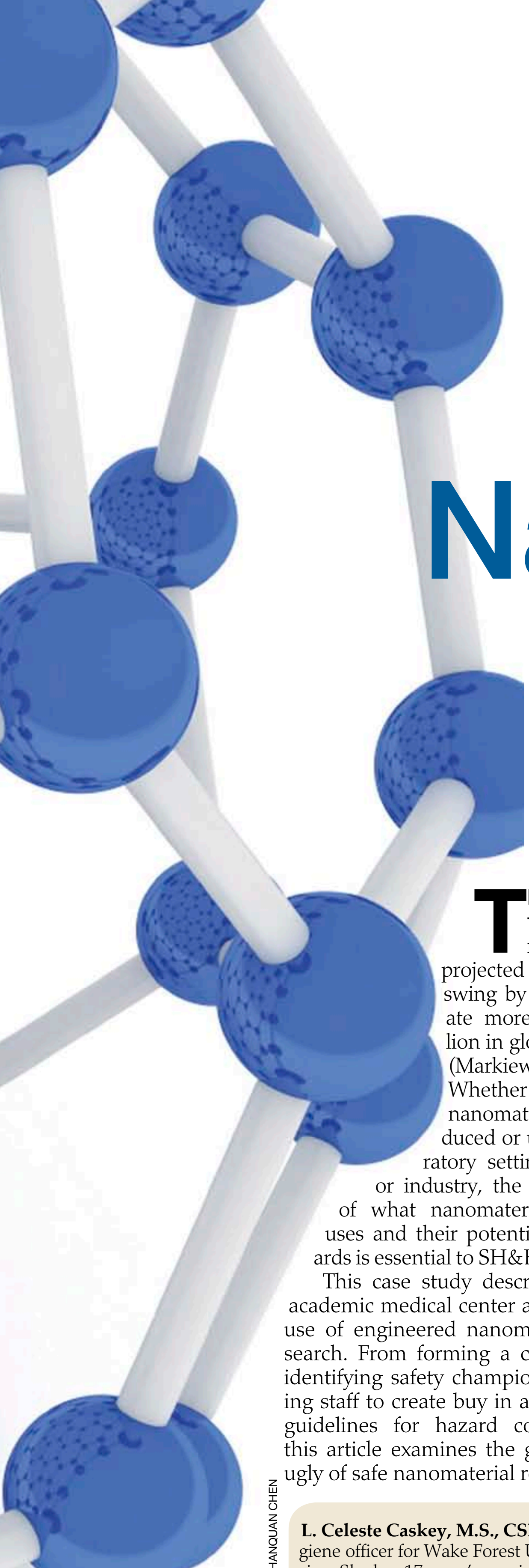

Nanomaterials

The Good, the Bad & the Ugly: A Case Study

By L. Celeste Caskey and Christopher Kolbash

The nanotechnology revolution is projected to be in full swing by 2015 and create more than $1 trillion in global commerce (Markiewicz, July 2009). Whether engineered nanomaterials are produced or used in a laboratory setting, pilot plant or industry, the understanding of what nanomaterials are, their uses and their potential SH&E hazards is essential to SH&E professionals.

This case study describes how one academic medical center approaches the use of engineered nanomaterials in research. From forming a committee and identifying safety champions, to educating staff to create buy in and developing guidelines for hazard communication, this article examines the good, bad and ugly of safe nanomaterial research.

Case Study: Wake Forest University

Wake Forest University Baptist Medical Center is an integrated healthcare system that operates 1,187 acute care, rehabilitation and long-term care beds, outpatient services, and community health and information centers. The center's component institutions carry out a joint mission of patient care, education, research and community service.

