J Nanopart Res (2009) 11:5–13
DOI 10.1007/s11051-008-9421-0

The Nanotoxicology Research Program in NIOSH

Vincent Castranova

Received: 28 April 2008 / Accepted: 9 May 2008 / Published online: 19 June 2008

Abstract The National Institute for Occupational Safety and Health through its Nanotechnology Research Center has developed a Strategic Plan for Nanotechnology Safety and Health Research. This Strategic Plan identified knowledge gaps and critical issues, which must be addressed to protect the health and safety of workers producing nanoparticles as well as those incorporating nanoparticles into commercial products or using nanomaterials in novel applications. This manuscript lists the projects that comprise the Nanotoxicology Program in NIOSH and provides a brief description of the goals and accomplishments of these projects.

Keywords Nanoparticles · Nanotoxicology · Pulmonary · Cardiovascular · Neural and dermal effects · Nanotechnology · Occupational health · EHS

Introduction

The National Nanotechnology Initiative (NNI) is a federal program established in 2001 to foster and coordinate nanotechnology research and development. Today, the NNI consists of the individual and cooperative nanotechnology-related activities of 25 federal agencies with a range of research and regulatory roles and responsibilities. In December 2004, the NNI Strategic Plan was published. This document set forth four strategic goals for the National Nanotechnology Initiative:

I. Advance a world-class nanotechnology research and development program.
II. Foster the transfer of the new technologies into products for commercial and public benefit.
III. Develop and sustain educational resources, a skilled workforce, and the supporting infrastructure and tools to advance nanotechnology.
IV. Support responsible development of nanotechnology.

The 21st Century Nanotechnology Research and Development Act of 2003 calls for the NNI Strategic Plan to be updated every third year. This update was published in December 2007. In this updated strategic plan, the four strategic goals were divided into eight major program components:

1. Discovery and development of fundamental knowledge pertaining to new phenomena that occur at the nanoscale. Elucidation of principles related to nanoscale structures, processes, and mechanisms.
2. Discovery of novel nanoscale and nanostructured materials and understanding of their properties.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessary represent the views of the National Institute for Occupational Safety and Health.

V. Castranova (✉)
Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA
e-mail: vic1@cdc.gov

Design and synthesize nanomaterials in a controlled manner with targeted properties.
3. Incorporation of nanoscale or nanostructured materials to achieve improved performance and/or functionality.
4. Advance development of instrumentation and standards for characterization, measurement, synthesis, and design of nanoscale or nanostructured structures and devices.
5. Enable development of reliable, cost-effective nanomanufacturing of materials, devices, and systems.
6. Development of user facilities and networks to foster nanotechnology research and development.
7. Understanding of the environmental, health, and safety impacts of nanotechnology development. Conduct risk assessment and implementation of risk management.
8. Development of educational and public communication efforts to evaluate the societal implications of nanotechnology.

The NNI Strategic Plan Update describes progress and activities in these major program areas.

Role of the National Institute for Occupational Safety and Health in NNI

The National Institute for Occupational Safety and Health (NIOSH) is a component of the Centers for Disease Control and Prevention (CDC) within the Department of Health and Human Services. NIOSH is mandated by law to conduct research and develop guidance on worker safety and health.

Nanotechnology is the understanding, control, and manipulation of matter at dimensions between 1 and 100 nm where unique phenomena enable novel applications. These novel applications include incorporation of nanoscale materials into a wide variety of new products, devices, and systems with new or improved performance. Such applicants include cosmetics, sunscreens, paints, coatings, electronics, structural materials, energy storage and transmission, sensors, medical imaging, and targeted drug delivery.

In light of the endless number of potential applications, nanotechnology is expected to grow into a trillion dollar industry employing millions of workers worldwide within the next decade (Roco 2004). Indeed, the explosive growth of nanotechnology is evidenced by more than 4800 patents classified under nanotechnology in the U.S. Patent and Trademark Office as of November, 2007. As with any new technology, the earliest and most extensive exposures to engineered nanoparticles are most likely to occur in the workplace among workers producing and handling nanoparticles as well as those incorporating nanoparticles into commercial products. Therefore, NIOSH is an active member of NNI. The NIOSH research activities in nanotechnology support NNI Goal 4, i.e., "support responsible development of nanotechnology." Among the eight program areas of NNI, NIOSH has active research in the following:

1. understanding the properties of nanoscale materials;
2. developing standardized techniques for measurement and characterization of nanomaterial exposures in the workplace;
3. understanding the health and safety impact of nanotechnology, conducting risk assessment, and recommending strategies for risk management;
4. dissemination of findings to labor, management, academia, and governmental regulatory agencies.

