Environmental, Health and Safety Services
459 Tech Center Drive (0423)
Blacksburg, Virginia 24061
540/231-2509 Fax: 540/231-3944
E-mail: nanosafety@vt.edu
www.ehss.vt.edu

Laboratory Guidelines for Nanomaterials Research

Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials, and devices. Nanotechnology at Virginia Tech has increased dramatically and continues to be an emerging industry with uncertain health and safety implications. There is currently no established safe level of exposure to nanomaterials and no State or Federal regulations that specifically address nanomaterials. However, many nanomaterials are regarded as “chemical substances” under the Toxic Substances Control Act (TSCA). This law provides the Environmental Protection Agency (EPA) with a strong regulatory framework for ensuring that new and existing chemical substances are manufactured and used in a manner that protects against unreasonable risks to human health and the environment. Furthermore, if a nanomaterial is produced from certain raw materials, it may be regulated by other agencies such as the Occupational Safety and Health Administration (OSHA) and have existing permissible exposure limits.

TSCA requires manufacturers of new chemical substances to provide specific information to the Agency for review prior to manufacturing chemicals or introducing them into commerce. The EPA can take action to ensure that those chemicals that pose an unreasonable risk to human health or the environment are effectively controlled. In addition, some of the environmental health and safety issues associated with nanotechnology processes may fall within existing state regulations that govern air, water, labeling, waste disposal and worker safety. Until the collaborative research efforts of our governing agencies and research institutions have produced legislation to provide specific guidelines for the safe use of nanomaterials, the following interim health and safety policies shall be implemented for all Virginia Tech research faculty, staff, and students to ensure that routine work processes involving the fabrication and use of nanomaterials are conducted in a safe and environmentally responsible manner using methods that will minimize any potential exposure risks.

What are Nanoparticles?

Nanoparticles are particles having a diameter between 1 to 100 nanometers (nm) that may or may not have size related intensive properties. The precise definition of particle diameter depends on particle shape as well as how the diameter is measured. These materials often exhibit unique physical and chemical properties as compared to their parent compounds. They may be suspended in a gas as a nanoaerosol, suspended in a liquid as a colloid or nanohydrosol, or embedded in a matrix as a nano-composite. Nanoparticles are classified based on their morphology. The following are some of the main categories of nanoparticles:

<u>Fullerenes</u> - comprised entirely of carbon and take the form of hollow spheres or tubes. The most common type has a molecular structure of C60 which takes the shape of a ball-shaped cage of carbon particles arranged in pentagons and hexagons that allow the formation of three-dimensional structures. The smallest fullerene, a 60 carbon molecule termed buckminsterfullerene (familiarly referred to as “buckyballs”), is the most common form of nanoparticles addressed in scientific

literature. Fullerenes have many potential medical applications as well as application in industrial coatings and fuel cells. However, in cell culture studies, different types of fullerenes produced cell death at concentrations of 1 to 15ppm in different mammalian cells when activated by light. Another study indicated that toxicity could be eliminated when water solubility on the fullerene surface was increased.

Quantum Dots (QD) – are nanocrystals sometimes referred to as artificial atoms containing 1,000-100,000 atoms and exhibiting unusual effects such as prolonged fluorescence. They are composed of metals/metal oxides or semiconductor materials and typically exhibit unconventional electronic, magnetic, optical, or catalytic properties. They are being investigated for use in immunostaining as alternatives to fluorescent dyes. Cadmium and selenium are the most common materials used for the core crystal. Both of these materials are known to be toxic to the cells and their exposure is regulated by OSHA.