The partnership includes three major members: Wake Forest University School of Medicine (WFUSM) and Wake Forest University

IN BRIEF

- **The nanotechnology field is quickly expanding and will create a large global industry in the coming years.**
- **The same characteristics that make nanomaterials such an exciting and fast-evolving field of use and research also create many unknowns for SH&E professionals.**
- **In an effort to create prudent practices in the absence of federal or state regulations, SH&E professionals can utilize existing and familiar risk management systems to categorize risks associated with nanomaterials.**
- **SH&E professionals must balance the need to protect workers and the environment with the need for innovation and discovery in a new field.**

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The PELs for some chemicals used to manufacture nanomaterials were established by OSHA without consideration for how these substances may interact with biological systems at the nanoscale.

Physicians—both part of Wake Forest University Health Sciences—and North Carolina Baptist Hospital. The center has 11,763 employees, of which 4,602 are WFUSM employees. The medical center operates a total of 5,778,108 sq ft of building space, of which 1,602,912 sq ft are designated as School of Medicine space. In total, the School of Medicine has 67 buildings spread out over three campuses, including 215,000 sq ft of animal facilities and 47,000 research animals covering 17 species.

Table 1

OSHA PEL

Substance	PEL
Aluminum oxide	5.0 mg/m^3
Carbon black	3.5 mg/m^3
Magnesium oxide	15.0 mg/m^3
Silver, metal	0.01 mg/m^3
Iron oxide	10.0 mg/m^3
Chromium, metal	1.0 mg/m^3
Copper, dusts	1.0 mg/m^3
Titanium dioxide	15.0 mg/m^3
Tin, metal	2.0 mg/m^3

Nanomaterials on Campus

One nanometer (nm) is one billionth of a meter. The nanoscale ranges from 100 nm down to the size of atoms (about 0.2 nm). At this scale, the properties of engineered nanomaterial are different from that of macro material with the same chemical composition. Nanomaterials have larger surface area when compared to an equal mass of the same material in macro form.

At the nanoscale, chemicals are more reactive and potentially more toxic. Strength and electrical properties are affected, and changes occur in the optical and the magnetic behavior of the materials (Markiewicz, July 2009). Many different types of nanomaterials exist, including carbon nanotubes (CNT), single-wall nanotubes (SWNT), double-wall nanotubes (DWNT) and multiwall nanotubes (MWNT), carbon black, fullerenes (C_{60}), nanoclays (silicon dioxide and titanium dioxide), polymeric, metals such as silver and gold nanoparticles and quantum dots.

WFUSM research staff procure nanomaterials in several ways. They may purchase nanomaterials from the Center for Nanotechnology and Molecular Materials at Wake Forest University, which manufactures SWNT, MWNT and fullerenes. Researchers also may opt to synthesize their own nanomaterials in their labs, obtain them from a collaborator at another university or purchase nanomaterials from various vendors. The type of nanomaterials currently being used at WFUSM are carbon nanotubes, carbon nanotubes with metals, fullerenes, silver and gold nanoparticles, and quantum dots.

Nanomaterials: The Good

Tremendous Opportunities

The good news about the nanotechnology revolution is that nanomaterial uses are truly broad in scope with myriad applications both occurring and envisioned. Many industries stand to benefit, from industrial to consumer goods to biomedicine. Molecular switches, lithium-ion batteries, solar cells, composites, super capacitors, reinforced plastics and semiconductors are a few of the applications for industrial use (Madl, Castranova & Pinkerton, 2009). For consumer use, nanomaterials can be found in appliances, food and beverages, textiles, filtration, sports equipment, electronics and cosmetics.

The Pew Charitable Trusts and the Woodrow Wilson International Center for Scholars track nanotechnology-based consumer products via the Project on Emerging Nanotechnologies (Rejeski, Kuiken, Polischuk, et al., 2011). As of March 11, 2011, their consumer products inventory listed 1,317 products containing nanomaterials. The inventory has grown 521% since its inception in March 2006, and it is projected to grow in a linear fashion through 2012.

The health and fitness consumer category accounts for nearly half of all known consumer products containing nanomaterials, with personal care, clothing and cosmetics making up the majority of the category. The most common nanomaterial types used in consumer products are silver-, carbon- and titanium-based nanomaterials; most of these products are sourced from the U.S. (Rejeski, et al., 2011).

