

Potential Risks of Nanomaterials and How to Safely Handle Materials of Uncertain Toxicity

"It is a mistake for someone to say nanoparticles are safe, and it is a mistake to say nanoparticles are dangerous. They are probably going to be somewhere in the middle. And it will depend very much on the specifics."

V. Colvin, Director of Center for Biological and Environmental Nanotechnology at Rice University, quoted in Technology Review

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Summary

In the last year and a half, there have been a number of research articles on the toxicity of different types of nanomaterials. These studies have suggested effects at the cellular level and in short-term animal tests. The effects seen depend on the base material of the nanoparticle, its size and structure, and its substituents and coatings. Additional toxicology testing is being funded or planned by the National Science Foundation (NSF), the National Toxicology Program, and other research organizations in the US and in Europe. Nanomaterials of uncertain toxicity can be handled using the same precautions currently used at MIT to handle toxic materials: use of exhaust ventilation (such as fume hoods and vented enclosures) to prevent inhalation exposure during procedures that may release aerosols or fibers and use of gloves to prevent dermal exposure. The EHS Office will continue to review health and safety information about nanomaterials as it becomes available and distribute it to the MIT community.

What are nanomaterials?

The ASTM Committee on Nanotechnology has defined a nanoparticle as a particle with lengths in 2 or 3 dimensions between 1 to 100 nm that may or may not have a size related intensive properties. Nanomaterials are generally in the 1-100 nm range and can be composed of many different base materials (carbon, silicon, and metals such as gold, cadmium, and selenium). Nanomaterials also have different shapes: referred to by terms such as nanotubes, nanowires, crystalline structures such as quantum dots, and fullerenes. Nanomaterials often exhibit very different properties from their respective bulk materials: greater strength, conductivity, and fluorescence, among other properties. About 40-50% of the atoms in nanoparticles are on the surface, resulting in greater reactivity than bulk materials.

Particles in the nanometer size range do occur both in nature and as a result of existing industrial processes. Nanosized particles are part of the range of atmospheric particles generated by natural events such as volcanic eruptions and forest fires. They also form part of the fumes generated during welding, metal smelting, automobile exhaust, and other industrial processes. One concern about small particles that are less than 10 μm is that they are respirable and reach the lungs.

The current nanotechnology revolution differs from past industrial processes because nanomaterials are being engineered and fabricated from the "bottom up", rather than occurring as a byproduct of other activities. The nanomaterials being engineered have different and unexpected properties compared to those of the parent compounds. Since their properties are different when they are small, it is expected that they will have different effects on the body and will need to be evaluated separately from the parent compounds for toxicity.

Currently nanomaterials have a limited commercial market. Some nanomaterials are used as catalyst supports in catalytic converters; nanosized titanium dioxide particles are used as a component of sunscreens; carbon nanotubes have been used to strengthen tennis rackets; components in silicon chips are reaching the 100 nm range. Research and industrial labs are working at the intersection of engineering and biology to extend uses to medicine as well as all areas of engineering. The impact is expected to revolutionize these areas. Government agencies in the US and Europe are beginning to fund toxicology research to understand the hazards of these materials before they become widely available.

What are the toxic effects of nanomaterials tested to date?

This article will give an overview of the testing done to date. A list of review articles and research citations are given at the end for further information.

Any toxic effects of nanomaterials will be very specific to the type of base material, size, ligands, and coatings. One of the earliest observations was that nanomaterials, also called ultrafine particles (<100 nm), showed greater toxicity than fine particulates (<2.5 µm) of the same material on a mass basis. This has been observed with different types of nanomaterials, including titanium dioxide, aluminum trioxide, carbon black, cobalt, and nickel. For example, Oberdorster et al. (1994) found that 21 nm titanium dioxide particles produced 43 fold more inflammation (as measured by the influx of polymorphonuclear leucocytes, a type of white blood cell, into the lung) than 250 nm particles. The increase in inflammation is believed to be due to the much greater surface area of the small particles for the same mass of material. Though multiple studies have shown that nano-sized particles may be more toxic than micro-sized particles, this is not always the case. Intrinsic surface reactivity may also be as important as surface area. Warheit et al. (2007) found that the toxicity for cytotoxic crystalline quartz did not relate to particle size, but did relate to surface reactivity as measured by hemoglobin release from cells in vitro. Warheit et al. (2006) also found that other types of crystalline anatase titanium dioxide did not show size intensive toxicity for nano sized particles.

