

NANOTECHNOLOGY and NANOPARTICLES

SAFE WORKING PRACTICES INFORMATION PAGE

(Revised: 7/17/09)

PURPOSE:

Research involving nanotechnology has recently been occurring at a pace that far exceeds the capabilities of regulatory agencies. In consideration of the lack of available governmental guidelines and established best practices, OEHS has developed the following guidelines to ensure that the VCU community conducts research involving nanotechnology in a safe and responsible manner.

BACKGROUND

Nanotechnology represents a rather broad interdisciplinary field of research and industrial activity involving particles less than 100 nanometers (nm) in diameter. Engineered materials made of such small particles exhibit novel properties that are distinctively different from their conventional forms and can affect their physical, chemical, and biological behavior. These nano-scale particles can be tubular (nanotubes), spherical, irregularly shaped, and may also exist in aggregated formations.

Nano-scale particles or nanoparticles (NPs) are not new to science and are ubiquitous in nature. Nanoparticles are by-products of fires, volcanic eruptions, and other natural processes. Nanoparticles are natural components of all living things: many proteins, enzymes, and RNA/DNA actually fit the criteria for NPs. In addition to natural NPs, a number of artificial or engineered NPs have recently been developed. Some of these engineered NPs are already in use in consumer products: titanium oxide, added to cosmetics; zinc oxides, added to sunscreen products. Future applications of NPs show promise in advancing the fields of medical treatment (gene therapy and targeted drug delivery), semiconductors, and environmental remediation technology.

Nanoparticles are classified based on their morphology and the main categories to date include the following: (Aitken, 2004)

Fullerenes: Discovered in the 1980s, these are comprised entirely of carbon and take the form of hollow spheres or tubes. These are similar in structure to graphite, forming a sheet of hexagonal carbon rings, but also contain pentagonal and heptagonal rings that allow the formation of three-dimensional structures. The smallest fullerene, a 60 carbon molecule termed buckminsterfullerene (familarly referred to as “buckyballs”), is the most familiar and recognizable form of NP addressed in common and scientific literature. Fullerenes are produced naturally in small amounts through burning of carbon-containing fuels and can be manufactured in commercial quantities through arching and combustion of graphite, coal, and various other hydrocarbons.

Nanotubes: This class of NP is actually comprised of fullerene-like (carbon) particles that are elongated to form tubular structures having a diameter of 1 to 2 nanometers. Carbon nanotubes have significant tensile strength, strength equaling or exceeding 100 times that of steel have been reported. In addition to great tensile strength, nanotubes also have the advantage of much lower weight in comparison to steel and most other commonly used structural materials: Other highly useful properties exhibited by nanotubes includes capacity for high conductivity, high molecular absorption, and other unique electrical properties.

Nanowires: These are essentially tiny interconnecting wires of a single crystalline structure that are constructed using approaches similar to semiconductor fabrication – template disposition.

Quantum dots: Sometimes referred to as artificial atoms, quantum dots are assemblies of materials between two to ten nanometers. They can be composed of metals, metal oxides, or semiconductor materials and typically exhibit unconventional electronic, magnetic, optical, or catalytic properties. These are constructed through chemical-colloidal disposition: a growth process that can be tuned to alter the structure's final characteristics. These particles are termed "quantum dots" because their final size (alone) can control their physical properties; irradiated fluorescent quantum dots for example, will emit different wavelengths of light depending on particle size.

OCCUPATIONAL EXPOSURE HAZARDS

Primary routes of occupational exposure to nanoparticles include: inhalation, trans-dermal absorption, and ingestion (Borm et al., 2006). Epidemiological data is limited for occupational exposure to nanoparticles. The available data suggests humans and laboratory animals exposed to nanoparticles may experience adverse health effects associated with chronic exposure. The observed effects are dependent on the route of exposure and the particular nanoparticle to which the individual has been exposed. The following observations have been recorded following chronic exposure to nanoparticles.

1. Carcinogenicity: Carcinogenicity data on nanoparticles is limited. Studies have shown that certain nanoparticles cause lung tumor development in experimental animals following a lung particle overload (Lee et al., 1985; Heinrich et al., 1995). In particular, carbon nanotubes (CNTs) exhibit a direct relationship between length and particle deposition and their effects on lung irritation, chronic lung inflammations, exacerbation of asthma, and the formation of granulomas consisting of macrophage-like multinucleated cells (Borm et al., 2006; Rehn et al., 2003).

