Review Article

Focused Actions to Protect Carbon Nanotube Workers

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There is still uncertainty about the potential health hazards of carbon nanotubes (CNTs) particularly involving carcinogenicity. However, the evidence is growing that some types of CNTs and nanofibers may have carcinogenic properties. The critical question is that while the carcinogenic potential of CNTs is being further investigated, what steps should be taken to protect workers who face exposure to CNTs, current and future, if CNTs are ultimately found to be carcinogenic? This paper addresses five areas to help focus action to protect workers: (i) review of the current evidence on the carcinogenic potential of CNTs; (ii) role of physical and chemical properties related to cancer development; (iii) CNT doses associated with genotoxicity in vitro and in vivo; (iv) workplace exposures to CNT; and (v) specific risk management actions needed to protect workers. Am. J. Ind. Med. *Published 2012. This article is a U.S. Government work and is in the public domain in the USA.*

KEY WORDS: cancer; mode of action; nanotechnology; risk management

INTRODUCTION

If some carbon nanotubes (CNTs) are ultimately shown to be carcinogenic, as the current limited experimental animal and in vitro data seem to suggest, are current workplace practices and exposure controls sufficient to prevent a significant risk of cancer in workers? This question hinges on a broad range of issues. For example, can we determine a safe level of exposure? What is the role of physical–chemical properties on the carcinogenic potential of various types of CNTs? What is the degree of risk based on what is known about various exposure scenarios? Are we effectively communicating to workers and employers what is known, and still uncertain, about the risks?

CNTs are widely regarded as having many important benefits to society, such as making materials stronger with less weight, making electronics faster, more powerful and more efficient, and contributing to significant medical, energy, transportation, and other useful advances. CNTs are hollow, rolled graphene sheets, with diameters of a 1–2 nm (single-wall CNTs or SWCNTs) or 2–100 nm (multi-wall CNTs or MWCNTs). As the number of graphene sheets comprising a MWCNT increases, so does its diameter and, thus, its stiffness. Therefore, preparations

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of MWCNTs appear straighter and less tangled than SWCNT [Mercer et al., 2008; Porter et al., 2010]. Indeed, MWCNT have been shown to penetrate the outer surface of the lungs and enter the intrapleural space [Mercer et al., 2010]. CNT length can range from <1 μm to tens of micrometers.[1] CNT manufacturing and use is increasing and so is the number of workers with potential exposures [Invernizzi, 2011].

The first step to protect workers' health for handling any potentially hazardous material is to put in place effective primary prevention measures. Elimination of the hazardous material and substitution to a less hazardous material are recommended as the top tiers in the hierarchy of controls to reduce or eliminate hazardous exposures. However, the extent that these steps are possible may depend on the material properties needed for specific applications and the societal value of those applications. If elimination and substitution are not feasible, then implementing effective engineering controls is the key to primary prevention, while the inclusion of safe workplace policies and appropriate use of personal protective equipment (PPE) may be required to provide additional protection. Secondary prevention measures—including medical surveillance, exposure registries, and epidemiological study—may also be needed to provide for the early detection of occupational disease so that intervention measures can be implemented to mitigate any adverse health effects. Implementing occupational safety and health programs including engineering controls and other preventive measures have a certain cost. However, it needs to be recognized that inaction also has a cost—often at the expense of workers' health and safety.

The question is, what level of evidence is needed to support risk management decisions? Risk assessment is a process to evaluate the scientific data and make evidence-based decisions about how to best protect workers. Yet data on CNTs are sparse (Table I). As nanotechnology develops, there remain opportunities to continue to incorporate precautionary steps in controlling exposures until more is known about the safety of these materials.

Concerns about the similarity of CNTs and asbestos have been raised [The Royal Society, The Royal Academy of Engineering, 2004; Jaurand et al., 2009; Donaldson et al., 2010], and calls for rapid implementation of exposure controls have been voiced, both to protect workers and to avoid problems that would preclude the safe incorporation of nanotechnology into society [Maynard, 2008]. It is now time to move from generalities to specifics—to determine what risk management actions are needed to protect workers based on the currently available evidence. The objectives of this paper are to examine the current scientific evidence on: (i) the carcinogenic potential of CNTs, including the possible biological modes of action; (ii) the physical–chemical properties of CNTs associated with the specific bioactivities related to cancer development; (iii) the CNT doses at which these effects have been observed in experimental systems, as well as estimated human-equivalent workplace exposures; (iv) workplace exposures to CNT; and (v) specific risk management actions needed to protect workers' health—if CNTs are ultimately shown to be carcinogenic. While the issue is being addressed, current evidence suggests a preventive approach is needed to protect workers from adverse health effects already demonstrated in animal studies.