Biomedical applications include nanomaterials utilized as drug carriers and delivery, biosensors, tumor imaging, cell-targeted therapy, cell sensors and microchips, enhanced electron-scanning microscopy imaging techniques, cell and tissue scaffolds, bone grafting and wound dressings (Madl, et al., 2009). On a much smaller scale, research involving the various types of nanomaterials at WFUSM seeks to find applications in the field of biomedicine and bioengineering.

Applicable Standards

In industry, employees who produce or develop nanomaterials are protected by SH&E regulations. Like industry, many of these regulations apply to universities and academic teaching facilities. Some common OSHA regulations that cover nanomaterial research are the general duty clause, HazCom and PPE.

A less-common standard that is pertinent to universities and teaching facilities is 29 CFR 1910.145, Occupational Exposure to Hazardous Chemicals in Laboratories (OSHA, 2006b). EPA regulations pertaining to hazardous waste, air emissions and effluent discharge may affect nanomaterial research. In addition, U.S. Department of Agriculture's Animal Welfare Information Center and the Department of Health and Human Services' Office of Laboratory Animal Welfare regulate animal research.

Various committees are chartered by the university to address chemical, biological, radiological and animal uses as required by the various agencies. WFUSM has a Chemical Safety Committee, an Institutional Biosafety Committee, Radiation Committee, and an Institutional Animal Care and Use Committee.

OSHA's 1910.145 standard requires the development of a chemical hygiene plan for laboratories and, if necessary, the formation of a chemical hygiene committee (OSHA, 2006b). At WFUSM, the SH&E department has developed a general chemical hygiene plan for all laboratories. The Chemical Safety Committee meets quarterly to review safety and health issues along with pertinent chemical research protocols. Research protocols are required for chemicals that are considered particularly hazardous (e.g., carcinogens, reproductive toxins, highly acute toxicity, select agent toxins, hazardous drugs, air or water reactive, and nanomaterials).

OSHA (2006a) has established permissible exposure limits (PEL) for many chemicals to protect employees from overexposure. However, no exposure standards have been established for nanomaterials.

Table 1 lists some chemicals used to manufacture nanomaterials. These PELs were established by OSHA without consideration for how these substances may interact with biological systems at the nanoscale. Historically, PELs, various federal, state and local regulations, toxicological data, and proven engineering controls, administrative controls and PPE have done an adequate job of protecting laboratory workers at WFUSM. However, nanomaterials present a dilemma.

Nanomaterials: The Bad

The Unknown Risks

The bad news about the nanotechnology revolution is that same properties that make nanomaterials such an exciting and fast-evolving field of use and research also create many unknowns for SH&E professionals. To date, no studies have shown adverse health effects in workers who produce or use CNTs; instead, most of the concern regarding CNTs and other nanomaterials stems from findings in animal studies (NIOSH, 2010).

NIOSH has reviewed studies showing that nanomaterials can be inhaled and deposited in the respiratory tract where, in animals, they have been found to translocate to the bloodstream and other organs. Experiments in rats and in cell culture have shown that nanomaterials have the ability to cause pulmonary inflammation and lung tumors (NIOSH, 2009).

Animal studies also have shown an asbestos-type pathology associated with exposure to longer, straighter CNT structures (NIOSH, 2010). In addition, MWNTs may migrate from pulmonary alveoli to the pleura, which is the same site where malignant mesothelioma develops due to asbestos exposure (NIOSH, 2010).

In fall 2008, WFUSM's Chemical Safety Committee received its initial set of chemical safety protocols for nanomaterials. This was the first time the subject of nanomaterial research had been brought forth for committee review. SH&E staff realized that the current protocol form did not do an adequate job of asking the principal investigator to delineate the hazards associated with nanomaterial research.

