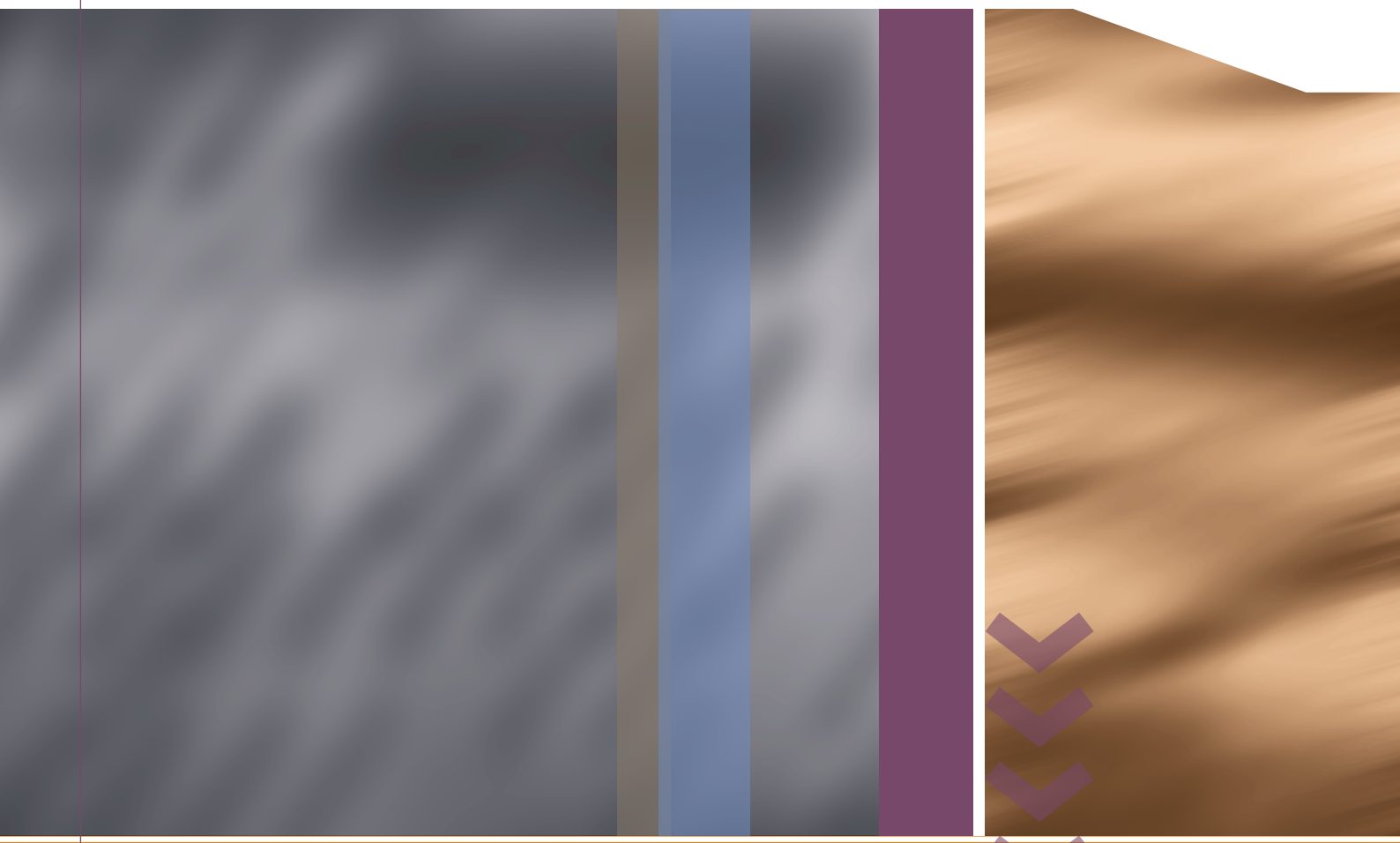
# A REVIEW OF THE POTENTIAL OCCUPATIONAL HEALTH & SAFETY IMPLICATIONS OF NANOTECHNOLOGY

July 2006



Australian Government
Australian Safety and Compensation Council

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**ISBN is: 0 642 32609 6 (web)**

**ISBN is: 0 642 32615 0 (print)**

# ‘A Review of the Potential Occupational Health and Safety Implications of Nanotechnology

for the

Department of Employment and Workplace Relations

Final Report

July 2006

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# Contents

## Acknowledgements

Drs. Peter Binks (CEO, Nanotechnology Victoria), Peter McCormick and Brian Priestly (Monash University) for valuable input and assistance.

Dr. Joe Shapter (Flinders University) for excellent technical advice.

Dr. Gunter Springholz (University of Linz) for generously providing information on quantum dot Ostwald ripening.

Dr. John Edwards (Flinders University) for critical review of the draft report.

Dr. Howard Morris (Office of the ASCC) for expert assistance and guidance in preparation of the final version of the report.

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# Glossary of Terms

**adenocarcinoma:** Any malignant growth of glandular tissue.

**aerodynamic diameter**: Diameter of a spherical particle with a density of 1000 kg/$m^3$, that has the same settling velocity as the particle under consideration; related to the inertial properties of aerosol particles.

**agglomerate:** Group of particles held together by relatively weak forces, including van der Waals forces, electrostatic forces and surface tension.

**aggregate:** Heterogeneous particle in which various components are not easily broken apart.

**anthropogenic:** Of human origin; man-made.

**apoptosis:** A form of *regulated* cell death initially identified from pathology but fully characterised as a genetically controlled program most often seen in development (a.k.a. "programmed cell death"). It is usually characterised by the ordered disassembly of the cell's contents, formation of smaller fragments known as "apoptotic bodies" and engulfment by neighbouring cells. Apoptosis, without secondary necrosis, is not inflammatory.

**atherosclerosis:** A form of vascular disease characterised by a fatty degeneration of the middle part of the artery wall.

**autophagy:** A physiological process of organelle degradation within the cell. When autophagy involves the total destruction of the cell it is called autophagic cell death and is a regulated process.

**blood-brain barrier:** A CNS (q.v.) epithelial (q.v.) cell barrier that is impermeant to all except lipophilic molecules (such as oxygen, carbon dioxide, and ethanol) and those with specific transporters (such as sugars and some amino acids). Substances with a molecular weight higher than 500 Daltons generally cannot cross the blood-brain barrier (incl. viruses and most drugs).

**bottom-up nanotechnology:** Mostly related to chemical synthesis – structure creation by connecting molecules.

**carbon nanotubes:** Tiny tubes about 10,000 times thinner than a human hair -- consist of rolled up sheets of carbon hexagons.

**ciliated:** Having microscopic hair-like structures which aid in the movement of fluids over membranes.

**cytochrome P-450:** Any of a large group of haem containing and electron-transferring enzymes that are involved in drug, steroid or chemical metabolism.

**CNS:** Central nervous system.

**dysmorphogenesis:** Abnormal structure formation during development.

**effective particle size:** Measure of a particle that characterises its properties or behaviour in a specific system.

**engineered nanoparticles:** Nanoparticles between 1 nm and 100 nm manufactured to have specific properties or composition.

**epithelial:** Relating to cells in close proximity and which line the surface of an organ or hollow internal structure without the need for connective tissue.

**equivalent diameter:** Diameter of a sphere which behaves like the observed particle relative to or deduced from a chosen property.

**fibrosis:** An abnormal (pathological) formation or development of excess fibrous connective tissue in an organ or tissue as a reparative or reactive process.

**fullerene:** A new allotrope of carbon characterized by a closed cage structure consisting of an even number of three coordinate carbon atoms without hydrogen atoms. This class was originally limited to closed-cage structures with twelve isolated five- membered rings, the rest being six- membered rings.

**glomerular:** Relating to the capillary structures that form the filtering unit of the kidney.

**granuloma:** Small nodules usually consisting of epithelioid macrophages surrounded by lymphocytes. When necrosis is evident internally this is termed 'caseating granulomas' - especially as observed with tuberculosis.

**graphene:** Individual layers of carbon atoms arranged in a honeycomb-like lattice, found in graphite.

**hepatocyte:** The main non-connective cell of the liver(*adj*. **hepatocellular**).

**homeostasis:** The maintenance of the body's normal operating conditions.

**humoral:** Relating to bodily fluids.

**hydrodynamic diameter:** Effective diameter of a particle in a liquid environment.

**hypertrophy:** An abnormal increase in organ size which is not usually cancerous.

**intraperitoneal:** Within the membrane that lines the abdominal cavity (peritoneum).

**ischaemia:** A period of reduced or absent blood flow to a tissue which can be caused by many different factors.

**keratinised:** Regarding the protein comprising the surface layer of the skin.

**lysosomal:** A cytoplasmic organelle containing hydrolytic ("degrading") enzymes and surrounded by a membrane.

**micellar:** Relating to polymer aggregates.

**mobility diameter:** Diameter of spherical particle with the same mobility as the particle under consideration.

**multi-walled carbon nanotubes:** Carbon nanotubes (q.v.) which consist of more than one nanotube completely contained within another

**MWCNTs:** Abbreviation for multi-walled carbon nanotubes.

**nano:** $10^{-9}$ or, alternatively, 0.000000001

**nanoaerosol:** A collection of nanoparticles suspended in a gas.

**nanocrystals:** A nanocrystal typically has a diameter of between 1 and 10 nm and may contain as few as a hundred or as many as tens of thousands of atoms. Many fundamental properties of nanocrystals depend strongly on their size Related term: **quantum dots.**

**nanoengineering:** The construction of nanostructures and their components.

**nanofluidics:** The movement of liquids on a nanoscale, e.g. by using

microscopic silicon devices, for uses such as counting, analysing and separating molecules.

**nanomanufacturing:** Is expected to be high- volume, high- rate, integrated assembly of nano-elements into commercial products. This involves controlling position, orientation, and interconnectivity of the nano- elements.

**nanomaterials:** Contain only a few thousand or tens of thousands of atoms, rather than the millions or billions of atoms in particles of their bulk counterparts.

**nanoparticle(s):** An engineered form of matter having at least one dimension (length, breadth or width) in the nanometre scale (<100 nm). Nanoparticles are considered distinct from UFPs (q.v.) for the purposes of this report only insomuch that UFPs are derived from "accidental" sources (human or natural).
Abbreviation: **NPs**.

**nanophase:** Discrete phase (i.e. material's physical state), within a material, which is at the nanoscale.

**nanopowder:** Dry nanoparticles.

**nanoscale:** 1 to 100 billionths of a metre.

**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.

**nanospheres:** Spheres ideally completely spherical and homogeneous in size and at the nanoscale.

**nanostructures:** Nanometre sized objects. Chemically, nanostructures are molecular assemblies of atoms numbering from $10^3$ to $10^9$ and of molecular weights of $10^4$ to $10^{10}$ Daltons. Thus, they are chemically large supramolecules. To molecular biologists, nanostructures have the size of objects such as proteins or viruses and cellular organelles. Material scientists and electrical engineers view nanostructures as the current limit of nanofabrication.

**nanotoxicology:** A new term which has been proposed to encompass the study of the adverse effects of nanoparticles (NPs) on health and the environment.

**nanotubes:** Nanometre-sized tubes composed of various substances including carbon, boron nitride, or nickel vanadate. Carbon nanotubes were discovered in 1991 by Sumio Iijima and resemble rolled up graphite.

**nanowires:** Molecular wires millions of times smaller in diameter than a human hair.

**necropsy:** The procedure of post-mortem examination.

**necrosis:** A form of cell death most often – but not entirely - occurring from acute cellular injury and generally considered to be unregulated ("*accidental cell death*"). It is usually characterised by a disruption of the cell's outer plasma membrane and release of internal contents which can then initiate inflammation.

**nephropathy:** Any damage or disease to the kidney.

**neutrophil:** A type of leucocyte or white blood cell.

**NPs:** Abbreviation for engineered nanoparticles (q.v.), c.f. UFPs (q.v.).

**oligonucleotides:** A string of up to approximately 30 DNA bases.

**oocyte:** A cell which gives rise to a mature egg cell ("ovum") by a process of cell division known as meiosis.

**particle size:** Size of a particle as determined by a specified measurement method.

**permissible Exposure Limit (PEL):** OSHA (USA) guideline/standard for maximum workplace exposure over an 8-hour time weighted average (TWA) exposure. Equivalent to Australian WES (Workplace Exposure Standard).

**peribronchial:** Relating to lung structures in vicinity of the bronchia (wind-pipe branches).

**phagosomal:** Relating to a specialised cellular structure formed during the internalisation of foreign particles by enclosing in the outer membrane. (*Verb*: **phagocytosis**).

**polyneuropathy:** A generalised disorder of the nerves.

**proteasome:** A complex barrel-shaped multi-protein structure inside the cell which functions to digest other proteins into short polypeptides and amino acids (either self or non-self). The proteasome system is essential for many cellular processes including cell cycle, signal transduction and regulation of gene expression.

**proteolytically:** Relating to the splitting of proteins or protein fragments by enzymes.

**quantum dots:** Nanometre sized fragments of semiconductor crystalline material.

**sarcoma:** A malignant tumour of non-epithelial tissue (e.g. connective tissue).

**sequestration:** The action or process of making unavailable without destroying or inactivating.

**semiconductor:** Material whose conductivity is normally in the range between that of metals and insulators and in which the electric charge carrier density can be changed by external means.

**single walled carbon nanotubes:** Carbon nanotubes (q.v.) which do not contain any material internally.

**specific surface area:** Ratio of the surface area to the mass of a nanopowder.

**squamous cell:** A morphologically thin and flattened cell of an epithelial layer.

**SWCNTs:** Abbreviation for single-walled carbon nanotubes.

**top-down nanotechnology:** Engineers taking existing devices, such as transistors, and making them smaller.

**UFPs:** Abbreviation for ultrafine particles (q.v.).

**transcription factor:** A protein which is involved in the control of new gene expression.

**trypanosome:** A parasitic protozoan that infects the blood of humans and animals.

**ultrafine particles:** An anthropogenic or natural form of nanoparticle which is usually derived from combustion processes. UFPs are distinguished by large variations in size and composition.

**workplace exposure standard (WES):** ASCC guideline/standard for maximum workplace exposure over an 8-hour time weighted average (TWA)

exposure. Equivalent to US PEL (Permissible Exposure Limit).

**xenobiotic:** A chemical foreign to the body and is not normally produced or expected to be present in it.

## Scope of this Report

This review of the literature has as its emphasis the occupational health and safety concerns of the nascent nanotechnology industry in Australia. More specifically, the primary focus is with the human exposure factors and toxicology of nanoparticles engineered for either commercial or research use.

Consequently, this *excludes* some areas of nanotechnology research and/or commercialisation except when this work is immediately applicable to such OHS considerations. The major areas excluded from this report include;

1. Life cycle analysis of products and by-products of nanoparticle manufacture and their effects on human and environmental health.
2. The therapeutic capabilities of NPs to be used in drug delivery, biomaterials (e.g. for implant), or for other biomedical uses. And,
3. the broader health impacts of ultrafine particles (UFPs) derived from general combustion and as by-products of industrial processes.

For example, previous reports (refer Section 2) have highlighted the need to distinguish between fixed and free NPs with the latter likely to pose a greater hazard to health and the environment. While this is likely to be true, at least in the shorter term, the prospect that widespread adoption of nanoparticles (NPs) into commercial products could ultimately result in large environmental burdens. As a consequence, it is conceivable that NPs may leach out from landfills and overload the sequestration capacities of our environment. At present there appears to be little resolve to address these issues from any of the major economies (e.g. UK Government Response 2005; Denison 2005). This appears to be principally a cost concern but at this early stage in the industry's development an example is worth citing. The manufacture of zinc oxide NPs (and manufacturing by-products containing Zn) for use in cosmetics and sun-screens are *currently* subject to disposal restrictions in Australia at the state level as inorganic Zn salts are known to be potent biocides.

More benign scenarios are also evident. Would the incorporation of UV-protective NPs and nanoclays into glass (Australian Govt. 2005; Nanotechnology Now 2005), for example, hinder or prevent these forms of glass from being recycled? Questions such as these, although beyond the scope of the present report, are ultimately worthy of consideration.

A portion of the nanotechnology and nano-manufacturing press remains speculative (at best) and fanciful (at worse). It is not the intention of this review to comment or evaluate the safety of these areas of "nanobots" or "self assembling nano-structures" as these remain too distant and theoretical a prospect to be of any immediate relevance to Australian OHS concerns.

# Executive Summary

Nanotechnologies and nanoparticles occur at the level of 0.000000001 metres and it is difficult to fully appreciate these remarkably small scales. The distinctive and oftentimes unique properties which are observed with nanoparticles have been proposed to revolutionise manufacturing and consuming in the future - much as the industrial revolution did in the late 18th and early 19th centuries. Only the future will confirm if these expectations will be realised.

In the intervening period, however, there will be large increases in the production, distribution and handling of nanoparticles. Various health and materials science specialists have expressed concern that the unique properties which have made nanoparticles attractive to industry may also have untoward effects on human and environmental health. The calls for proactive steps to be taken to characterise any hazardous properties of nanoparticles have come from both industry and the public arena. These calls for action have been evident not only from overseas but also from within Australia.

Nanoparticles - with names such as fullerenes ("buckyballs"), nanotubes and quantum dots - will contribute to such diverse areas of manufacture as electronics, aerospace and transport engineering, textiles, food and beverage, energy and the environment. At this early stage the potential applications of nanoparticles seem to be limited only by the imagination. Although difficult to predict the long term commercial potential for carbon nanotubes, quantum dots and their derivatives appear to be the most promising. As a result preferential emphasis should be given to assessing the health and environmental effects of these types of nanoparticles.

The occupational health and safety effects of engineered nanoscale particles are mostly unknown. This can be attributed to the relatively recent development of the nanotechnology sector and, as a result, the lack of available information on human exposures and working conditions. As a consequence our abilities to accurately predict the impact of nanoparticle exposures on worker health are limited at this time. In particular our abilities to measure nanoparticles in the workplace (or more generally) are limited by current technologies. Nanotechnology presents us with new challenges as the properties of nanoparticles now depend on size and shape as much as the more conventional factors of chemical structure and composition. The measurement of these additional attributes will be necessary to accurately assess nanoparticle concentrations in the workplace. In addition, the capability of the human body to recognise and appropriately respond to these tiny entities is essentially unknown at the moment.

Findings from animal and in vitro test systems have provided some valuable information and these results indicate that human and environmental health

consequences are possible from nanoparticle use and exposures. However, given the inadequate number and variability of the studies reported to date, the confidence in extrapolating to the assessment of occupational risks is minimal. Several credible animal studies suggest that lung pathologies (such as cancers, inflammation, granuloma formation, fibrosis and breathing difficulties) may be expected with exposures to carbon nanotubes and metal oxide nanopowders. The ambient air concentrations and extent of exposures in workplace settings to nanotubes and quantum dot nanoparticles are not well established.

Quantum dot nanoparticles generally consist of an inner metal or metalloid core. The stability of this core is of toxicological concern as any breakdown of the lattice releases kidney-, liver-, reproductive- and brain damaging chemicals such as cadmium, arsenic and lead.

The manufacture of nanoparticles are typically carried out in closed systems. Although the opportunity exists that exposures may occur from catastrophic equipment failure this appears to be less likely than other workplace exposure settings. Consequently, the possibilities for human exposure are greatest during the handling and transport of nanomaterials following manufacture and release into more open environments. In addition, all manufacturing processes require equipment maintenance procedures and the potential for human exposure to nanoparticles in these situations are also expected to be higher.

This report considers that the greatest gaps in our present knowledge, and those requiring attention as a matter of priority, are:

- the development of cost-effective and robust ambient air monitoring systems for nanotubes, nanopowders and quantum dots in workplace environments that can provide accurate information on worker exposures (ideally in real-time).
- Setting of priorities to acquire the necessary information for the determination of meaningful workplace exposure standards and adequate worker protection. Information relevant to nanotubes, metal nanopowders and quantum dots should be given priority. Some of the more necessary OHS issues that should be examined here are:
    - ⇒ assessments of inhalational and dermal absorption and uptake under as realistic conditions as possible.
    - ⇒ Effectiveness of personal protection and control measures for the workplace (e.g. the effectiveness of various types of respirators and clothing to nanotubes and quantum dots).
    - ⇒ The determination of worker exposure potentials especially during the vulnerable procedures of material handling and transport and maintenance of production machinery.

- Chronic exposure studies in appropriate in vivo test systems, and particularly for organic nanoparticles and quantum dots, should be undertaken so as to generate information on the long term health effects of these compounds and as a precursor to any future epidemiological studies.

These gaps in our knowledge will best be addressed at a multidisciplinary level. Human health toxicologists, molecular biologists and biochemists, clinical pathologists, occupational health practitioners and - eventually as the industry grows – epidemiologists - all have vital roles to play in safeguarding health in this fast-moving field. The authors consider that collaborative studies - ideally coordinated with overseas colleagues - are essential in order to provide the critical information required within a reasonable time frame.

The nanotechnology industry is still in its infancy. The industry will expand rapidly and contribute to the nation's wealth in both predictable and unforeseen ways. Strategic - but relatively small - investments in this area by the Australian Federal Government can be considered to be investments in the knowledge economy of this burgeoning sector. Existing governmental agencies already provide the support infrastructures that are required to foster the future growth of this industry. Nonetheless, there are calls from within the private and public sectors for considerations to be given to existing regulatory frameworks to examine their effectiveness in dealing with the advent of nanotechnology. For example, new chemical safety assessments for engineered nanoparticles may need to be revised and not be undertaken based on CAS# alone as it is known that the nanoscale compound will likely differ substantially from its bulk mass counterpart. It is the view of the authors that government will need to act proactively to protect the health of its community (including those within the nanotechnology industry) and, in so doing, contribute to the international body of toxicology knowledge in this area.

# 1. Introduction

## 1.1 History

The theoretical possibilities of the nanometre scale, and its exploitation, are widely attributed to the American Nobel Prize winning physicist Richard Feynman. In a 1959 talk he advocated the manipulation of matter with the end result of producing unique and ultimately useful physical and chemical properties. That these processes are now reaching commercialisation is a vindication of his foresight.