The Nanotechnology Research Program within NIOSH

In 2004, NIOSH created a Nanotechnology Research Center (NTRC). The charge of the NTRC, under its Director Dr. Paul Schulte, is to:

1. identify knowledge gaps and critical occupational safety and health issues for nanotechnology;
2. develop strategic goals and objectives to address those gaps and issues;
3. develop a timeline for accomplishing these goals and objectives;
4. guide, organize, and coordinate NIOSH research efforts in nanotechnology;
5. coordinate the dissemination of research findings and recommendations;
6. develop a NIOSH Nanotechnology Web Page.

The NIOSH Nanotechnology Web Page address is:

http://www/cdc/gov/niosh/topics/nanotech/.

On this web page, one can obtain the following:

I. Strategic Plan NIOSH Nanotechnology Research: Filling Knowledge Gaps—This plan lists four strategic goals for NIOSH in the area of nanotechnology:

1. determine if nanoparticles or nanomaterials pose risks for work-related injuries and illnesses;
2. conduct research on the applications of nanotechnology for the prevention of work-related injuries and illnesses;
3. promote healthy workplaces through interventions, recommendations, and capacity building;
4. enhance global workplace safety and health through national and international collaborations on nanotechnology research and guidance.

The strategic plan outlines critical gaps and issues in the following areas:

toxicity and internal dose, risk assessment, epidemiology and surveillance, engineering controls and personal protective equipment, measurement methods, exposure assessment, fire and explosion safety, recommendations and guidance, communication and education, and applications.

II. Nanoparticles Information Library—This site lists available information for selected nanoparticles, such as, composition, method of production, size, surface area, morphology, etc. Scientists throughout the world are encouraged to contribute information to this site.

III. Approaches to Safe Nanotechnology: An Information Exchange with NIOSH—This site presents an overview of potential health and safety concerns in dealing with nanomaterials, describes exposure assessment and characterization instrumentation and methods, reviews current information on the effectiveness of control technologies, and presents advice for the prudent handling of nanomaterials in light of limited information concerning risk.

IV. Evaluation of Health Hazard and Recommendation for Occupational Exposure to Titanium Dioxide—This document reviews current research data on workers and from rat inhalation studies concerning the incidence of lung cancer following exposure to fine or ultrafine titanium dioxide. A quantitative risk assessment is conducted, and separate exposure limits for fine and ultrafine TiO_2 are recommended.

V. Progress toward Safe Nanotechnology in the Workplace—This document is an extensive list of the nanotechnology research activities in NIOSH. It lists more than 20 research projects. It provides the project title and PI and gives the accomplishments, publications, and presentations for each project.

The Nanotoxicology Research Program in NIOSH

Under the NIOSH Nanotechnology Research Program within the Nanotechnology Research Center, NIOSH has an active research program in nanotoxicology. The nanotoxicology program in NIOSH addresses issues of toxicology and internal dose identified in the Strategic Plan for NIOSH Nanotechnology Research. These studies pose the following questions:

A. Pulmonary toxicity

   1. What is the deposition pattern in the lung following exposure to nanoparticles? Are nanoparticles effectively cleared by alveolar macrophages? Do nanoparticles rapidly enter the alveolar interstitium?
   2. What are the pulmonary effects of exposure to nanoparticles?
   3. Do intratracheal instillation or aspiration exposure techniques reflect responses to inhaled nanoparticles?
   4. Are nanoparticles genotoxic or carcinogenic?
   5. Are exposure doses used in animal studies relevant to anticipated or measured occupational exposures?

B. Systemic effects

   1. Do nanoparticles translocate from the lung to systemic sites?
   2. What are the systemic effects (cardiovascular or central nervous system) following pulmonary exposure to nanoparticles?

C. Dermal effects

   1. Do nanoparticles penetrate the skin?
   2. What are the effects of topical exposure to nanoparticles on dermal inflammation?

D. Predictive algorithms

   1. What is the best dose metric for nanoparticles (mass, surface area, or particle number)?
   2. What are the effects of size, shape, surface area, surface functionalization, surface charge, chemical composition, etc. on bioactivity?