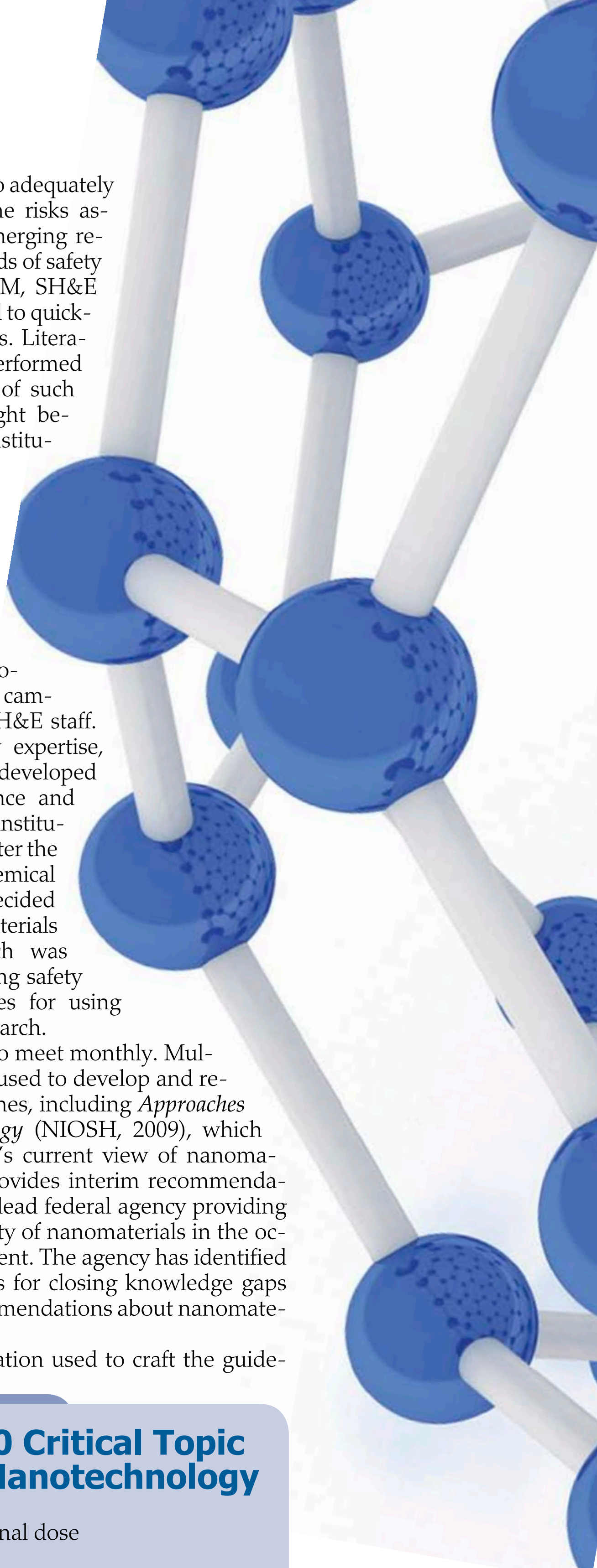
Many committee members, including SH&E staff, lacked the expertise to adequately review and assess the risks associated with this emerging research. As the stewards of safety and health at WFUSM, SH&E staff realized they had to quickly educate themselves. Literature reviews were performed and the importance of such education was brought before the various institutional committees.

Developing Guidelines

Around this time a researcher who had experience with nanomaterials and wanted to use nanomaterials arrived on campus and contacted SH&E staff. Through this faculty expertise, a presentation was developed to explain nanoscience and SH&E concerns to institutional committees. After the presentation, the Chemical Safety Committee decided to form a Nanomaterials Subcommittee, which was tasked with developing safety and health guidelines for using nanomaterials in research.

The group began to meet monthly. Multiple resources were used to develop and refine the draft guidelines, including *Approaches to Safe Nanotechnology* (NIOSH, 2009), which summarizes NIOSH's current view of nanomaterial toxicity and provides interim recommendations. NIOSH is the lead federal agency providing guidance on the safety of nanomaterials in the occupational environment. The agency has identified 10 critical topic areas for closing knowledge gaps and providing recommendations about nanomaterials (sidebar below).