Carbon Nanotubes (CNT) – represent one of the fastest developing nanomaterials as production has increased rapidly because of its useful properties. CNT can have either single or multiple layers of carbon atoms arranged in a cylinder. Single wall CNT are about 1-2 nm in diameter by 0.1um in length. Multiple wall CNT are 20 nm in diameter and 1mm long. CNT have a tendency to form tangles and ropes. It has been reported that CNT have great tensile strength and are potentially the strongest, smallest fibers known equaling or exceeding 100 times the strength of steel, yet of much lower weight than steel and other commonly used structural materials. CNT may behave like fibers in the lungs. Short-term animal tests of pulmonary toxicity suggest the potential for lung toxicity though questions have been raised about the nature of the toxicity observed and the doses used.

Potential Health Concerns

Nanomaterials have the greatest potential to enter the body if they are in the form of nanoparticles, agglomerates of nanoparticles, and particles from nanostructure materials that become airborne or come into contact with the skin. Preliminary conclusions drawn from the toxicology studies to date are that some types of nanomaterials can be toxic if they are not bound up in a substrate and they are available to the body.

Inhalation

Based on particle physics and studies of ultra-fine atmospheric pollutants, nanoparticles are in the size range that remains suspended for days to weeks if released into the air. Animal and human studies have shown that airborne nanomaterials can be inhaled and deposited in all regions of the respiratory tract, with approximately 35% being deposited into the deep alveolar region of the lungs.

Once in the body, nanoparticles can enter the blood stream and translocate to other organs including the central nervous system. Silver, albumin, and carbon nanoparticles all showed systemic availability after inhalation exposure. A 2004 study showed that 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. Nanoscale titanium dioxide joins several other types of nanomaterials such as manganese oxide, nano carbon and some viruses that can enter the brain directly by means of the olfactory pathway from the nose. The ability of nanomaterials to move about the body may depend on their chemical reactivity, surface characteristics, and ability to bind to body proteins.

Ingestion

Ingestion of nanoparticles may occur from unintentional hand to mouth transfer of materials; this can occur with traditional materials, and it is scientifically reasonable to assume that it also could happen during handling of materials that contain nanoparticles. Ingestion may also accompany inhalation exposure because particles that are cleared from the respiratory tract via the mucociliary escalator may be swallowed. Little is known about the possible adverse effects from the ingestion of nanoparticles.

Skin Contact

A 2006 study reported that nanoparticles with varying physiochemical properties were able to penetrate the intact skin of pigs. These nanoparticles were quantum dots of different size, shape, and surface coatings. They were reported to penetrate the stratum corneum barrier by passive diffusion and localize within the epidermal and dermal layers within 8 to 24 hours. The dosing solutions were two-to-four-fold dilutions of quantum dots as commercially supplied and thus represent occupationally relevant doses. This study suggests that the skin is a potential route of exposure for nanoparticles. However, it remains unclear how these findings may be extrapolated to a potential occupational risk. Research on the dermal exposure of nanoparticles is ongoing.

Engineering Controls

Working safely with nanomaterials involves adhering to standard procedures that would be followed for any particulate material with known or uncertain toxicity. Control techniques such as source enclosure and local exhaust ventilation systems should be effective for capturing airborne nanoparticles based on what is known about their motion and behavior in air.

Current studies indicate that a well designed exhaust ventilation system with a high-efficiency particulate air filter (HEPA) should effectively remove nanoparticles. If a HEPA filter is used in the dust collection system, make sure that it is properly seated in a well-designed filter housing. Nanoparticles have the potential to bypass the filter decreasing filter efficiency and possibly becoming re-entrained into the atmosphere.

Syringes used for nanoparticle injections must be safety engineered (self-sheathing syringes, luer-lock syringes, etc.). When injections or other dosing methods are administered to animals, they shall be appropriately restrained and/or sedated prior to the procedure in accordance with applicable Virginia Tech policy and approved protocols.

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. A safety shower will also be required if corrosive chemicals are being used.