2. Genotoxicity: Radicals may form on the surface of some nanoparticles that may have toxicological consequences resulting in the formation of reactive oxygen species (Albrecht et al., 2006). Reactive oxygen species have been shown to interact with DNA (Dung et al., 2006) resulting in DNA damage that causes fibrosis and lung cancer (Albrecht et al., 2006). In addition, molecular components of the nanoparticles are likely to have aromatic ring systems which have the size and shape to interact with DNA, and thus have potential to promote DNA damage or cancer (Freites, 2003). Many of the genotoxic effects associated with nanoparticles

may be directly related to the characteristics of the nanoparticle surface: functionality, charge, and induced charge (Oberdöster et al., 2005). These characteristics create unique properties that may result in genotoxic interactions.

3. Cytotoxicity: Cytotoxicity depends upon the geometric shapes of the carbon nanomaterial (Jia et al., 2005). Carbon nanotubes (CNTs) have been studied for their cytotoxic properties and have been shown to cause a time- and dose-dependent relationship resulting in apoptosis of various human cell lines (Massimo et al., 2005; Cui et al., 2005; Monerio-Riviere et al., 2005). The CNTs are thought to cause cellular toxicity by a non-specific association with hydrophobic regions of the cell surface and internalization by endocytosis, and accumulation in the cytoplasm of the cell. DNA then wraps around the CNTs resulting in cell death (Hoet et al., 2004).

4. Toxicity: There is limited toxicological data in relation to nanoparticles. Most of the experimental work has involved a limited set of more common NPs, such as carbon black, titanium dioxide, iron oxide, and amorphous silica. Inhaled nanoparticles have the potential to aggravate existing respiratory condition, such as asthma or bronchitis (Oberdöster, 2001), and cause lung inflammation or tumors (Borm et al., 2006). Inhaled nanoparticles have been shown to translocate from the lungs toward other organs within 24 hours after exposure (Hoet et al., 2004). Specific consequences of the translocation are largely unknown. However, preliminary results indicated that affected organ systems may show inflammation, altered heart rate and functions, and oxidative stress (Brown et al., 2004; Stone et al., 1994; Campen et al., 2003; Campbell et al., 2005). Ingested nanoparticles may be absorbed through the intestinal lining and translocate into the blood stream where they undergo first pass metabolism in the liver (Jani et al., 1994; Bockmann et al., 2000). Again, the effects of this translocation are largely unknown. Dermal uptake is limited and does not appear to produce systemic effects (Borm et al., 2006).

SAFE WORK METHODS

The list of potential nanoparticle-related health hazards identified above necessitates the need for principal investigators to conduct thorough risk assessments individually for all nanoparticles and processes involved, and to prepare protocols which include measures for minimizing staff exposure potential. No governmental regulations currently exist regarding nanotechnology use. In lieu of available regulatory guidance, VCU and OEHS recommend that the course for principal investigators to follow is to either eliminate or reduce exposure potential as much as feasible through implementation of the safe work methods listed below.

1. Administrative Controls.

a. Management considerations for nanoparticles and other potentially hazardous chemicals must be included in the laboratory [Chemical Hygiene Plan](#).

b. Protocols involving the *in vivo* use of nanoparticles must include completion of [IACUC Hazardous Chemical Information Page](#) and approval through the [Institutional Animal Care and Use Committee](#) and the [Institutional Biosafety Committee](#)

c. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff will prepare/administer nanoparticles with minimal exposure.

d. All tasks having potential for occupational exposure to nanoparticles will only be conducted by competent staff who have received appropriate training (OSHA: “Worker Right to Know”) regarding the specific nanoparticle-related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

e. Laboratory personnel using nanoparticles in any procedure are required to complete all applicable sections of the [VCU Laboratory Safety Training Modules](#).

f. Laboratory personnel must be instructed to use extreme caution when performing injections involving nanoparticles since accidental needle stick presents an exposure threat.

g. Exposures involving nanoparticles or any other acutely hazardous material should be reported to Employee Health as soon as possible.

2. Personal Protective Equipment. Nanoparticle exposure may often be attributable to the wearing of inadequate PPE. Staff involved in any tasks where potential exposure to nanoparticles exposure exists must don the following PPE:

a. Examination gloves: One particular type of glove will not always provide adequate protection and glove selections is best determined by the risk assessment and the chemicals used for the procedure. Nitrile or rubber examination gloves which cover hands and wrists completely through overlapping sleeve of lab coat when working with nanoparticles may provide adequate protection. Wearing of two sets of gloves (“double gloving”) is advised whenever performing tasks involving nanoparticles and other hazardous substances. Laboratory personnel should thoroughly wash hands with soap and water before and immediately upon removal of examination gloves.

b. Safety glasses or safety goggles (ANSI Z-87 approved) are considered the minimum appropriate level of eye protection. The IBC recommends donning of full-face shield when conducting tasks posing potential for any generation of aerosol or droplets.

c. Lab coats or disposable coveralls that provide complete coverage of skin not otherwise protected by PPE and/or attire. Laboratory personnel whose clothing has been contaminated by nanoparticles should change into clean clothing promptly. Do not take contaminated work clothes home – contaminated clothing should be disposed of as hazardous waste.