Nanoparticles (<0.1 µm) are generally similar in size to proteins in the body. They are considerably smaller than many cells in the body. Human alveolar macrophages are 24 µm in diameter and red blood cells are 7-8 µm in diameter. Cells growing in tissue culture will pick up most nanoparticles.

The ability to be taken up by cells is being used to develop nanosized drug delivery systems and does not inherently indicate toxicity. One study by Goodman et al. (2004) found that cellular toxicity depended upon cationic charge of side chains substituted onto nanoparticles with a 2 nm gold core. Gold nanoparticles are being investigated as transfection agents, DNA-binding agents, protein inhibitors and other biomedical applications. Goldman et al. found that positively charged gold particles with quaternary ammonium substituted side chains were toxic to two types of mammalian cells (red blood cells and Cos-1 cells) and E. coli. bacteria, causing 50% of the cell to die at 1-3 µM concentrations. Negatively charged cells with carboxylate substituted side chains did not show cellular toxicity even when tested at much higher concentrations. The researchers attributed the cell lysis to binding by cationic particles to negatively charged cell membranes and subsequent membrane leakage. They are currently designing nanoparticles with different properties to prevent this type of toxicity.

Once in the body, some types of nanoparticles may have the ability to translocate and be distributed to other organs, including the central nervous system. Silver, albumin, and carbon nanoparticles all showed systemic availability after inhalation exposure. Significant amounts of ¹³C labeled carbon particles (22-30 nm in diameter) were found in the livers of rats after 6 hours of inhalation exposure to 80 or 180 µg/m³ (Oberdorster et al. 2002). In contrast, only very small

amounts of ^{192}Ir particles (15 nm) were found systemically. Oberdorster et al. (2004) also found that inhaled ^{13}C labeled carbon particles reached the olfactory bulb and also the cerebrum and cerebellum, suggesting that translocation to the brain occurred through the nasal mucosa along the olfactory nerve to the brain. The ability of nanomaterials to move about the body may depend on their chemical reactivity, surface characteristics, and ability to bind to body proteins.

There is currently no consensus about the ability of nanoparticles to penetrate through the skin. Particles in the micrometer range are generally thought to be unable to penetrate through the skin. The outer skin consists of a 10 μm thick, tough layer of dead keratinized cells (stratum corneum) that is difficult to pass for particles, ionic compounds, and water soluble compounds. Tinkle et al. (2003) found that 0.5 and 1 μm dextran spheres penetrated "flexed" human skin in an in vitro experiment. Particles penetrated into the epidermis and a few entered the dermis only during flexing of the skin. Particles 2 and 4 μm in diameter did not penetrate. Rymen-Rasmussen et al. (2006) also found that quantum dots penetrated through pig skin and into living dermis using an in vitro pig skin bioassay which is considered a good model for human skin.

Micronized titanium dioxide (40 nm) is currently being used in sunscreens and cosmetics as sun protection. The nm particles are transparent and do not give the cosmetics the white, chalky appearance that coarser preparations did. The nm particles have been found to penetrate into the stratum corneum and more deeply into hair follicles and sweat glands than μm particles though they did not reach the epidermis layer and dermis layers (Laddeman et al., 1999). There is also a concern that nm titanium dioxide particles have higher photo-reactivity than coarser particles and may generate free radicals that can cause cell damage. Some manufacturers have addressed this issue by coating the particles to prevent free radical formation. The FDA has reviewed available information and determined that nm titanium dioxide particles are not a new ingredient but a specific grade of the original product (Luther, 2004).

Quantum dots (QD) are nanocrystals containing 1000 to 100,000 atoms and exhibiting unusual "quantum effects" such as prolonged fluorescence. They are being investigated for use in immunostaining as alternatives to fluorescent dyes. The most commonly used material for the core crystal is cadmium-selenium, which exhibits bright fluorescence and high photostability. Both bulk cadmium and selenium are toxic to cells. One of the primary sites of cadmium toxicity in vivo is the liver.

