Best Practices for Handling Nanoparticles in Laboratories

Introduction

The purpose of this document is to provide a readily-accessible summary of information currently available on safe work practices for research laboratories working with engineered nanomaterials at Missouri State University. This interim guidance has been compiled from guidance from governmental agencies and universities currently engaged in nanomaterial research sources such as:

- The Center for Disease Control (CDC),
- The National Institute for Occupational Safety and Health (NIOSH),
- The Occupational Safety and Health Administration (OSHA),
- Department of Energy (DOE),
- Massachusetts Institute of Technology (MIT),
- Virginia Tech, and
- University of Florida.

A list of sources can be found in the References section at the end of this document.

It should be recognized that rapid changes in the understanding of these risks and management techniques may occur in this field, and researchers are strongly encouraged to stay abreast of these developments. It is anticipated that the internal MSU documents will be used in conjunction with the researcher's Departmental (or University general) Chemical Hygiene Plan (CHP), and that this guidance is subject to revision as new information or regulatory guidance becomes available.

Nanomaterial Definitions

Nanoparticles are particles having a diameter of 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 nanocomposite. Nanoparticles are classified based on their morphology. The following are some of the main categories of nanoparticles:

Carbon Nanotubes (CNTs) – CNTs can have either single or multiple layers of carbon atoms arranged in a cylinder. CNTs reportedly have great tensile strength and are potentially the strongest, smallest fibers known equaling or exceeding the strength of steel, with a much lower weight. They represent one of the fastest developing nanomaterials; production has increased rapidly because of its useful properties. Consequently, a number of toxicologic studies of CNTs have been performed in recent years. These studies indicate that the toxicity of CNT may differ from that of other nanomaterials of similar chemical composition (CDC, 2009). CNTs have a tendency to form tangles and ropes, and may behave like fibers in the lungs. With regard to human health hazards, the USEPA notes that "the major health concerns [for CNTs] are for potential pulmonary toxicity, fibrosis and cancer to workers exposed via inhalation.

There are also data suggesting that pulmonary deposition of some nanoscale materials, including carbon nano[tubes] in the agglomerated form, may induce cardiovascular toxicity when these nanoscale materials are inhaled" (USEPA, 2010).

Fullerenes- Fullerenes are comprised entirely of carbon and take the form of hollow spheres or tubes. The most common molecular structure is 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 15 ppm 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. 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.

Potential Health Concerns/ Exposure Routes

Nanomaterials have the most potential to enter the body when in the form of nanoparticles (or particles of nanostructures) that become airborne or come into contact with bare skin. Some early research indicates that some types of nanomaterials can be toxic if not bound up with a substrate. Routes of entry into the body include:

Inhalation

If released to the air nanoparticles may remain suspended for days to weeks, creating the potential for inhalation and deposition into all parts of the respiratory system, including the lungs. Research has also shown a potential for translocation of nanoparticles through nasal passages to the brain.

Ingestion

Ingestion of nanoparticles may occur as a result of hand-to-mouth transfer of materials; it may also occur in conjunction with inhalation into nasal passages. Potential health effects resulting from ingestion of nanoparticles are not well defined.

Skin Contact

At least one study has shown that nanoparticles were able to penetrate intact animal skin, indicating that skin contact is a potential exposure route. The risk associated with this type of exposure is poorly understood at present.

Current Best Laboratory Practices for Nanomaterial Research

A graded approach to controls discussed in this document is suggested. Work with dry nanomaterials requires a much higher level of control than work with nanomaterials imbedded in solid materials. From the perspective of laboratory worker safety, the preferred order for handling nanomaterials is:

1. Solid materials with imbedded nanostructures
2. Solid nanomaterials with nanostructures fixed to the material's surface
3. Nanoparticles suspended in liquids
4. Dry, dispersible (engineered) nanoparticles, nanoparticle agglomerates, or nanoparticle aggregates

The following sections provide a summary of best practices and work controls currently being recommended by the CDC/NIOSH, OSHA, and a number of universities.