d. Appropriate laboratory attire: laboratory personnel handling nanoparticles should don attire which when worn in combination with lab coat and other PPE provides entire coverage of the body. Short pants/dresses and open-toed shoes are not appropriate laboratory attire.

e. If an aerosol exposure threat exists, all procedures should be conducted in an approved chemical fume hood whenever possible (see Engineering Controls below). If an approved chemical fume hood cannot be utilized, an appropriate air-purifying respirator must be utilized

for all procedures where exposure potential is present. A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use. Prior to instituting respiratory protection to personnel, the laboratory must participate in the university [Respiratory Protection Program](#).

3. Work Practices:

- a. Procedures with the potential for producing nanoparticle aerosols should be conducted within an approved chemical fume hood whenever possible.
- b. Needles used for nanoparticle injection will be disposed of in approved sharps containers immediately following use.
- c. Needles used for nanoparticle injection should never be bent, sheared, or recapped. If recapping is absolutely necessary, a "[Needle Recapping Waiver](#)" must be submitted for IBC review/approval prior to proceeding.
- d. Bench paper utilized during preparation of nanoparticle stock should be lined with an impervious backing to limit potential for contamination of work surfaces in the event of a minor spills.
- e. Areas where nanoparticles are prepared and/or administered should be cleaned and decontaminated immediately following each task. Bench tops, BSC interiors, equipment, and laboratory surfaces with potential for nanoparticles contamination should be routinely cleaned.
- f. Do not eat, smoke, or drink where nanoparticles is handled, processed, or stored, since exposure may occur via ingestion. Wash hands carefully before eating, drinking, applying cosmetics, smoking, or using the restroom.

4. Engineering Controls:

- a. Use of a chemical fume hood is recommended for all tasks with potential of aerosolizing nanoparticles. In all cases where engineering controls alone do not sufficiently reduce exposure potential, provision of appropriate PPE for suitably minimizing hazard will be required.
- b. Syringes used for nanoparticles injection must be safety engineered (self-sheathing syringes, luer-lock syringes, etc.). Exceptions will be considered by the IBC on a case-by-case basis.
- c. Animals should be appropriately restrained and/or sedated prior to administering injections and other dosing methods.
- d. Laboratories and other spaces where handling of nanoparticles occurs must be equipped with an eyewash station that meets American National Standards Institute (ANSI) and OSHA requirements.

5. Waste Disposal:

a. Nanoparticles are potentially hazardous materials, surplus stocks and other waste materials containing greater than trace contamination must be disposed of through the university hazardous waste disposal program.

b. Metabolism and excretion of nanoparticles are dependent upon the route of absorption and the particle surface properties. Inorganic nanoparticles, such as titanium dioxide, are unlikely to be altered. However any chemical group added to the inorganic particle's surface could be modified enzymatically or non-enzymatically within the body (Borm et al., 2006). Polymers of nanoparticles will most likely undergo enzymatic alteration but will be based on the chemical composition and specific properties of the polymer. Some carbonaceous nanoparticles have been metabolized in aquatic systems and it is therefore assumed that those with branched side chains or hydrophilic groups are targets for normal human metabolic machinery which is driven by oxidative enzymes (Sayers et al., 2004). It has been shown in animal models that certain polymer based nanoparticles are excreted via urine (Nigavkar et al., 2004). Radiolabeled nanoparticles administered to laboratory animals were found to be secreted in bile. Therefore it can be implied, depending upon the properties of the nanoparticle, that the feces of contaminated individuals will contain nanoparticles or nanoparticle metabolites (Nefzger et al., 1984). The metabolism and potential risks associated with nanoparticle use requires that all potential contaminated carcasses, bedding, and other materials be disposed of through incineration.

c. All contaminated sharps waste materials must be placed in proper sharps container and disposed of as RMW.

6. Spills: Laboratory personnel must don appropriate PPE prior to attempting to manage any spill involving hazardous agents. University policy for addressing spills involving nanoparticles is provided below:

a. Small spills (typically involving less than 5 mg of material) of nanoparticle-containing powder should be wet-wiped with cloth/gauze that is dampened with soapy water. Affected surfaces should be thoroughly wet-wiped three times over with appropriate cleaning agent determined by the laboratory risk assessment – with clean damp cloth used for each wipe down. Following completion all cloth and other materials utilized during spill clean-up with potential for nanoparticles contamination must be disposed of as hazardous waste.

b. Small spills (typically involving less 5 ml of material) of nanoparticle-containing solutions should be covered/absorbed with absorbent material. Areas affected by liquid spills should be triple cleaned with soap and water following removal of absorbent paper.

c. For larger spills of nanoparticles, contact the OEHS emergency line (828-9834) for assistance.

