RJ, Creely KS, Tran CL (2004) Nanoparticles: An occupational hygiene review Prepared by the Institute of Occupational Medicine for the Health and Safety Executive 2004. Research Report No 274. Available at <http://www.hse.gov.uk/research/rrpdf/rr274.pdf> Last accessed 10.03.'08
- Alivisatos AP, Gu W, Larabell C (2005) Quantum dots as cellular probes. *Ann. Rev. Biomed. Eng.* 7:55-76
- Baccarelli, A., and colleagues, 2007. Effects of exposure to air pollution on blood coagulation. *J Thromb Haemost* 5, 252-260.
- Behrens, I., Pena, A.I., Alonso, M.J., Kissel, T., 2002. Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm Res* 19, 1185-1193.
- Best Practices:
- Progress Towards safe Nanotechnology in the workplace (2007) (<http://www.cdc.gov/niosh/docs/2007-123/pdfs/2007-123.pdf>)
 - Guidance for Handling and Use of Nanomaterials at the Workplace (2007) (http://www.baua.de/nn_49456/en/Topics-from-A-to-Z/Hazardous-Substances/Nanotechnology/pdf/guidance.pdf)
 - Guide to safe handling and disposal of manufactured nanomaterials (2007) (<http://www.bsi-global.com/en/Standards-and-Publications/Industry-Sectors/Nanotechnologies/PD-6699-2/Download-PD6699-2-2007/>)
 - Department of Energy, Nanoscale Science Research Center: Approach to Nanomaterial ES&H (2007) (<http://orise.orau.gov/ihos/Nanotechnology/files/NSRC%20ESH%20Approach%20Doc%20Rev2.pdf>)
 - Nanotechnology Consensus Workplace Safety Guidelines (<http://www.orc-dc.com/Nano.Guidelines.Matrix.htm>)
 - Interim Best Practices for Working with Nanoparticles (2007) (www.turi.org/.../4128/48845/file/Best%20Practices%20for%20Working%20with%20Nanoparticles%20Version%200.pdf)
 - (http://www.nsec.wisc.edu/NanoRisks/NS--HS_Protocols_BestPractices.php)
- Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D and Oberdorster E (2006) The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and Fibre Toxicology*, 3:11
- Brant JA, Lecoanet H, Wiesner MR (2005) Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. *J. Nano. Res.* 7:545-553.
- Brown, S.C., Kamal, M., Nasreen, N., Baumuratov, A., Sharma, P., Antony, V.B., Moudgil, B.M., 2007. Influence of shape, adhesion and simulated lung mechanics on amorphous silica nanoparticle toxicity. *Advanced Powder Technology* 18, 69-79.

- Cheng X., Kan A, Tomson M (2004) Naphthalene adsorption and desorption from aqueous C60 fullerene. *J. Chem. Eng. Data* 49:657-83.
- Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work. OJ L183, 29.6.1989, p. 1
- Council Directive 91/689/EEC of 12 December 1991 on hazardous waste. OJ L 377, 31. 12. 1991, pp. 20-27
- Davoren, M., Herzog, E., Casey, A., Cottineau, B., Chambers, G., Byrne, H.J., Lyng, F.M., 2006. In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicol In Vitro*.
- Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC) (codified version) OJ L158, 30.4.2004, p. 50.
- Dror I, Baram D, Berkowitz B (2005) Use of nanosized catalysts for transformation of chloro-organic pollutants. *Environ. Sci. Technol.* 39:1823-1290
- Donaldson, K. & Borm P. (Ed) (2007) *Particle Toxicology*, CRC Press, Boca Raton, Florida
- Donaldson, K., Stone, V., 2003. Current hypotheses on the mechanisms of toxicity of ultrafine particles. *Ann Ist Super Sanita* 39, 405-410.
- Donaldson, K., Stone, V., Clouter, A., Renwick, L., MacNee, W., 2001. Ultrafine particles. *Occup Environ Med* 58, 211-6, 199.
- Elder, A., and colleagues, 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect* 114, 1172-1178.
- European NanOSH Conference –Nanotechnologies: A Critical Area in Occupational Safety and Health 3–5 December 2007, Marina Congress Center, Helsinki, Finland
- Ferin, J., Oberdorster, G., Penney, D.P., 1992. Pulmonary retention of ultrafine and fine particles in rats. *Am J Respir Cell Mol Biol* 6, 535-542.