3. Does agglomeration affect bioactivity? Are there effective methods to disperse nanoparticles for toxicity testing?
4. Are in vitro assays predictive of in vivo bioactivity? Is oxidant generation predictive for all classes of nanoparticles?

Current progress in nanotoxicology research

A. Pulmonary toxicity

A primary route of potential occupational exposure to nanoparticles is anticipated to be inhalation exposure to the lung. Therefore, NIOSH currently has six projects evaluating the pulmonary effects of exposure to a variety of nanoparticles.

I. Pulmonary toxicity of carbon nanotube particles, Dr. Shvedova, PI—This project is evaluating the pulmonary response to single-walled carbon nanotubes (SWCNT) produced by the high-pressure CO disproportionation process (HiP_{CO}). This process employs metal nanoparticles (usually iron) as a catalyst. Therefore, raw SWCNT contain high levels of metal contamination (30% by weight). Raw SWCNT generate reactive oxygen species in the presence of bronchial epithelial cells (Shvedova et al. 2004). This oxidant stress results in a dose-dependent cytotoxicity, which is reversed by the addition of the iron chelator, deferoxamine. High temperature acid treatment effectively removes the contamination iron from the SWCNT (Gorelik et al. 2000). Shvedova and colleagues found that this purified SWCNT sample fails to generate oxygen radicals in vitro and is much less toxic to bronchial epithelial cells in culture.

   Shvedova et al. (2005) reported the effects of pharyngeal aspiration of a suspension of purified SWCNT in a mouse model. They demonstrated that the aspirated suspension contained both large agglomerates of micrometer size and more dispersed nanorope structures. Aspiration of SWCNT resulted in a rapid dose-dependent inflammatory response marked by the elevation of pro-inflammatory cytokines (tumor necrosis factor-alpha and interleukin-1 beta) and polymorphonuclear leukocytes in bronchoalveolar lavage fluid, rapid lung cell damage (elevated lactate dehydrogenase activity in lavage fluid), alveolar/blood barrier damage (protein in lavage fluid), and oxidant stress (decreased lung tissue glutathione levels). This inflammatory/damage response was not persistent and returned toward control levels 7 days after a single exposure. Large agglomerates of SWCNT deposited at the terminal bronchioles and proximal alveoli. These agglomerates were encapsulated by epithelioid macrophages within 7 days post-exposure. Distal alveolar regions of the lung did not contain agglomerates visible by light microscopy. However, these distal alveolar walls were the sites of rapid (within 7 days), dose-dependent interstitial fibrosis marked by thickening of the alveolar septa and increased Sirius red staining for collagen. This interstitial fibrotic response progressed with time post-exposure in the absence of persistent inflammation.

   Mice with vitamin E deficiency (given a vitamin E-deficient diet for 24 weeks prior to exposure to SWCNT) exhibited significantly greater acute inflammation, more prolonged oxidant stress, and greater interstitial fibrosis in response to aspiration of purified SWCNT than mice on a normal diet (Shvedova et al. 2007b). Pharyngeal aspiration of purified SWCNT has also been shown to enhance the susceptibility of mice to pulmonary infection (Shvedova et al. 2008a).

   Recently, Shvedova et al. (2008b) have successfully generated an aerosol of SWCNT and conducted an inhalation exposure study in mice. Aerosolization of dry SWCNT samples resulted in exposure to more dispersed SWCNT structures than was achieved by pharyngeal aspiration of a SWCNT suspension. Pulmonary responses to inhaled SWCNT were qualitatively similar to those reported for aspiration. If anything, inhalation of a more dispersed SWCNT preparation resulted in a less dramatic granulomatous response but greater interstitial fibrotic reaction.

II. Evaluation of the pulmonary deposition and translocation of nanomaterials, Dr. Mercer, PI—This project successfully labeled purified single-walled carbon nanotubes with colloidal gold nanoparticles (Mercer et al. 2007). Silver

staining of mouse lungs after pharyngeal aspiration of gold-labeled SWCNT allowed both agglomerated and more dispersed SWCNT structures to be visualized by light microscopy. Results indicate that dispersed SWCNT deposit in the distal alveoli. They are not avidly phagocytized by alveolar macrophages. Rather, they rapidly (within 3 h post-exposure) cross the alveolar epithelium and enter the alveolar interstitium. These alveolar septa represent the sites of excess collagen production and diffuse interstitial fibrosis reported by Shvedova et al. (2005).