However, it was to take another 26 years before the first practical examples caught up with the theory. In 1985 Kroto, Curl, Smalley and colleagues discovered the third physical form of carbon at Rice University (Houston) by using laser vapourisation of graphite (Kroto et al 1985). This unique allotrope was a pure form of carbon consisting of 60 atoms and termed "$C_{60}$", "fullerene(s)" or "buckyballs". For their efforts they also received the Nobel Prize, in 1996. Fullerenes are known to usually take the shape of a hollow sphere or ellipsoid and are structurally related to carbon nanotubes (refer Sections 2.1 & 2.2). Their distinctive properties, which also have a bearing on their expected human health effects, are derived from their shape which imposes constraints on electronic structures. The highest occupied $C_{60}$ molecular orbitals (HOMO) are occupied whereas the lowest unoccupied molecular orbitals (LUMO) - which can hold 6 electrons – are completely empty. This means that $C_{60}$ has intrinsic properties of an electron *acceptor* or an "electrophile". These distinctive electronic properties are expected to allow fullerenes to be used in many new and commercially viable ways (Jensen et al 1996; Nova 2005). More specifically, fullerenes will likely contribute to important areas such as molecular electronics and medical imaging (e.g. nuclear magnetic resonance – more commonly referred to as "NMR").

Although the possible uses for fullerenes have been continuously expanding there have been some limitations to their immediate application. Firstly, the cost of producing pure fullerenes in large quantities has been an obstacle to commercialisation. Nonetheless, there are now several reports of commercial production facilities in both Japan and the USA providing advances in cost-efficient manufacture (Mitsubishi Monitor 2005; Nano-C 2005a; Colvin, 2003). Secondly, $C_{60}$ is an extremely stable compound able to withstand high temperatures and pressures but which can react chemically when exposed to ultraviolet light (i.e. $C_{60}$ "photosensitive" properties). The aqueous solubility of unmodified fullerenes, and hence potential for biological use, is known to be limited due to the hydrophobicity of its enclosed structure. The considerable hydrophobicity of fullerenes and carbon nanotubes (CNTs) is also a major factor in the "biopersistence" of these compounds and this has implications for human toxicology (refer Sections 4.3 & 4.4).

## 1.2 What is Nanotechnology? Definitions of Nanoparticles and Nanotechnology Relevant to this Report.

Nanometre scales are at the level of $10^{-9}$ (or 0.000000001) metres. The Royal Society/Royal Society of Engineers (U.K.) has in their comprehensive report on the field (Royal Society & The Royal Academy of Engineering 2004) defined nanoscience and nanotechnology , i.e. human endeavour at the nanometre scale, as:

> "…the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale; and …… the design, characterisation, production and application of structures, devices and systems by controlling shape and size at the nanometre scale. …from 100nm down to the size of atoms (approximately 0.2nm)…"
>
> *The Royal Society/The Royal Society of Engineers (2004).*

Likewise, the NNI (National Nanotechnology Initiative, USA) defines nanotechnology as:

> "Research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1 - 100 nanometer range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices and systems that have novel properties and functions because of their small and/or intermediate size. The novel and differentiating properties and functions are developed at a critical length scale of matter typically under 100 nm. Nanotechnology research and development includes manipulation under control of the nanoscale structures and their integration into larger material components, systems and architectures. …… In some particular cases, the critical length scale for novel properties and phenomena may be under 1 nm (e.g., manipulation of atoms at ~0.1 nm) or be larger than 100 nm (e.g., nanoparticle-reinforced polymers have the unique feature at ~ 200 - 300 nm as a function of the local bridges or bonds between the nano particles and the polymer)."
>
> *Interagency Subcommittee on Nanoscale Science, Engineering and Technology (NSET); U.S. Federal Office of Science and Technology Policy.*

*For the purposes of this review we have limited ourselves to nanotechnology industries which are defined as those purposefully manufacturing nanoparticles, nanostructures or nanoconstructs with at least one dimension less than 100 nm and with an expected end use in mind.*

It cannot be over emphasised that the properties of NPs (both beneficial and adverse) are related to their size and that these properties are acquired as a result of this size. It is oftentimes difficult to fully appreciate the nanometre scale. Previously other major reports have provided excellent examples to demonstrate these relative scales. Figure 1 provides an alternative perspective and is discussed further below.

Biological and non-biological NPs are not new and have been in existence for millennia. Certainly humans have been exposed to "accidental" combustion and biological NPs (e.g. wood smoke, viruses and bacteria) for a very long

time. These exposures have exerted selective pressures on human biology and the acquisition of defence systems such as the cytochrome P-450 superfamily of monooxygenases (CYP) and humoral/cellular immunity are two prominent examples. Three important points are relevant here. *Firstly*, exposures to non-anthropogenic NPs are invariably intermittent. *Secondly*, the selection of advantageous traits to exposures of naturally-derived NPs is multigenerational. *Thirdly*, human exposures to NPs have increased markedly with the advent of the industrial age due to the predominance of carbon-based fuels as an energy source.

Remarkable advances in nanotechnology, which have been likened to a new "industrial age", are set to increase future human exposures to NPs even further. Many of our defence mechanisms, developed over evolutionary time scales, may be inadequate to deal with this increased burden (refer Sections 4.1 & 4.4) and there may be human health costs to this increased exposure. These are not surprising conclusions but instead the foreseeable down side to the benefits associated with the advent of any new technology (Greenpeace 2003; Nicotera 2005).

Naturally occurring NPs include human viruses which can be up to several hundred nanometres in length or diameter (Fig. 1). Although greater than the size range of interest to this report it has been shown that both human viruses and NPs can be taken up into the nervous system in unexpected ways (e.g. polio virus; refer Oderdörster et al 2005a&b). Accordingly, it is worthwhile keeping in mind the biology of human systems when considering the potential health impacts of exposure to NPs.

By way of comparison, two important human cell types (the epithelial cell and oocyte) are approximately one to two orders of magnitude larger than most eukaryotic viruses. Both of these cell types serve prominent functions. Epithelial cells act as one of the primary internal barriers to non-host infiltration into the systemic circulation and the human egg cell or ovum functions in reproduction. *Ninety-five percent* of all cancer deaths are known to originate from tumours of epithelia (Fraumeni et al 1989). This is the case for cancers of the lung, colon, breast, prostate, liver and many other organs. The epithelial cell population of these organs share in common a high proliferative rate and dense packing (consistent with their compartmenting functions within the body). Therefore it is of interest to determine any short term epithelial damage to target organs as this may act as a precursor for more chronic sequelae.

Similarly, the oocyte's central function in reproduction imposes an imperative on the scientific community to determine the effects of NPs on potentially exposed workers and their progeny (Section 4.4). What then are the size differences between an oocyte and NPs? The oocyte, which is admittedly a large cell by any standards, is approximately 700,000 times larger than a single fullerene. If we were to assume that a single fullerene was one metre in diameter an oocyte's diameter in comparison would be approximately *the distance from Melbourne to Sydney* (700 km) (Fig. 1).

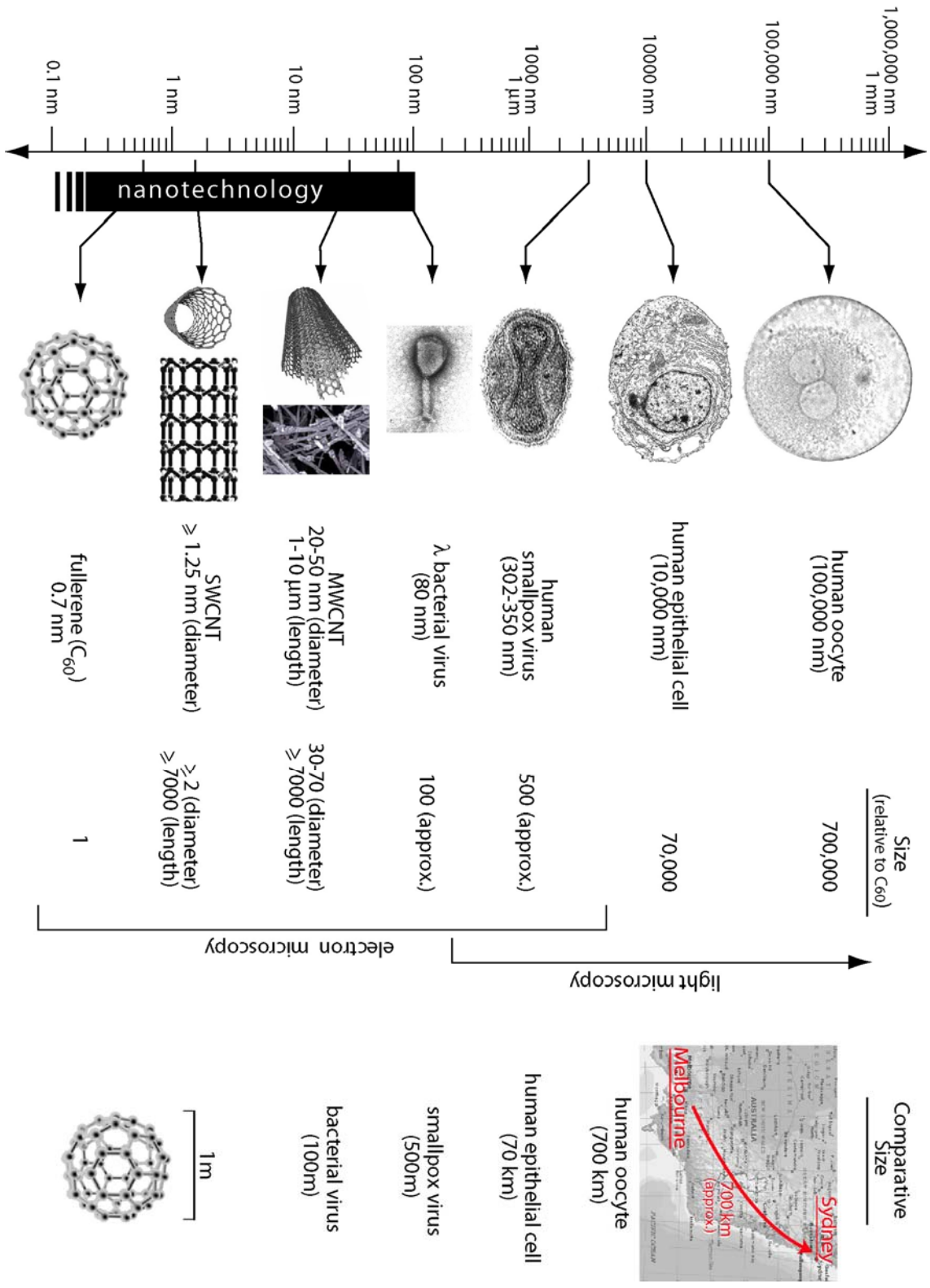



**Figure 1:** A comparison of the sizes of selected engineered nanoparticles with natural biological structures of interest. A 'real-world' analogy is provided as a comparison of the relative scales of these chosen examples (see text for details).

This highlights a crucial point for assessments of human exposure. On the basis of (molecular) mass alone the exposures encountered in an occupational setting may be considered minor but from another perspective such as surface area the body burden to NPs may be much greater (refer Oberdörster et al 2005 for a comprehensive review). Hardman (2006) provides a striking example. If only *2 grams* of 100 nm diameter NPs were to be evenly distributed there would be enough to provide every human worldwide with 300,000 particles each (Hardman 2006). Based on these sorts of considerations there now appears to be general agreement in the literature that human exposure assessments should include non-mass attributes in the determination of resultant biological and toxicological potencies (refer Section 4.5 for more detail including the intrinsic difficulties with such measurements).

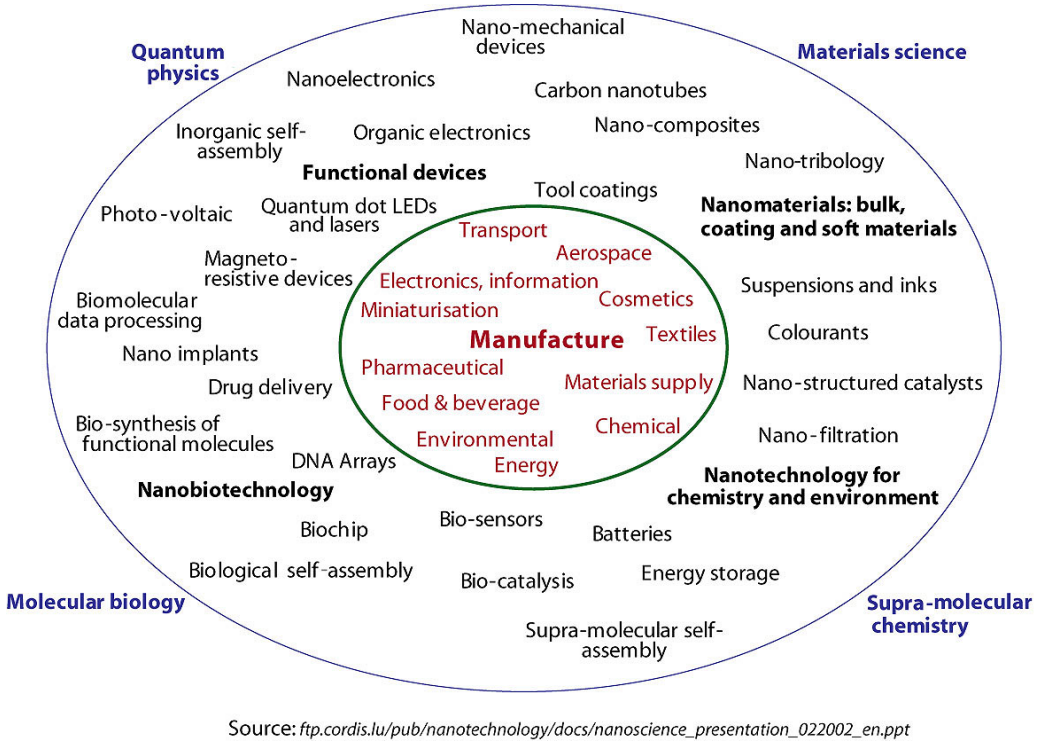
1. Nanotechnology presents new challenges as the properties of NPs now depend on size and shape as much as the more conventional factors of chemical structure and composition.
2. These challenges are true for all facets of the industry; *viz*. materials characterisation, end product quality control, employee exposure and health assessments.
3. The novel chemical properties of engineered NPs are expected to be distinct from what humans have been exposed to previously.
4. The capability of human cells to recognise, and appropriately respond to, NPs is mostly unknown at this stage.

## 1.3 Nanoparticle Classifications and Current Applications

Nanoparticles, and their associated nanotechnologies, are extremely broad in application. The European Union has classified the current and predicted applications of NPs and associated industries and this is presented in Figure 2 (below). NPs of all types are expected to find uses in the electronic, magnetic, optical, telecommunications, biomedical, pharmaceutical, cosmetic, energy, catalytic and materials industries. To date revenues are being realised from the commercial application of NPs to sunscreens, toothpastes, automotive exhaust catalysts, conductive coatings and optical fibres (for example). In the medical and biological arenas engineered NPs have found, or will soon find, application in cancer chemotherapeutics, biodegradable supports for drug delivery, radio-frequency shielding of equipment, luminescent biomarkers, numerous antimicrobial applications, micellar encapsulation (e.g. of drugs) and UV protection (Salata 2004; Nanotechnology Now 2005).

Nonetheless, the term ‘nanotechnology’ should not imply the idea of a single industry. Likewise, and perhaps more importantly, NPs are structurally and chemically diverse and should not be considered as a group of similar compounds. Even within a subcategory (e.g. SWCNTs) there is the

possibility of numerous modifications which add functionalities to the otherwise underivatised parent compound. Such an oversimplification could have potentially dangerous ramifications. For example, the toxicological properties of a particular NP – or the absence thereof - might be inaccurately assumed based on the properties of another despite apparently similar chemical structures.




Source: *ftp.cordis.lu/pub/nanotechnology/docs/nanoscience_presentation_022002_en.ppt*

**Figure 2:** A perspective of nanotechnology applications as related to discipline. Adapted from the EU Sixth Framework Programme (*www.cordis.lu/nmp/home.html*).

## 1.4 Future Outlook

It is a truism that the future is very difficult to predict. This would appear to be particularly so for areas at the forefront of technological advances. For example, at the introduction of DNA manipulation techniques in the 1970's and early 1980's only a few visionary people might have been able to predict, for example, DNA analysis in forensic medicine or the genetic duplication of an entire mammal from a differentiated adult cell (i.e. "Dolly" the sheep; Campbell et al 1996).

Likewise, the potential applications for engineered NPs such as fullerenes, CNTs, quantum dots and nanowires seem only to be limited by the human imagination. Reports appearing frequently and in prestigious journals bear witness to this. Applications spanning nanowire circuitry, quantum computing and "nanofluidics" (movement of small quantities of fluid) have recently been reported (Beckman et al 2005; Chen et al 2005a; Chen et al 2005b; Grigorenko et al 2005). These scientific reports give some indication

of the massive pipeline of potential new products that may arise from the basic research now being undertaken in the nanotechnology arena.

From an economic standpoint a major overseas report has suggested that the real impact of nanotechnologies on national wealth and consumer lifestyles will come when these technologies are used for purposes other than to just enhance the known properties of products already on the market (e.g. a stronger tennis racket or a more durable bowling ball; Innovest 2005). The future of nanotechnologies may incorporate radical shifts in product functionalities. For example, flexible and weather-proof clothing may incorporate solar cells and be linked to microprocessors for mobile computing. Alternatively, a wine glass may have gas chromatographic properties and detect the breath alcohol level of its owner, accurately extrapolate to blood levels with an inbuilt "nano-processor", and then automatically call for a taxi with an embedded "nano-telecommunications" capability.

# 2. Toxicology of Nanoparticles.

The general physical and chemical properties of nanoparticles are discussed below according to the type of nanoparticle. Where appropriate general toxicological principles or known hazard properties have been discussed under each classification. More specific and recent advances for each of the nanoparticle categories are also dealt with further in Sections 4.4 & 5).

## 2.1 Single-Walled and Multi-Walled Carbon Nanotubes (CNTs).

Carbon nanotubes (CNTs) are cylinders of either single- (SWCNTs) or multi-walled (MWCNTs) carbon atoms covalently bonded in a hexagonal network of dimensions approximately 1-50 nm (diameter) by ≤100 μm (length; refer Fig. 1). The characteristic CNT structure contributes to its tremendous mechanical strength which is many times greater than steel (Luther 2004). Furthermore, it is the combination of large surface areas, electrical conductivity and mechanical strength which holds promise for the eventual use of CNTs in composite materials or nanoscale devices. Distinctive electron transport and magnetic properties have also been observed thus providing further potential uses in nanoelectromechanical devices. CNTs can be further modified by chemical reactions (i.e. "derivatised") which alter the original properties of the otherwise mostly chemically inert CNTs.

Three major techniques are reported in the scientific literature for producing CNTs, viz., carbon arc-discharge, laser-ablation and chemical vapour deposition. All three preparation methods require a catalyst which is usually a first-row transition metal (e.g. Co, Ni, or Fe) or combination of these (e.g. Ni–Co, Co–yttrium, or Ni–yttrium). As a result, CNT production results in a product carrying impurities of which the residual metal catalyst is a contributor (Sinha & Yeow 2005; Maser et al 2002). Various purification steps have been reported and these include sonication, filtration and acid/non-acid oxidation and washing processes. To date all purification procedures have the capability of structurally modifying CNTs thereby resulting in the loss or alteration of their distinctive properties (Sinha & Yeow 2005). More recently, commercial production of SWCNTs has been reported using a relatively inexpensive combustion process but this process is also dependent on the presence of metal catalyst in the synthesis phase (Nano-C 2005b).

The residual metal component of purified or unpurified CNTs represents an area of concern for many toxicologists (Lam et al 2006). For example, it is well-established that transition metals, and especially $Fe^{2+}$, are capable of producing potent reactive oxygen species *in situ* (e.g. hydroxyl radical) by what is termed the 'Fenton reaction' (IUPAC 2005; Boesterli 2005). From a chemical standpoint the Fenton reaction is as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

(where $OH^{\bullet}$ represents hydroxyl radical and $OH^{-}$ represents hydroxyl ion).

Moreover, it is also generally accepted that non-physiological levels of reactive oxygen species (ROS) are contributory factors in human disease and in the experimental animal models which are used to study them (Dalle-Donne et al 2005).[†] Oxidative stress occurs if intracellular ROS production exceeds the cell's antioxidant defence systems. Under these conditions many, if not all, of the cell's components (inc. proteins, lipids and DNA) are capable of being structurally modified and their function lost. Cell killing can be the end result of this process and, if excessive, irreparable target organ damage is the consequence.

From a toxicological perspective it is worthwhile considering some of the structural properties of CNTs. Single SWCNTs are of approximately 1.25 nm (width) by several microns in length (Fig. 1). However, SWCNTs do not usually exist as individual tubes but aggregate by hydrophobic forces into microscopic bundles. These, in turn, agglomerate loosely into small clumps or "ropes" of typically 20 to 50 tubes with a collective diameter of approximately 20 nm and up to several microns in length (Lam et al 2006). Aggregation and agglomeration processes are evident for MWCNTs as well. These can take MWCNTs from an original diameter of approximately 20 nm to aggregates with sizes ranging from 0.4 to 2 μm (Lam et al 2006).

In addition, there are two further factors which result in CNT size heterogeneity. Firstly, as can be expected for all chemical synthesis methods, inefficiencies in manufacture will result in partial-length products. Secondly, and a potentially significant issue for worker health, is that of the physical breakdown products of CNTs. Although not generally evident in the literature it is common knowledge amongst materials scientists (J. Shapter *pers. comm.*) that CNTs are capable of physically breaking down and do so in a distinctive way (Figure 3). Shearing forces (F) applied to CNTs result in breakage along the shorter axis with a resultant decrease in fibre length. In comparison, asbestos fibres subject to similar forces are known to break along the long axis with little or no reduction in fibre length ***and*** little or no reduction in the biopersistence and carcinogenic potential of the fibre (Kaplan-Ashiri et al 2006). It is not yet known if these test tube findings are of relevance to human exposure situations and, if they are, whether a decrease in CNT biopersistence results from this form of degradation. Further research work may seek to understand if the normal forces found in the pleural cavity during breathing are enough to shear CNTs in the manner described and, if so, whether decreases in inflammation, fibrosis and granulomas are the result.

[†] This is an oversimplification as reactive nitrogen species (RNS) are often also observed in many of the same pathological situations but, at present, it is technically difficult to separate the exact contributions of ROS and RNS to the final extent of cell and tissue damage (Dalle-Donne et al 2005; Donaldson et al 2005).