Early studies found that Cd-Se quantum dots were not toxic to immortalized cell lines used for these studies. Recently Shiohara et al. (2004) found that three types mercapto-undecanoic acid (MUA) substituted Cd-Se quantum dots decrease viability in three types of cells in vitro (monkey kidney, HeLa cells, and human hepatocytes) and caused cell death after 4-6 hours of incubation. One type of MUA-QD was less toxic than the other two. Derfus et al. (2004) also found that Cd-Se QDs were toxic to liver hepatocytes if exposed to air or UV light, as a result of oxygen combining with Se and releasing free Cd^{+2} from the crystal lattice. They found that coating the Cd-Se QDs with ZnS, polyethylene glycol, or other coatings prevented toxicity during a two week incubation with hepatocytes. They concluded that Cd-Se QDs can be made nontoxic with appropriate surface coatings but future use in vivo must be carefully evaluated to rule out release of Cd^{+2} over time.

Carbon nanotubes (CNT) can have either single or multiple layers of carbon atoms arranged in a cylinder. The dimensions of single wall carbon nanotubes (SWCNT) are about 1-2 nm in diameter by 0.1 μm in length. Multiple wall carbon nanotubes (MWCNT) are 20 nm in diameter and 1 mm long. CNT behave like fibers in the lung. They have properties very different from bulk carbon or graphite. They have great tensile strength and are potentially the strongest, smallest fibers known. CNT have been tested in short term animal tests of pulmonary toxicity and the results suggest the potential for lung toxicity though there are questions about the nature of the toxicity observed and the doses used.

Lam et al. (2004) instilled three types of SWCNT, as well as silica and carbon black, into rat lungs and found granulomas, a type of cellular accumulation in the lung in which clumps of fibers were surrounded by mononuclear macrophages. Silica, a dust known to be very toxic to the lungs, also produced granulomas but carbon black did not. Warheit et al. (2004), using a different type of SWCNT, also found granulomas but did not see increases in other markers of pulmonary

inflammation whereas quartz produced both macrophage accumulation and increased pulmonary inflammation. Warheit et al. interpreted their SWCNT results as possibly of limited physiological relevance but requiring further inhalation studies.

Shvedova et al. (2005) using more physiologically relevant doses, found granulomas, fibrosis, and increased markers of inflammation from both SWCNT and quartz. SWCNT also affected lung function: breathing rate and the ability to clear bacteria were decreased. More extensive inhalation studies are currently underway in several research centers. One mitigating factor regarding lung toxicity is that CNTs have a tendency to clump together to form nanoropes, which are large, non-respirable clumps, and may prevent inhalation exposure in many instances (see discussion below Maynard et al. [2004] study).

The addition of functional groups such as phenyl-sulfite and phenyl-carboxylic acid onto CNTs can decrease toxicity, as demonstrated using in vitro tests by Sayes et al. (2006). Other in vitro tests have found inhibited cell growth and viability. Good recent reviews of CNT toxicity which cover pulmonary toxicity and also in vitro testing and environmental considerations are provided by Donaldson et al. (2006) and Helland et al. (2007). A recent report by Zheng Li et al. (2007) found that instillation of CNTs produced cardiovascular effects in transgenic arteriosclerosis prone mice; the mice developed accelerated plaque formation after four doses of CNTs over an 8 week period.