Mercer et al. (2007) also reported that it was practical to significantly improve the dispersion of SWCNT prior to aspiration. Aspiration of a more dispersed sample of SWCNT resulted in fewer granulomas (a result of deposition of large agglomerates) and a four-fold more potent interstitial fibrotic response (a result of greater deposition of nanoropes in the distal alveoli). The fibrogenic potency of dispersed SWCNT via aspiration was similar to that reported after inhalation of a dispersed SWCNT preparation (Shvedova et al. 2008b). Using peak airborne levels of 53 $\mu g/m^3$ found in a laboratory scale SWCNT production site (Maynard et al. 2004), lung burdens of SWCNT, which caused diffuse interstitial fibrosis in the mouse would be possible in workers after 2 years of exposure. Therefore, results from the mouse exposures have relevance to possible occupational exposures (Mercer et al. 2007).

III. In vivo investigation of multi-walled carbon nanotube toxicity, Dr. Porter, PI—This project will address the influence of CNT dimension on pulmonary response. Mice will be exposed by pharyngeal aspiration to a well-dispersed preparation of MWCNT and the response compared to that for SWCNT. Thus far, results indicate that the pulmonary aspiration of MWCNT causes acute lung inflammation and damage, which resolves with time post-exposure (Sriram et al. 2007). These responses are qualitatively similar to those for SWCNT. Porter and colleagues have recently completed the design, construction, and testing of a system to generate an aerosol of MWCNT for inhalation studies. Such inhalation exposures to MWCNT are ongoing, and results will be compared to pulmonary responses to the same MWCNT administered by pharyngeal aspiration.

IV. Potential aneuploidy following exposure to carbon nanotubes, Dr. Sargent, PI—In this project, bronchial epithelial cells will be exposed in vitro to SWCNT, and effects on mitosis will be monitored. In addition, mice will be exposed by pharyngeal aspiration to SWCNT under conditions similar to those reported by Shvedova et al. (2005). Mice will be sacrificed at various times post-exposure, and chromosome number per cell will be evaluated. The hypothesis to be tested is that SWCNT, being fiber-like in dimension, may interfere with mitotic spindles during cell division. Such a phenomenon has been reported after exposure of cells to asbestos fibers and has been linked to oncogenesis (Oshimura et al, 1984; Barrett et al. 1989).

V. Pulmonary toxicity of metal oxide nanospheres and nanowires, Dr. Porter, PI—This project will expose rats by intratracheal instillation to titanium oxide nanospheres and nanowires. The experimental design will allow evaluation of the contribution of shape to pulmonary response. In addition, in vivo inflammatory response will be compared with the in vitro potency in generating oxygen radicals. This will help in evaluating the potential of in vitro oxidant generation as a predictive screening assay for bioactivity. Thus far, metal oxide nanoparticles have been shown to generate hydroxyl radicals in vitro and produce acute inflammation in vivo (Porter et al. 2008).

VI. Potential effects of silicon-based nanowires on lung toxicity, Drs. Leonard and Roberts, PIs—This project will test the ability of well characterized silicon-based nanowires to generate radicals in vitro and to cause pulmonary inflammatory response in vivo. The effect of various modifications to the nanowire surface on oxidant generation and bioactivity will be determined.

B. Systemic effects

Epidemiologic information has linked pulmonary exposure to ambient particulate matter with elevated cardiovascular morbidity and mortality (Dockery et al.

1995; Pope et al. 1995). Therefore, NIOSH has two projects investigating cardiovascular responses following exposure of the lung to nanoparticles. Furthermore, it has been proposed recently that nanoparticles may enter olfactory neurons in the nasal cavity and migrate the neurons to the brain (Oberdorster et al. 2005). Therefore, NIOSH is also evaluating whether pulmonary exposure to nanoparticles would alter inflammatory or blood/brain barrier markers in the brain.