TABLE I. Current Evidence Concerning Carcinogenicity of Carbon Nanotubes

Type of evidence	Level of evidence
Human studies	None
Animal studies	Some (mesothelioma following abdominal exposure)
Mechanistic data (in vitro; short-term in vivo)	Strong

REVIEW OF CURRENT EVIDENCE ON THE CARCINOGENIC POTENTIAL OF CNT

Modes of Action

The evidence on currently recognized modes of action of particle and fiber carcinogenicity (Fig. 1) is likely to apply to CNTs that possess the requisite physical and chemical properties. In addition, evidence from studies in animals (in vivo) and cell cultures (in vitro) has suggested new mechanisms on how CNTs of certain dimensions can interfere with normal cell function, notably cell division, which could lead to carcinogenic adverse effects (Fig. 1). CNT structures are often heterogeneous, ranging from individual fibers, to nanoropes, to loose ''birds nest'' agglomerates, or to tighter spherical structures (Fig. 2), and the particular form of CNTs may influence their carcinogenic potential. Multiwalled CNTs are ''stiffer'' than single walled CNTs and stiffness may affect the carcinogenic potential [Mercer et al., 2010]. In addition, contaminants that adhere to the particle surface (e.g., PAHs) or the residual metal content from catalysis could play a role in the lung effects including carcinogenicity of particles and fibers. Mode of action is typically used to refer to general

[1] Although nomenclature varies, ISO defines CNT as hollow carbon nanofibers (CNFs), and carbon nanorods (CNR) as solid CNF [ISO, 2008]. This article primarily discusses CNTs, as these have been studied the most to date, although some studies of worker exposures and a few toxicology studies in animals have referred to CNF (possibly meaning CNR). Moreover, production processes can result in a mixture of hollow and solid CNF structures.