Fullerenes are another category of carbon based nanoparticles. The most common type has a molecular structure of C₆₀ which take the shape of a ball shaped cage of carbon particles arranged in pentagons or hexagons. Fullerenes have many potential medical applications as well as applications in industrial coatings and fuel cells, so a number of preliminary toxicology studies have been done with them. In cell culture, different types of fullerenes produced cell death at concentrations of 1 to 15 ppm in different mammalian cells when activated by light (as discussed in Colvin, 2003). Sayes et al. (2004) found that toxicity could be eliminated when carboxyl groups were substituted on the fullerene surface to increase water solubility. Cell death in this study appeared to be a function of damage to the cell membranes. In an in vivo study, Chen et al. (2004) found that water soluble polyalkylsulfonated C₆₀ produced no deaths in rats when given orally but was moderately toxic when administered intraperitoneally (LD₅₀=600 mg/kg). Doses of 100 to 600 mg/kg also produced an unusual form of kidney toxicity. Finally, in the first study investigating aquatic toxicology, Oberdorster (2004) found that 48 hours of exposure to 0.5 and 1.0 ppm of uncoated pure C₆₀ produced cell membrane lipid peroxidation in the brains of fish (juvenile large mouth bass). The changes in the brain as a result of the short exposure did not appear to affect the behavior of the fish but were an indication of oxidative stress. An additional concern generated by this study is the effects of release of durable carbon nanomaterials into the environment.

How to Work Safely with Nanomaterials

The preliminary conclusions to be drawn from the toxicology studies to date is that some types of nanomaterials can be toxic, if they are not bound up in a substrate and they are available to the body. Multiple government organizations are working to fund and assemble toxicology information on these materials. In the interim, MIT researchers must use procedures that prevent inhalation and dermal exposures because at this time nanotoxicology information is limited.

Based on particles physics and studies of fine atmospheric pollutants, the nanoparticle size range is the range of minimum settling. This means that once released into air, nanoparticles will remain airborne for considerable periods of time. Nanoparticles can be inhaled and will be collected in all regions of the respiratory tract; about 35% will deposit in the deep alveolar region of the lungs.

Because they are so small, nanoparticles follow airstreams more easily than larger particles, so they will be easily collected and retained in standard ventilated enclosures such as fume hoods. In addition, nanoparticles are readily collected by HEPA filters. Respirators with HEPA filters will be adequate protection for nanoparticles in case of spills of large amounts of material.

Working safely with nanomaterials involves following standard procedures that would be followed for any particulate material with known or uncertain toxicity: preventing inhalation, dermal, and ingestion exposure. Many nanomaterials are synthesized in enclosed reactors or glove boxes. The enclosures are under vacuum or exhaust ventilation, which prevent exposure during the actual synthesis. Inhalation exposure can occur during additional processing of materials removed from reactors, and this processing should be done in fume hoods. In addition, maintenance on reactor parts that may release residual particles in the air should be done in fume hoods. Another process, the synthesis of particles using sol-gel chemistry, should be carried out in ventilated fume hoods or glove boxes.

The type of surface coating on nanoparticles often causes them to clump together so that few particles are actually released when particles are removed from reactors. In one of the few workplace industrial hygiene studies of nanoparticles, Maynard et al. (2004) found almost no release of fibers when carbon nanotubes were removed from a reactor and transferred into a secondary container. The SWCNT clumped together into nanoropes and remained attached to the substrate as it was removed from the reactor. Maynard et al. (2004) also found that it took considerable energy to break up the nanoropes and release them into air: the highest settings on a fluidized bed vortex shaker were needed to produce aerosol release. The type of SWCNT investigated in this study were uncoated with about 30% Fe catalyst remaining as part of the nanoropes. Researchers are attempting to coat CNT and other nanoparticles with materials that make them less sticky and more easily dispersed; if successful, this would make them more easily aerosolized and require additional care when handling.

Concerning skin contact, Maynard et al. found clumps of nanoropes on the gloves of workers removing the synthesized materials from the reactors. Since the ability of nanoparticles to penetrate the skin is uncertain at this point, gloves should be worn when handling particulate and solutions containing particles. A glove having good chemical resistance to any solution the particles are suspended in should be used. If working with dry particulate, a sturdy glove with good integrity should be used. Disposable nitrile gloves commonly used in many labs would provide good protection from nanoparticles for most procedures that don't involve extensive skin contact. Two pairs of gloves can be worn if extensive skin contact is anticipated, as well as gloves with gauntlets or extended sleeve nitrile gloves, to prevent contamination of lab coats or clothing.