I. Role of carbon nanotubes in cardiovascular inflammation and COPA-related disease, Dr. Simeonova, PI—In this project, mice were exposed once by pharyngeal aspiration to SWCNT under conditions similar to those reported by Shvedova et al. (2005). Oxidant stress in aortic and cardiac tissue was monitored 1, 7, and 28 days post-exposure. There was no evidence of oxidant stress of cardiovascular tissue 1 day after exposure. However, oxidant damage to mitochondrial DNA and heme oxygenase 1 activity were elevated in aortic and cardiac tissue 7 days post-exposure. This oxidant-stress following a single aspiration was transient, with markers of oxidant injury returning to control values 28 days post-exposure (Li et al. 2007).
   This project also evaluated the cardiovascular response to multiple pulmonary exposures of SWCNT. This study used ApoE−/− mice, which are genetically susceptible to atherosclerosis. ApoE−/− mice exhibit spontaneous aortic plaque formation when fed a high fat diet. The number and extent of aortic plaques was significantly elevated in mice receiving multiple exposures (20 μg/mouse, every 2 weeks, for 2 months) to SWCNT (Li et al. 2007).
II. Systemic microvascular dysfunction effects of ultrafine vs. fine particles, Dr. Castranova, PI—The ability of the systemic microvasculature to respond to vasodilators is vital to decreasing peripheral resistance in response to exercise. Systemic microvascular dilation is controlled by the release of nitric oxide from arteriolar endothelial cells, which relaxes arteriolar smooth muscle. Systemic microvascular dysfunction would result in high peripheral resistance, stress on the heart, and in those with compromised cardiac function, a possible cardiac event.
   This project investigated the effect of intratracheally instilled particles on the ability of the microvessels in the spinotrapezius muscle to respond to a dilator, calcium ionophore (A23187), which was evaluated by in vivo microscopy of the microvessels. Pulmonary exposure to residual oil fly ash (ROFA; 2 μm in diameter) caused a dose-dependent inhibition of the responsiveness of the systemic microvessels to a micro infusion of A23187 at 24 h post-exposure. Interestingly, significant systemic microvascular dysfunction was observed at relatively low pulmonary exposures to particles, which did not significantly elevate the number of inflammatory cells or proteins measured in bronchoalveolar lavage fluid 24 h post-exposure. This response appears due to particles and not soluble metal because fine TiO_2 (1 μm) exhibited identical potency as ROFA (Nurkiewicz et al. 2004). This microvascular dysfunction was not due to alternation of the responsiveness of arteriolar smooth muscle to nitric oxide. Rather, it was associated with adherence of polymorphonulcear leukocytes to the microvessel walls and local production of reactive oxygen species (Nurkiewicz et al. 2006). Recent investigations have compared the microvascular effect of inhalation of fine TiO_2 (count median diameter = 710 nm) to inhalation of equivalent mass lung burdens of ultrafine TiO_2 (count median diameter = 100 nm). Inhalation of ultrafine TiO_2 was 10-fold more potent in inhibiting the ability of systemic microvessels to dilate in response to A23187 compared to fine TiO_2 (Nurkiewicz et al. 2008). A 70% inhibition of microvessel dilation was noted at a lung burden of 10 μg of ultrafine TiO_2, i.e., a lung burden that caused no increase in lavage markers of pulmonary inflammation or damage. At a lung burden of 19 μg ultrafine TiO_2, significant vasoconstriction was observed 24 h post-exposure. Such vasoconstriction was likely the result of leakage of A23187 through the endothelial barrier damaged by oxidant stress. Indeed, both inhibition of dilation and vasoconstriction in response of ultrafine TiO_2 can be reversed by infusion of antioxidants.
III. Occupational exposures and potential neurological risks, Dr. Sriram, PI—This project will evaluate changes in inflammatory cytokine and blood/brain barrier damage markers in various

regions of the brain following pulmonary exposure to nanoparticles. Initial studies indicate that mRNA expression for inflammatory cytokines (TNF-alpha and CCL-2) and a marker of endothelial cell damage (E-selectin) were significantly elevated in the olfactory bulb, hippocampus, and frontal cortex 24 h after pharyngeal aspiration of mice with multi-walled carbon nanotubes (Sriram et al. 2007). Preliminary studies indicate qualitatively similar central nervous system changes following pharyngeal aspiration of mice with titanium dioxide nanowires (Porter et al. 2008).

C. Dermal effects

The skin is another target organ and route of entry for engineered nanoparticles. Issues of concern are direct dermal effects of exposure and whether engineered nanoparticles can penetrate the skin and have effects on the immune system.