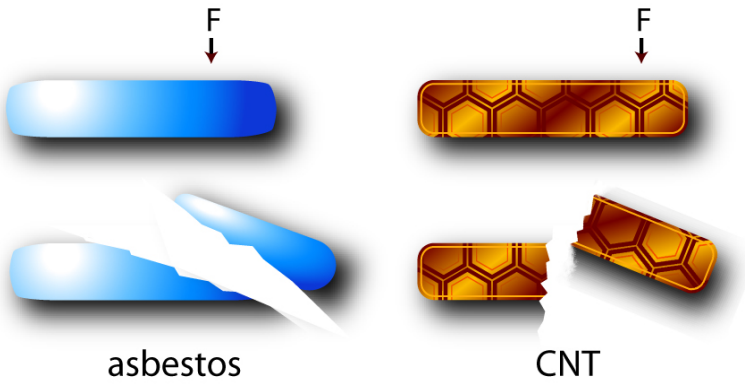



**Figure 3:** Currently understood physical breakdown products of asbestos in comparison to CNTs (refer text for details).

Consequently, from these considerations it can be seen that the morphologies of CNTs will overlap - at least to some extent - with the physical dimensions of asbestos fibres. It is well established that particular forms of asbestos are causative factors in otherwise rare, occupationally-derived, malignant mesotheliomas (Robinson & Lake 2005) and other lung disorders (inc. pulmonary interstitial fibrosis, pleural plaques, calcification and thickening) (US CDC 2001). Numerous toxicological studies have established that the absolute physical size of an asbestos fibre is secondary to an aspect (i.e. length to width) ratio of ≥3:1 in mesothelioma formation (see below for more details). Current manufacturing techniques can achieve similar aspect ratios of 3:1 or greater and micron lengths for both SWCNTs and MWCNTs. The physical and chemical properties of CNTs - and their aggregation and breakdown products - are still under active investigation but the biopersistence properties and physical similarities to asbestos are noteworthy.

The historical basis for fibre toxicity was set over 30 years ago by Stanton and colleagues who determined relative rates of mesothelioma formation in experimental animals in response to precisely sized glass or asbestos fibres (Stanton & Wrench 1972; Stanton et al 1977). These, and subsequent studies, have indicated that asbestos fibres with lengths ≥5 µm and length: width ratios ≥3:1 are of the most concern in the aetiology of the conditions listed above (Berman et al 1995; Churg & Wiggs 1984). There appears to be considerably greater lung cancer risk following inhalation of fibres longer than 10 µm but some debate still surrounds the potential human health effects of fibres with lengths <5 µm. For example, a recent report has suggested that short, thin, asbestos fibres (≤ 250 nm in width by ≤ 5 micron in length) appear to contribute to the causation of human malignant mesotheliomas (Suzuki et al 2005).

The 11$^{th}$ Report on Carcinogens lists glass wool of respirable size as "*reasonably anticipated to be a human carcinogen*" (US RoC 2005). The IARC classifies some special purpose glass fibres such as '104E' and 'JM475' as "*possibly carcinogenic to humans (Group 2B)*" (IARC 2002). However, for other categories of glass fibres (e.g. glass wool and continuous glass filament) there is inadequate evidence for carcinogenicity in humans.

Insulation glass wool and continuous glass filament have been rated as "*not classifiable as to their carcinogenicity to humans (Group 3)*" (IARC 2002). More recent epidemiological studies have incorporated confounding variables (e.g. smoking) and weakened the evidence for these associations (Baan & Grosse 2004). Consequently, final toxicological evaluations are dependent on animal bioassay and carcinogenicity studies (Baan & Grosse 2004). Rats and hamsters receiving glass wool (length of <3.2 to <7 μm; diameter of <0.18 to <1 μm) by intratracheal instillation have been shown to develop adenocarcinomas, squamous cell carcinomas, bronchoalveolar tumours, lung carcinomas, mesotheliomas and sarcomas. When administered by intraperitoneal injection (length of <2.4 to <30 μm; diameter of <0.18 to <1 μm) mesotheliomas were induced (US RoC 2005).

It should be re-emphasised that it is not known at this stage if these findings with asbestos and glass wool are directly relevant to SWCNTs and MWCNTs. However, a precedent has been set with inert fibres from a biological perspective. It is the author's opinion that only chronic exposure studies in appropriate *in vivo* systems will provide some of the necessary answers to these urgent toxicological concerns. For these chronic studies to be relevant to occupational exposure scenarios this report considers that attention will need to be given to the following variables:

- What are the most likely routes and locations of human occupational and environmental exposure?
- What are the molecular mechanisms of toxicity induced by exposure (with particular attention given to known pathologies)?
- Does any observed toxicity correlate most to size, shape, and/or composition of the CNTs being studied?
- Is there a dose-dependent relationship between exposure and toxicity? And,
- are any of the by-products of production or decomposition toxic?

These types of mechanistic in vivo studies are required to provide the obligatory information fundamental to the determination of safe work environments and practices and are generally thought of as standard toxicological fare. The expertise to undertake these types of studies should be available within Australia.

## 2.2 Fullerenes (e.g. $C_{60}$).

Even though discovered in 1985 fullerenes were not more generally available for study and characterisation until approximately 1991. Fullerenes as a chemical group are also known to contain other examples (e.g. $C_{70}$, $C_{76}$, $C_{78}$, and $C_{84}$) which may prove to have even more interesting chemical and toxicological properties. All the major research efforts have focussed on $C_{60}$ and, as a consequence, this form is reviewed here. Nonetheless, with the fast-paced progress of the field it will be necessary to monitor any new applications of non-$C_{60}$ fullerenes and their associated health effects.

### 2.2.1 Health Effects of Fullerenes

Underivatised fullerenes are electron deficient and prefer to act as electrophiles (i.e. interact with electron rich substrates; Diederich & Thilgen 1996; as discussed in Section 1.1). Perhaps counter intuitively some earlier reports, principally from two independent groups, proposed that fullerenes may be useful as antioxidants in biological systems (Huang et al 2001; Dugan et al 1997, 2001; Gan et al 2002). Proposals were put forward for their use in the treatment of chronic diseases with oxygen stress aetiologies (e.g. cerebral ischaemia and Parkinson's: Huang et al 2001; Corona-Morales et al 2003; Dugan et al 1997, 2001; Ali et al 2004).

Other reports indicate that using fullerenes (or fullerene derivatives) in such therapeutic capacities will not be straightforward. For example, water-soluble polyarylsulphonated fullerenes are capable of inducing an unusual form of nephropathy in rats. The injury has features of a type of kidney damage involving autophagy and is widespread throughout the organ but preferentially targets the epithelial cells of the proximal convoluted tubule. The authors suggested that the damage appeared to be mediated intracellularly by a phagosomal and/or lysosomal pathway (Chen et al 1997, 1998). On necropsy there was also evidence of hepatocyte necrosis and hypertrophy of the liver's resident macrophage cell type (the Kupffer cell; Chen et al 1997). Unmodified $C_{60}$ has also been shown to have embryotoxic effects using both in vitro and in vivo mouse test systems (Tsuchiya et al 1996). It will be of interest to see if these studies can be independently confirmed by other groups. More recently, and especially in the last year or two, studies have appeared which address the molecular mechanisms of fullerene interactions with the mammalian cell. These are discussed further in Section 4.4).

### 2.2.2 Fullerenes and Environmental Health

Fullerene properties have also been investigated for uses in environmental remediation (Cheng et al 2004 & 2005; Savage & Diallo 2005). Despite being only sparingly soluble fullerenes can form stable colloidal aggregates in water at approximately 10 p.p.m. (or approximately 10 mg per litre of water). Although this may seem a relatively low concentration it is 100 times the solubility limit of polyaromatic hydrocarbons (e.g. dioxin) which, when found in groundwater, are known to have considerable health and environmental impacts. It is unknown at present if the biopersistence and environmental impact of fullerenes has any similarities to PAHs but there has been some anxiety expressed (Colvin 2003). In the only study of its type Oberdörster (2004) has shown that fullerene aqueous levels of only 0.5 p.p.m. can damage the CNS of largemouth bass but these studies have been criticised as being attributable to the tetrahydrofuran solvent vehicle used. Other studies have indicated that the formation of $C_{60}$ aggregates is altered in the presence of even relatively weak salt solutions which act to precipitate fullerenes out of solution (Brant et al 2005). Only further work will resolve these unknowns which are of interest not only to environmental health studies but also to the stability of fullerenes in the workplace.

### 2.2.3 Major Areas for Future Research

Progress in the last decade has increasingly seen chemical modifications made to the basic alkene-like fullerene structure. These modifications add new functionalities to the parent molecule and increase the number of potential uses for these compounds in the market. However, it is still essentially unknown how $C_{60}$ and its derivatives interact with the human body - even at the most basic level. In the author's opinion it is particularly disconcerting that some studies show that fullerenes are cleared only very slowly and are retained for extended periods in vivo (Yamago et al 1995; Roberts et al 2005). Another report appears to indicate that fullerenes inhibit the very metabolic systems (i.e. the cytochrome P450 enzymes) which are required to convert them into less harmful chemicals (Ueng et al 1997). This relative lack of metabolism and clearance of fullerenes adds to their biopersistence properties and toxicological hazard potential. These represent significant gaps in our understanding of the chemical, environmental and human health effects of these potentially important molecules. This report considers that there is now a need to determine more systematically the biological properties of the fullerenes.

## 2.3 Metal Oxide Nanopowders (e.g. $TiO_2$, ZnO).

At the present time the most commercially important class of engineered nanoparticles are the metal oxide nanopowders which include those of zinc (ZnO), silicon ($SiO_2$), titanium ($TiO_2$), aluminium $Al_2O_3$) and iron ($Fe_3O_4$ or $Fe_2O_3$). The more purified forms of; for example, $TiO_2$, ZnO and $Al_2O_3$ are being used extensively in products such as sun-screen lotions, cosmetics and as chemical catalysts. In addition, an increasing interest is being shown in other metal-based NPs such as the precious metals and those required for the manufacture of quantum dots (QDs, e.g. cadmium selenide [CdSe], cadmium telluride [CdTe] and gallium arsenide [GaAs]; refer Section 2.4; Grigorenko et al 2005). Many of the metal containing NPs have their origin in the semiconductor industry where their primary use is in the stabilisation of energy levels within substrate materials (J. Shapter, *pers. comm*.). As a result, these types of NPs are generally characterised as being of relatively high purity and homogeneity (both size and composition).

### 2.3.1 Studies with 'Environmental' Ultrafine Particles (UFPs) and Significance to Engineered Nanopowders.

This contrasts with non-engineered – either anthropogenic or 'accidental' - UFPs of nanometre dimensions which are far more complex in structure. Combustion-related UFPs consist mostly of a core of elemental carbon, other organic compounds, nitrates, sulphates and a large percentage of metals (incl. iron, copper, zinc, lead, and nickel: Delfino et al 2005; Pöschl 2005; Lippmann et al 2003). It is believed that not all of the UFP components are linked to the strong epidemiological evidence for acute and chronic respiratory and cardiovascular effects in humans but the exact contributions of each of the components has not been established (Brook et

al 2004; Xia et al 2004; Donaldson et al 2005). Combustion related emissions are considered to contribute to the majority of adverse health effects products by nanoparticles, especially in susceptible subpopulations (e.g. the aged or asthmatics). Nonetheless, it is plausible that the localised release of manufactured NPs might be sufficiently high in a workplace setting and, as a result, be of concern.

Toxicological and epidemiological UFP-related studies are well-developed and have immediate, although not absolute, relevance to the impact of nanopowders and nanoparticles on human health. Epidemiologic studies carried out over the past 15 years have increasingly shown that ambient air particulate matter, including the UFP fraction, is associated with adverse respiratory and cardiovascular health effects (e.g. daily and annual mortality, hospital emergency room admissions and time lost from work). In the USA air quality guidance standards were also revised to take into account new evidence for an excess mortality that is more strongly associated with particles having aerodynamic diameters less than 2.5 μm.[#] This fraction also represents a portion of the UFPs which have aggregated and agglomerated in the atmosphere. In particular, studies have concentrated on the respiratory toxicology of UFP aerosols as this is the most likely route of occupational and incidental exposures. By definition an aerosol is composed of both the particulate and gas-phase components but in practice, and for the purposes of this report, the term refers to the semi- and non-volatile particles of the gas phase. Once inhaled both NPs and UFPs can be taken up into the human bloodstream by several different mechanisms (Oberdörster et al 2005; Hoet 2004; Nemmar et al 2002; Oberdörster et al 2002). This systemic distribution of NPs or UFPs is disconcerting from a human health perspective and forms the basis for the downstream events that are known to occur. The most widely accepted hypothesis for the adverse health effects following environmental UFP or NP exposure is that of an 'oxidative/nitrosative stress-mediated inflammation' to specific target tissues and to the body as a whole (Delphino et al 2005; Brook et al 2004; Donaldson 2005; Knaapen et al 2004).

Despite the fact that many of the precise details are still not known excellent studies do exist which indicate some of the mechanisms of adverse health effects in response to anthropogenic UFPs. Following deposition at the alveolar epithelial barrier UFPs can activate macrophage and neutrophil functions which are invariably associated with the production of reactive oxygen and/or nitrogen radical species (Driscoll et al 2002; Brook et al 2004). Within hours cytokine and chemokine mediators are released which signal the compensatory cellular movements that are typically connected with inflammation. A critical point to note here is that *the increased relative surface area of UFPs and engineered NPs* in comparison to larger particles *contributes to an amplified inflammatory response* per given unit of mass (Oberdörster et al 2005). Only when the measured responses, such as neutrophil migration to the site of injury, are normalised to particulate

[#] It is interesting to note that these air quality guidance standards are currently under scientific pressure to be further reduced (E. Stokstad (2006) Science 311: 27).

surface area do the inflammatory responses resemble the equivalent for bulk materials (Hext et al 2005; Oberdörster et al 2005 and references therein).

In an excellent recent study these types of inflammatory responses have been shown to be causally linked to the development of peribronchial fibrosis and allergen-induced airway remodelling (Broide et al 2005). In addition, the development of lung fibrosis is well known to correlate with eventual declines in lung function. Finally, inhalation of environmental UFPs has been shown to be associated with acute and chronic cardiovascular effects, such as vascular thrombosis and heart rate changes. These pathological changes have been proposed to be linked to the initial inflammatory responses and tissue damage following exposure to nanoscale particulates (Delfino et al 2005; Mar et al 2005; Radomski et al 2005; Brook et al 2004; Brown et al 2004).

The literature has proposed a multifaceted and complex cascade of interrelationships to explain the biological effects of UFP exposures and a version is represented in Figure 4. Although the responses to NPs are as not as well known as for UFPs *it is widely anticipated that at least some of the health effects that have been observed to date with environmental particulates will also be pertinent to human nanopowder and CNT exposures.*

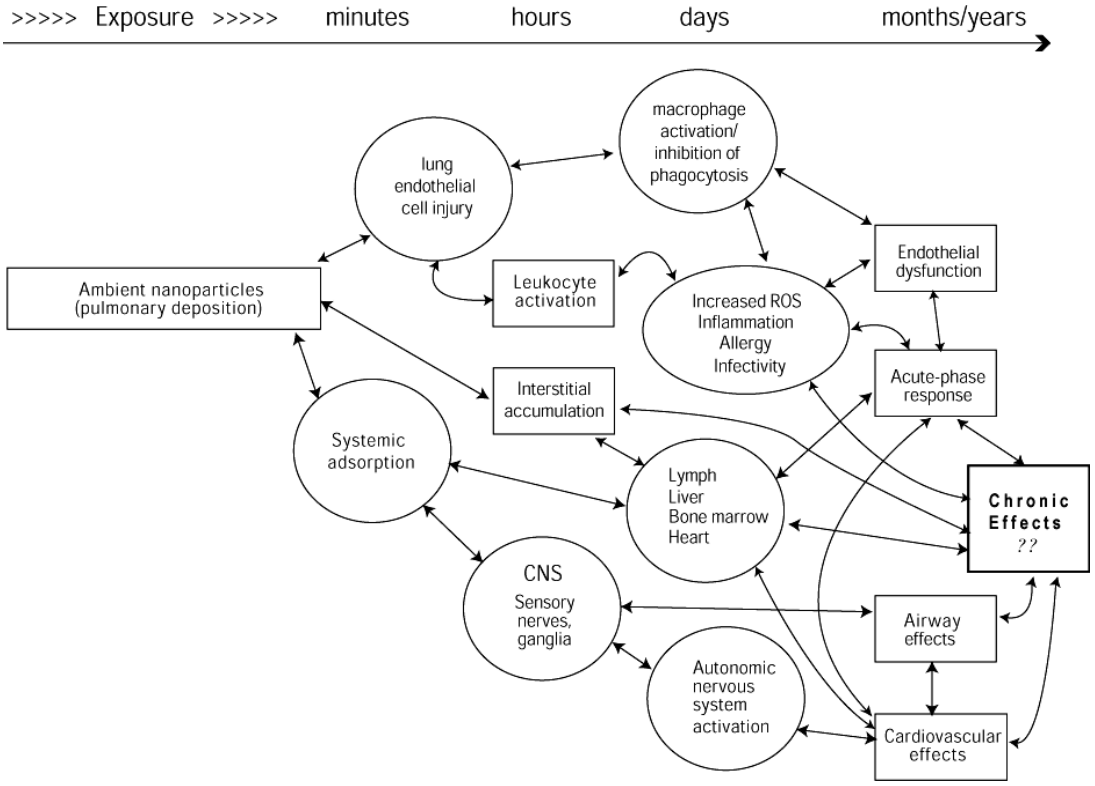



**Figure 4:** The complex and interdependent cascade of biological sequelae following exposure of humans and experimental animals to anthropogenic and environmentally ubiquitous UFPs.

### 2.3.2 Summary of Studies with Metal Oxide NPs.

Of the nanopowders $TiO_2$ is possibly the archetypal example of what is generally considered to be a low toxicity dust. The toxicological and risk assessment studies for this compound are relatively advanced (e.g. US NIOSH 2005). $TiO_2$ is a white, crystalline and insoluble powder which has different mineral forms in the natural state including anatase and rutile. These forms of $TiO_2$ are not pure and may contain iron, tin, vanadium, chromium and niobium. Particle size is an important determinant of the properties of $TiO_2$-based pigments and other products. Commercial preparations of $TiO_2$ typically range in average particle size from 0.2 μm to 0.3 μm and are used in products such as paints, paper, plastic, ceramics, rubber and printing inks (US NIOSH 2005). In most circumstances the primary particle size indicated above represents significantly aggregated $TiO_2$ at physical equilibrium.

Both the US National Toxicology Program (US NTP) and the IARC rate $TiO_2$ as an improbable human carcinogen based on the available information. However, there is some evidence for lung neoplasia formation in long-term, high-dose bioassays and a genotoxic potential in (perhaps contrived) in vitro assay systems (Heinrich et al 1995; Lee et 1985; Lu et al 1998). Heinrich et al. (1995) in particular observed a statistically significant increase in adenocarcinomas and benign squamous-cell tumours in rats exposed to ultrafine $TiO_2$ of size 15 to 40 nm (10 mg/$m^3$ for 2 years). Additionally, human case reports have indicated the development of pneumoconiosis, alveolar proteinosis, fibrosis and reversible inflammatory changes (US NIOSH 2005). In general, however, the epidemiological evidence has been considered equivocal. A comprehensive review of the occupational hazards of $TiO_2$ has recently been made public in draft form and is available online (US NIOSH 2005).

The major conclusions of the NIOSH draft report are nonetheless relevant to this report. Although it was concluded that the animal and human data regarding $TiO_2$–mediated carcinogenicity were not chemical specific the observed effects were related more specifically to *particle size and surface area*. It was further concluded that a secondary mechanism related to $TiO_2$-induced chronic inflammation was also a factor. Consequently, the available data indicates that fine and ultrafine $TiO_2$ particles share commonalities with environmental UFPs as discussed above (Section 2.3.1 & Fig. 4).

## 2.4 Nanocrystals, Quantum Dots (consisting of CdTe, CdSe, ZnSe and related metal lattices).

Quantum dots (QDs) are typically composed of combinations of group II and VI or III and V elements of the periodic table and are defined as having physical dimensions smaller than the exciton Bohr radius. As with all NPs this size restriction leads to unique properties. In the case of QDs these are mostly optical or electronic. For example, QDs can absorb white light and

then re-emit it at a specific wavelength only a couple of nanoseconds later. By varying the size and composition of the QDs the emission wavelength can be predetermined from blue to near infrared. The flexibility of QDs allows for nanoscale applications where light is used to process information such as multicolour optical coding in gene expression studies, high throughput screening, and bioimaging. Furthermore, the large surface area-to-volume ratio of QDs makes them suitable for tertiary chemical modification with small surface functional groups, conjugating proteins, antibodies, and oligonucleotides (Michalet et al 2005). Modified QDs have found, or are being developed for, uses in important areas such as directed drug delivery, diagnostic procedures and non-organic solar cells (Åkerman et al 2002; Giepmans et al 2005; Gur et al 2005; Michalet et al 2005; Fig. 5). Consequently, the flexibility and number of potential uses for underivatised and derivatised QDs is enormous. Figure 5 represents an idealised representation of some quantum dot structures which are characteristically composed of a metal/metalloid core and can be capped for stability and/or functionality.

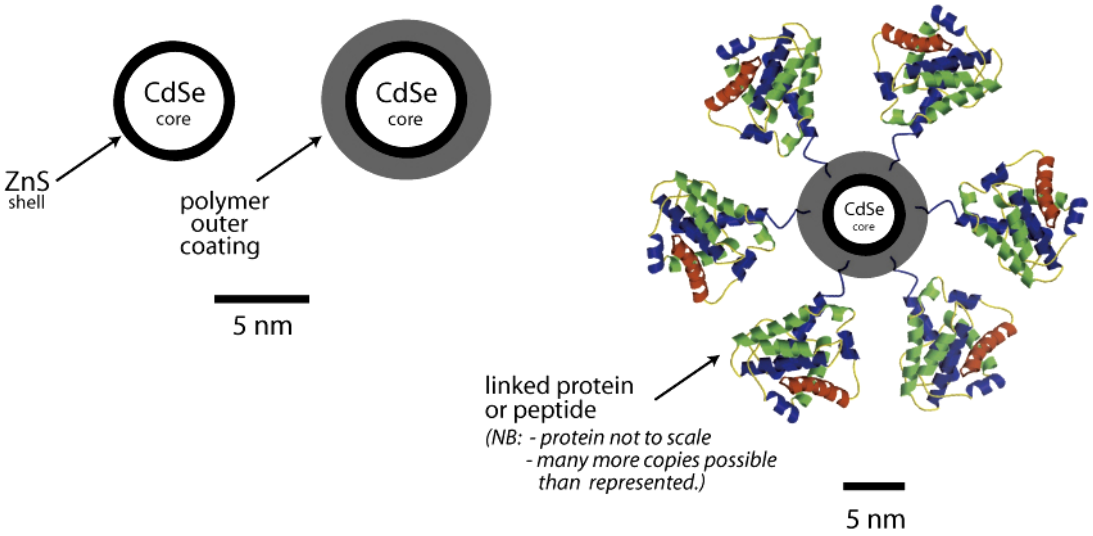



**Figure 5:** Schematic representation of some quantum dot structures. The inner core (far left) generally consists of group II/IV or III/V elemental groupings such as CdTe, CdSe, GaAs (Hardman 2006). Further modifications are possible to the inner metalloid core by adding a "cap" or "shell", a polymer outer coating, or functionalising by linking small molecular weight chemical groups or higher mass biological molecules (e.g. proteins or DNA).