- Fortner JD, Lyon DY, Sayes CM, Boyd AM, Falkner JC, Hotze EM, Alemany LB, Tao YJ, Guo W, Ausman KD, Colvin VL, Hughes JB (2005) C60 in water: nanocrystal formation and microbial response. *Environ. Sci. Technol.* 39:4307-4316.
- Gilmour, P.S., and colleagues, 2004. Pulmonary and systemic effects of short-term inhalation exposure to ultrafine carbon black particles. *Toxicol Appl Pharmacol* 195, 35-44.
- Guzman KAD, Taylor MR, Banfield JF (2006) Environmental risks of nanotechnology: National nanotechnology initiative funding, 2000-2004 40(5):1401-1407.
- Hardman R (2006) A toxicological review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.* 114:165-172
- Helland A, Scheringer M, Siegrist M, Kastenholz HG, Wiek A, Scholz RW (2008) Risk assessment of engineered nanomaterials: A survey of industrial approaches. *Environ. Sci. Technol.* 42, 2: 640-646.
- Hoek, G., Brunekreef, B., Fischer, P., van, W.J., 2001. The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. *Epidemiology* 12, 355-357.
- Hoet, P.H., Bruske-Hohlfeld, I., Salata, O.V., 2004. Nanoparticles - known and unknown health risks. *J Nanobiotechnology* 2, 12.
- Hoet PHM, A Nemmar, Nemery B (2004) Health impact of nanomaterials? *Nature Biotechnol.* 22(1): 19.
- Hood E (2004) Nanotechnology: looking as we leap. *Environ. Health Perspect.* 112(13), A740-A749.
- Howard C V (2003) Nano-particles and toxicity. *Occ. Paper Series* 7:15-19 http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

- Hyung Y, Fortner JD, Hughes JB, Jae H (2007) Natural Organic Matter Stabilizes Carbon Nanotubes in the Aqueous Phase. *Environ. Sci. Technol.* 41:179-184.
- IRSST (2006) Nanoparticles - Actual Knowledge about Occupational Health and Safety Risks and Prevention Measures. Research projects R470: IRSST, Quebec, p. 100 (<http://www.irsst.qc.ca/files/documents/PubIRSST/R-470.pdf>)
- Kreuter, J., 2001. Nanoparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev* 47, 65-81.
- Kuempel ED, Geraci CL, Schulte PA (2007) Risk Assessment Approaches and Research Needs for Nanomaterials: An examination of data and information from current studies in Nanotechnology 119–145 in *Toxicological Issues and Environmental Safety*, Springer, Netherlands.
- Lam C-W, James JT, McCluskey R, Hunter RL (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol. Sci.* 77:126-134.
- Lecoanet HF, Bottero JY, Wiesner MR (2004) Laboratory assessment of the mobility of nanomaterials in porous media. *Environ. Sci. Technol.* 38:5164.
- Li, X.Y., Gilmour, P.S., Donaldson, K., MacNee, W., 1996. Free radical activity and pro-inflammatory effects of particulate air pollution (PM10) in vivo and in vitro. *Thorax* 51, 1216-1222.
- Lovern SB, Klaper R (2006) *Daphnia magna* mortality when exposed to Titanium Dioxide and Fullerene. *Environ. Toxicol. Chem.* 25 :1132-1137.
- Masciangioli T, Zhang WX (2003) Environmental technologies at the nanoscale. *Environ. Sci. Technol.* 37:102A-108A.
- Maynard, A.D., and colleagues, 2006. Safe handling of nanotechnology. *Nature* 444, 267-9.
- Monteiller, C., Tran, L., MacNee, W., Faux, S., Jones, A., Miller, B., Donaldson, K., 2007. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. *Occup Environ Med* 64, 609-615.
- Murr LE, Soto KF, Guerrero PA, Lopez DA, Ramirez DA (2004b) Carbon nanotubes and other fullerene-related nanocrystals in the environment: A TEM study. *JOM* 56, 28.
- Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. *Science* 311, 622-627.
- Nemmar, A., Hoet, P.H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., Nemery, B., 2002. Passage of inhaled particles into the blood circulation in humans. *Circulation* 105, 411-414.
- Nemmar, A., Hoylaerts, M.F., Hoet, P.H., Nemery, B., 2004. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. *Toxicol Lett* 149, 243-253.
- NIOSH [2006] last accessed 10.03.'2008 at http://0-www.cdc.gov.pugwash.lib.warwick.ac.uk/niosh/topics/nanotech/safenano/pdfs/approaches_to_safe_nanotechnology_28november2006_updated.pdf
- Nowack B, Bucheli TD (2007) Occurrence, behaviour and effects of nanoparticles in the environment. *Environ. Poll.* 150, 5-22.
- Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C., 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16, 437-445.
- Oberdörster E (2004) Manufactured nanomaterials (Fullerenes, C₆₀) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Persp.* 112:1058-1062.
- Oberdörster E, Zhu S, Blickley TM, McClellan-Green P, Haasch ML (2006) Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerenes (C₆₀) on aquatic organisms. *Carbon* 44:1112-1120.

- Oberdorster, G., Oberdorster, E., Oberdorster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113, 823-839.
- Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., Cox, C., 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health A* 65, 1531-1543.
- Oberdorster, G., Utell, M.J., 2002. Ultrafine particles in the urban air: to the respiratory tract--and beyond? *Environ Health Perspect* 110, A440-A441.
- OECD Environment, Health and Safety Publications Series on the Safety of Manufactured Nanomaterials No. 6 Working Party on Manufactured Nanomaterials: List of manufactured nanomaterials and list of endpoints for phase one of the OECD testing programme. Environment Directorate. Organisation for Economic Co-Operation and Development. Paris, 2008. ENV/JM/MONO(2008)13
- Owen R, Depledge M (2005) Nanotechnology and the environment: Risks and rewards. *Marine Poll. Bull.* 50, 6: 609-612.
- Poland, C.A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W.A.H., Seaton, A., Stone, V., Brown, S., MacNee, W., Donaldson, K., 2008. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nano* advanced online publication.
- Pope, C.A., III, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R., Dockery, D.W., 1999. Heart rate variability associated with particulate air pollution. *Am Heart J* 138, 890-899.
- Powell MC, Kanarek MS (2006) Nanomaterial Health Effects – Part 1:Background and Current Knowledge *Wisconsin Medical Journal* 105(2):16-20.
- Pulskamp, K., Diabate, S., Krug, H.F., 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol Lett* 168, 58-74.
- Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC
- Remskar M (2004) Inorganic nanotubes. *Adv. Mater.* 16, 1497-1504
- Royal Society Report (2004) Nanoscience and Nanotechnologies: Opportunities and Uncertainties - Summary and Recommendations. The Royal Society & Royal Academy of Engineering, UK.
- Samet, J.M., Dominici, F., Curriero, F.C., Coursac, I., Zeger, S.L., 2000. Fine particulate air pollution and mortality in 20 U.S. cities, 1987-1994. *N Engl J Med* 343, 1742-1749.
- Saito R, Dresselhaus G, Dresselhaus MS (1998) Physical properties of carbon nanotubes. Imperial College Press, London
- Sayes, C.M., Reed, K.L., Warheit, D.B., 2007. Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. *Toxicol Sci* 97, 163-180.
- Schins, R.P., Knaapen, A.M., 2007. Genotoxicity of poorly soluble particles. *Inhal Toxicol* 19 Suppl 1, 189-198.
- Singh S, Nalwa HS (2007) Nanotechnology and health safety – toxicity and risk assessments of nanostructured materials human health. *Journal of Nanoscience and Nanotechnology* 7(9): 3048-3070.
- Tenne R, Margulis L, Genut M, Hodes G (1992) Polyhedral and cylindrical structures of tungsten disulphide. *Nature* 360:444-446

- Warheit, D.B., Webb, T.R., Reed, K.L., Frerichs, S., Sayes, C.M., 2007. Pulmonary toxicity study in rats with three forms of ultrafine-TiO₂ particles: differential responses related to surface properties. *Toxicology* 230, 90-104.
- Warheit, D.B., Webb, T.R., Sayes, C.M., Colvin, V.L., Reed, K.L., 2006. Pulmonary instillation studies with nanoscale TiO₂ rods and dots in rats: toxicity is not dependent upon particle size and surface area. *Toxicol Sci* 91, 227-236.
- Wick, P., Manser, P., Limbach, L.K., Dettlaff-Weglikowska, U., Krumeich, F., Roth, S., Stark, W.J., Bruinink, A., 2007. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol Lett* 168, 121-31.
- Worle-Knirsch, J.M., Pulskamp, K., Krug, H.F., 2006. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett* 6, 1261-8.
- Zhu YQ, Sekine T, Li YH, Wang WX, Fay MW, Edwards H, Brown PD, Fleischer N, Tenne R (2005) WS₂ and MoS₂ inorganic fullerenes - super shock absorbers at very high pressures. *Adv. Mater.* 17:1500-1503