One potential safety concern with nanoparticles is fires and explosions if large quantities of dust are generated during reactions or production. This is expected to become more of a concern when reactions are scaled up to pilot plant or production levels. Both carbonaceous and metal dusts can burn and explode if an oxidant such as air and an ignition source are present. Nanodusts can be anticipated to have a greater potential for explosivity than larger particles. Determination of lower flammability limits using standard test bomb protocols is being planned in Europe. There are currently no government occupational exposure standards for nanomaterials. When they are eventually developed, different standards for different types of nanomaterials will be needed. One should also be aware that Material Safety Data Sheets (MSDS) may not have accurate information at this point in time. For example, the MSDSs that are accompanying some commercially available carbon nanotubes are referring to the graphite Permissible Exposure Limit as a relevant exposure standard. Both graphite and carbon nanotubes are composed of carbon arranged in a honeycomb pattern. However, graphite is composed of coarse particles while CNTs are shaped like fibers and have much different tensile and conductive properties than graphite. CNTs are much more toxic in the short-term animal tests that have been performed to date. Consequently, the graphite PEL and toxicity information is not appropriate for MSDSs of CNTs. CNTs should be treated as potentially toxic fibers, if capable of being released into the air and not bound up in a substrate, and should be handled with appropriate controls as described previously.

Nanomaterial Waste Management

As nanotechnology emerges and evolves, potential environmental applications and human health and environmental implications are under consideration by the EPA and local regulators.

EPA has a number of different offices coordinating their review of this rapidly evolving technology. The EPA is currently trying a voluntary approach to testing and developing a stewardship program.

There are currently no guidelines from the EPA specifically addressing disposal of waste nanomaterials. It seems that regulation at some level is inevitable. Some political subdivisions, including the City of Cambridge, are already evaluating local regulation.

MIT is taking a cautious approach to nano waste management. It is our belief that regulation is inevitable. In order to better understand the potential volumes and characteristics of these waste streams we are advising that all waste materials potentially contaminated with nano materials be identified and evaluated or collected for special waste disposal. On the content section note that it contains nano sized particles and indicate what they are.

The following waste management guidance applies to nanomaterial-bearing waste streams consisting of:

- Pure nanomaterials (e.g., carbon nanotubes)
- Items contaminated with nanomaterials (e.g., wipes/PPE)
- Liquid suspensions containing nanomaterials
- Solid matrixes with nanomaterials that are friable or have a nanostructure loosely attached to the surface such that they can reasonably be expected to break free or leach out when in contact with air or water, or when subjected to reasonably foreseeable mechanical forces.

The guidance does not apply to nanomaterials embedded in a solid matrix that cannot reasonably be expected to break free or leach out when they contact air or water, but would apply to dusts and fines generated when cutting or milling such materials.

DO NOT put material from nanomaterial – bearing waste streams into the regular trash or down the drain. Before disposal of any waste contaminated with nanomaterial, call the EHS Office (452-3477) for a waste determination.

Collect paper, wipes, PPE and other items with loose contamination in a plastic bag or other sealing container stored in the laboratory hood. When the bag is full, close it, take it out of the hood and place it into a second plastic bag or other sealing container. Label the outer bag with the laboratory's proper waste label. On the content section note that it contains nano sized particles and indicate what they are.

Currently the disposal requirements for the base materials should be considered first when characterizing these materials. If the base material is toxic, such as silver or cadmium, or the carrier is a hazardous waste, such as a flammable solvent or acid, clearly they should carry those identifiers. Many nanoparticles may also be otherwise joined with toxic metals or chemicals. Bulk carbon is considered a flammable solid, so even carbon based nanomaterials should be collected for determination as hazardous waste characteristics.

Additional Sources of Information

Below are additional information sources for nanomaterials (web sites, review articles, and individual research articles). The EHS Office plans to screen new information regularly and alert the MIT community about additional toxicology studies as they become available. We also request that MIT researchers alert us about studies that they learn of so we can distribute them to the MIT community. We would like to observe handling procedures in different labs so we can share good practice information within the MIT community. Many of the articles listed below can be accessed electronically through the MIT Libraries if an electronic subscription is available. Web sites are also provided where available.

Web Sites that Post Current Information about Nanotoxicology

Gradient Corp. Monthly EH&S Nano News at www.gradient.com

International Council on Nanotechnology at: <http://icon.rice.edu>. Up-to-date postings on nanotoxicology worldwide.