Safe Work Practices

The following practices shall be used when laboratory activities include the use of nanoparticles:

- The preferred method for nanoparticle manipulation shall be in solution. Once in solution, it may be handled on the lab bench using the same precautions as is necessary for other chemical solutions.
- A chemical fume hood or a biological safety cabinet is recommended for all tasks that have the potential for producing nanoparticle aerosolization.
- Nanoparticles shall be synthesized in enclosed reactors or glove boxes that are under vacuum or exhaust ventilation.
- A well designed local exhaust ventilation system with a HEPA filter shall be used to effectively remove nanomaterials in the ambient air and the breathing zone of the laboratory technician.
- Additional processing of materials removed from reactors shall be conducted inside of a fume hood or biological safety cabinet to reduce inhalation exposures.
- Any maintenance on reactor parts shall be done in a fume hood.
- Work areas should be cleaned at the end of each shift with a hepa-filtered vacuum and appropriate wet wiping method.
- Dry sweeping or air hoses are prohibited for use when cleaning work areas potentially contaminated with nanomaterials. Cleanup should be conducted in a manner that prevents worker contact with waste.
- Syringes used for nanoparticle injection will be disposed of in approved sharps container immediately following use.
- 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 minor spills.
- The storage and consumption of food or beverages in the laboratory or workplace where nanomaterials are being handled is prohibited.
- Hand-washing facilities should be provided and workers encouraged to use them before eating, smoking, or leaving the laboratory.
- Facilities for showering and changing clothes should be provided to prevent the inadvertent contamination of other areas caused by the transfer of nanoparticles on clothing and skin.

Personal Protective Equipment

Nanoparticle exposure may often be attributed to inadequate use of PPE. All persons involved in any tasks where there is a potential to be exposed to nanoparticles must use the following PPE:

Appropriate attire – Laboratory personnel handling nanoparticles should be wearing 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.

Examination gloves – Gloves should be worn when handling nanoparticles and solutions containing nanoparticles. Glove selection may best be determined by choosing one that has good chemical resistance to the solution the particles are suspended in. Research has shown that particle penetration of commercially available latex gloves was significant in the 30 to 80 nanometer particle size range. Thus, scientist have advised double gloving whenever performing tasks involving nanoparticles. Latex gloves from Kimberly Clark performed best in tests results. Vinyl or nitrile gloves which cover the hands and wrists completely through overlapping the sleeve of the lab coat should provide adequate protection as well. Laboratory personnel should thoroughly wash their hands with soap and water before and immediately upon removal of examination gloves.

Safety glasses or goggles – ANSI Z-87 approved eye wear are considered the minimum appropriate level of eye protection. A full-face shield should be used when it is anticipated that a task may potentially generate nanoparticle-containing aerosols or droplets.

Lab coats or disposable coveralls - should provide complete coverage of the skin that is not otherwise protected using other PPE. Current research has shown that high-density polyethylene textile (Tyvek Suit) or non-woven fabrics are more efficient against nanoparticles than cotton or polypropylene. Cotton fabrics should be avoided. If lab personnel clothing becomes contaminated with nanoparticles, they should change their clothes immediately and dispose in a segregated container designated for nanomaterial waste. Clothing contaminated with a nanomaterial containing a classified hazardous chemical, should be disposed of in accordance with the guidelines governing that hazardous waste.

Respiratory protection – The use of respirators is not generally required for worker protection provided that adequate engineering controls are in place. Some research has shown that N-series filtering facepieces and respirators equipped with HEPA cartridges or canisters are efficient for capturing particles in the nanoscale range. For circumstances in which respirator use is required, the appropriate selection of respirators must be coordinated through EHSS to ensure compliance with the OSHA Respiratory Protection Standard, 29 CFR 1910.134.

Spills

Standard approaches to cleaning up powder and/or liquid spills include the use of HEPA-filtered vacuum cleaners, wetting powders down, using dampened cloths to wipe up powders and applying absorbent materials/liquid traps for containment. Damp cleaning methods with soaps or cleaning oils are preferred. Cleaning cloths should be properly disposed. Drying and reuse of contaminated cloths can result in re-dispersion of nanoparticles. Use of commercially available wet or electrostatic microfiber cleaning cloths may also be effective in removing particles from surfaces with minimal dispersion into the air.