1. **Know Your Material** – Familiarize yourself with toxicity information available for the nanomaterial you are working with. Be aware that many Safety Data Sheets (SDSs) currently shipped with nanomaterials refer to the bulk material toxicity information, which is not the same as the toxicity of the nanomaterial. If no information is available for your materials or the toxicity information is limited or uncertain, handle the material as if it is toxic or an unknown. The International Council on Nanomaterials (ICON) database collects toxicity and environmental information by nanoparticle type and can be a good resource (see References for link). Another database resource is the National Center for Biotechnology Information's (NCBI) PubMed database (note that PubMed search results will be much broader than ICON).

2. **Preplan Experiment Procedures and Determine Appropriate Equipment -** Factor in equipment and procedures to prevent worker exposure and/or laboratory contamination, and allow for proper disposal of all nanomaterial waste. Equipment setup may require additional exhaust ventilation.

3. **Prevent Inhalation Exposure during All Handling of Nanomaterials** Work with free particulate nanomaterials, or with suspensions of nanoparticles that are subjected to processes that generate aerosols, should be conducted in exhausted enclosures that to ensure that no release to the laboratory air occurs. Manipulation of free nanoparticulates on the lab bench is strongly discouraged. Exhausted enclosures may include: fume hoods, glove boxes, Class II Type A2, B1 or B2 biosafety cabinets, reactors and furnaces.

    a. **Fume Hood/ Glove Box** When using a fume hood to contain dust or aerosols of nanomaterials, follow good fume hood use practices such as working 6" back from sash, working with sash below the chin, removing arms slowly from hoods to prevent dragging out contaminants, and not blocking the lower back slot with equipment.
    b. **Biosafety Cabinets(BsC)** Only Class II type A2, B1 or B2 biosafety cabinets which are exhausted into the building ventilation system may be used for nanomaterials work. BSCs that recirculate into the room may not be used. There is recirculation of air inside type A2 and B1 cabinets; care should be taken not to perform extremely dusty processes in these cabinets as the internal fans of the BSC are not explosion proof. The air in the type B2 cabinet is 100% exhausted and standard amounts of nanomaterials and solvents may be used in this type of enclosure.
    c. **Ventilation for furnaces and reactors** should be provided to exhaust gasses generated by this equipment. If possible, the exhaust gasses should be run through a liquid filled bubbler to catch particulate before it enters the building ventilation system. Parts removed from reactors or furnaces for cleaning that may be contaminated with nanomaterial residue should be repaired or cleaned in a fume hood or other type of exhausted enclosure.
    d. **Ventilation for large equipment or engineering processes** Equipment that is too large to be enclosed in a fume hood can be set up such that specially designed local exhaust

ventilation can capture contaminants at points where emission is possible. Also custom enclosures can also be designed by local vendors to contain potential emissions.

e. **Respirators** When adequate ventilation controls are in place (as described above) the use of respirators is generally not necessary for worker protection. While not definitive, some research has shown that N-series filtering respirators, when equipped with HEPA cartridges/canisters can efficiently capture nanoscale range particles. Be aware that respirator use must be in compliance with the OSHA Respiratory Protection Standard (29 CFR 1910.134), involving a formal program with medical clearance, training, and recordkeeping requirements. Check with Environmental Management for details.

4. **Skin Contact Prevention** With some specific considerations, typical laboratory personal protective equipment (PPE) should be sufficient to prevent skin contact exposures:

   a. **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 double-gloving whenever performing tasks involving nanoparticles is advised. Laboratory personnel should thoroughly wash their hands with soap and water before and immediately upon removal of examination gloves. [*Supplemental comments from Virginia Polytechnic: 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.*]

   b. **Laboratory Coats** Lab coats or disposable coveralls should provide complete coverage of the skin that is not otherwise protected using other PPE. Research has shown that high-density polyethylene textile (i.e.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 as a hazardous waste.

   c. **Eye Protection** Wear eye protection appropriate to the experimental conditions (for example, safety glasses, goggles, or face shields). Safety glasses or face shields alone cannot protect against aerosols released with pressure, so goggles may be necessary for some nanomaterial processes. An eyewash station that meets ANSI or OSHA requirements is required in labs where nanoparticles are being handled.