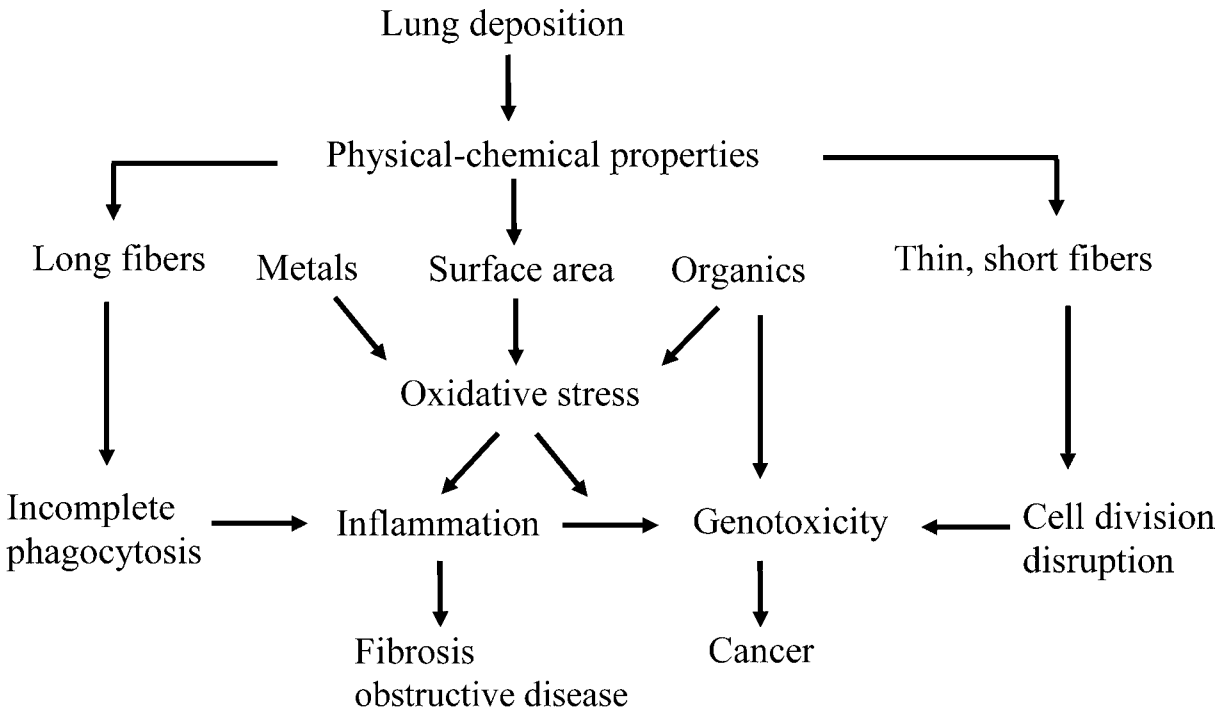

FIGURE 1. Possible modes of action for lung injury and disease development from inhaled particles and fibers (Adapted from Donaldson et al., 2005, 2006).

pathways leading to a disease, such as cancer, while mechanisms of action are considered to be based on more specific data such as the substance–cell interactions within those pathways. The evidence on the modes and mechanisms that may apply to CNT carcinogenicity is discussed below.

Particle-Induced Secondary Genotoxicity and Cancer

Inhaled poorly soluble particles may be carcinogenic through a secondary (or indirect) mode of action involving persistent pulmonary inflammation at sufficiently high lung doses as observed in rats [Schins and Knaapen, 2007; NIOSH, 2011]. Particles that deposit in the alveolar (gas exchange) region of the lungs are normally engulfed (phagocytized) by alveolar macrophages and cleared to the tracheobronchial airways via the ''mucociliary escalator.'' Particles that escape phagocytosis can interact with the alveolar epithelial cells, resulting in recruitment of inflammatory cells from pulmonary capillaries [polymorphonuclear leukocytes (PMNs) or additional macrophages] for clearance of particles from the alveolar airspaces. These cells produce reactive oxygen and nitrogen species (ROS/RNS), defenses aimed at destroying bacterial cell membranes and other organic materials that enter the lungs. In addition, the surface activity of some particles (e.g., crystalline silica) may also generate oxidants, which can increase considerably the particle toxicity [Castranova, 2000; NIOSH, 2011]. When the dose of particles (including poorly-soluble low toxicity particles) is sufficiently high, the alveolar macrophage-mediated clearance becomes impaired, resulting in increased buildup of particles in the lungs, as observed in rats and mice [Morrow, 1988; Bellmann et al., 1991]. This effect has been associated with either the particle volume [Morrow, 1988; Bellmann et al., 1991; Pauluhn, 2011] or the particle surface area dose [Tran et al., 2000; Elder et al., 2005] across a range of particle sizes and densities. Nanoparticles, with greater surface area and number per unit mass, may elicit greater inflammatory response from lung cells and reduce clearance by macrophages at lower-mass doses than larger, respirable particles [Oberdörster et al., 1994; Tran et al., 2000]. Loosely agglomerated CNTs exhibit high total volume or surface area per unit mass. Respirable particles ($<$10 μm aerodynamic diameter) are those that are capable of reaching the alveolar region of the lungs, where gas exchange occurs. Nanoparticles including CNTs that are detected in workplace air are typically agglomerated, although these agglomerates can still be of respirable size [Maynard et al., 2004; Methner et al., 2010]. The extent to which agglomerated CNT structures may dissociate in the