From a toxicological perspective it is useful to briefly consider the potential health effects of the bulk components of QDs as there is little information currently available on the stability of QDs - either in the environment or within the human body. Consequently, it is the author's opinion that until further information becomes available, the hazard potential of the metal/metalloid ions released from QDs should be assumed to be equivalent to the same material in the bulk form.

### 2.4.1 Potential Toxicities of QDs Constituents.

#### 2.4.1.1 Cadmium and Cadmium Containing Compounds.

Cadmium and cadmium containing compounds are *known* to be human carcinogens (US CDC 2005; US RoC 2005; IARC 1976). There is sufficient evidence of multisite cadmium-induced carcinogenicity in humans based on cohort studies. The epidemiological evidence, including case control and geographic distribution studies, indicate an association between cadmium exposure and cancer of the lung, liver, prostate, kidney and bladder (US RoC 2005; Jarup & Alfven 2004). Moreover, extensive mechanistic in vivo studies using multiple animal species provide the basis for an understanding of cancer formation with the free ionic form of cadmium as the active and genotoxic form (US CDC 2005). Chronic in vivo exposures are known to result in preferential accumulation in the liver and the kidney. The estimated biological half-life of cadmium in the kidney and other organs is very long – from one to four *decades* (Boesterli 2005; US CDC 2005). For this reason cadmium is considered especially dangerous both in the workplace and as an environmental pollutant. The inhalational route of exposure is considered the most important. In comparison intestinal absorption of cadmium is low. Occupational exposures are also known to result in renal tubular and glomerular damage which likely represent precursors to neoplasias at these sights (US CDC 2005).

#### 2.4.1.2 Arsenic and Lead.

There is ample epidemiological evidence in humans to indicate that inorganic arsenic compounds are carcinogenic. These studies are also supported by results from animal and in vitro systems (US RoC 2005). Arsenic and arsenic containing compounds can induce cancer at multiple sites including the skin, lung, digestive tract, liver, bladder, kidney, and lymphatic and hematopoietic systems. Exposure to arsenic found in drinking water has been associated with cancer at additional sites including the prostate and nasopharyngeal areas (Cantor 1997). Animal studies have reproduced many of the neoplasias observed with exposure to metallic arsenic and arsenic salts in humans (Dopp et al 2005).

Following inhalation or ingestion lead (Pb) is readily absorbed and binds to erythrocytes and distributed initially to multiple soft tissues and to the bone matrix more slowly. Lead can also cross the placenta and poses a potential hazard to the foetus. Lead is cleared relatively slowly from the human body with a half-life of *years* to *decades* from the skeleton in comparison to only 1-2 months from the blood and soft connective tissues.

It is the ability of Pb to interfere with the normal functions of other physiologic cations (such as Ca, Zn, and Fe), the functions of some enzymes and regulatory proteins and the ability to generate ROS that contributes to its biological potency (US CDC 2005). Large amounts of Pb in the body can cause anaemia, kidney injury, abdominal pain, seizures, encephalopathy, and paralysis. Low level environmental lead exposure has also been

associated with decreased neurocognitive effects in children and adults. At higher levels these subtle symptoms become more pronounced (US CDC 2005). In occupational and environmental studies consistent associations between blood lead concentrations and symptoms has been observed in adults. There is also evidence of reproductive toxicities in both females and males (US CDC 2005). Both the IARC and the US NTP consider lead as a possible human carcinogen pending more definitive epidemiological data (US NTP 2005).

#### 2.4.1.3 Selenium Sulphide.

If QDs prove to be unstable under some circumstances then the possibility exists that the CdSe core may reconstitute with sulphur from the ZnS shell in QDs of this composition (Fig. 5). Under these circumstances it is prudent to consider the toxicology of SeS. Unlike other forms of selenium, selenium sulphide is recognised as a "*probable"* human carcinogen according to the US EPA and US NTP (US EPA 2005a). When administered orally, SeS produces hepatocellular carcinomas in both sexes of F344 rats and female $B6C3F_1$ mice and alveolar/bronchiolar carcinomas or adenomas in female $B6C3F_1$ mice. Selenium sulphide is generally considered chemically distinct from the organic and inorganic selenium compounds typically found in foods and in the environment. In fact, forms of selenium other than SeS are considered to have beneficial health effects at physiological levels.

#### 2.4.1.4 Other Quantum Dot Metals/Metalloids.

These include metalloids and "poor metals" (i.e. post-transition metals) such as gallium, thallium, indium and tellurium. Along with cadmium these are generally used in combination to achieve suitable semiconductor or other properties and are grouped here for consideration. The literature acknowledges that – given their widespread use – there has been inadequate attention paid to the human health effects of these compounds. Gallium arsenide (GaAs) is the most widely used in the microelectronics industry and indium arsenide (InAs) and aluminium gallium arsenide (AlGaAs) have also found practical applications. Other combinations not listed here have also been reported.

Gallium has been investigated for therapeutic uses against certain cancer types including non-Hodgkin's lymphoma (Jakupec & Keppler 2004). GaAs and InAs particles administered intratracheally elicited acute pulmonary inflammatory lesions and fibrosis (US NTP 2000a; Tanaka 2004). Short-term indices such as non-neoplastic pulmonary lesions, fibrosis and the incidence of hyperplasias of the lung epithelium increased in a dose dependent manner in these inhalation studies (US NTP 2000a). More chronic effects included sex-specific formation of benign and malignant lung tumours (US NTP 2000a). Lung tumour formation increased with increasing GaAs dose (0.01, 0.1, or 1.0 $mg/m^3$). *In comparison*, indium phosphide itself has been shown to be capable of producing alveolar and bronchiolar adenomas and bronchiolar carcinomas suggesting that the arsenide component of InAs is not a contributing factor to the lung pathologies observed (US NTP 2000b;

Gottschling et al 2001). Multispecies testicular toxicity has also been reported after either intratracheal GaAs or InAs dosing and this has been associated with sperm dysmorphogenesis and a reduced sperm count (Tanaka 2004 & references within).

Thallium and its compounds are considered *highly* toxic and in mammals approximately comparable to mercury on a molar basis. A median lethal dose in humans is only in the vicinity of 8 to 12 mg/kg producing gastroenteritis, polyneuropathy, and hair loss. Accumulation after absorption is widespread in bone, renal medulla, and the central nervous system. Thallium (and related compounds) appear not to be genotoxic but long-term carcinogenicity studies are lacking. Consequently, the US NTP, IARC, and the US EPA have not classified thallium as to its human carcinogenicity potential. Thallium and like compounds are also recognised as being widely toxic to fish and plants indicating that precautions are necessary for release into the environment (Mulkey & Oehme 1993; Zitko 1975).

Tellurium has widespread industrial uses such as in the microelectronic industry, rubber vulcanisation and metal oxidising applications. It may be the most innocuous of the quantum dot metalloid/metal components but its use and is steadily increasing. Descriptions of human toxicity from tellurium inhalation or ingestion are rare but available. Symptoms are generally mild (e.g. vomiting, black discolouration of the oral mucosa, and a garlic odour to the breath) but can be fatal (Yarema & Curry 2005; Harrison et al 2006). More recent toxicological studies indicate that tellurium-dioxide induces maternal toxicity and teratogenic effects in the rat (Perez-D'Gregorio et al 1988). In comparison, other reports have failed to find strong evidence for teratogenicity or reproductive toxicity in the rat or rabbit test systems (Johnson et al 1988; Harris et al 1994).

#### 2.4.1.5 Preliminary Conclusions on Quantum Dot Hazards.

We can conclude that the hazards to humans and the environment posed by the cadmium, arsenic and lead components of QDs are potentially very high. Although the human health effects of QDs specifically are not known there is reason to suspect that the components will break down to elemental forms under favourable or unfavourable conditions. Consequently, human health and environment hazard potentials are possible. At present the commercial production of materials for QDs, in absolute mass terms, is dwarfed by the current environmental levels of these metals (e.g. from refining and smelting). Nonetheless, if the popularity of QD applications increases as the literature would suggest, there remains a potential for localised high levels of release in occupational settings.

The physical stability of the quantum dot inner core after manufacture also becomes of concern as it will be a factor in the degree of human exposure to the metal core components or, alternatively, to selenium sulphide. No available information on the relative efficiency of the capping process or the stability of the core itself in a biological medium (e.g. within the human

body or the cell) has been identified by the authors. A preliminary report has observed that surfactant treated ZnS coated CdSe QDs were only minimally distributed to lymph nodes after subcutaneous injection in mice even after 48 hours (Roberts et al 2005). This suggests that the skin serves as a "depot" for QDs and that QDs of this composition are stable in the short term. Given the lack of information in this area the authors' consider that more systematic and longer term studies are needed. The potential release of elemental core metal is of toxicological significance and has been mentioned previously in the peer-reviewed literature (Hardman 2006; Lovrić et al 2005).

Quantum dot technologies appear to be the most flexible and adaptive of the nanotechnologies. Their potential uses are numerous but understanding and modifying the interactions of QDs with components of the mammalian cell will be necessary to circumvent QD-initiated damage (Moghimi et al 2005; Hardman 2006). Important areas for QD applications in the future include uses in photodynamic cancer therapies and bioimaging (Michalet et al 2005).

## 2.5 Other Examples

Other examples of NPs exist in the peer-reviewed and popular literature. These include – but are not limited to - nanowires of varying composition (silicon wires, ZnO, Ni, $Gd_2O_3$, Fe/Mo, CdS, etc.), "pea-pods" (metal ions or fullerenes embedded in CNTs), inorganic fullerene-like nanostructures and nanotubes, atom-thin graphene sheets, dendrimers and ordered DNA nanostrand arrays (Moghimi et al 2005, Guan & Lee 2005; Kaplan-Ashiri et al 2006). These applications are less well established than those discussed above. The toxicological hazards posed by these examples of engineered NPs are likely to be a subset or combination of the hazards identified in Sections 2.1 to 2.4 and, although not considered further in this report, are worthy of being kept under surveillance.

# 3. Potential OHS Considerations. A Summary of Developments to Mid 2004.

During 2004 a number of significant major reports were published which provided a comprehensive summary of concerns related to the potential occupational health and safety impacts of the emerging nanotechnology industry.

These reports have included the following:

- Nanoparticles: An Occupational Hygiene Review, Research Report 274, prepared by the Institute of Occupational Medicine for the Health and Safety Executive (UK HSE 2004);
- Nanoscience and Nanotechnologies: Opportunities and Uncertainties, produced by the Royal Society and the Royal Academy of Engineering (Luther 2004);
- Technological Analysis - Industrial Application of Nanomaterials – Chances and Risks, prepared by Future Technologies Division of VDI Technologiezentrum GmBH, Germany;
- Nanotechnologies: A Preliminary Risk Analysis On The Basis of A Workshop Organised in Brussels On 1-2 March By The Health And Consumer Protection Directorate Of The European Commission;
- Small Sizes That Matter: Opportunities and Risks Of Nanotechnologies, A report prepared by The Alliance Centre For Technology and Alliance Global Risk in cooperation with the OECD International Futures Program.
- Swiss Reinsurance Company (Zurich, Switzerland). Nanotechnology; Small Matter, Many Unknowns. (Author: A. Hett), and,
- First International Symposium on Occupational Health Implications of Nanomaterials (12-14 October 2004, Buxton, Derbyshire, UK).

A review of this material has made it clear that nanotechnology is now an extremely broad area of research and industrial activity which has grown rapidly over the last decade or so. Even though the potential health and environmental benefits of nanotechnologies have been widely publicised a number of concerns have also been expressed regarding the nature of NPs. These include the high surface reactivities and their potential to cross cell membranes (Royal Society & The Royal Academy of Engineering 2004; refer also Section 4.4). It has been predicted that these distinctive properties could produce detrimental health effects and could even result in greater toxicity relative to the equivalent macro-scale compound. Conventional compounds which may normally be considered harmless may well prove to be dangerous on a nanometre scale (Luther 2004).

There is a considerable body of epidemiological work which shows an association between particulate air pollution and health (refer Section 2.3.1). Most epidemiological studies have been conducted in an environmental context with industrial and traffic emissions being the main source of particulate matter in the ambient environment. To date, and in part due to the early stage of nanotechnology development across the

world, comparable epidemiological and workplace exposure studies to engineered NPs have not been reported.

From an occupational hygiene perspective the major conclusions drawn from the Health and Safety Executive (UK) report entitled "Nanoparticles: An Occupational Hygiene Review" (UK HSE 2004) are:

- That there are four major nanoparticle production processes, gas-phase, vapour deposition, colloidal and attrition, all of which may potentially result in exposure by, inhalation, dermal or ingestion routes;
- Nanoparticle production processes are similar to a range of existing chemical processes;
- Only gas-phase processes have the potential to cause exposure to primary NPs by inhalation during the production phase. All processes may give rise to exposure (by inhalation, dermal and ingestion) to agglomerated particles during recovery, powder handling or product processing;
- There are existing controls and methods which are likely to be effective in nanoparticle processes;
- For dermal and ingestion exposures, control methods based upon personal protective equipment may not be as effective as they are in existing processes;
- Particle surface area may provide the most suitable criterion for assessing inhalational exposure. Currently however, there are no effective methods available by which particle surface area can be assessed in the workplace;
- Current knowledge is inadequate for risk assessment purposes; and
- There is little evidence to suggest that the exposure of workers arising from the production of nanoparticles has been adequately assessed.

It has been suggested that emerging nanotechnologies will not introduce new health or safety problems – *although* the evidence currently available is inadequate to be definitive. Where NPs are physically contained or embedded any potential hazards associate may will be minimised. The main concerns however are primarily with free NPs which are thought to be more available for absorption and distribution within the body (Royal Society & The Royal Academy of Engineering 2004). With an expectation of such properties it is probably wise that NPs be treated with a degree of caution. For production on a large scale any hazards ought to be characterised and any likely worker exposure be assessed so that identified risks can be minimized.

Whilst there has been increasing attention paid to the potential health effects of exposure to NPs and, in particular, during the production and handling of nanopowders very little consideration has been given to their explosive potential. In order to address this knowledge gap the HSE commissioned a literature review to examine the potential explosion hazards of nanopowders (Pritchard 2004), the main findings of which were;

- An increasing range of materials capable of acting as explosive dusts are being produced as nanopowders;
- The potential explosion hazards of nanopowders have not been adequately characterised; and
- The explosion characteristics of micron-scale powders (particle sizes from 10 – 500 μm) are well known but there was no data for nanopowders (particle sizes from 1 – 100 nm). It was concluded that any extrapolation of the data available for larger particles to nano-sized particles could not be carried out with any certainty.

As an overall summary to mid-2004 the predominant view is that - despite the wide range of benefits likely to emerge from nanotechnology industry - there are a significant number of risk and regulatory concerns that should be considered. Further research into the biological properties of NPs are necessary to determine this more precisely so that a more definitive regulatory framework and public education program can be developed. This view is advocated by experts in the field, for example "we currently know enough to treat engineered nanoparticles with caution, but not enough to predict their potential health impact" (Maynard 2004).

The table below highlights the major issues which were identified by three European reports and requiring attention (Adapted from Michelson 2004).

| Issue | Switzerland | UK | Germany |
|---|---|---|---|
| **Health Risk Analysis** | • Little data available<br>• Defects occur over time<br>• Could damage lung, heart and brain<br>• Analogy with asbestos used to inform regulatory framework | • Little data available<br>• Need new tests to understand toxicity<br>• Analogy with asbestos used to inform regulatory framework | • Most risky form is inhaling aerosol particles<br>• Could damage lung, heart and brain |
| **Worker Risk Analysis** | • Reduce exposure limits<br>• Safety devices not robust<br>• Need 'best 'handling and transportation practices | • Reduce exposure limits<br>• Review accident management procedures | • Need worker training on protective measures<br>• Need 'best 'handling and transportation practices |
| **Regulatory Framework** | • Nanoparticles should be a new class of materials<br>• Common international standards required | • Nanoparticles should be a new class of materials<br>• Continually adaptable and emerging regulatory framework<br>• Include provisions for future applications | • Regulatory approach needs to be adapted<br>• Common international standards required |

| | | | |
|---|---|---|---|
| **Regulatory Philosophy** | • Precautionary principle supported | • Cautious, though specifically states there is no need for a moratorium on production | • Careful consideration of hazards required |
| **General Education** | • Window of time available to shape public perception<br>• Do not wait for negative event to shape public opinion | • Fund research into public attitudes<br>• Suggest bi-annual review of new nanotechnologies | • Communicate risks to the public<br>• Initiate public discussion including all stakeholders |

The primary conclusions that these major reports have in common are:

1. NPs typically have distinct physical and chemical properties by virtue of their extremely small size.
2. These properties **cannot** be predicted *a priori* from the properties of the larger scale equivalent (i.e. the "bulk material").
3. There exists remarkable engineering flexibility and adaptability in process-dependent uses for NPs.
4. Modifications to NPs (e.g. chemically by "derivatisation") for specific uses further alters their physical and chemical properties from that of the original nanoparticle.
   (*N.B. This has been referred to as a NPs "tunable" properties.*)
5. The safety of NPs represents a significant uncertainty factor.

Based on these conclusions the following recommendations are gaining widespread acceptance in the scientific literature:

1. NPs **cannot** be considered as one large group of compounds.
2. NPs should be regulated on an **individual** basis with the initial premise that each has unique physical, chemical and - hence - biological properties distinct from the bulk material.
3. The likely human health consequences of exposure to NPs may lie in each NPs distinctive physical, chemical and biological characteristics.

# 4. Summary of Developments from mid 2004 to Present

## 4.1 Essential Background

It is worthwhile considering briefly the biology of the human body's defences against xenobiotics in order to fully appreciate how exposure to nanoparticles may affect the health of an individual. Similar summaries can be found in each of the major reports released to date (Royal Society & The Royal Academy of Engineering 2004; Luther 2004). Nonetheless, reiterating the numerous cellular and tissue defensive capabilities to foreign toxic insults is of fundamental importance and one that determines the maintenance of health and, ultimately, worker productivity. The following description provides an overview only of the processes involved. At a more comprehensive level the human body's responses to xenobiotic insult and the maintenance of homeostasis are nuanced and the details are still the subject of active research by specialists.

The body responds to NPs and other environmental particulates by presenting a multilayered defence system essentially as it does against all infectious bacteria and viruses. Each succeeding layer recruits stronger and more varied processes in an effort to maintain the body's well-being. The first important level is a physical barrier provided by the epithelial cell and keratinised layers of the skin. As a result, the physical integrity of the skin is an important factor in determining the level of non-inhalational NP exposures. It is often cited that exposures are prevented by the keratinised layer of the skin and in normal circumstances this may be true (although it cannot be excluded completely for NPs at the lower end of the size scale given the poor state of knowledge in this area). However, little mention is made of the consequences of a breakdown in the skin's physical barrier functions. Conditions of occupational dermatitis affect "virtually all industries and businesses" (UK HSE 2002) and include symptoms such as skin rashes, swelling, thickening, blisters, hives, and cracks. These symptoms are an indication that a chemical has penetrated the surface layer of the skin and provoked a reaction in the underlying epithelial layers. Even low-level, but usually chronic, environmental exposures can elicit inflammatory responses (e.g. dioxin-induced chloracne). Once the skin's ability to provide a contiguous physical barrier has been compromised exposures are subsequently increased. This is as relevant to occupational nanoparticle exposures as it is to the voluntary application of nanoparticle-containing cosmetics in sensitive individuals.

Secondly, epithelial cells and associated fluids also present a barrier. The scientific consensus is that ambient air exposure will likely represent the major route of concern for occupational exposures. The best information currently available comes from inhalational studies with metal oxide or radiolabelled NPs which show differential deposition patterns within the lung. For example, 1 nm particles are preferentially deposited higher in the nasopharyngeal region while 5 nm particles are deposited throughout the

lung (Oberdörster et al 2005a). The alveolar gas exchange region of the distal lung is the site of greatest 20 nm NP accumulation (Oberdörster et al 2005a). Once deposited foreign material must cross a tightly packed epithelial layer before access to the bloodstream is possible. Mucus fluid secretions lining the airways and ciliated epithelial cells are important elements of this barrier system and compromise the "mucociliary escalator". The physiological actions of coughing and sneezing also act to remove larger particles which have been trapped in lung fluid.

Finally, if NPs are able to enter the tissues or cells other more powerful defences are initiated. The cellular environment consists of "scavenger" cells (usually termed macrophages or neutrophils) which attempt to internalise, proteolytically degrade and/or present the degraded foreign material on the cell's surface as examples of "non-self". This is typically part of more generalised inflammatory and acute phase responses which incorporate the release of signalling molecules such as chemokines, cytokines, C-reactive protein and fibrinogen (a coagulant). Macrophage and neutrophil activation is also associated with the production of reactive oxygen species (such as hydrogen peroxide; refer Section 2.1). As a result, cell migration from the bloodstream to the site of injury occurs and this is a reliable indicator of localised tissue damage. An often under-appreciated point is that the inflammatory response need not be entirely protective and, if uncontrolled, may further damage the target tissue (Bruschi 2005).

The process of macrophage ingestion and (attempted) proteolytic breakdown may also go awry. In addition, NPs may become internalised in non-immune-related cell types (e.g. parenchymal cells) – by either conventional or unconventional means (refer Section 4.3). Under these circumstances intracellular molecular defences are initiated which result in the new expression of protective genes. The number and type of genes newly expressed in response to exposure are thought to be *indicative and diagnostic for the type of cellular damage*. Several broad intracellular protective responses are known to be induced with the most prominent being:

- the drug and chemical metabolising enzymes,
- the stress (or "heat shock") response, and,
- the antioxidant-response element-inducible genes and proteins.