National Institute for Occupational Safety and Health (NIOSH) Nanotechnology Topic Page at www.cdc.gov/niosh/topics/nanotech

National Nanotechnology Infrastructure Network (NNIN) at: <http://www.nnin.org/>

National Center for Biotechnology Information (NCBI) Pub Med at: <http://www.ncbi.nlm.nih.gov/entrez>. [Can search for articles on nanoparticle toxicity.]

Review Articles or Reports About Nanotoxicology

Borm P JA, Robbins D, Haubold S et al. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fiber Toxicol 3:11-35 2006.

Colvin VL. The potential environmental impact of engineered nanomaterials. Nature Biotechnology 21:1166-1170 2003. [Note: Excellent and succinct overview of nanotoxicology.]

Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. Environmental Health Perspectives 113:823-839 2005.

Health and Safety Executive (UK). Health effects of particles produced for nanotechnologies. Document EH75/6. 35 pp. December 2004. Available at: www.hse.gov.uk. [Search for EH75/6]

Health and Safety Executive (UK). Nanoparticles: an occupational hygiene review. Research Report 274. 100 pp. 2004. Available at: www.hse.gov.uk. [Search for RR274]

BIA. Workshop on ultrafine aerosols at workplaces. Held August 2002 in Germany. 208 pp. Available at: <http://www.cdc.gov/niosh/topics/nanotech>. [Go to Nanotechnology Topic Page. Report is listed in section Non-US Governmental Resources]

Research Articles on Nanotoxicology

[Many articles are available electronically through [MIT Libraries](#)]

Chen HH, Yu C, Ueng TH, Chen S et al. Acute and subacute toxicity study of water soluble polyalkylsulfonated C60 in rats. Toxicol Pathol 26:143-151 1998.

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

Derfus AM, Chan WC, Bhatia SN. Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 4:11-18 2004.

Donaldson K, Aitken R, Tran L, et al. Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol Sci 92:5-22 2006.

Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjugate Chem 15:897-900 2004.

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Lam CW, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol Sci* 77:126-134 2004.

Li Z, Hulderman T, Salmen R, Chapman R, et al. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. *Environ Hlth Perspec* 115:377-382 2007.

Maynard AD, Baron PA, Foley M, Shvedova AA et al. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. *J Toxicol Environ Hlth, Part A*, 67:87-107 2004.

Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY et al. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol Lett* 155:377-384 2005.

Oberdorster E. Manufactured nanomaterials (fullerenes) induce oxidative stress in the brain of juvenile largemouth bass. *Environ Hlth Perspec* 112:1058-1062 2004.

Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence and lung injury. *Env Hlth Perspec* 102 (suppl 5):173-179 2004a.

Oberdorster G, Sharp Z, Atudorei V, Elder A et al. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Hlth Part A* 65:1531-1543 2002.

Oberdorster G, Sharp Z, Atudorei V, Elder A et al. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16:453-459 2004b.

Rymen-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicol Sci* 91:159-165 2006.

Sayes CM, Fortner JD, Guo W, Lyon D et al. The differential cytotoxicity of water-soluble fullerenes. *Nano Lett* 4:1881-1887 2004

Sayes CM, Liang F, Hudson JL et al. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. *Toxicol Lett* 161:135-142 2006

Shvedova AA, Kisin ER, Mercer R, Murray AR, et al. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol* 289:L698-L708 2005.

Shiohara A, Hshino A, Hanaki K, Suzuki K, et al. On the cyto-toxicity caused by quantum dots. *Microbiol Immunol* 48:669-675 2004.

Tinkle SS, Antonini JM, Rich BA, Roberts JR et al. Skin as a route of exposure and sensitization in chronic beryllium disease. *Env Hlth Perspec* 111:1202-1208 2003.

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

Warheit DB, Webb TR, Colvin VC, et al. Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. *Toxicol Sci* 95:270-280 2007.

Warheit DB, Webb TR, Sayes CM et al. Pulmonary instillation studies with nanoscale TiO₂ rods and dots in rats: toxicity is not dependent upon particle size and surface area. *Toxicol Sci* 91:227-236 2006.