Additional information used to craft the guide-



NIOSH's 10 Critical Topic Areas for Nanotechnology

- Toxicity and internal dose
- Risk assessment
- Engineering controls and PPE
- Measurement methods
- Exposure assessment
- Fire and explosion safety
- Recommendations and guidance
- Communication and information
- Applications
- Epidemiology and surveillance

Figure 1
Nanosafety Level System

Level	FORM	TYPE OF NANOMATERIAL
Green - NSL1 — Nanomaterials consist of no to little harm – known to be inert	Wet	PDot (poly(3,4-dioctyloxythiophene)) Polymer nanotubes
	Dry	PDot (poly(3,4-dioctyloxythiophene)) Polymer nanotubes
	Required Engineering Controls: General Ventilation	
	Required PPE: Lab coat, safety glasses/goggles, single glove with nitrile gloves	
Yellow - NSL 2 — Nanomaterials consist of Potential Hazard(s)	**FORM**	**TYPE OF NANOMATERIAL**
	Water	Silver or Gold coated nanotubes; Quantum Dots; Silver or gold nanoparticles; carbon nanotubes
	Polymer Matrix	Silver or gold nanoparticles; carbon nanotubes; Hydroxyapatite Nanoparticles
	PEG Liquid	Hydroxyapatite Nanoparticles
	Other Non Toxic Solvents	Carbon Black; Silver or Gold nanoparticles; Carbon nanotubes; fullerences (C_{60})
	Required Engineering Controls: Fume hood or BSC	
	Required PPE: Lab coat, safety glasses/goggles, single glove with nitrile gloves	
Orange - NSL 3 — Nanomaterials - limited information is known	**FORM**	**TYPE OF NANOMATERIAL**
	Dry	Carbon nanotubes; gold or silver nanoparticles; Hydroxyapatite Nanoparticles; carbon black
	Required Engineering Controls: Glove Box, fume hood with HEPA or hard ducted BSC	
	Required PPE: Lab coat, safety glasses/goggles, double glove with nitrile gloves	
Red - NSL 4 — Nanomaterial information is unknown - inhalation hazard	**FORM**	**TYPE OF NANOMATERIAL**
	In Solvents and/ or dry Nanomaterial + carcinogenic, teratrogen, mutagen, highly toxic, etc. in aqueous form	Carbon Nanotubes; Gold or silver particles or Quantum dots + oxaliplatin, doxorubicin, mitomycin C, cyclophosphamide or daunomycin
	Required Engineering Controls: Glove Box, fume hood with HEPA or hard ducted BSC	
	Required PPE: Lab coat, safety glasses/goggles, double glove with nitrile gloves and N95/N100 respirator	

The NSL system allows any employee on campus to quickly identify the hazard based on the color codes.

lines was culled from the European Commission's NanoSafe project (Brun, 2009); the International Council on Nanotechnology's GoodNanoGuide (Jaffe, 2010); AIHA (Madl, et al., 2009); programs from peer institutions (Massachusetts Institute of Technology, 2010; Texas A&M Engineering, 2010); and multiple publications from various journals (Springston, 2008; Knowles, 2006; McShane, 2006; Rengasamy, King, Elmer, et al., 2008).

In addition, SH&E staff attended conference sessions on nanomaterials (AIHA, 2009; ASSE, 2009). The additional information had opposing effects on subsequent versions of the guidelines. For example, confirming that HEPA filters capture nanomaterials (NIOSH, 2009; Wang & Kasper, 1981; Lee & Liu, 1981) allowed SH&E staff to relax the ventilation requirements specified in the guidelines. However, absorption information (NIOSH, 2009) confirmed the need to increase dermal protection and other PPE requirements in laboratories and animal research spaces.