NPs can also enter the body via the digestive tract by either ingestion or by the mucociliary escalator after inhalation. The physiological environment presented through this route is very different from that encountered in the lung. In comparison to the thin alveolar epithelium required for gas exchange the gut consists of a (mostly) very acidic environment and a thick mucous barrier lining. Ingestion may therefore represent a more benign exposure route.

All the physical barriers mentioned above can be circumvented by the direct intravenous administration of NPs (e.g. therapeutically). Although this is not discussed further here it is of interest to point out that earlier studies

have found evidence of fullerene renal toxicity following i.v. (and i.p.) administration but not after ingestion of the same compounds (Chen et al 1997, 1998). Consequently, multiple administration routes will undoubtedly be required in future work to adequately examine the bioavailability and toxicological profiles of NPs of interest.

From this necessarily brief outline of the human biological responses following xenobiotic exposure it can be concluded that a failure in any of these protective layers may result in adverse human health effects. Consequently, it is of toxicological and OHS interest to monitor for effects such as atypical inflammatory responses and gene inductions as these are likely to represent precursors of downstream pathological conditions.

1. The human body presents a hierarchical set of defences to combat foreign particles and maintain homeostasis.
2. Any changes to the normal physiology of these defences are an indication that the body is under stress and that disease may result if repair pathways are overwhelmed.
3. Nanoparticle-induced changes can occur at the level of a body's organs, cells and/or molecules.

## 4.2 Human Inhalational Exposure Studies

Older studies have indicated that under controlled conditions the inhalation of radiolabelled, inorganic or carbon-based nanoscale particulates can enter the systemic circulation - usually within hours (Nemmar et al 2002; Donaldson et al 2005). For combustion-derived UFPs the evidence is strong that these exposures can impair normal health. For example, inflammatory responses have been shown to short-term UFP exposures in otherwise healthy volunteers (Englert 2004; Savli et al 1999 & 2000). Similar findings have been reported for pulmonary deposition of carbon black NPs which decreased heart rate in experimental animals and prolonged cardiac repolarisation in young healthy individuals (Frampton et al 2004). Consequently, the literature indicates that engineered NPs and combustion-derived UFPs share a capacity to be absorbed rapidly and distribute throughout the human body.

The biological consequences of engineered NPs entering the systemic circulation are mostly unknown. Several recent studies address this issue. Beckett et al (2005) were unable to find significant respiratory, haematologic or cardiovascular effects following inhalation of 500 $\mu g/m^3$ UFP ZnO for 2 hours in healthy human volunteers. The authors concluded that this acute dose was far below the toxicological threshold. Furthermore, an epidemiological meta-analysis of UFP $TiO_2$ dusts evaluated the mortality statistics at eleven European and four US $TiO_2$ manufacturing plants and concluded that there was no indication of $TiO_2$–mediated carcinogenic effects associated with workplace exposures (Hext et al 2005). How these observations relate to the authors findings of tumour formation with experimental animals is uncertain (refer Section 4.3 below).

Given that the nanotechnology industry in Australia and elsewhere is still in its infancy it is perhaps not surprising that there have been no reports on human inhalational exposures to CNTs, fullerenes or other NPs. As commercial applications and production of these types of NPs become more common this situation may change. If this occurs it will be of necessary to monitor occupational exposures as an adjunct to any concurrent epidemiological work.

Key observations from recent human inhalational and corresponding *in vivo* studies:

1. Non-physiological or pathological effects have **not** been observed in humans following short term (acute) exposures to nanopowders.
2. Lung neoplasias in test animals from inhaled $TiO_2$ NPs are dependent on particle size and surface area (e.g. US NIOSH 2005).
3. Lung neoplasias are observed in the rat only.
4. The relevance to humans of this rat-specific phenomenon is not known at present.
5. A recent epidemiological meta-analysis suggests that occupational exposures to $TiO_2$ NPs do not represent a hazard to human health.

## 4.3 Recent In Vivo and In Vitro Studies

As mentioned in the previous section Hext et al (2005) demonstrated clear species specific differences in the tumourigenic potential of UFP $TiO_2$ which could not be adequately explained by total lung burdens and clearance rates. Instead, when lung burdens and clearance rates were expressed as a function of the surface area of the administered UFP $TiO_2$ there was a much closer correlation with biological responses. Lung neutrophil infiltrations were seen to be a sensitive marker for UFP $TiO_2$–induced pulmonary inflammatory changes and correlated well with tissue histopathologies. The preferential development of lung tumours in the rat but not other animal models raises unresolved questions as to the relevance of these findings to human exposure situations (Hext et al 2005; US NIOSH 2005). Nonetheless, the general principle of biopersistence and body burden is maintained in as much as an overloaded rat lung may also model potentially harmful bioaccumulation effects in humans exposed to UFP $TiO_2$, CNTs or other NPs (Bermudez et al 2004).

The inhalational toxicity of CNTs has been examined by several groups. Most recently Castranova and colleagues (US NIOSH) comprehensively examined the pulmonary responses to SWCNTs in both in vivo and in vitro test systems (Shvedova et al 2005). Significantly for the field these authors utilised purified SWCNTs, i.e. purified of the residual metal catalyst used in synthesis. Clear dose-related increases in organ toxicity and oxidative stress markers were observed following pharyngeal administration in mice from a Black 6 genetic background. Early and significant lymphocyte, macrophage and neutrophil responses were evident for up to 60 days post exposure *and* were remarkably dose-dependent. Likewise, alterations to inflammatory cytokines and in vitro macrophage functions were observed. Epithelial

hypertrophy, interstitial fibrosis and compromised respiratory function were also seen as longer term consequences of SWCNT deposition.

The inflammatory responses, fibrosis and granuloma formation at the sites of CNT accumulation found by Shvedova and colleagues were in agreement with other previous studies which have also examined the toxicity of CNTs but which did not use purified CNTs (Warheit et al 2004; Lam et al 2004). The authors concluded that the early inflammatory and fibrotic responses observed in their studies with SWCNTs were indicative of a human hazard potential at the current US OSHA permissible exposure levels (PEL) for graphite particles and that under these conditions the formation of some lung lesions were possible (Shvedova et al 2005).

In the only work of its type the same group also examined the relative ability of CNTs to aerosolise in a realistic and operational production facility (Maynard et al 2004). The air-borne CNT levels and likely exposure potentials were concluded to be minimal in this small-scale workplace setting. Further studies relevant to CNTs exposure in realistic workplace scenarios will undoubtedly be eagerly awaited.

In comparison to the above study of Shvedova and co-workers the cytotoxic (i.e. cell killing) properties of more hydrophilic, phenylated, derivatives of SWCNTs were investigated by Sayes et al (2006). Although these more water soluble SWCNTs appeared to be less cytotoxic to several human cell lines important Pluronic™-only experimental controls were absent. The water soluble derivatives were administered without the need for Pluronic™ in comparison to unmodified SWCNTs which required 1% (v/v) Puronic™ for aqueous solubility. This is of importance as Pluronic™ - as a non-ionic detergent - is known to be a plasma membrane disrupting agent. Another recent study has also been reported indicating that both SWCNTs and MWCNTs are cytotoxic after only 6 hours of exposure to alveolar macrophages in the following order of potency:- SWCNTs > MWCNTs > quartz > fullerenes (Jia et al 2005). Importantly, using intracellular enzyme leakage as a viability measure, fullerenes ($C_{60}$) were shown to have dose-related cytotoxic effects to human dermal fibroblasts but not to human neuronal astrocytes or transformed human hepatocytes under identical conditions (Sayes et al 2005). These non-intuitive findings will require additional studies to resolve.

In related studies fullerenes were shown to kill mammalian cells at the exceptionally low in vitro concentrations of 20 ppb (or approximately 0.8 micromolar; Sayes et al 2004). Cell death was proposed by the authors to be mediated through oxidative stress and lipid peroxidation mechanisms. Fullerenes which had been extensively chemically modified to become more water soluble were not able to generate reactive oxygen species and, as a result, were not as cytotoxic (Sayes et al 2004). Independent verification of these findings will be of interest to many in this field. Separate morphological studies using electron microscopy have confirmed that MWCNTs can be internalised within skin keratinocytes (Monteiro-Riviere et al 2005) and other authors have shown that SWCNTs can inhibit basic cell

functions including division and substratum adhesion in a dose-dependent manner (Cui et al 2005).

The toxicological properties of NPs received considerable interest at a recent professional meeting[¶] but these preliminary reports have not yet reached the peer-reviewed literature. It is nonetheless worthwhile commenting on some of these reports. James & Lam (2005) observed dose-dependent pulmonary granulomas and inflammation in $B6C3F_1$ mice (Black 6 genetic background) following intratracheal instillation of 0.1 or 0.5 mg/animal of SWCNTs. This contrasted with quartz administration which produced inflammation but no granulomas after 90 days. Significantly, mortality was observed in a few animals. The authors chose these dosages based on the current PEL (US OSHA) for synthetic graphite and concluded that "*carbon nanotubes cannot be regarded as relatively non-hazardous carbonaceous dust……prudent to treat the hazard from carbon nanotube dust similarly to the hazard from quartz dust.*" These observations were confirmed and extended by two groups who also measured oxidative and mitochondrial DNA damage in the heart and vasculature. Focal granulomatous lesions in the alveolar regions were also assessed over a period of 60 days. The damage to mitochondrial DNA observed in CNT-treated mice persisted for *at least* 6 months and was a good predictor for the eventual onset of atherosclerosis. Additionally, substantial oxidative damage (a known atherosclerosis risk factor) was found – along with compensatory gene expression - in the cardiac, pulmonary and aortic tissues after exposure (Kisin et al 2005; Li et al 2005). With further implications for cardiovascular disease, ultrafine carbon particles have been shown to have thrombogenic potential in rodents (Khandoga et al 2004; Silva et al 2005). These findings will provide possible mechanistic explanations for any chronic injuries developed by CNTs if these should be ultimately observed in future studies.

1. Dose-dependent inflammatory responses, pulmonary granuloma and fibrosis formation have been reported in experimental animals by several groups.
2. Crucial chronic <u>in vivo</u> exposure studies - to any of the forms of engineered NPs - have not yet been published.

3. In vivo toxicological studies suggest that short term exposures to SWCNTs and MWCNTs have toxicological properties of concern to human health.
4. The inherent inhalational hazard potential of SWCNTs and MWCNTs may be mitigated by a relative *inability* to aerosolise.
5. Factors influencing the stability of fullerenes and CNTs in aqueous solution are poorly defined, especially in a workplace setting.

[¶] Society of Toxicology 44th Annual Meeting and ToxExpo. March 6-10, 2005. New Orleans.

## 4.4 Recent Molecular and Genetic Studies

It is the authors' opinion that perhaps the most disquieting of the recent developments regarding the toxicology of engineered NPs have been in the molecular aspects of their behaviour. Pubic health concerns may be generated if these findings prove to have analogous correlates in exposed human populations.

As indicated in Section 4.1 (above) the human body's defences have evolved at a cellular level to combat invasion by biological entities such as bacteria and viruses (some with nanometre dimensions). These processes include internalisation and compartmentation of the foreign particle by procedures known as endocytosis and phagocytosis. These processes have been refined over evolutionary time to be (generally) effective defensive pathways. Human infectious agents such as human immunodeficiency virus (HIV), *mycobacterium tuberculosis* and trypanosomes are particularly virulent in that these intracellular pathogens can evade or utilise normal cellular processes as a basis for infectivity (Smith & Helenius 2004).

What then if – by virtue of a nanoparticle's atomic rather than biological scale – these cellular defence mechanisms are circumvented completely? Under these circumstances there exists a potential for internalised particles to bypass the normal quality control and defence pathways of the cell. Processes of recognition, proteolytic cleavage (if applicable to NPs) and removal from the target cell will no longer be relevant. From an OHS perspective the exposures to NPs and/or UFPs are more constant – something that phagocytic pathways have not evolved to recognise (especially if the internalised particle is not capable of being proteolytically digested as a viral particle is; refer Sections 1.2 & 4.1). In addition, an inability to "hand-ball" to the proteasome and breakdown NPs within the cell adds to the "biopersistence" burden on the human body (observed as a relevant phenomena in UFP $TiO_2$-induced neoplasias in rats; Hext et al 2005; Bermudez et al 2004). We still do not have sufficient information to conclude that these considerations apply but several recent reports in the last year are of interest.

Geiser et al (2005) were the first to show the direct transfer of a large portion of dosed $TiO_2$ NPs (≈ 4 nm,> 20%) across the alveolar endothelial cell membranes and into the connective tissue and microvasculature of rats. Additionally, NPs were shown to cross the plasma membrane of cultured macrophages and freshly isolated red blood cells. Using labour-intensive analytical techniques (energy filtered electron microscopy) the localisations of titanium containing NPs were determined and found <u>not</u> to be associated with any cellular structures thereby casting doubt on internalisation by endo- or phagocytosis. The authors instead concluded that the non-biological processes of thermodynamics, unbalanced capillary forces and adhesion effects were the basis for these particle movements. Perhaps the most significant finding from this study was that there was <u>no</u> difference in particle uptake that could be attributed to surface composition or chemistry (Geiser et al 2005). These findings are in agreement with the major

conclusions of the US National Institute of Occupational Safety and Health and other studies which have concluded that particle size is a primary factor in the carcinogenic potential of metal oxide nanopowders (Limbach et al 2005; US NIOSH 2005; refer also Sections 2.3.2 & 4.5.5). Pending the release of confirmatory studies (e.g. Shimada et al 2005 using UFP carbon black) these otherwise detailed studies by Geiser and co-workers should sound a cautionary note.

In comparable studies using radiolabelled isotopic NPs (e.g. $^{192}$Ir or $^{99}$Tc) some authors have proposed that the transepithelial movement of NPs in the lung occurs as a result of the inflammatory release of histamine and other chemokines and cytokines (Meiring et al 2005; Nemmar et al 2005). Similar conclusions have been reached by Shimada and colleagues in their preliminary report (Shimada et al 2005). Although the mechanisms of pulmonary nanoparticle movements have yet to be fully established the translocation into the systemic circulation is a toxicological issue of importance (as evidenced from the acquired knowledge with combustion related UFPs; Delphino et al 2005; refer Section 2.3.1). Some adverse effects on the heart, vasculature, liver and other organs can be expected following systemic absorption if the internalised nanoparticle dose is in the toxicological range. As already reported the systemic translocation of fullerenes into the brain is associated with cellular effects in that organ (Oberdörster 2004; Oberdörster et al 2004).

Intracellular localisations are also evident for other nanoparticle forms. Water soluble, carboxylated, derivatives of fullerenes have been shown to cross the plasma membranes of human fibroblast and monkey kidney cells in vitro and preferentially locate to the mitochondrial fraction (Foley et al 2002; refer Section 5). Translocation into the nucleus was not assessed in this study but might be of interest given a recent report which proposes that fullerenes are theoretically capable of strongly intercalating with DNA and disrupting structure and/or function (Zhao et al 2005). Consequently, empirical studies are required to assess the genotoxic potentials of derivatised and underivatised fullerenes.

A more relevant category for occupational exposure concerns is the degree to which NPs undergo dermal absorption. Unfortunately, the literature is sporadic and often contradictory. There exists a need for the standardisation of techniques amongst those working in this area. In general, there is a lack of information on whether topically administered or exposed carbonaceous NPs articles can be absorbed across the skin's outer stratum corneum layer. From the extensive pharmacological literature SWCNTs have been investigated for the purposes of drug delivery but under these circumstances absorption is enhanced with co-administration of many types of solvent or other vehicles (incl. polyethylene glycol derivatives, lipids and/or peptides). As a result these types of studies are not immediately relevant to workplace exposure situations. From the studies that are more immediately relevant, however, submicron fluorospheres and beryllium particles have been shown to penetrate the cornified layer of flexed human skin thereby suggesting a potential route for immune system

activation following dermal exposure (Tinkle 2004). Clearly, further studies are required in this area before any conclusions can be made as to the dermal toxicity of carbonaceous and non- carbonaceous NPs. It is important to note here - as with any other nanotechnology area where there is a paucity of data - that *the absence of any evidence of harm does* **not** *necessarily imply that there is no potential for harm.*

In a pioneering study Ding and colleagues (2005) have utilised microarray techniques to examine the genes induced in response to MWCNTs and structurally similar compounds in human skin fibroblasts. In addition, other functional criteria such as cell viability and the type of cell death were measured. Although there appears to be problems associated with the dosing of cultured cells in this study it still serves as a model for the toxicogenomic studies that will be necessary in the future in order to define the mechanisms by which NPs achieve their biological effects. Ding et al (2005) were able to confirm the induction of inflammatory, metabolic and proteolytic genes after MWCNT exposure in this cell type. Of the inflammatory genes which were induced the interferons and viral resistance genes were prominent suggesting that these are default genetic responses to CNT exposure. These genomic findings are consistent with the whole animal studies mentioned previously in this section. To conclude, a generalised cellular "stress response" was evident in this study.

In conceptually similar proteomic studies, *macroscopic* GaAs and InAs particles have been shown to induce the major "stress proteins" in renal proximal tubule cells of hamsters consistent with the known nephrotoxicity of the semiconductor metals/metalloids (Fowler et al 2005; see also Section 2.4.1.1 & 2.4.1.2). The authors concluded that the observed inductions in renal cell gene expression patterns in response to in vivo treatment with GaAs or InAs particles represented an early response by the cell to injury and preceded overt cell death and organ injury. Of note was that the biochemical changes which were observed occurred at sites removed from the subcutaneous sites of administration for these compounds (Fowler et al 2005). The work of Fowler et al (2005) is supported by an excellent study which defines the molecular basis of CdTe cytotoxicity as reactive oxygen mediated and with preferential damage to the nuclear and mitochondrial subcellular compartments (Lovrić et al 2005). The authors are unsure as to why these photo-responsive quantum dots components can induce ROS production in the absence of light. This may have considerably less to do with the chemical composition and surface properties of CdTe per se and more to do with the biology of the target cell (see Section 5). In any event, these molecular studies (and others) provide further evidence that the inherent hazard potential of quantum dot metals lies in an ability to degrade under photolytic, mechanical or oxidative conditions and, as a result, diffuse from the structural core (Hardman 2006; Kirchner et al 2005a, 2005b; Shiohara et al 2004, Soto et al 2005).

Finally, in the only study of its type so far, the potential reproductive toxicity of several metal oxide nanopowders (Ag, $MoO_3$ and Al; either 15 nm or 30 nm) has been reported (Bradydich-Stolle et al 2005). Using cell

culture methods evidence of dose-related spermatogonial cell apoptosis was found with these nanoscale metal compounds which did not necessary correlate with overt necrosis (as would be expected). Most disturbingly, however, the authors observed clear, significant and dose-dependent inhibitions of mitochondrial function to each of the nanopowders examined in this in vitro test system. The almost complete absence of studies which have investigated the human reproductive toxicity of any of the nanoparticles is a concern. Further work in this area is required to confirm or refute this initial study, and also that of fullerene-induced embryotoxicity in mice (Tsuchiya et al 1996).

Important findings from recent *in vitro* and molecular studies:

1. Several studies, using animal test systems, now indicate clear dose-related increases in lung toxicities following CNT administration.
2. One study (Shvedova et al 2005) has correlated these pathological findings to compromised respiratory function in the longer term.

3. Nano-sized particles can translocate across the plasma membrane of epithelial cells by unconventional means (i.e. directly avoiding phagocytosis or endocytosis).
4. NPs have also been shown to translocate along neuronal axons and dendrites.
5. NPs have been observed in the brain compartment and the systemic circulation (perhaps as a result of 1 & 2, above).

6. The capacity of engineered NPs to cross human skin is mostly unknown.
7. The mitochondria are preferred subcellular structures for the accumulation of some NPs.
8. Inhibition of mitochondrial function has been associated with exposure to NPs in experiment animal and culture systems. *In the author's opinion this likely represents a 'smoking-gun' from a molecular toxicology perspective.*

9. Core and shell components of QDs have the potential to be unstable in biological settings.
10. Decomposition of QDs in situ will release elemental metals and possibly selenium sulphide.
11. Renal damage and cancer formation (at different sites) will be dependent on the both the extent of exposure and the relative stability and excretion of QDs.

12. Only two published reports address nanoparticle-mediated reproductive toxicities. The relative absence of information in this area is a concern.

## 4.5 OHS Considerations.

Information regarding the health and safety risks associated with occupational exposure to NPs is limited. In particular, there are relatively few publications which specifically address the OHS issues of nanoparticle manufacture and handling. Many governmental agencies are formulating guidelines and inviting feedback but this area is still in its infancy (US NIOSH 2006). As a consequence, the discussion in this section draws heavily from relatively few previous reports, and notably from *Approaches to Safe Nanotechnology* (US NIOSH 2005b).

It would appear that in general OHS practitioners recognise that there is a lack of reliable and consistent guidance for the safe handling of nanomaterials. The main reason for this is the unknown risks that nanomaterials pose to workers. If NPs involve any of the same characteristics that have been associated with UFPs - where they have been shown to cause increases in pulmonary inflammation and other systemic effects (refer Section 2.3.1) - then they may raise similar concerns for worker's health. It has been suggested that until preliminary findings and hypotheses regarding these concerns are confirmed a precautionary approach to the use of NPs should be adopted (US NIOSH 2005b).

In order to maximise the likelihood that workers are protected it would appear wise, at least initially, to consider using control measures that are known to work for larger particles. Ideally - and as previously shown with most hazards encountered in the workplace - it would be useful for those working with nanomaterials to utilize a broad range of control measures to ensure that exposure is kept to a minimum.

### 4.5.1 Potential For Occupational Exposure.

Exposure opportunities may exist during the manufacture, storage, handling and transport of nanomaterials. Inhaled NPs in particular may represent a potential health risk. Occupationally generated aerosols containing NPs may come form a variety of sources depending on the type of activity and processes taking place. Given that most nanoparticle production processes occur in closed reaction chambers it is more likely that the potential for exposure will occur following the manufacturing process. Exceptions to this could be in cases where there are work process and or system failures which result in particle leakage into the work environment.

A recent study from the USA examined manufacturing processes and came to the conclusion that, from an insurance industry perspective at least, the manufacturing procedures *per se* were not especially hazardous (Robichaud et al 2005). This assessment of the relative risk of nanomaterials production encompassed diverse processes but may not have fully included the broader manufacturing aspects of end- and by-product handling and distribution. For the purposes of this report the authors consider these to be important areas of future concern for occupational exposures.