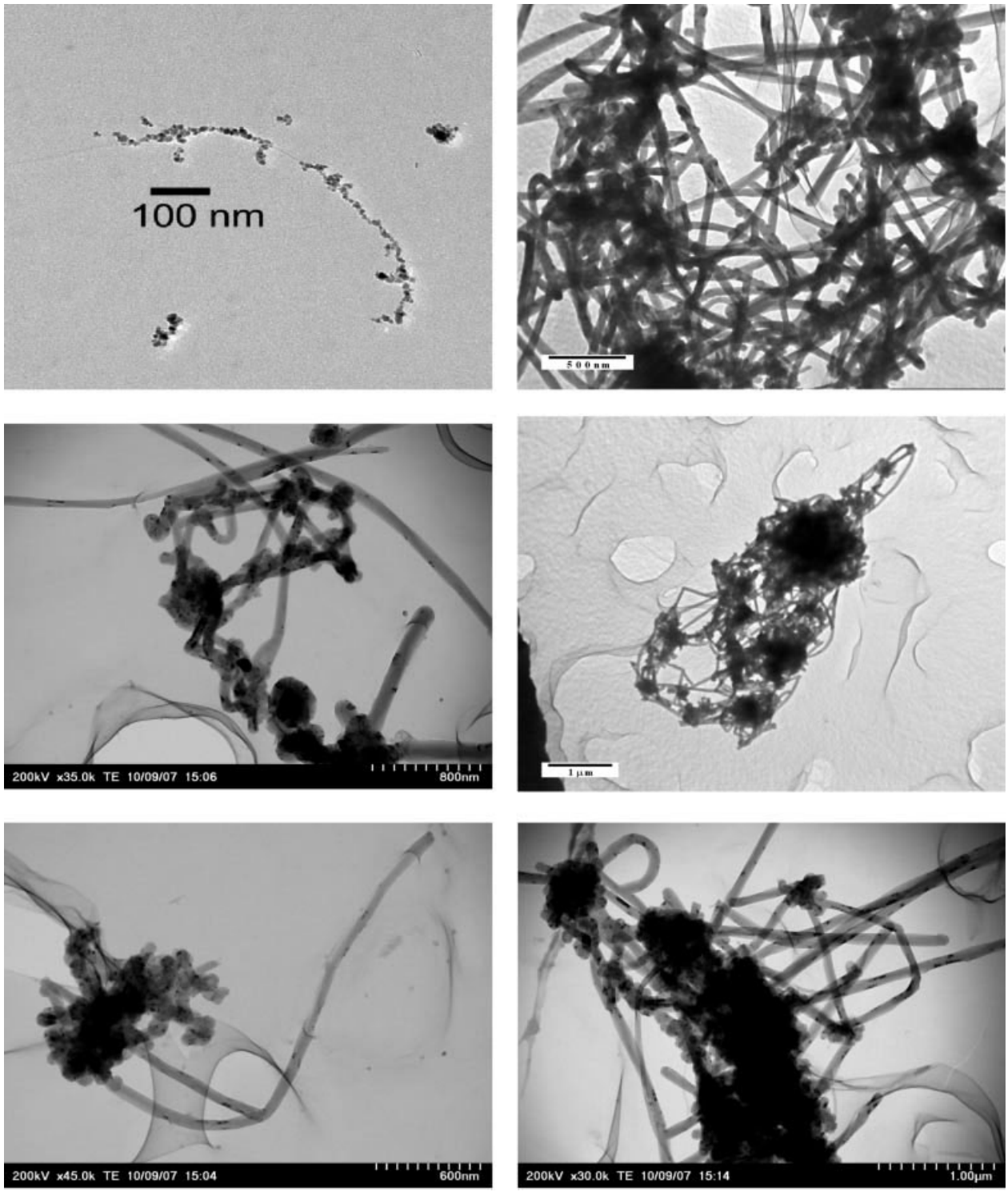

FIGURE 2. Examples of carbon nanotube structures in airborne samples. All images (except top left) were generated by NIOSH by sampling a laboratory-generated aerosol of multi-wall carbon nanotubes (MWCNT). Top left image was generated by sampling the exhaust from lab-scale high-temperature synthesis of MWCNT (Reprinted with permission from Tsai et al. [2009]. Copyright 2009 American Chemical Society).

lungs is not well known, although some evidence suggests they do, and the individual structures (few nm in diameter) can rapidly elicit interstitial fibrosis [Mercer et al., 2008, 2011].

Based on the poorly-soluble particle paradigm, dispersed or loosely agglomerated CNTs would be expected to be more potent on a mass basis at inducing inflammation compared to larger particles with lower surface area or volume per unit mass [Oberdörster et al., 1994; Driscoll, 1996; Pauluhn, 2011]. Persistent inflammation has been associated with oxidative DNA damage, increased cell proliferation, and cancer in rats [Driscoll et al., 1997; Schins and Knaapen, 2007] (Fig. 1). Because of normal antioxidant responses, there may be a certain dose (threshold) below which this inflammation-related mode of action is unlikely to occur, as evidenced by the nonlinear dose–response relationship between particle surface area dose and rat lung inflammation or tumors [NIOSH, 2011]. However, there may also be considerable variability in the distribution of threshold responses in a population, which needs to be considered in a risk assessment.