I. Dermal effects of nanoparticles, Drs. Shvedova and Ding, PIs—In vitro exposure of keratinocytes to raw SWCNT (with 30% contamination iron) results in the generation of hydroxyl radicals, which is reversible with the addition of the metal chelator, deferoxamine (Shvedova et al. 2003). This oxidant generation resulted in dose-dependent damage and cytotoxicity to the cells. Purified SWCNT (0.2% iron) did not generate hydroxyl radicals and were far less cytotoxic to keratinocytes in vitro. Topical application of a suspension of raw SWCNT to the skin of nude mice resulted in dermal inflammation, while purified SWCNT did not induce inflammation (Murray et al. 2007).
This project will also evaluate the effects of nano metal oxides on keratinocytes. Results indicate that in vitro exposure of dermal cells to ultrafine titanium dioxide generates hydroxyl radicals and activates the transcription factor, AP-1, via oxidant-dependent phosphorylation of MAPK pathways (Ding et al. 2006).

D. Predictive algorithms

It is clear that the number of types of engineered nanomaterials currently in production or being evaluated for commercial application far exceeds the ability to conduct in vivo tests for toxicity. Therefore, it is critical to move toward the development of predictive algorithms for biological activity that relate specific physicochemical properties with bioactivity. Properties of engineered nanoparticles to be considered include: surface area, shape, oxidant generation, surface charge, solubility, surface functionalization, and chemical composition.

I. Particle surface area as a dose metric, Dr. Castranova, PI—A widely held hypothesis is that nano-sized particles are more biologically active on an equivalent mass basis than fine particles of the same chemical composition in both in vitro and in vivo test systems due to the high particle surface area of nanoparticles (Oberdorster et al. 2005). A technical problem in conducting in vitro assays as well as pulmonary exposures to particle suspensions delivered by intratracheal instillation or pharyngeal aspiration is that engineered nanoparticles tend to agglomerate into μm-sized structures when suspended in aqueous media. Therefore, development of an appropriate medium to disperse nanoparticles without affecting the biological activity of the surface is critical for hazard assessment in nanotoxicology.
It is argued that upon inhalation of nanoparticles, these structures would come in contact with the alveolar lining fluid in the respiratory zone of the lung. Therefore, this study evaluated whether diluted alveolar lining fluid obtained by bronchoalveolar lavage (a single lavage with 6 mL of PBS) of a naïve rat would be an effective dispersant media for nanoparticles. Sager et al. (2007) demonstrated that ultrafine TiO_2 or ultrafine carbon black suspended in PBS would form μm-sized agglomerates. However, the use of bronchoalveolar lavage fluid as a suspension medium greatly improved the dispersion of the nanoparticles. Use of PBS containing disaturated phosphatidycholine (10 μg/mL) plus albumin (0.6 mg/mL) at concentrations found in lavage fluid was also an effective dispersant. Sager et al. (2007) also demonstrated that this lipid/protein dispersant did not mask the biological activity of the particle surface. Further studies indicate that improved dispersion of nanoparticles increased their biological activity upon intratracheal

instillation in a rat model. Indeed, ultrafine carbon black dispersed in bronchoalveolar lavage fluid was 2–3 fold more potent in causing pulmonary inflammation and damage 24 h post-exposure than equivalent doses of poorly dispersed ultrafine carbon black suspended in PBS (Shvedova et al. 2007a).

This project also evaluated mass vs particle surface area as a dose metric. Intratracheal instillation of ultrafine carbon black or ultrafine TiO_2 caused more lung inflammation and damage 24 h after intratracheal instillation in rats than fine-sized carbon black or TiO_2, respectively, when exposure dose was expressed on an equivalent mass basis (Sager et al. 2008). Conversion of exposure dose to total particle surface area/alveolar epithelial surface area significantly, but not completely, eliminated the potency difference between the ultrafine vs fine size of carbon black or TiO_2. These data suggest that particle surface area may be a more appropriate dose metric than mass for nanoparticles.

II. Specific biomarkers for unusual toxicity of nanoparticles, Dr. Rojanasakul, PI—The ability of nanoparticles to generate reactive oxidant species in vitro has been proposed as a rapid screening assay for potential biological activity and nanotoxicity (Nel et al. 2006). However, purified SWCNT (low iron contamination) do not generate reactive oxygen species in vitro or cause sustained oxidant stress in vivo, yet induce rapid and progressive interstitial fibrosis (Shvedova et al. 2005). Therefore, the goal of this project is to develop an in vitro assay to evaluate the potential fibrogencity of engineered nanoparticles. Preliminary data indicate that exposure of fibroblast cells to SWCNT results in significant enhancement of cell proliferation and collagen production (Wang et al. 2008). It appears that this biological activity of SWCNT is a property of its shape and its tendency to form a mesh-like network of the nanomaterial.