There have been very few studies that have measured human exposure to NPs. In general it is most likely that processes which generate nanomaterials in the gas phase, or using or producing nanomaterials such as powders or solutions probably pose the greatest risk for releasing NPs. Maintenance on production systems may also provide an opportunity for exposure, particularly during the cleaning and disposal of materials from dust collection systems (Lam et al 2006; Luther 2004). Exposures associated with waste containing nanomaterials may also occur (US NIOSH 2005b).

Although it is generally recognised that the most likely exposure route for NPs is inhalation other opportunities for exposure are also evident (e.g. via ingestion or through the skin). Ingestion could occur from unintentional hand to mouth transfer of materials. Ingestion may also occur following inhalation exposure as particles may be cleared from the respiratory tract via the muco-ciliary escalator could then be swallowed. Very little is known about the effects from the ingestion of any of the nanoparticles with the possible exception of the metal/metalloid components of QDs (refer Sections 2.4.1). The dermal absorption of carbonaceous and non-carbonaceous NPs has been the subject of many studies (the majority being in drug delivery; refer Section 4.4). However, the situation currently is that these findings cannot yet be extrapolated to an occupational risk environment.

### 4.5.2 Potential Safety Hazards

Not much is known regarding the safety risks of NPs although some data exist suggesting that NPs share some properties in common with the more traditional bulk materials. These are primarily catalytic effects and fire and/or explosion hazards. Even though there is still insufficient information to predict specific fire and explosion risks associated with nanopowders, nanoscale combustible material could present a higher risk than a similar quantity of coarser material, given its unique properties (UK HSE 2004).

For many years it has been recognised that some NPs and nanostructured porous materials can be used as effective catalysts in chemical reactions for decreasing the temperature for these reactions to occur (either as gases or liquids) or for increasing rates or yields of products. It is possible that some nanomaterials may initiate catalytic reactions that would not otherwise be anticipated and contingencies should be made for such possibilities (Pritchard 2004).

### 4.5.3 Chemical Identification And Characterisation.

The identification and characterisation of chemical substances and materials is an important first step in the assessment of their risk. Chemical properties that are important in the characterisation of discrete chemical substances include - but are not limited to - molecular weight, melting point, boiling point, vapour pressure, octanol:water partition coefficient, water solubility, reactivity and stability. The diversity and complexity of

nanomaterials, however, makes chemical identification and characterisation difficult. A much broader spectrum of properties will be required to sufficiently characterise a given nanomaterial for the purposes of evaluating hazard and risk (US EPA 2005b). Chemical properties such as those listed above may be important for some nanomaterials, but others such as particle size and distribution, surface/volume ratio, coatings, and conductivity are expected to become more important for most nanomaterials. As well as the chemical characteristics there are many other requirements that need to be taken into account during the characterisation processes of nanoparticle exposure assessment. Some of these other considerations are outlined below (Figure 6).

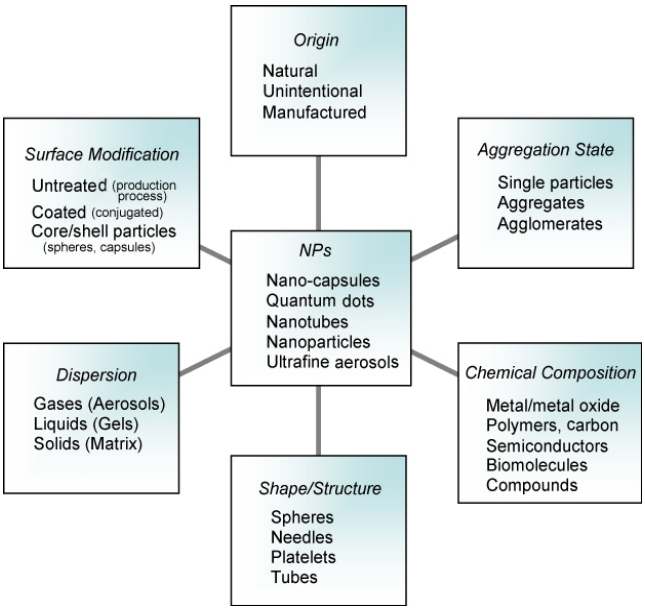



**Figure 6:** A representation of some physical, chemical and other attributes of nanoparticles which can be included as variables during assessments of occupational exposures. Adapted from Innovest (2005).

### 4.5.4 Exposure Assessment.

Factors affecting exposure to NPs will include the production, transfer, transportation and cleanup processes in use, the amount of material being used and whether material can be easily dispersed (e.g. in the case of a powder) or from airborne sprays or droplets (in the case of suspensions). The degree of containment will also impact on the likelihood of exposure. In the case of airborne materials, particle or droplet size will determine whether the material can enter the respiratory tract and where it is most likely to be deposited.

Some of the factors that have been reported to influence the potential for exposure to nanomaterials in the workplace include (US NIOSH 2005b):

- the risk of skin exposure may be increased if working with nanomaterials in liquid media without suitable protection (e.g. without the use of gloves or overalls);

- the likelihood of forming inhalable and respirable aerosols may be increased during pouring, agitation or mixing of liquid media containing nanomaterials;
- the likelihood of aerosol release into the workplace is increased if nanoparticles are generated in the gas phase in non-enclosed systems
- equipment maintenance and the cleanup of spilled nanomaterials pose enhanced potentials for worker exposure (Luther 2004);
- the transfer of nanomaterials in open systems is likely to increase exposure potentials even for relatively hydrophobic NPs (e.g. Lam et al 2006);
- the cleaning of "dust" collection systems used to capture NPs presents a further potential for both skin and inhalational exposures.

Currently, there exists little information or broad agreement regarding the characteristics and opportunities for workplace exposures to NPs. In addition, the measurement techniques that should be utilised to monitor ambient concentrations and exposures in the workplace are also not well understood. Existing research indicates that mass and bulk chemistries may be less important than the particle size and surface area of NPs (Oberdörster et al 1992 & 1994). The relative importance of the various particle characteristics and how they relate to the accurate assessment of nanoparticle exposures is being actively investigated at present, particularly by US NIOSH.

Monitoring concentrations of NPs in the workplace will be a critical component in any exposure assessment process. However, there is currently no single sampling method that can be generally utilised to measure all nanoscale aerosols. Therefore, any attempt to characterise workplace exposure to NPs should probably include a range of sampling techniques that encompass the physical and chemical properties of the NPs of concern. However, the current situation is that many of the most accurate measuring techniques currently available are complex, time-consuming and costly and as a result are not readily applicable to routine exposure monitoring (e.g. electron microscopy).

To add a further dimension of complexity associated with measuring the concentration and subsequent worker exposure to nanoaerosols is the concept of "Ostwald ripening". This is illustrated by a study which examined the thermal stability of facetted self-assembled CdSe quantum dots during annealing (Raab & Springholtz 2001). The authors reported that with increasing annealing time the dot density was found to decrease rapidly with a simultaneous increase of the average dot volume (Figure 7).

The Ostwald ripening properties of NPs presents a significant challenge for the monitoring of NPs in a workplace environment. It poses a fundamental question for exposure assessments - how does one measure the exposure to particles that are constantly changing in the ambient air? The need for robust and reliable procedures to measure and detect nanoparticles and nanotubes - ideally in real time - is seen as a difficult task but one that is of

fundamental importance. The accurate monitoring of exposures to NPs of varying chemical compositions and physical properties will be required for occupational and toxicological investigations as well as, eventually, for risk assessments and epidemiology. These concerns have been previously highlighted (Royal Society & The Royal Academy of Engineering 2004; Denison 2005).

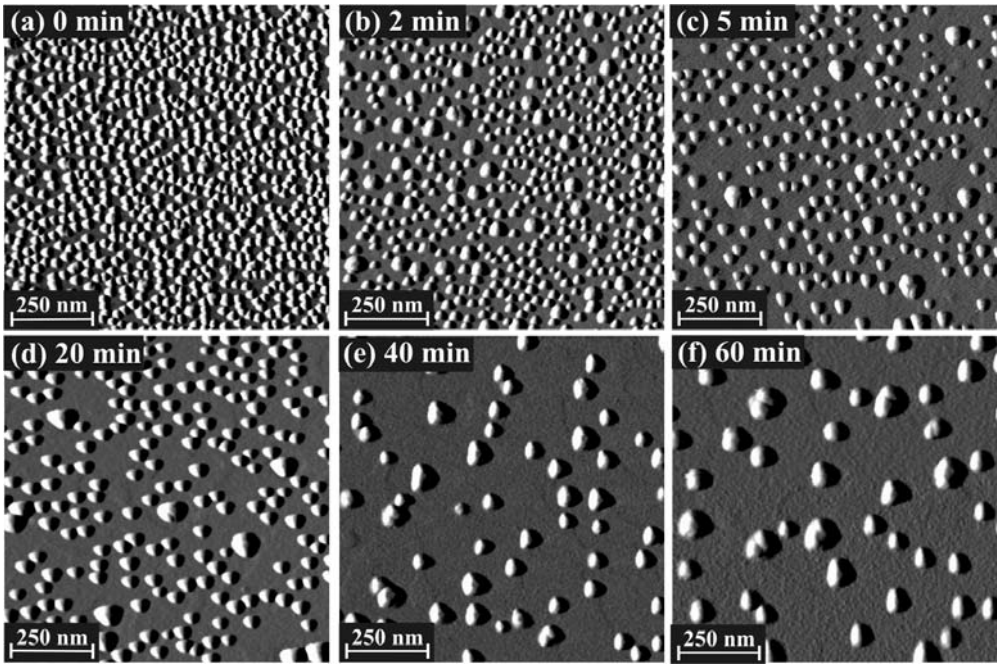



**Figure 7:** Real-time analysis of CdSe quantum dot aggregation and agglomeration events ("Ostwald ripening") indicating the change in physical properties which are likely to be encountered in an occupational environment. Data kindly provided by Dr. Gunter Springholz (University of Linz) with permission.

Further to this point is the developing consensus that currently used mass measurements are inadequate for measuring nanoparticle exposures. It is expected that supplementing Australia's Material Safety Data Sheets (MSDSs) system will be required at some stage to acknowledge that a material originated as a nanoparticulate and, hence, may have unique properties. Since MSDSs invariably provide guidelines based on the bulk-scale chemical counterpart (e.g. 'soot' for CNTs) and may not consider the high surface area and increased reactivity of current and future nanomaterials, the current procedures could prove inadequate to serve the safety requirements of workers.

A standardised approach to measuring exposures is needed but, in order to achieve this, new measuring technologies will need to be developed (Royal Society & The Royal Academy of Engineering 2004).

### 4.5.5 Determination and Variation of Titanium Dioxide Nanoparticle Exposure Limits.

During November 2005 NIOSH released a draft Current Intelligence Bulletin titled: "Evaluation of Health hazard and Recommendations for Occupational Exposure to Titanium Dioxide" (US NIOSH 2005). This extensive bulletin sets out the rationale for the exposure limits of 1.5 milligrams per cubic metre (TWA 10 hours) for fine $TiO_2$ and 0.1 milligrams per cubic metre (TWA 10 hours) for ultrafine particles during a 40 hour week.

As a basis for setting these limits NIOSH reviewed the available human, animal and in vitro studies on $TiO_2$ and provided a quantitative risk assessment using relevant dose-response data in rats for both cancer (lung tumours) and non-cancer (pulmonary inflammation) responses and extrapolation to humans with lung dosimetry modelling. Based on this approach it was clearly shown that $TiO_2$ and other poorly soluble, low toxicity dusts (PSLT) show a consistent dose-response relationship for pulmonary responses in rats, including persistent pulmonary inflammation and lung tumours, when dose is expressed as particle surface area. It was concluded that the increased mass-based potency of ultrafine $TiO_2$ compared to the larger (i.e. "fine") particles of $TiO_2$ is associated with a relatively greater surface area for a given mass.

In addition, while a dose-response relationship has been determined in test animals more research is still required on the measurement of workplace airborne exposures to ultrafine $TiO_2$ in workplaces producing or using $TiO_2$. In the authors' opinion further research needed includes, exposure-response relationship assessments between ultrafine PSLT particles and human health effects, the fate of UFPs such as $TiO_2$ in the lungs and the associated pulmonary responses and effectiveness of engineering controls for controlling exposures to fine and ultrafine $TiO_2$. Such gaps in knowledge also apply to a range of other ultrafine particles as well as to engineered nanoparticles.

### 4.5.6 Risk Controls

Information regarding the health risks associated with occupational exposure to NPs is limited. In general, given the lack of information, a precautionary approach should be adopted regarding work practices. For most processes associated with the manufacture of NPs, the control of airborne exposure can probably be accomplished quite well using a wide variety of engineering controls similar to those used to reduce exposures to conventional aerosols (Ratherman 1996, Burton 1997). This at least will address the immediate issues of worker exposures but may not adequately address broader environmental exposures if these prove to be significant.

When considering measures to reduce exposure to NPs this report considers that it is important to employ a broad based risk management program using the *As Low As Reasonably Achievable* (ALARA) approach to ensure that

worker exposure is kept to an absolute minimum. The generally accepted approach is the application of a hierarchy of risk controls which incorporate the broad elements of elimination, substitution, engineering controls, administrative controls and – finally - the use of personal protective equipment. Elements of such a program should include the following (in order of preference):

Elimination

Given the absence of information regarding the relative toxicity of engineered nanoparticles and consequently suitable workplace exposure standards (WESs), this report considers it will be prudent to apply the ALARA principle to potential workplace exposures, and eliminate workers' exposure wherever possible during the manufacturing and handling of manufactured nanoparticles.

Substitution

The unique chemical and physical characteristics of individual NPs are likely to prevent any possibilities for substitution. In this regard this report views that this form of hazard minimisation is unlikely to be adopted in any widespread way in facilities of nanomaterial manufacture. After all, it is this uniqueness which will likely determine their application and commercial usefulness.

Engineering Controls

Based on the available information, the authors' consider that current control measures such as enclosure and local exhaust ventilation should be effective for capturing airborne nanoparticles, given what is generally understood about the motion and behaviour of NPs in air. Until further evidence becomes available ventilation systems should be designed, tested and maintained using American Conference of Governmental Industrial Ventilation guidelines (ACGIH 2001) to remain optimally effective. In addition, high efficiency particulate (HEPA) filters are generally expected to effectively remove NPs in the air (Hinds 1999). Such filters are normally tested using particles that have the lowest probability of being captured (usually around 300 nm) with collection efficiency rising above and below this diameter (US NIOSH 2005b). However, information on HEPA filter efficiencies is lacking for discrete particles that are only a few nanometres in diameter and further research is still required in this area.

Administrative Controls

Good industrial hygiene practices will also undoubtedly assist in minimising worker exposures to NPs. Some acknowledged types of good workplace practices might include the following (adapted from US NIOSH 2005b):

- Cleaning work areas following each shift using a HEPA vacuum cleaner and wet wiping methods. Cleanup and disposal should be conducted in a way that prevents worker contact with waste products;
- Prevention of the storage and consumption of food and drink in work areas;

- Provision of hand-washing facilities. Workers should be encouraged to use them prior to eating, drinking, smoking and before leaving the workplace;
- Provision of showering and change facilities to prevent transfer of contamination to the home environment; and
- While there is no specific guidance related to the cleanup of nanoparticle spillages, based on available information, it is advisable to use HEPA vacuum cleanup methods to prevent further dispersal and subsequent exposure.

Personal Protective Equipment (PPE)

The provision of personal protective clothing will no doubt be a factor in assisting to prevent exposure to nanoparticles, particularly skin exposure. However, there are currently no guidelines available regarding the selection of such clothing. In addition it is also recognised that for powders at the macro scale such equipment is limited in its effectiveness (Schneider et al 2000).

Finally, in relation to the hierarchy of controls, respirators may be necessary when engineering and administrative controls do not adequately keep worker exposures to an airborne contaminant below a regulatory limit. At this time there are no specific workplace exposure standards (WESs) available for airborne concentrations of NPs (of any type), although they do exist for larger particles of similar chemical composition. It should be noted that preliminary evidence suggests that nanoparticles may be biologically more reactive than larger particles of similar chemical composition and therefore may pose a greater health risk when inhaled.

NIOSH tests and certifies respirator filters using particles that are about 300 nm in diameter in order to determine the collection efficiency at 95% to at least 99.97% of the available material. Particles at this size have historically been considered to be the most penetrating although the efficiencies of respirator filters to nanoscale particles is being actively investigated by NIOSH. Current data indicate that the penetration of 300 nm particles represents the worst case scenario for respirator filters but this may be revised as current NIOSH studies become publicly available.

It should be noted that recent reviews have indicated that current knowledge is limited regarding whether existing occupational hygiene practices are adequate to assure worker health and safety in the nanotechnology industry (Aitken et al 2004; The Royal Society and Royal Academy of Engineering 2004). Although not necessarily the view held by all in the nanotechnology industry there is the opinion that: *"Until a number of key knowledge gaps can be filled, it is unlikely that rigorous, specific guidelines or work practices can be fully developed"* (Maynard et al 2005).

### 4.5.7 Regulatory Issues

Most would agree that there is currently insufficient information available to properly assess the risk of nanomaterials causing harm to human health. It should be noted, however, that as the production of nanomaterials increases, exposure is also likely to increase. The authors' consider therefore that production should be monitored and, as new markets for nanomaterials are developed, the relevant health and safety impact assessments should be undertaken. In order to undertake these assessments and to appropriately regulate the manufacture of nanomaterials, investment in the research necessary to effectively support regulation is needed (Service 2005; Innovest 2005; The Royal Society–Science Council of Japan 2005; Hardman 2006).

Finally all stakeholders including the general public should be engaged at an early stage as it is likely that this process would more effectively support appropriate regulations.

Notable exposure-related issues include:

1. Opportunities for exposure are unlikely to be during manufacture.
2. Greater chance of worker exposure during transfer prior to storage and shipping.
3. Further exposures are possible during spillage and cleanup situations.
4. Additional worker exposures are likely during routine equipment maintenance procedures.
5. The relative risk to health from each scenario is unknown.
6. The most likely route of workplace exposure is inhalation with ingestion and dermal exposure being of relatively minor importance.
7. Current technologies do not allow for the accurate measurement of NPs in the occupational environment.

## 5. Critical Evaluation of the More Recent Literature

The physical and chemical properties of the fullerenes have provided some impetus for groups to examine these NPs as therapeutic agents (e.g. in cerebral ischaemia and Parkinson's disease; Dugan et al 1996). These studies now appear to have reached an impasse and the reason may be apparent if one takes an overview of the current state of knowledge (imperfect as it is) on the health effects of nanomaterials (Table 1).

One of the unifying themes emerging from studies released in the last year is that fullerenes (and even other NPs of different structural classes) induce ROS formation and interact with cells and the mammalian body in a pro-oxidative manner (Table 1). There are likely to be several plausible explanations for these findings. For example, the inherent chemical properties of NPs may only represent a minor contribution to the (human) biological responses to NPs. A primary example is the activation of resident macrophages and release of ROS and other species. The phagosome has also been shown to play an important role in the release of chemokines and cytokines and the pro-oxidative conditions associated with macrophage and other immune cell activations (Murray et al 2005). In addition, the internal oxidising environments of the endoplasmic reticulum and the endosome are well known (e.g. Mellman et al 1986; Austin et al 2005).

Almost all of the studies summarised in Table 1 document some form of interaction with the cell's outer plasma membrane and also some form or another of cell killing (either in vivo or in vitro). Many of these studies report the concurrent production of ROS (either directly or indirectly). This is an indication that the intrinsic chemical features of NPs may be minor in comparison to the broader biological responses of the exposed individual.

Another theme which surprisingly emerges from the recent literature is the preferential localisation of different NPs into mitochondria (Table 1). If not examined directly then most of the remaining studies found evidence of perturbations to mitochondrial function(s). This is an important observation because it is well established that mitochondrial damage can be a direct cause of fatal disease (e.g. as in human genetic disorders of metabolism). In these reported studies the preferential targeting of this organelle has been shown to be accompanied by enhanced intracellular ROS production and susceptibility to injury and cell death.

Mitochondria are increasingly being recognised as important subcellular targets in drug- and chemical-induced tissue injuries (Ho et al 2005a; Duchen 2004). For example, mitochondrial dysfunction can have a significant impact on overall cell survival if critical intramitochondrial functions of cellular respiration are perturbed. Many groups have also shown that important proteins related to the control of apoptosis are found

**Table 1:** Summary of selected recent and significant reports on the in vitro and in vivo toxicities of engineered and anthropogenic nanoparticles. A tick indicates a positive observation, a question mark indicates some evidence and a blank cell indicates that the category was not directly examined (i.e. NOT necessarily a negative observation, represented as X).

| | | | Cell membrane) [a] | ROS Production | NRF2 Induction | Signal Transduction | Mitochondrial Involvement | Cell Killing | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **Fullerenes** | $C_{60}$ | | ✓ | ✓ | | | | ? | Oberdörster (2004) [b] |
| | $C_{60}$ & derivatives | | ✓ | ✓ | ? | ? | ✓ | ✓ | Sayes et al (2004; 2005) [c] |
| | $C_{61}(CO_2H)_2$ | | ✓ | ? | | | ✓ | ? | Foley et al (2002) [d] |
| | $C_{60}((CH_2)_4SO_3Na)_{4\text{-}8}$ | | ? | | | | | ✓ | Chen et al (1997; 1998) [e] |
| **SWCNTs** | purified | | ✓ | ✓ | | ✓ | | ✓ | Li et al (2005) [f] |
| | unpurified | | ✓ | ✓ | | | ? | ✓ | Shvedova et al (2003) |
| | SWCNTs & derivatives | | ✓ | | | | ✓ | ✓ | Sayes et al (2006) [g] |
| **MWCNTs** | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | Ding et al (2005) [h] |
| | | | ✓ | | | | | ✓ | Nemanich et al (2005) |
| **MWCNTs & SWCNTs** | | unpurified | ✓ | | | | ✓ | ✓ | Soto et al (2005) [i] |
| **QDs** | CdTe | | ✓ | ✓ | | ? | ✓ | ? | Lovric et al (2005) [j] |
| | CdTe | | ? | | | | | ? | Kirchner et al (2005a,b) [k] |
| | CdSe/ZnS | | ✓ | | | | | ✓ | Shiohara et al (2004) [l] |
| | CdSe/ZnS | | | ✓ | | | | | Green & Howman (2005) |
| **Nano-particulates or UFPs** | $TiO_2$ NPs | | ✓ | | | | | | Geiser et al (2005) [m] |
| | Ag, Al, Mo NPs | | ✓ | | | | ✓ | ✓ | Bracydich-Stolle et al (2005) [n] |
| | DEP | | ✓ | ✓ | | ✓ | ✓ | ✓ | Xia et al (2004) [o] |
| | DEP | | ? | ✓ | ✓ | ✓ | ? | ? | Li et al (2004) [p] |
| | UFP – carbon | | ? | | | ✓ | ? | ? | Brown et al (2004) |
| | conc. UFPs | | ? | ✓ | | | ✓ | | Li et al (2003) [p] |

**Notes:** [a] Cell membrane damage *and/or* atypical transfer. [b] In vivo (largemouth bass). [c] $C_{60}$ more cytotoxic than hydrophilic derivatives; HDF human skin fibroblast, HepG2 liver carcinoma & astrocytes. [d] HS68 human fibroblast, COS-7 monkey kidney. [e] In vivo. Atypical nephropathy (Sprague-Dewley rat); necrosis & evidence of autophagy. [f] C57BL/6 mice (Black 6 background) & RAW 264.7 macrophage (in vitro). Inflammation plus dose-dependent fibrosis, granulomas & LDH release (in vivo). Equivocal apoptosis to SWCNT "nanotrope" (in vitro). [g] Cytotoxicity in human dermal fibroblasts inversely proportional to phenyl-$SO_3$(H/Na) functionalisation. Lacking Pluronic F108 only control. [h] Normal skin fibroblast [i] Cytotoxicity index greater than or comparable to chrysotile asbestos. MTT(mitochondrial) function inhibited. In vitro study (macrophage). [j] Caspase-independent apoptosis (MCF-7, human breast cancer). [k] Oncosis-related adherent cell measurement parameter. NRK fibroblast, MDA breast cancer, CHO & RBL cells. [l] Dose-dependent effects. African green monkey & HeLa human hepatocyte. [m] WKY/NCrl BR rat & porcine lung macrophage. [n] Apoptosis; in vitro (Spermatogonie-derived germ cell line). [o] Apoptosis; in vitro (RAW 264.7 macrophage). [p] Human bronchial epithelial BEAS-2B & murine bronchial epithelial LA-4, murine macrophage RAW 264.7.

within this organelle. Release of these proteins is now acknowledged to alter cellular viability and organ health (Green & Kroemer 2004).