5. **Exposure Monitoring** Traditional methods of air sampling that measure mass are not appropriate for nanomaterials. Measurement methods that count nanoparticles or measure

surface area are being developed for nanomaterials. If you feel this is an issue, please contact the Environmental Management office.

6. **Signage and Labeling**: In areas where easily dispersible nanoparticles are in use, post signs indicating the hazards, control procedures, and personal protection equipment required. If warranted, establish a Designated Area as described in the Chemical Hygiene Plan. Nanomaterial storage containers should have a designation that the material is 'nanonscale' or a 'nanomaterial', such as 'nanoscale titanium dioxide'.

7. **Transportation of Nanomaterials**

    a. **Within the Lab** Nanomaterials removed from furnaces, reactors, or other enclosures should be put in sealed containers for transport to other locations. If nanomaterial product from a reactor is bound or adhered to a substrate, the substrate may be removed and put in a transport container. If the nanomaterials product is unbound and easily dispersible (such as in CNT synthesis using aerosolized catalyst), the removal from a reactor should be done with supplementary exhaust ventilation or a glove bag connected to a HEPA vacuum.
    b. **Off-Site** Transportation of nanomaterials to offsite locations and other universities or laboratories outside of MSU may be covered by DOT regulations. Improper packaging and/or transportation could lead to regulatory action and fines.

8. **Prevent Contamination of Laboratory Surfaces** Fume hood or enclosure surfaces should be wet-wiped after each use or at the end of the day. Alternatively, bench liners may be used to prevent contamination. Bench liners, if contaminated, must be disposed of as hazardous waste. Do not dry sweep or use compressed air for cleanup.

9. **Fire and Explosion Hazards** Nanoscale particles are thought to have a greater explosive potential than micron-sized particles, because of their increased reactivity. They may also have greater catalytic potential, resulting in greater fire/explosion potential than would be otherwise be anticipated from chemical composition alone. Fire and explosions concerns increase as the scale of the activity increases.

10. **Spill Cleanup** Have appropriate spill materials on hand before beginning your work, and train appropriate personnel in cleanup procedures. Depending upon the quantity of nanomaterials in use in the lab, each lab should consider having the following items in a nanoparticle spill kit:
    a. barricade tape,
    b. nitrile gloves,
    c. disposable P100 respirators,
    d. adsorbent material,
    e. wipes,

f. sealable plastic bags, and
g. Walk-off mat (e.g. Tacki-Mat™).

Minor spills or small quantities of nanomaterial can be wiped up using wet wiping for solid material and absorbent wipes for suspensions. Larger spills can be cleaned using a vacuum cleaner specially fitted with a HEPA filter on the exhaust to prevent dispersion into lab air (the Nilfisk GM80CR is reportedly a reliable model). A log of HEPA vacuum use should be maintained so that incompatible materials are not collected on the HEPA filter. HEPA filter change-out should be done in a fume hood. Contact the Environmental Management office for assistance with cleanup of major nanomaterial spills.

11. **Nanomaterial Waste Management** There are no specific federal or state regulations that apply to nanomaterial waste. Most research facilities are taking a cautious approach and handling nanomaterial waste as hazardous. The following waste management guidance applies to nanomaterial-bearing waste streams consisting of:
    a. Pure nanomaterials (e.g., carbon nanotubes),
    a. Items contaminated with nanomaterials (e.g., wipes/PPE),
    b. Liquid suspensions containing nanomaterials,
    c. Solid matrixes with nanomaterials that:
        i. are friable, or
        ii. have a nanostructure loosely attached to the surface such that they can reasonably be expected to break free, or
        iii. leach out when in contact with air/water, or
        iv. may break loose 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 in contact air or water, but would apply to dusts and fines generated when cutting or milling such materials.