Further reading:

Aitken, RJ *et al.* Manufacture and use of nanomaterials: current status in the UK and global trends. (In-depth review) *Occupational Medicine* 2006; 56: 300-306.

International Council on Nanotechnology <http://icon.rice.edu/>

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

The National Nanotechnology Initiative in the United States www.nano.gov

References

RJ Aitken, KS Creely, CL Tran (2004). Nanoparticles: An occupational hygiene review. *Institute of Occupational Medicine. Health and Safety Executive*, Research Report 274.
Retrieved October 6, 2006, from <http://www.hse.gov.uk/research/rrpdf/rr274.pdf>

Albrecht MA, Evans CW, and Raston CL. Green Chemistry and the Health Implications of Nanoparticles. *Green Chemistry* 2006; 8: 417-432

Bockmann J, Lahl H, Eckert T, and Unterhalt B: [Titanium blood levels of dialysis patients compared to healthy volunteers]. *Pharmazie*. 2000; 55: 468.

Borm PJA, Robbins D , Haubold S , Kuhlbusch T, Fissan H, Donaldson K, Schins RPF, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, and Oberdorster E. The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and Fibre Toxicology* 2006; 3:11

Brown DM, Donaldson K, Borm PJ, Schins RP, Dehnhardt M, Gilmour P, *et al.* 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* 2004; 286:L344-L353.

Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C, *et al.*: Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology*. 2005; 26: 133-140.

Campen MJ, McDonald JD, Gigliotti AP, Seilkop SK, Reed MD, and Benson JM: Cardiovascular effects of inhaled diesel exhaust in spontaneously hypertensive rats. *Cardiovasc Toxicol* 2003; 3: 353-361.

Cui DX, Tian FR, Ozkan CS, Wang M, and Gao HJ. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicol Lett*. 2005; 155: 73-85

Dung CH, Wu SC, and Yen SC. Genotoxicity and oxidative stress of mutagenic compounds found in fumes of heated soybean oil, sunflower oil and lard. *Toxico. In Vitro*. 2006; 20: 439-437

Freites RA. *Nanomedicine*, vol IIA: Biocompatibility, Landes Bioscience, 2003

Heinrich U, Fuhst R, Ritteinghausen S, et al. Chronic inhalation exposure of wistar rats and 2 different strains of mice to diesel-engine exhaust, carbon black, and titanium-dioxide. *Inhalation Toxicology*, 1995; 7:533-556

Hoet PHM, Druske-holdeld I, and Salata OV. Nanoparticles – known and unknown health risks. *J. Nanobiotechnol.*, 2004; 2: 12

Jani PU, McCarthy DE, and Florence AT: Titanium dioxide (rutile) particles uptake from the rat GI tract and translocation to systemic organs after oral administration. *J Pharm*. 1994; 105: 157-168.

Jia, G., H. Wang, et al. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. *Environ Sci Technol*, 2005; 5: 1378-83.

Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, Gaub HE, Stolzle S, Fertig N, and Parak WJ. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano lett*. 2005; 5:331-338

Lee KP, Trochimowicz HJ, and Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide by inhalation for two years. *Toxicol Apply Pharmacol.*, 1985; 79:179-192

Massimo B, Buckner S, Konstantina N, et al. Multi-walled carbon nanotubes induced T lymphocyte apoptosis. *Toxicol. Lett*. 2006; 160: 121-126

Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol Lett*. 2005; 3: 377-84

Nefzger M, Kreuter J, Voges R, Liehl E, Czok R. Distribution and elimination of polymethyl methacrylate nanoparticles after peroral administration to rats. *J Pharm Sci*. 1984; 73: 1309-1311.

Nigavekar SS, Sung LY, Llanes M, El Jawahri A, Lawrence TS, Becker CW, et al: 3H dendrimer nanoparticle organ/tumor distribution. *Pharm Res*. 2004; 21: 476-483.

Oberdorster G. Pulmonary effects of inhaled ultrafine particles. *Int Arch Occup Environ Health*. 2001; 1:1-8.

Oberdorster G, Oberdorster E, and Oberdorster J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect* 2005; 113: 823-839

Rehn B, Seiler F, Rehn S, Bruch J, and Maier M. Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. *Toxicology and Applied Pharmacology*, 2003; 189:84-95

Sayes CM, Fortner JD, Guo W, Lyon D, and Colvin VL. The differential cytotoxicity of water-soluble fullerenes. *Nano Letters*. 2004; 4: 1881-1887.

Stone V, Johnson GD, Wilton JC, Coleman R, and Chipman JK. Effect of oxidative stress and disruption of Ca²⁺ homeostasis on hepatocyte canalicular function in vitro. *Biochem Pharmacol*. 1994; 47: 625-632.