Conclusion

In summary, NIOSH currently is pursing an extensive research program in nanotoxicology. This nanotoxicology program includes projects addressing issues of pulmonary toxicity, systemic effects following

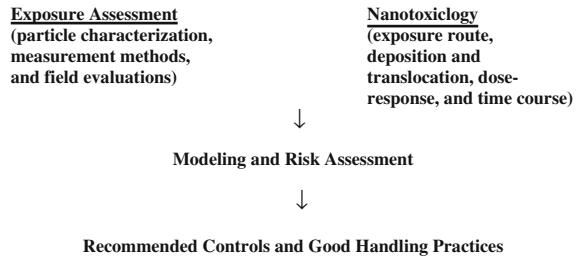

Fig. 1 Integration of nanotoxicology, exposure assessment, and modeling and risk assessment programs in the NIOSH Nanotechnology Research Center to develop recommendations for control and good handling practices to prevent adverse occupational health and safety effects from nanomaterials

pulmonary exposure, dermal effects, and development of predictive algorithms for nanotoxicology. Engineered nanoparticles currently under investigation include: SWCNT, MWCNT, carbon black, silicon nanowires, and nano-metal oxides. The effects of size and shape are being evaluated by comparing fine and ultrafine particles of the same composition (i.e., carbon black and TiO_2), comparing nano-sized carbon materials of different shape (i.e., carbon black, SWCNT, and MWCNT), and comparing nano-metal oxides of different shapes (nanospheres and nanowires). The effect of composition and ability to generate reactive oxidant species on in vivo toxicity is also being evaluated.

The objectives of the nanotoxicology program in NIOSH are to increase knowledge in the areas of exposure route, deposition and translocation, dose-response relationships and time course of responses, and nanoparticle properties which drive bioactivity. Results of the nanotoxicology program will be used with those from the exposure assessment program in NIOSH (covering issues such as, particle characterization, measurement methods, and field evaluations) to conduct modeling and risk assessment. (Fig. 1). The net result of the NIOSH Nanotechnology Program is to determine the potential health and safety risks to nanotechnology workers and to recommend effective control measures and prudent handling practices to reduce risk and prevent adverse health effects.

References

Barrett JC, Lamb PW, Wiseman RW (1989) Multiple mechanisms for the carcinogenic effects of asbestos and other mineral fibers. Environ Health Perspect 81:81–89

Ding M, Lu Y, Bowman L, Leonard S, Castranova V, Vallyathan V (2006) Titanium nanoparticles induce AP-1

activation through ROS and MAPKs pathways. In: Proceedings of the first international conference on toxicology of nanomaterials: biomedical aspects. Miami, 29 January–1 February, 2006, p 101

Dockery DW, Pope CA, Xu X, Spengler JD, Ware SH, Fay ME, Ferris BG, Speizer FE (1995) Mortality risks of air pollution: a prospective cohort study. New Engl J Med 329:1953–1759

Gorelik O, Nikolaev P, Arepulli S (2000) Purification procedures for single-walled carbon nanotubes. NASA Contractor Report. NASA/CR – 2000-208926

Li Z, Hulderman T, Salman R, Chapman R, Leonard SS, Yong S-H, Shvedova A, Luster MI, Simeonova PP (2007) Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ Health Perspect 115: 377–382

Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V (2004) Exposure to carbon nanotube material during the handling of unrefined single walled carbon nanotube material. J Toxicol Environ Health Part A 67:87–107

Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, Shvedova AA, Castranova V (2007) Alternation of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model. Am J Physiol Lung Cell Mol Physiol. doi:10.1152/ajplun.00186.2007

Murray AR, Kisin E, Kommineni C, Kagen VE, Castranova V, Shvedova AA (2007) Single-walled carbon nanotubes induce oxidative stress and inflammation in skin. Toxicologist 91:A1406

Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311:622–627

Nurkiewicz TR, Porter DW, Barger M, Castranova V, Boegehold MA (2004) Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation. Environ Health Perspect 112:1299–1306

Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao KM, Marvar PJ, Hubbs AF, Castranova V, Boegehold M (2006) Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environ Health Perspect 114:412–419

Nurkiewicz TR, Porter DW, Hubbs AF, Cumpston JL, Chen BT, Frazer DG, Castranova V (2008) Nanoparticle inhalation augments particle dependent systemic microvascular dysfunction. Part Fibre Toxicol 5:1. doi:10 1186/1743-8977-5-1

Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839

Oshimura M, Hesterberg TW, Tsutsui T, Barrett JC (1984) Correlation of asbestos-induced cytogentic effects with cell transformation of Syrian hamster embryo cells in culture. Cancer Res 44:5017–5022

Pope CA, Thun MS, Namboodiri MM, Dockery DW, Evans JS, Speizer FE, Health CW (1995) Particulate air pollution as a predictor of mortality in a prospective study of US adults. Am J Respir Crit Care Med 151:669–674

Porter DW, Holian A, Sriram K, Wu N, Wolfarth M, Hamilton R, Buford M (2008) Engineered titanium dioxide nanowire toxicity in vitro and in vivo. Toxicologist 102:A1492

Roco MC (2004) Science and technology integration for increased human potential and societal outcomes. Ann NY Acad Sci 1013:1–6

Sager T, Porter D, Robinson V, Lindsley WG, Schwegler-Berry DE, Castranova V (2007) An improved method to disperse nanoparticles for in vitro and in vivo investigation of toxicity. Nanotoxicology 1:118–129

Sager T, Porter DW, Castranova V (2008) Pulmonary response to intratracheal instillation of fine or ultrafine carbon black or titanium dioxide: role of surface area. Toxicologist 102:A1491

Shvedova AA, Kisin ER, Murray AR, Schwegler-Berry D, Gandelsman VZ, Maynard A, Baron P, Castranova V (2003) Exposure to carbon nanotube material: assessment of the biological effects of nanotube materials using human keratinocytes. J Toxicol Environ Health Part A 66:1901–1926

Shvedova AA, Kisin E, Murray A, Schwegler-Berry D, Gandelsman VZ, Baron P, Maynard A, Gunter MR, Castranova V (2004) Exposure of human bronchial epithelial cells to carbon nanotubes caused oxidative stress and cytotoxicity. In: Proceedings of the society of free radical research meeting, Ioannina, Greece, 26–29 June 2003, pp 91–103

Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, Hubbs AF, Antonini J, Evans DE, Ku B-K, Ramey D, Maynard A, Kagan VE, Castranova V, Baron P (2005) Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol 289:698–708

Shvedova AA, Sager T, Murray A, Kisin E, Porter DW, Leonard SS, Schwegler-Berry D, Robinson VA, Castranova V (2007a) Critical issues in the evaluation of possible effects resulting from airborne nanoparticles. In: Monteiro-Riviere N, Tran L (eds) Nanotechnology: characterization, dosing, and health effects chap 14. Informa Healthcare, Philadelphia, pp 221–232

Shvedova AA, Kisin ER, Murray AR, Gorelik O, Arepalli S, Castranova V, Young S-H, Gao F, Tyurina YY, Oury TD, Kagan V (2007b) Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57Bl/6 mice. Toxicol Appl Pharmacol 221:339–348

Shvedova AA, Fabisiak JP, Kisin ER, Murray AR, Robert JR, Antonini JA, Kommineni C, Reynolds J, Barchowsky A, Castranova V, Kagan V (2008a) Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. Am J Respir Cell Mol Biol 38:579–590

Shvedova AA, Kisin E, Murray AR, Johnson V, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Stone S, Frazer D, Chen T-H, Deye G, Maynard, A, Baron P, Mason R, Kadiiska M, Stadler K, Mouithys-Mickadlad A, Castranova V, Kagan VE (2008b) Inhalation of carbon nanotubes induces oxidative stress and cytokine response causing respiratory impairment and pulmonary fibrosis in mice. Toxicologist 102:A1497

Sriram K, Porter DW, Tsuruoka S, Endo M, Jefferson AM, Wolfarth MG, Roger GM, Castranova V, Luster MI (2007) Neuroinflammatory response following exposure to engineered nanomaterials. Toxicologist 96:A1390

Wang L, Castranova V, Rojanaskul Y, Lu Y, Scabilloni JF, Mercer RR (2008) Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts. Toxicologist 102:A1499