Collect and contain <u>all</u> waste materials potentially contaminated with nanomaterials. Aqueous waste should be contained in properly labeled containers. <u>**Do not**</u> discharge nanomaterial waste to sinks or drains, or place in regular trash containers. Solid waste materials must be kept moist to avoid aerosolizing, and should be managed as follows:

1) Paper, wipes, PPE and other items with loose contamination are collected in a *sealable* plastic bag in a laboratory hood or appropriate work station
2) Wet the inside of the bag slightly prior to adding waste.
3) When full (or at the end of the day/shift), seal the bag (an additional "wetting" may be done to ensure that the contents of the bag do not dry out prior to closing).
4) Place the sealed bag into the satellite accumulation container
5) Close the satellite accumulation container
6) Ensure that an appropriately completed label is present on the container.

Use of sealed bags within the within the satellite accumulation bucket will ensure that dry nanoparticles are not aerosolized when the satellite bucket is opened to add waste. Satellite accumulation labels are available from the Environmental Management office.

Submit your waste for pickup through the University's electronic submittal system (www.MissouriState.edu/Environmental)

12. **Animal Studies** It is currently unknown how animals dosed with nanoparticles will excrete them. Metabolism and excretion of nanoparticles are dependent upon the route of absorption and the particle surface properties. Current research findings indicate:
    a. 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).
    b. 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 metabolic processes driven by oxidative enzymes (Sayers et al., 2004).
    c. It has been shown in animal models that certain polymer based nanoparticles are excreted via urine (Nigavkar et al., 2004).
    d. 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 animals 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 in accordance with current regulations.

Dosing and necropsies shall be conducted within an exhausted hood, preferably one with HEPA filtration if available. Additionally, the composition of the nanoparticles will vary amongst animal study (IACUC) protocols, e.g. chemical composition, radiolabelled nanoparticles, nanoparticle structure, etc., so potential toxicity and safety control measures may also vary with each IACUC proposal. IACUC proposals involving nanoparticles in animals should include safety controls based on the University/Departmental Chemical Hygiene Plan (CHP).

Animal Housing, Waste and Bedding

a. Nanoparticle dosing methodology will determine how animals should be housed. If animals are subjected to an aerosol containing nanoparticles then they should be housed in "environmentally controlled cages". If the animals are dosed with nano-particles via ingestion or injection then they can be housed in conventional housing.

b. Animal care staff should wear appropriate PPE to prevent exposure to airborne materials or materials on surfaces from urine, feces etc.
c. Workers who are disposing of contaminated bedding should also wear the appropriate PPE, including but not be limited to; protective eye wear, disposable gloves, dust mask, closed front disposable gown, hair cover and shoe covers.

References

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NIOSH [2013]. Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 2013–145

NIOSH [2012]. General Safe Practices for Working with Engineered Nanomaterials in Research Laboratories. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 2012–147 [http://www.cdc.gov/niosh/docs/2012-147/pdfs/2012-147.pdf].

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MIT Best practices for handling nanomaterials in laboratories. http://ehs.mit.edu/site/sites/default/files/files/University_Best_Practices.pdf

Summary of EPA's Current Assessments of Health and Environmental Effects of Carbon Nanotubes. U.S. Environmental Protection Agency, June 2010. Docket Identification Number EPA-HQ-OPPT-2009-0686.

Virginia Tech guideline for nanomaterial research http://www.ehss.vt.edu/programs/nano_safety.php

University of Florida guideline for nanomaterial research http://www.ehs.ufl.edu/programs/lab/nano/

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Suggested Education and Training Resources for Nanomaterial and Occupational Health

GoodNanoGuide, Oregon State University: www.goodnanoguide.org/Short+Courses

Other Nanomaterial Information Sources

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

National Center for Biotechnology Information (NCBI) Pub Med at: http://www.ncbi.nlm.gov/entrez .

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

University of Massachusetts-Lowell, Department of Work Environment, Center for High Rate Nanomanufacturing. Interim Best Practices for Working with Nanoparticles. November 2007.

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