The foundation for the guidelines is based on a hierarchical approach to control measures in WFUSM research and animal spaces. The subcommittee convened to address the greatest concern—respiratory absorption by WFUSM personnel. The guidelines indicate that all free particulate nanomaterials should be manipulated in exhausted enclosures (e.g., fume hoods, glove boxes, Class II Type A2, B1 or B2 biosafety cabinets).

Controlling Exposures

One challenge of specifying exhausted enclosures is that ventilation and fume hood design can vary depending on the research building. Variable air volume (VAV) fume hoods and hard-ducted Class II Type B2 biosafety cabinets were identified as the preferred engineering controls due to their inherent safety benefits over other engineering control alternatives.

When compared to constant volume fume hoods, VAV fume hoods are less likely to lose containment even at elevated sash heights (National Academy Press, 1995). VAV fume hoods maintain a constant 100 ft per minute face velocity, regardless of sash height. Constant volume hoods cannot maintain 100 ft per minute face velocity when the sash is raised beyond certain set points, potentially allowing for the release of nanomaterials into the laboratory and exposing the lab worker (National Academy Press).

Class II Type B2 biosafety cabinets have the advantage of being a 100% exhausted enclosure, whose exhaust stream passes through a HEPA filter before leaving the building (CDC, 2009). Other types of biosafety cabinets recirculate a portion of air back into the cabinet and are not recommended for use with volatile toxic chemicals.

Unfortunately, identifying VAV fume hoods and Class II Type B2 biosafety cabinets as preferred engineering controls limits the locations on campus where free nanoparticles can be manipulated. The lack of VAV hoods and hard-ducted biosafety cabinets may become a challenge for the institution as nanomaterials research on campus grows.

The guidelines detail specific administrative controls and PPE requirements as well. Administratively, biosafety cabinets being used for nanomaterial research are now certified semiannually, instead of annually. Prefilters and HEPA filters used in biosafety cabinets are to be changed and serviced by vendors using a bag in/bag out process to minimize exposure to any toxic substance the filters may have trapped.

With regard to PPE, staff identified research showing that dry nanomaterials can pass through the woven fabric typical of lab coats worn throughout WFUSM labs. The subcommittee determined that if researchers are using dry nanomaterials, they must wear disposable Tyvek lab coats or disposable Tyvek sleeves and double nitrile gloves

(NIOSH, 2009). Again, the added requirements of the guidelines create challenges for departments with limited budgets.

Nanomaterials: The Ugly

Categorizing & Communicating the Risks

The ugly news is that even with a set of guidelines in place, numerous questions remained about how to categorize the risks associated with different nanomaterial research at WFUSM. How should hazard information be communicated to lab personnel, animal research personnel and visitors? How should research and animal resources staff be protected from nanomaterials that may be shed from research animals? Where should larger animals that may be injected with and excrete nanomaterials be housed? How should employee exposure to nanomaterials be monitored? And, if employees are monitored, are current PELs applicable for determining exposure to nanomaterials?

While the WFUSM guidelines provide a basic hierarchical structure for controlling nanomaterial hazards, the subcommittee lacked a method for categorizing the risks associated with the nanomaterial protocols being submitted for review. Instead of creating a new risk assessment and communication system, the subcommittee decided to graft a system onto existing, proven frameworks. The system is called Nanosafety Levels (NSL); it is a hybrid of the four biosafety levels described by CDC (2009) and Department of Homeland Security's (2002) color-coded advisory system.

The biosafety levels and color-coded threat levels were familiar to researchers on the committee and employees working with nanomaterials. Essentially, the NSL system is a way to group nanomaterials into categories of risk based on their properties, any known associated health hazards, and the hierarchy of controls used to minimize personal and environmental exposure (Paik, Zalk & Swuste, 2008; Zalk, Paik & Swuste, 2009).