Furthermore, a couple of studies worth discussing have observed the induction of an important stress-responsive transcription factor known as "Nrf2" after CNT or diesel exhaust particulate exposures (Ding et al 2005; Li et al 2004). Nrf2 induction can be seen as a protective response to chemical exposure and this can be to *both* oxidative or non-oxidative damage (Itoh et al 2004; Ho et al 2005b). As a result the induction of Nrf2 can be considered to be indicative of cells or animals under xenobiotic stress. In an excellent recent review Nel and colleagues have addressed the hierarchical responses of an organism to foreign nanoparticle intrusions (Nel et al 2006). Although this is not a new concept (refer Section 4.1) its application to discussions of the effects of NPs on biological systems will remain relevant for some time to come.

# 6. Social, Ethical, Corporate & Governmental Impacts of Nanotechnology and Related Nanotoxicology.

The PMSEIC has identified nanotechnology as a national priority with due cause (PMSEIC 2005; Invest Australia 2004). However, there is a broad international consensus that the lack of current information on the human, occupational and environmental health impacts of nanoparticles is a severe impediment to the advancement of these technologies even to the point of realising economic benefit. This view comes from both within the nanotechnology sector and from outside (Service 2005; Denison 2005; Innovest 2005; Gaidos 2005; Hardman 2006; P. Binks *pers. comm*.). Many experts have indicated that the current levels of funding for nanotoxicology are insufficient to protect the huge investments already outlaid. For example, The US National Science Foundation expects that by 2015 nanotechnology will have a $US 1 trillion impact on the world economy and employ 2 million workers worldwide. Today, global spending on nanotechnology R&D is approximately $US 9 billion a year but in the USA only a *minor* fraction is allocated to studies targeted at understanding the effects of nanoparticles on human health and environment (i.e. of $US1 billion/year in federal government funds only $US39 million is devoted to this; Service 2005). Proportionally the amount spent in other industrial countries, including Australia, may be even less.

This highlights a pressing need and one advocated by many that the field is in need of an influx of biological, toxicological, pathological and other expertise in order for advances to continue. More importantly, however, the strategic fostering of work in this area will be seen by the public, and ultimately the consuming public, as investments in the safety and acceptability of nanoparticle containing products.

Innovest Strategic Value Advisors (Inc) is an internationally recognised investment research and advisory firm based in the USA which provides specialised company performance indices on financial, environmental, social, and strategic governance issues. In a comprehensive report released last year several key determinants in investor confidence in the nanotechnology industry were identified. A significant portion of the more than sixty US companies interviewed by Innovest indicated that *science-based* regulation would be more equitable for companies in the industry. In addition, the lack of adequate funding for toxicology research was mentioned repeatedly as an important issue. Furthermore, the Innovest report “strongly supported” calls made by others in the investment community for increased US government funding of toxicology research. It viewed the National Nanotechnology Initiative’s (NNI USA) lack of priority for toxicology funding as a missed opportunity to minimize uncertainty (Innovest 2005).

Similar calls are also being heard in Australia – from within and from outside of the industry (refer below). Any increases in toxicological research within

Australia will be seen as a contribution to an international effort proposed by David Rejeski (Director, Woodrow Wilson International Center for Scholars). In addition, it is increasingly recognised that the contributions being made to nanotoxicology by materials scientists are in need of being supplemented by more diverse expertise (Hardman 2006). Efforts have already begun in Australia with consortiums such as "Nanotox Australia" and "TetraQ" which are positioning themselves to provide such skills.

During preparation of this report the authors', in consultation with Dr. Peter Binks (CEO, Nanotechnology Victoria; Nov. 2005), have identified several key issues regarding the Australian nanotechnology industry. The nascent nanotechnology industry understands the need to create the right environment for its growth and ultimate economic success. Companies and investors are aware of the need for "due diligence" in technology development, product introduction and marketing, and corporate affairs. It is the opinion of the industry that in order to anticipate and manage potential consumer reactions to the nanotechnology sector, these companies seek involvement and guidance from the federal regulatory and policy mechanisms. The feeling from within the industry is that issues posed by nanotechnology developments need to be assessed by parties with the requisite skills and incentives. This may mean broadening or enhancing the activities and powers of established vehicles such as NICNAS, the TGA, or the Federal and State OHS authorities. While not a preferred outcome, it may be appropriate to establish a new independent body in the future to overview the safe production and use of engineered nanoparticles, drawing upon the skills and frameworks of established governmental mechanisms.

Further discussions with the Australian private sector have identified that any strategic investments by the federal government in the nanotechnology-directed toxicology and OHS areas would be viewed as critical to the safe development of the industry and should be implemented as soon as possible. It is the consideration of this report that such investments need not be substantive in amount BUT must be carefully directed. It is the view of the industry itself that any investments must be seen to be independent of the commercial interests of the industry and cannot be perceived as a mechanism for manipulation of public opinion. Federally-supported and directed toxicology work will sustain the emerging companies in this field who – at present - lack the expertise and resources to fund this type of research themselves. Importantly, the provision of both research and regulation will encourage partnerships and investment by the major international firms seeking to grow nanotechnology product portfolios (examples, BASF, DuPont, GSK, Intel), and for whom Australia is in all other respects an attractive investment environment.

Finally, this report believes that federal government support for OHS and toxicology research is an investment in the knowledge economy and the work practices of the emerging nanotechnology industry. By designing and launching such efforts at an early stage in industry development, the Australian government ensures that an Australian skill base and expertise is developed when needed most (i.e. when the industry is still being

established). These are topical issues currently being grappled with by all countries supporting the growth of the nanotechnology industry within their own boundaries.

The advent of every new technology has brought with it a cost as well as benefit(s). History tells us that it would be naïve to consider otherwise. Many of the major nanotechnology reports mentioned in Section 3 cite historical examples of public backlash if risks were not adequately identified and addressed. A recent example is with the lack in perceived transparency by companies wishing to introduce genetically modified foods and organisms onto the market (Editorial (Anon) 2005). The public clearly identifies with safety testing and the release of pertinent information as an effort to build public trust.

# 7. Final Conclusions

## 7.1 Human health effects of nanoparticles.

The major conclusions drawn from this review with regard to the potential human health effects of nanoparticles - especially as they relate to occupational exposure issues - are as follows:

- There exists sufficient uncertainty in the human health effects of all nanoparticles, following either short or long term exposure, for an immediate adoption of precautionary measures in workplace settings.
- Bioaccumulation and biopersistence are common characteristics of nanoparticles and there exist little information in the literature. As these attributes have implications for long term worker health further information in this area would be of immediate benefit.
- Chronic exposure studies in appropriate animal test systems are urgently needed as a first step in evaluating long-term human health risk.

## 7.2 OHS considerations.

The major conclusions drawn from this review with regard to the potential occupational health and safety issues of nanoparticle exposures are as follows:

- Our abilities to monitor and measure nanoparticles in the workplace are limited by the current state of technology.
- Further technological advances are required in order to develop robust and reproducible methods to measure airborne nanoparticle concentrations in workplace settings.
- These measurement advances are necessary for applications “across-the board” - such as meaningful WES assessments and evaluation of toxicological risks.
- Given the current uncertainty with regard to worker health effects it is recommended that a precautionary approach be adopted especially in the handling, transfer and transport of all nanoparticles until further information becomes available.

## 7.3 Major recommendations of this report.

- Maintain a constant watching brief on this rapidly developing area.
- Given the current state of knowledge it is scientifically not justifiable to base an MSDS for any nanoparticle on the corresponding bulk matter counterpart. A movement away from this practice should be facilitated. The most facile solution may be to simply designate the origin of the material as a nanoparticle and to use more stringent handling procedures than required for the corresponding bulk material equivalent.

- Fund research in the development of robust and reproducible nanoparticle measurement systems suitable for occupational settings.
- Actively pursue interagency avenues to facilitate advancements in OHS and toxicological data necessary to establish nanoparticle workplace exposure standards and safe work practices.
- The prevailing regulatory frameworks of government agencies should be examined to determine appropriateness to nanoparticles.

# 8. References (in alphabetical order).

ACGIH (2001). Industrial Ventilation, a manual of recommended practice.

Åkerman ME, Chan WCW, Laakkonen P, Bhatia SN & Ruoslahti ER (2002). Nanocrystal targeting in vivo. Proc Natl Acad Sci (USA). 99(20): 12617–12621.

Ali SS, Hardt JI, Quick KL, Kim-Han JS, Erlanger BF, Huang TT, Epstein CJ & Dugan LL (2004). A biologically effective fullerene ($C_{60}$) derivative with superoxide dismutase mimetic properties. Free Radical Biology & Medicine 37(8): 1191–1202.

Austin CD, Wen X, Gazzard L, Nelson C, Scheller RH & Scales SJ (2005). Oxidizing potential of endosomes and lysosomes limits intracellular cleavage of disulfide-based antibody-drug conjugates. Proc Natl Acad Sci (USA). 102: 17987-1792.

Australian Government (2005). Australian Nanotechnology: Capability & commercial potential (2nd Ed.) www.investaustralia.gov.au/media/IR_Nano_NanotechReport.pdf Accessed Dec 02, 2005.

Baan RA & Grosse Y (2004). Man-made (vitreous) fibres: evaluations of cancer hazards by the IARC Monographs Programme. Mutation Research 553: 43-58.

Beckett WS, Chalupa DF, Pauly-Brown A, Speers DM, Stewart JC, Frampton MW, Utell MJ, Huang LS, Cox C, Zareba W & Oberdörster G (2005). Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults: A human inhalation study. Am J Respir Crit Care Med 171: 1129–1135.

Beckman R, Johnston-Halperin E, Luo Y, Green JE & Heath (2005). Bridging dimensions: Demultiplexing ultrahigh-density nanowire circuits. Science 310: 465-468.

Berman DW, Crump KS, Chatfield EJ, Davis JM & Jones AD (1995). The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation. Risk Anal. 15(2): 181-195.

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB & Everitt JI (2004). Pulmonary responses of mice, rats and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicological Sciences 77: 347-357.

Boelsterli UA (2005). Mechanistic toxicology. Taylor & Francis. New York (NY).

Bradydich-Stolle L, Hussain S, Schlager JJ & Hofmann M-C (2005). In vitro cytotoxicity of nanoparticles in mammalian stem cells. Toxicol Sci. 88(2): 412-419.

Brant J, Lecoanet H & Wiesner MR (2005). Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. J. Nanoparticle Res. 7: 545-553.

Broide DH, Lawrence T, Doherty T, Cho JY, Miller M, McElwain K, McElwain S & Karin M (2005). Allergen-induced peribronchial fibrosis and mucus production mediated by I□B kinase □-dependent genes in airway epithelium. Proc Natl Acad Sci (USA) 102(6): 17723-17728.

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R, Mittleman M, Samet J, Smith SC & Tager I (2004). Air pollution and cardiovascular disease: A statement for healthcare professionals from the expert panel on Population and Prevention Science of the American Heart Association. Circulation 109: 2655-2671.

Brown DM, Donaldson K, Borm PJ, Schins RP, Dehnhardt M, Gilmour P, Jimenez LA & Stone V (2004). Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles. Am J Physiol Lung Cell Mol Physiol 286: L344-L353.

Bruschi SA (2005) Methods and approaches to study metabolism and toxicity of acetaminophen. In: *Drug Metabolism and Transport: Molecular methods and mechanisms*; L. H. Lash, Edirtor). Humana Press (Totowa, NJ).

Burton J (1997). General methods for the control of airborne hazards. In: Dinardi SR (Ed.) The occupational environment – its evaluation and control. Fairfax (VA). American Industrial Hygiene Association.

Cantor KP (1997). Drinking water and cancer. Cancer Causes Control 8(3): 292-308.

Casarett LJ & Doull J (1996). Toxicology: The basic science of poisons. Fifth Edition. McGraw-Hill Co., Inc. New York (NY).

Campbell, KHS, McWhir, J, Ritchie, WA & Wilmut, A (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature 380: 64-66.

Chen HH, Yu C, Ueng TH, Chen S, Chen BJ, Huang KJ & Chiang LY (1998). Acute and subacute toxicity study of water-soluble polyalkylsulfonated $C_{60}$ in rats. Toxicol Pathol. 26(1): 143-151.

Chen HH, Yu C, Ueng TH, Liang CT, Chen BJ, Hong CC & Chiang LY (1997). Renal effects of water-soluble polyalkylsulfonated $C_{60}$ in rats with an acute toxicity study. Toxicol Pathol. 26(1): 143-151.

Chen JY, Kutana A, Collier CP & Giapis KP (2005a). Electrowetting in carbon nanotubes. Science 310: 1480-1483.

Chen J, Perebeinos V, Freitag M, Tsang J, Fu Q, Liu J & Avouris P (2005b). Bright infrared emission from electrically induced excitons in carbon nanotubes. Science 310: 1171-1174.

Cheng X, Kan AT & Tomson MB (2004). Naphthalene adsorption and desorption from aqueous $C_{60}$ fullerene. J. Chem. Eng. Data 49: 675-683

Cheng XK, Kan AT & Tomson MB (2005). Uptake and sequestration of naphthalene and 1,2-dichlorobenzene by C-60. J. Nanoparticle Res. 7: 555-567.

Churg A & Wiggs B (1984). Fiber size and number in amphibole asbestos-induced mesothelioma. Am J Pathol. 115: 437-442.

Colvin VL (2003). The potential environmental impact of engineered nanomaterials. Nature Biotechnology 21(10): 1166-1170.

Corona-Morales AA, Castell A, Escobar A, Drucker-Colin R & Zhang L (2003). Fullerene C60 and ascorbic acid protect cultured chromaffin cells against levodopa toxicity. J Neurosci Res. 71(1): 121-126.

Cui D, Tian F, Ozkan CS, Wang M & Gao H (2005). Effect of single-wall carbon nanotubes on human HEK293 cells. Toxicol Lett. 155(1): 73-85.

Dalle-Donne I, Scaloni A, Giustarini D, Cavarra E, Tell G, Lungarella G, Colombo R, Rossi R & Milzani A (2005). Proteins as biomarkers of oxidative/nitrosative stress in diseases: the contribution of redox proteomics. Mass Spectrom Rev. 24(1): 55-99.

Delfino RJ, Sioutas C & Malik S (2005). Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. Environ. Health. Perspect. 113: 934-946.

Denison RA (2005). Statement before the U.S. House of Representatives concerning Environmental and Safety impacts of Nanotechnology. www.house.gov/science/hearings/full05/nov%2017/Denison.pdf. Accessed Jan 19, 2006.

Diederich F & Thilgen C (1996). Covalent fullerene chemistry. Science 271: 371-323.

Ding L, Stilwell J, Zhang T, Elboudwarej O, Jiang H, Selegue JP, Cooke PA, Gray JW & Chen FF (2005). Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. Nano Letters 5(12), 2448-2464.

Donaldson K, Stone V, Tran CL, Kreyling W & Borm PJA (2004). Nanotoxicology. Occup Environ Med 61: 727-728.

Donaldson K, Tran L, Jimenez L, Duffin R, Newby DE, Mills N, MacNee W & Stone V (2005). Combustion-derived nanoparticles: A review of their toxicology following inhalational exposure. Particle & Fibre Toxicology 2:10 doi:10.1186/1743-8977-2-10 (BioMed Central).

Dopp E, Hartmann LM, von Recklinghausen U, Florea AM, Rabieh S, Zimmermann U, Shokouhi B, Yadev S, Hirner AV & Rettenmeier (2005). Forced uptake of trivalent and pentavalent methylated and inorganic arsenic and its cyto-/genotoxicity in fibroblasts and hepatoma cells. Toxicol Sci. 87, 46-56.

Driscoll KE, Carter JM & Borm PJA (2002). Antioxidant defense mechanisms and the toxicity of fibrous and nonfibrous particles. Inhal Toxicol. 14: 101-118.

Duchen MR (2004). Roles of mitochondria in health and disease. Diabetes 53: S96-S102.

Dugan LL, Gabrielsen JK, Yu SP, Lin TS & Choi DW (1996). Buckminsterfullerenol free radical scavengers reduce excitotoxic and apoptotic death of cultured cortical neurons. Neurobiol Dis. 3(2): 129-135.

Dugan LL, Lovett EG, Quick KL, Lotharius J, Lin TT, O'Malley KL (2001). Fullerene-based antioxidants and neurodegenerative disorders. Parkinsonism Relat Disord. 7(3): 243-246.

Dugan LL, Turetsky DM, Du C, Lobner D, Wheeler M, Almli CR, Shen CKF, Luh TY, Choi DW & Lin TS (1997). Carboxyfullerenes as neuroprotective agents. Proc. Natl. Acad. Sci. USA 94: 9434–9439.

Editorial (Anon) (2005). Value-free nanotech? Nature 437: 451-452.

Englert N (2004). Fine particles and human health – a review of epidemiological studies. Toxicology Letters 149: 235-242.

Foley S, Crowley C, Smaihi M, Bonfils C, Erlanger BF, Seta P & Larroqueb C (2002). Cellular localisation of a water-soluble fullerene derivative. Biochem. and Biophys. Res. Commun. 294: 116–119.

Fowler BA, Conner EA & Yamauchi H (2005). Metabolomic and proteomic biomarkers for III-V semiconductors: chemical-specific porphyrinurias and proteinurias. Toxicol Appl Pharmacol. 206(2): 121-30.

Frampton MW, Utell MJ, Zareba W, Oberdörster G, Cox C, Huang LS, Morrow PE, Lee FE, Chalupa D, Frasier LM, Speers DM & Stewart J (2004). Effects of exposure to ultrafine carbon particles in healthy subjects and subjects with asthma. Res Rep Health Eff Inst. 126(Dec): 1-47.

Fraumeni JF, Hoover RN, Devesa SS & Kinlen LJ (1989). Epidemiology of cancer. *In*: Cancer; Principles and Practice of Oncology. (Devita VT, Hellman S & Rosenberg SA, Eds.) Lippincott, Philadelphia, PA. pp. 196-209.

Gaidos S (2005). Time to regulate nanoparticle safety? An EPA-hosted meeting in June lays the groundwork for a new toxicity-reporting scheme. The Scientist 19(15): 29(1).

Gan L, Huang S, Zhang, X, Zhang A, Cheng H, Li X & Shang G (2002). Fullerenes as a tert–butylperoxy radical trap, metal catalyzed reaction of tert–butyl hydroperoxide with fullerenes, and formation of the first fullerene mixed peroxides $C_{60}(O)(OO^tBu)_4$ and $C_{70}(OO^tBu)_{10}$. J Am Chem Soc. 124: 13384–13385.

Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J & Gehr P (2005). Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Env Health Persp. 113(11): 1555-1560.

Giepmans BNG, Deerinck TJ, Smarr BL, Jones YZ & Ellisman MH (2005). Correlated light and electron microscopic imaging of multiple endogenous proteins using quantum dots. Nature Methods 2(10): 743-749.

Gottschling BC, Maranpot RR, Hailey JR, Peddada S, Moomaw CR, Klaunig JE & Nyska A (2001). The role of oxidative stress in indium phosphide-induced lung carcinogenesis in rats. Toxicol Sci. 64, 28–40.

Green DR & Kroemer G (2004). The pathophysiology of mitochondrial cell death. Science 305: 626-629.

Green M & Howman E (2005) Semiconductor quantum dots and free radical induced DNA nicking. Chem Commun (Camb). Jan 7;(1):121-123.

Greenpeace (2003). Future technologies, Today's choices. Nanotechnology, artificial intelligence and robotics: A technical, political and institutional map of emerging technologies. Arnall AH (Imperial College London). A report for the Greenpeace Environmental Trust. www.greenpeace.org.uk/MultimediaFiles/Live/FullReport/5886.pdf. Accessed Jan 05, 2006.

Grigorenko AN, Geim AK, Gleeson HF, Zhang Y, Firsov AA, Khrushchev IY & Petrovic J (2005). Nanofabricated media with negative permeability at visible frequencies. Nature 438: 335-338.

Guan J & Lee LJ (2005). Generating highly ordered DNA nanostrand arrays. Proc Natl Acad Sci (USA) 102: 18321-18325.

Gur I, Fromer NA, Geier ML & Alivisatos AP (2005). Air-stable all-inororganic nanocrystal sole cells processed from solution. Science 310: 462-464.