Dry sweeping or the use of compressed air is prohibited. As in the case of any material spill or cleaning of contaminated surfaces, handling and disposal of the waste material should follow Federal, State, or local regulations.

Inhalation and dermal exposure will likely present the greatest risks during clean up of spills. Therefore, consideration should be given to the appropriate level of PPE.

Waste Management and Disposal

There are currently no guidelines from the EPA specifically addressing disposal of waste nanomaterials. However, regulation at some level is inevitable and certain nanomaterial waste may, by definition, be hazardous waste. Therefore, Virginia Tech is taking the cautious approach to nanomaterials waste management and requiring that all waste materials potentially contaminated with nanoparticles be identified, evaluated, and managed for disposal through EHSS. See www.ehss.vt.edu for specific guidance.

The following waste management guidance applies to potentially contaminated nanomaterial waste streams consisting of:

- Pure nanomaterials (e.g., carbon nanotubes)
- Items contaminated with nanomaterials (e.g., wipes, pipettes, culture plates, PPE, etc.)
- Liquid suspensions containing nanomaterials
- Solid matrices 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.

This 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 fine particles generated when cutting or milling such materials.

DO NOT put nanomaterial-bearing waste streams into regular trash or down the drain. Before disposal of any waste contaminated with nanoparticles, call the EHSS office at 231-2982 for a waste characterization and disposal determination. All waste paper, wipes, PPE and other items with loose contamination should be stored in a plastic bag or other sealing container and stored in the laboratory fume hood. The container should be sealed daily. When the container is filled, close it, take it out of the fume hood and place it into a second plastic bag or other sealing container. Label the outer bag with the laboratory's proper waste label. In the content section of the label, note that it contains nano-sized particles and indicate the source of the nanomaterial.

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, they should carry those identifiers.

Potential Fire and Explosion Hazard

Although insufficient information exists to predict the fire and explosion risk associated with nanoscale powders, nanoscale combustible material could present a higher risk than coarser material of similar quantity given its unique properties. Decreasing the particle size of combustible materials can reduce minimum ignition energy and increase combustion potential and combustion rate, leading to the possibility of relatively inert materials becoming highly combustible. Dispersion of combustible nanomaterials in air may present a greater safety risk than dispersions of non-nanomaterials with similar compositions. Some nanomaterials are designed to generate heat through the progression of reactions at the nanoscale. Such materials may present a fire hazard that is unique to engineered nanomaterials. In the case of some metals, explosion risk can increase significantly as particle size decreases.

Nanoscale particles and nanostructure-porous materials have been used for many years as effective catalysts for increasing the rate of reactions or decreasing the necessary temperature for reactions to occur in liquids and gases. Depending on their composition and structure, some nanomaterials may initiate catalytic reactions and increase their fire and explosion potential that would not otherwise be anticipated from their chemical composition alone. Laboratory research technicians are to take precaution when working with nanoscale combustible materials.

Nanomaterials Risk Level Management System

EHS has established an on-line registration database for all research projects that involve the use of nanomaterials. If you are using nanomaterials for your research, please go to the following link to read about our control banding tool. In order to access the database, please forward a description of your research and your email pid to Nanosafety@vt.edu. You will be logged into the system to gain access to the database for completion of your registration and to obtain an overall risk level to ensure proper engineering controls are in place. If the use of your nanomaterials is not related to research, please contact our office so that we may conduct an exposure assessment to ensure proper controls are in place for your project. Please send email request to nanosafety@vt.edu.

Go to http://www.ehss.vt.edu/programs/nano_tool.php to learn more about the NRLM system.

Signature Page

My signature below is acknowledgement that I have read, understand and will implement the guidelines presented in this document to the best of my abilities while participating in nanotechnology research here at Virginia Tech.

Principal Investigator/ Lead Researcher	**Graduate Student**	**UG Student**	**Date**
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