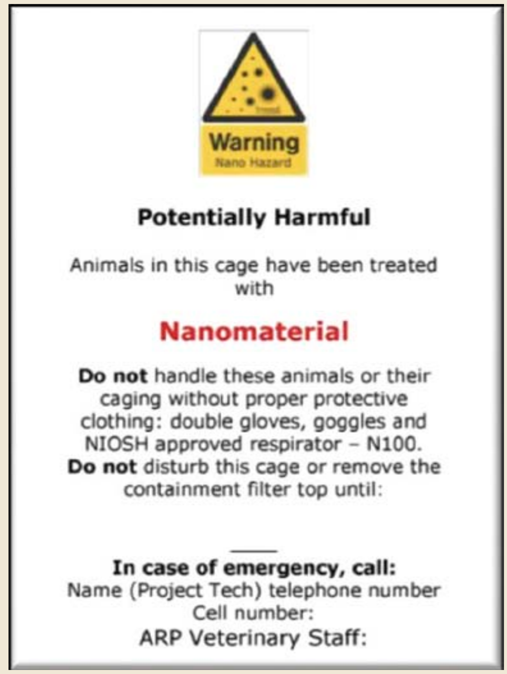
The subcommittee decided to prioritize the characterization of specific types of nanomaterials by beginning with nanomaterials currently used on campus. Researchers were asked to report the properties of the nanomaterials they use (or plan to use).

Ultimately, four levels were created, each with its own color: NSL 1 (green), NSL 2 (yellow), NSL 3 (orange) and NSL 4 (red). The criteria used to categorize nanomaterials into the four levels include form of nanomaterial and known knowledge of material. NSL 1 materials present minimal hazards to employees and the environment and, therefore, require the least controls. NSL 4 materials present substantial hazards to employees and the environment and, therefore, require stringent engineering, administrative and PPE controls (Balazy, Toivola, Reponen, et al., 2006; Rengasamy, et al., 2008). Figure 1 presents the NSL chart.

WFUSM laboratories have specific NSL levels and animal resource areas have separate, specific NSL levels. The subcommittee relied heavily on subject-matter experts to make reasonable and

Figure 2

Cage Card



SH&E personnel create cage cards for animal cages to communicate to animal resources personnel that the animal within the cage has been injected with a highly toxic material.

prudent decisions when knowledge gaps existed regarding the potential health hazards of specific nanomaterials.

The NSL system allows any employee on campus to quickly identify the hazard based on the color codes. Whether a researcher working with nanomaterials or a member of housekeeping staff, the color codes communicate the hazards associated with the nanomaterials being used in lab or animal areas quickly and effectively. Since new information on nanomaterials is generated quickly and evolves constantly, the subcommittee's task continues. The guidelines and the system are routinely reviewed, evaluated and updated as new information becomes available.

Another unknown for the subcommittee was the question of how to protect research staff and animal resources staff from nanomaterials that may be excreted from research animals. The animal resources program employs several engineering controls to minimize personal exposure. These controls are animal-dependent and cater more toward small animals. Micro-VENT mouse racks are HEPA-filtered and create a closed ventilation system, effectively eliminating exposure to shed nanomaterials until the cages must be cleaned.

To avoid exposure during cage cleaning, disposable cages could be used to house small animals. Unfortunately, switching to all disposable cages would be cost prohibitive and wasteful. Reusable cages can be changed underneath a hard-ducted Class 2 Type B2 biosafety cabinet, but WFUSM only has a few of these hoods on campus.

Potential Hazards Associated With Nanomaterials in Animal Studies

- Long and straight CNTs show asbestos-type pathology in animal lungs
- Adverse respiratory effects, such as pulmonary inflammation and fibrosis
- Genotoxicity in in vitro cell studies
- Inhaled nanomaterials deposit in the respiratory tract and migrate to the bloodstream and other organs
- Certain nanomaterials can be absorbed through skin
- Increased risk of dust explosions due to larger surface area of nanomaterials

Additional complications occur when nanomaterial research involves larger animals that cannot be housed in ventilated cage rack systems and require far more hands-on husbandry. In addition to the engineering controls in place, several administrative controls were created to help reduce exposures.