Hardman R (2006). A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect. 114(2): 165-172.

Harris MW, Chapin RE, Haskins EA, Allen JD, Wilson RE, Ward SM, Davis BJ, Collins BJ, Lockhart AC & Moskowitz PD (1994). The general and reproductive toxicity of the photovoltaic material cadmium telluride (CdTe). Toxicologist 14(1):267 (abstr.).

Harrison WN, Bradberry SM & Vale JA (2006). Tellurium: UKPID Monograph. IPCSINTOX Databank: http://www.intox.org/databank/documents/chemical/tellur/ukpid84.htm. Accessed Jan 09, 2006.

Heinrich RF, Driscoll KE, Harkema JR, Lindenschmidt RC, Chang IY, Maples KR & Barr EB (1995). A comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. Fund Appl Toxicol. 24: 183–197.

Hext PM, Tomenson JA & Thompson P (2005). Titanium dioxide: inhalation toxicology and epidemiology. Ann Occup. Hyg. 49(6): 461–472.

Hinds WC (1999). Aerosol Technology; properties, behaviour and measurement of airborne particles. 2nd ed, New York; Wiley-Interscience.

Ho HK, Hu ZH, Tzung SP, Hockenbery DM, Fausto N, Nelson SD & Bruschi SA (2005a). BCL-xL overexpression effectively protects against tetrafluoroethylcysteine-induced intramitochondrial damage and cell death. Biochem Pharmacol. 69(1): 147-157.

Ho HK, White CC, Fernandez C, Fausto N, Kavanagh TJ, Nelson SD & Bruschi SA (2005b). Nrf2 activation involves an oxidative-stress independent pathway in tetrafluoroethylcysteine-induced cytotoxicity. Toxicol Sci. 86(2): 354-64.

Hoet PM, Brüske-Hohlfeld I & Salata OV (2004). Nanoparticles – known and unknown health risks. J Nanobiotech. 2:12 doi:10.1186/1477-3155-2-12 (BioMed Central).

Hoffman RS (2003). Thallium toxicity and the role of Prussian blue in therapy. Toxicol Rev. 22(1): 29-40.

Holsapple MP, Farland WH, Landry TD, Monteiro-Riviere NA et al (2005). Research strategies for safety evaluation of nanomaterials, Part II: Toxicological and safety evaluation of nanomaterials; Current challenges and data needs. Toxicological Sciences 88(1): 12-17.

Huang SS, Tsai SK, Chih CL, Chiang LY, Hsieh HM, Teng CM & Tsai MC (2001). Neuroprotective effect of hexasulfobutylated C60 on rats subjected to focal cerebral ischemia. Free Radic. Biol. Med. 30: 643– 649.

IARC (1976). IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: cadmium, nickel, some epoxides, miscellaneous industrial chemicals and general consideration on volatile anaesthetics. IARC Monogr Eval Carcinog Risk Chem Man. 11: 1-293. ISBN 92 832 1211 8

IARC (2002). IARC monographs on the evaluation of the carcinogenic risk to humans: Man-made vitreous fibres. IARC Monogr Eval Carcinog Hum. 81: 1-418. ISBN 92 832 1281 9.

Invest Australia (2004). Nanotechnology Australia: Capability & Commercial Potential: 2nd Ed., http://investaustralia.hyperlink.net.au/media/IR_Nano_NanotechReport.pdf Accessed Dec 09 2005.

Innovest (2005). Nanotechnology: Non-traditional methods for valuation of nanotechnology producers. (H. Langsner, Snr. Analyst). www.innovestgroup.com/pdfs/Nanotechnology_Report.pdf Accessed Dec 18 2005.

Itoh K, Tong KI & Yamamoto M (2004). Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med. 36(10):1208-1213.

IUPAC (2005). http://www.iupac.org/publications/compendium/F.html Accessed Dec 30 2005.

Jakupec MA & Keppler BK (2004). Gallium in cancer treatment. Curr Top Med Chem. 4: 1575-1583.

James JT & Lam C (2005). Pulmonary toxicity of carbon nanotubes in mice and implications for human risk assessment. Toxicologist 84: 133. Abstract# 650.

Jarup L & Alfven T (2004). Low level cadmium exposure, renal and bone effects--the OSCAR study. Biometals 17(5): 505-509.

Jensen AW, Wilson SR & Schuster DI (1996). Biological applications of fullerenes. Bioorg Med Chem. 4(6):767-779.

Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, Zhao Y & Guo X (2005). Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Env Sci Technol. 39(5): 1378-1383.

Johnson EM, Christian MS, Hoberman AM, DeMarco CJ Kilpper R & Mermelstein R (1988) Developmental toxicology investigation of tellurium. Fund Appl Toxicol. 11: 691-702.

Kaplan-Ashiri I, Cohen SR, Gartsman K, Ivanovskaya V, Heine T, Seifert G, Wiesel I, Wagner HD & Tenne R (2006). On the mechanical behavior of $WS_2$ nanotubes under axial tension and compression. Proc Natl Acad Sci (USA) 103(3): 523-528.

Khandoga A, Stampfl A, Takenaka S, Schulz H, Radykewicz, Kreyling W & Krombach F (2004). Ultrafine particles exert prothrombic but not inflammatory effects on the hepatic microcirculation in healthy mice in vivo. Circulation 109: 1320-1325.

Kipen HM, Laskin DL (2005). Smaller is not always better: nanotechnology yields nanotoxicology. An J Physiol Lung Cell Mol Physiol 289: L696-697.

Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, Gaub HE, Stolzle S, Fertig N & Parak WJ (2005a). Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Letters 5(2): 331-338.

Kirchner C, Muñoz Javier A, Susha AS, Rogach AL, Kreft O, Sukhorukov GB & Parak WJ (2005b). Cytotoxicity of nanoparticle-loaded polymer capsules. Talanta 67: 486-491.

Kisin E, Murray AR, Johnson V, Gorelik O, Arepalli S, Gandelsman VZ, Hobbs AE, Mercer AF, Mercer RR, Baron P, Kagan VE, Castranova V & Shvedova AA (2005). Pulmonary toxicity of carbon nanotubes. Toxicologist 84: 212. Abstract# 1041.

Knaapen AM, Borm PJA, Albrecht C & Schins PF (2004). Inhaled particles and lung cancer. Part A: Mechanisms. Int J Cancer 109: 799-809.

Kroto HW, Heath JR, O'Brien SC, Curl RF & Smalley RE (1985). $C_{60}$: Buckminsterfullerene. Nature 318: 162-163.

Lam CW, James JT, McCluskey R, Arepalli S & Hunter RL (2006). A review of carbon nanotube toxicity and assessment of potential and environmental health risks. Crit. Rev. Toxicol. 36: 189-217.

Lam CW, 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.

Lee KP, Trochimowicz HJ & Reinhardt CF (1985). Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol Appl Pharmacol. 79: 179–192.

Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, Slaughter N, Killeen E, Wang X, Huang A, Wang M, Miguel AH, Cho A, Sioutas C & Nel AE. (2004). Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. J Immunol. 173(5): 3467-3481.

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J & Nel A (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health. Perspect. 111: 455-460.

Li Z, Salmen T, Huldermen T, Kisin E, Shvedova A, Luster MI & Simeonova PP (2005). Pulmonary exposure to carbon nanotubes induces vascular toxicity. Toxicologist 84: 213. Abstract# 1045.

Limbach LK, Li Y, Grass RN, Brunner TJ, Hintermann MA, Muller M, Gunther D & Stark WJ (2005). Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. Environ Sci Technol. 39(23): 9370-9376.

Lippmann M, Frampton M, Schwartz J, Dockery D, Schlesinger R, Koutrakis P, Froines J, Nel A, Finkelstein J, Godleski J, Kaufman J, Koenig J, Larson T, Luchtel D, Liu SLJ, Oberdörster D, Peters A, Sarnat J, Sioutas C, Suh H, Sullivan J, Utell M, Wichmann E & Zelikoff J (2003). The U.S. Environmental Protection Agency particulate matter health effects research centers program: A Midcourse report of status, progress, and plans. Environ. Health. Perspect. 111: 1074-1092.

Lovrić J, Cho SJ, Winnik FM & Maysinger D (2005). Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. Chem & Biol. 12: 1227-1234.

Lu P-J, Ho IC & Lee TC (1998). Induction of sister chromatid exchanges and micronuclei by titanium dioxide in Chinese hamster ovary-K1 cells. Mutat Res. 414(1–3): 15–20.

Luther W (Ed.) (2004). Industrial application of nanomaterials: Future Technologies No. 54. – chances and risks: Technology analysis. VDI Technologiezentrum GmbH, Dűsseldorf, Germany.

Mar TF, Koenig JQ, Jansen K, Sullivan J, Kaufman J, Trenga CA, Siahpush SH, Liu LJ & Neas L (2005). Fine particulate air pollution and cardiorespiratory effects in the elderly. Epidemiology. 16(5): 681-687.

Maser WK, Benito AM & Martínez MT (2002). Production of carbon nanotubes: the light approach. Carbon 40: 1685–1695.

Maynard AD (2004). Responsible Nanotech at Work. Nanotoday, 56.

Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER & Castranova V (2004). Exposure to carbon nanotube material: Aerosol release during the handling of unrefined single walled carbon nanotube material. J Toxicol Environ Health 2004, 67: 87-107.

Maynard AD & Kuempel ED (2005). Airborne Nanostructured Particles and Occupational Health. Nanoparticle Research 7: 587-614.

Meiring JJ, Borm PJA, Bagate K, Semmler M, Seitz J, Takenaka S & Kreyling WG (2005). The influence of hydrogen peroxide and histamine on lung permeability and translocation of iridium nanoparticles in the isolated perfused rat lung. Particle and Fibre Toxicology 2:3 doi:10.1186/1743-8977-2-3.

Mellman I, Fuchs R & Helenius A (1986). Acidification of the endocytic and exocytic pathways. Annu Rev Biochem. 55: 663-700.

Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS & Weiss S (2005). Quantum dots for live cells, in vivo imaging and diagnostics. Science 307: 538-544.

Michelson E (2004). Analysing The European Approach To Nanotechnology, Occasional Paper on Nanotechnology Woodrow Wilson International Centre for Scholars.

Mitsubishi Monitor (2005). http://www.mitsubishi.or.jp/e/monitor/0306/NP.html#d Accessed Dec 30, 2005.

Moghimi SM, Hunter AC & Murray JC (2005). Nanomedicine: current status and future prospects. FASEB J. 19: 311-330.

Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE (2005). Mulit-walled carbon nanotube interactions in human epidermal keratinocytes. Toxicology Letters 155(3): 377-384.

Mulkey JP & Oehme FW (1993). A Review of Thallium Toxicity. Vet Human Toxicol. 35(5): 445-453.

Murray RZ, Kay JG, Sangermani DG & Stow JL (2005). A role for the phagosome in cytokine secretion. Science 310: 1492-1495.

Nano-C (2005a) http://www.nano-c.com/ Accessed Dec 28, 2005.

Nano-C (2005b.) http://www.nano-c.com/newsrelease10.asp Accessed Dec 29, 2005.

Nanotechnology Now (2005). http://www.nanotech-now.com/current-uses.htm Accessed Dec 28, 2005.

Nel A, Xia T, Mädler L & Li N (2006). Toxic potentials of materials at the nanolevel. Science 311, 622-627.

Nemanich RJ, Inman AO, Wang YY & Riviere JE (2005). Multi-walled carbon nanotubes interactions with human epidermal keratinocytes. Toxicol Letts. 155: 377-384.

Nemmar A, Hamoir J, Nemery B, Gustin P (2005). Evaluation of particle translocation across the alveolo-capillary barrier in isolated perfused rabbit lung model. Toxicology 208(1): 105-113.

Nemmar A, Hoet PHM, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L & Nemery B (2002). Passage of inhaled particles into the blood circulation in humans. Circulation 105: 411-414.

Nicotera P (2005). Mixed reactions. Nature 437, 1093.

Nova: Science in the news (2005). http://www.science.org.au/nova/024/024box02.htm Accessed Dec. 27, 2005.

Oberdörster E (2004). Manufactured nanomaterials (Fullerene, $C_{60}$) induce oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect. 112: 1058-1062.

Oberdörster G, Ferin J, Gelein R, Soderholm SC & Finkelstein J (1992). Role of the alveolar macrophage in lung injury – studies with ultrafine particles. Environ Health Perspect. 97: 193-199.

Oberdörster G, Ferin J, Lehnert BE (1994). Correlation between particle- size in- vivo particle persistence and lung injury. Environ Health Perspect 102(S5);173-179.

Oberdörster G, Oberdörster E & Oberdörster J (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspect. 13(7): 823-839.

Oberdörster G, Oberdörster E & Oberdörster J (2005). Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. Environmental Health Perspect. 13(7): 823-839. Supplemental Web Sections pp. 1-31.

Oberdörster 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(6-7): 437-445.

Oberdörster 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 65: 1531–1543.

Perez-D'Gregorio RE, Miller RK & Baggs RB (1988). Maternal toxicity and teratogenicity of tellurium dioxide in the Wistar rat. Reproductive Toxicol. 2(1): 55-61.

PMSEIC (2005). Nanotechnology: Enabling technologies for Australian innovative industries. http://www.dest.gov.au/sectors/science_innovation/publications_resources/profiles/nanotechnology_enabling_innovative_industries.htm Accessed Dec 28 2005.

Pöschl U (2005). Atmospheric Aerosols: Composition, transformation, climate and health effects. Angew. Chem. Int. Ed. 44, 7520–7540.

Pritchard DK (2004). Literature Review – Explosion Hazards Associated With Nanopowders, Health & Safety Laboratory, HSL/2004/12.

Raab A & Springholz G (2000).. Ostwald ripening and shape transitions of self-assembled PbSe quantum dots during annealing. Applied Physics Letters 77, 2991-2995.

Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, Malinski T & Radomski MW (2005). Nanoparticle-induced platelet aggregation and vascular thrombosis. Br J Pharmacol. 146(6):882-893.

Ratherman S (1996). Methods of control. In: Plog B (Ed.) Fundamentals of industrial hygiene. Itasca IL: National Safety Council.

Roberts DW, Gopee NV, Miller BJ, Norton D, Warbritton AR, Bucher JR, Yu WW Sayes CM, Colvin VL & Howard PC (2005). Localization of intradermally injected quantum dot nanoparticles in regional lymph nodes. Toxicologist 84: 448. Abstract# 2185.

Robichaud CO, Tanzil D, Weilenmann U & Wiesner MR (2005). Relative risk analysis of several manufactured nanomaterials: an insurance industry context. Environ Sci Technol. 39(22):8985-94.

Robinson BW & Lake RA (2005). Advances in malignant mesothelioma. N Engl J Med. 353(15): 1591-603.

Royal Society & The Royal Academy of Engineering (2004). Possible adverse health,, environmental and Safety impacts. Nanoscience and nanotechnologies pp. 35-48.

The Royal Society – Science Council of Japan (2005), Report of a joint workshop on the potential health, environmental and societal impacts of nanotechnologies.

Salata OV (2004). Application of nanoparticles in biology and medicine. J. Nanobiotechnology 2(1): 3.

Salvi S, Blomberg A, Rudell B, Kelly F, Sandström T, Holgate ST, Frew A (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159: 702–709.

Salvi S, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, Wilson S, Sandström T, Holgate ST, Frew AJ (2000). Acute exposure to diesel exhaust increases IL-8 and GRO-a production in healthy human airways. Am J Respir Crit Care Med 161: 550–557.

Savage N, Diallo MS (2005). Nanomaterials and water purification: Opportunities and challenges. J. Nanoparticle Res. 7: 331-342.

Sayes CM, Fortner JD, Go W, Lyon D et al (2004). The differential cytotoxicity of water-soluble fullerenes. Nano Letters 4(10): 1881-1887.

Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL & Colvin VL (2005). Nano-$C_{60}$ cytotoxicity is due to lipid peroxidation. Biomaterials 26(16): 7587-7595.

Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, Beach JM, Moore VC, Doyle CD, West JL, Billups WE, Ausman KD & Colvin VL (2006). Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicol Lett. 161(2): 135-42.

Schneider T, Cherrie IW, Vermeulen R & Krombout H ( 2000). Dermal Exposure Assessment. Ann Occup Hyg 44(7): 493-499.

Service RF (2005). Calls rise for more research on toxicology of nanomaterials. Science 310: 1609.

Shimada A, Kawamura N, Kaewamatawong T, Okajima M, Sawada M & Morita T (2005). Electron microscopic study on the translocation of ultrafine carbon black particles at the airway-capillary barrier in lung. Toxicologist 84: 212. Abstract# 1040.

Shiohara A, Hoshino A, Hanaki K, Suzuki K & Yamamoto K (2004). On the cyto-toxicity caused by quantum dots. Microbiol Immunol. 48(9): 669-675.

Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, Hubbs AF, Antonini J, Evans DE, Ku BK, Ramsey D, Maynard A, Kagan VE, Castranova V & Baron P. (2005). Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. An J Physiol Lung Cell Mol Physiol. 289: L698-L708.

Shvedova AA, Kisin ER, Murray AR, Gandelsman VZ, Maynard A & Baron P (2003). Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. Journal of Toxicology and Environmental Health, Part A 66: 1909-1926.

Silva VM, Corson N, Elder A, Gelein R & Oberdörster G (2005). Exposure to ultrafine elemental carbon particles (UCP) significantly increase thrombogenesis. Toxicologist 84: 212. Abstract# 1038.

Sinha N & Yeow JTW (2005). Carbon Nanotubes for Biomedical Applications. IEEE Trans. Nanobiosci. 4(2): 180-195.

Smith AE & Helenius A (2004). How viruses enter animal cells. Science. 304: 237-242.

Soto KF, Carrasco A, Powell TG, Garza KM & Murr LE (2005). Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. Journal of Nanoparticle Research 7: 145-169.

Stanton MF & Wrench C (1972). Mechanisms of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst. 48: 797-821.

Stanton MF, Laynard M, Tegeris A, Miller E, May M & Kent E (1977). Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. J Natl Cancer Inst. 58: 587-603.

Suzuki Y, Yuen SR & Ashley R (2005). Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. Int J Hyg Environ Health. 208(3):201-210.

Tanaka A (2004). Toxicity of indium arsenide, gallium arsenide, and aluminium gallium arsenide. Toxicol Appl Pharmacol. 198: 405-411.

Tinkle S (2004). Nanomaterials: A risk to health at work? First International Symposium on Occupational Health Implications of Nanomaterials. 12-14 October. (D. Mark, Chair) Palace Hotel, Buxton, Derbyshire, UK. pp: 47-52.

Tsuchiya T, Oguri I, Yamakoshi YN & Miyata N (1996). Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. FEBS Letts. 393: 139-145.

Ueng TH, Kang JJ, Wang HW, Cheng YW & Chiang LY (1997). Suppression of microsomal cytochrome P450-dependent monooxygenases and mitochondrial phosphorylation by fullerol, a polyhydroxylated fullerene C60. Toxicol Letts. 93, 29-37.

UK Government Response (2005). http://www.ost.gov.uk/policy/issues/index.htm#Nanotechnology Accessed Dec 05, 2005.

UK HSE (2002). Skin problems in the printing industry. IACL101(rev1) Reprinted 6/04.

UK HSE (2004). Nanoparticles: An Occupational Hygiene Review, Research Report 274. Prepared by the Institute of Occupational Medicine for the Health and Safety Executive (HSE).

UK HSE (2004). Health and Safety Executive information note: Nanotechnology. Horizons Scanning Information Note No HSIN1: 1-4.

US CDC (2001). http://www.atsdr.cdc.gov/asbestos/doc_tremolite.html#Mechanisms Accessed Dec 30 2005.

US CDC (2005). Third National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services, Centers for Disease Control and Prevention. http://www.cdc.gov/exposurereport/ Accessed Dec 12, 2005.

US EPA (2005a). http://www.epa.gov/IRIS/subst/0458.htm Accessed Dec 15, 2005.

US EPA (2005b). External Review Draft, Nanotechnology White Paper. http://www.epa.gov/osa/nanotech.htm Accessed Feb 05 2006.

US NIOSH (2005). NIOSH Current Intelligence Bulletin: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide. http://www.cdc.gov/niosh/docs/preprint/tio2 Accessed Dec 07, 2005.

US NIOSH (2005b). Approaches To Safe Nanotechnology, An Information Exchange with NIOSH. http://www.cdc.gov/niosh/topics/nanotech/nano_exchange.html Accessed Feb 05 2006.

US NIOSH (2006). Strategic plan for research on several occupational safety and health aspects of nanotechnology. http://www.cdc.gov/niosh/topics/nanotech/strat_plan.html. Accessed July 07 2006.

US NTP (2000a). Toxicology and carcinogenicity studies of gallium arsenide (CAS No. 1303-00-0) in F344/N rats and B6C3F1 mice (inhalation studies), Department of Health and Human Services, Public Health Services, Bethesda, MD.

US NTP (2000b). Toxicology and carcinogenesis studies of indium phosphide (CAS No. 22398-80-7) in F344 rats and B6C3 F1 mice (inhalation studies). Department of Health and Human Services, Public Health Services, Bethesda.

US NTP (2005). 11th Report on Carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp.niehs.nih.gov/ntp/roc/toc11.html Accessed Dec 116, 2005.

US RoC (2005). 11th Report on Carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp.niehs.nih.gov/ntp/roc/toc11.html Accessed Jan 10-13, 2006

Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR (2004). Comparative pulmonary toxicity assessment of single wall carbon nanotubes in rats. Toxicol Sci 77: 117-125.

Xia T, Korge P, Weiss JN, Li N, Venkatesen G, Sioutas C & Nel A (2004). Quinines and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: Implications for ultrafine particle toxicity. Env. Health Perspectives 112(14): 1347-1358.

Yamago S, Tokuyama H, Nakamura E, Kikuchi K, Kananishi S, Sueki K, Nakahara H, Enomoto S & Ambe F (1995). In vivo biological behavior of a water-miscible fullerene: $^{14}C$ labeling, absorption, distribution, excretion and acute toxicity. Chem. Biol. 2, 385-389.

Yarema MC & Curry SC (2005). Acute tellurium toxicity from ingestion of metal-oxidizing solutions. Pediatrics 116(2):e319-e321.

Zhao X, Striolo A, Cummings PT (2005). $C_{60}$ binds to and deforms nucleotides. Biophys J. 89(6): 3856-3862.

Zitko V (1975). Toxicity and pollution potential of thallium. Science Total Env. 4: 185-192.