Following a similar pattern established by the creation of the NSL, the subcommittee grafted a system onto an existing, proven framework. SH&E personnel create cage cards (Figure 2, p. 53) for animal cages to communicate to animal resources personnel that the animal within the cage has been injected with a highly toxic material. The cage cards are used when animals have been treated with a carcinogen, mutagen, reproductive toxin, chemotherapeutic agent or, now, nanomaterials.

The system is integrated with the NSL system, so the NSL level dictates the cage card needed on the housing. This way personnel know at a glance that the animal has been treated with nanomaterials and that they must be vigilant when handling, changing, and cleaning the cage and bedding.

While the NSL and cage cards communicate the risk associated with specific nanomaterial research on campus, questions remained regarding how to properly quantify personal exposure to nanomaterials in campus laboratories and animal care facilities. NIOSH (2009) describes a proposed sampling strategy, but it relies primarily on area sampling, which creates uncertainty when estimating worker exposure. If personal exposure could be quantified reliably, then which PEL should be referenced?

Without significant toxicological data and proven PELs, the group agreed to strive to keep exposures as low as reasonably achievable, a longstanding exposure principle in radiation protection. Moving forward, SH&E staff plans to perform wipe sampling in labs using nanomaterials to see whether surface contamination remains after laboratory personnel have completed their procedures and cleaned the work surface.

Wipe samples will be gathered immediately after the lab worker finishes a procedure. Samples will be analyzed using a transmission electron microscope (TEM). The TEM method was chosen because the TEM can provide structural and chemical information at the atomic scale (Weyland, Midgley, Brydson, et al., 2008).

The wipe sampling will be a crude, qualitative method, but it should shed light on the relative hygiene of nanomaterial research and lab cleaning methods. Wipe sampling also may help identify hot spots of contamination and improve administrative controls enacted with the approval of the researcher's chemical safety protocol.

Beyond wipe sampling, SH&E staff would like to evaluate NIOSH's new mass-based airborne concentration measurement for monitoring laboratory exposures to carbon nanotubes (NIOSH, 2010). Using a 37 mm quartz fiber cassette and a personal sampling pump operating between 2 and 4 L/min, the samples would be analyzed for elemental carbon (NIOSH method No. 5040) and judged against NIOSH's recommended exposure limit of 7 µg/m^3. Staff will continue to refine area and personal sampling procedures as new techniques become available and best practices are established.

Conclusion

The nanotechnology field is quickly expanding and will create a large global industry in the coming years. As an academic research institution, WFUSM procures, creates and researches nanomaterials on a laboratory scale. While the quantities may be smaller than those found in industry, the variety of nanomaterials on campus may exceed that found in industrial settings. To create prudent practices in the absence of federal or state regulations, WFUSM SH&E staff augmented and expanded an existing and familiar risk management system (chemical research protocols) for engineered nanomaterials.

The expanded risk management approach included the development of general guidelines for working with nanomaterials safely on campus, the creation of the NLS system and the addition of nanomaterial cage cards for animal research areas.

The Nanomaterials Subcommittee, formed to create the prudent practices described, continues to evaluate and update the guidelines and nanosafety levels as new SH&E information becomes available. **PS**

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Key Links on Nanomaterial Safety

1) Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials (**www.cdc.gov/niosh/docs/2009-125**)
2) Occupational Exposure to Carbon Nanotubes and Nanofibers (**www.cdc.gov/niosh/docket/review/docket161A/pdfs/carbonNanotubeCIB_PublicReviewOfDraft.pdf**)
3) GoodNanoGuide (**http://goodnanoguide.org/tiki-index.php?page=HomePage**)
4) International Council on Nanotechnology (**http://icon.rice.edu**)
5) Risk Analysis: Special Series on Nanotechnology (**http://onlinelibrary.wiley.com/doi/10.1111/risk.2010.30.issue-11/issuetoc**)
6) The Project on Emerging Nanotechnologies **(www.nanotechproject.org**)