Some evidence suggests that, because of the carbon composition of CNTs, these materials are not as easily recognized as foreign bodies, and the inflammatory process subsides within a few weeks after a short-term exposure [Shvedova et al., 2005, 2008; Porter et al., 2010]. Despite the decline in the inflammation, pulmonary fibrosis develops rapidly and persists or progresses after the end of exposure [Shvedova et al., 2005, 2008; Mercer et al., 2008, 2011], suggesting that the mechanism of action involves the thin CNT acting like the basement membrane, on which fibroblast cells grow [Wang et al., 2010a,b]. Such effect may be useful in medical applications such as bone grafts or artificial hips [Christenson et al., 2007; Li et al., 2009], but the effect is disruptive to normal lung architecture and function. In summary, CNTs, which are respirable, poorly soluble, and biopersistent, may follow a secondary genotoxic mode of action based on particle surface area if they elicit persistent inflammation.

Fiber-Specific Carcinogenesis

The high aspect ratio of many CNTs (i.e., narrow diameters and long lengths) and relatively high biopersistence of some types of CNTs support concern that the fiber paradigm of carcinogenesis might apply [Stanton, 1973; Davis et al., 1986; Donaldson et al., 2006, 2010; Donaldson, 2009; Jaurand et al., 2009; Sanchez et al., 2009]. Inhaled fibers may be ineffectively cleared from the lungs in part because the alveolar macrophages are not able to fully phagocytize (engulf) the longer fiber structures. Instead, these structures can extend from or pierce the cell and cause ''frustrated phagocytosis'' and leakage of ROS/RNS, which elicit generation of pro-inflammatory cytokines and cell growth mediators (Fig. 1). Inability to clear these structures, combined with low solubility, results in biopersistence at the site of deposition in the lungs (bifurcations of airways, in particular, as well as peripheral regions, with potential for retention in the interstitium or migration to the parietal and peritoneal pleura (linings of lung and abdomen, respectively). Biopersistent fibers such as asbestos have been associated with lung cancer in workers, especially long, thin fibers [Stayner et al., 2008] and with mesothelioma [Donaldson et al., 2010]. A hypothesis for the mechanism of action of mesothelioma is that the longer fibers (>5 μm) are physically unable to navigate through the stomata (openings) of the parietal and peritoneal pleura to be cleared into the lymph system; resulting in persistent inflammation, and eventually, mesothelioma [Donaldson et al., 2010; Murphy et al., 2011].

Experimental animal data show that inhaled SWCNTs and MWCNTs can reach the alveoli, penetrate the interstitium of the lung, and reach the subpleural tissue [Mercer et al., 2008, 2010; Shvedova et al., 2008; Ryman-Rasmussen et al., 2009; Porter et al., 2010]. Straight, rigid MWCNTs can migrate to the outer lining of the lungs and enter the intrapleural space [Mercer et al., 2010]. For some MWCNTs (median size 3.9 μm in length and 50 nm in diameter), transport to the subpleural tissue in mice was shown to be ''rapid and direct'' (0.6% within 24 hr). At 20 μg/lung, MWCNTs exhibited pleural penetration at >1 CNT for each mesothelial cell. A 20-μg dose contained approximately 15 million CNTs, which resulted in approximately 75 million CNTs per g of lung tissue (given a mouse lung weight of 0.2 g) [Mercer et al., 2010]. No adverse responses of the mesothelial cells were reported over a 2-month post-exposure period in that study [Mercer et al., 2010], although a 40-μg lung dose of MWCNTs (by pharyngeal aspiration) in mice caused early-onset persistent pulmonary fibrosis [Mercer et al., 2011].

Studies that have administered CNTs in the peritoneal cavity and intrascrotally have shown inflammation changes and granulomatous lesions of the abdominal wall and diaphragm [Poland et al., 2008; Takagi et al., 2008; Sakamoto et al., 2009; Kanno et al., 2010]. Takagi et al. [2008] have shown mesothelioma following intraperitoneal injection of 3 mg of MWCNTs (containing ~30% of structures >5 μm in length and 100 nm average diameter) in a sensitive mouse strain (p53+/−). The injected mass dose of 3 mg is considered quite high [Ichihara et al., 2008]. For example, 3 mg is several orders of magnitude greater than would be estimated to translocate to the pleura following chronic inhalation of 5 mg/m^3 in mice, assuming 1% alveolar deposition, and 1% penetration of CNTs from lung to pleura (estimated from Mercer et al.

[2010]). However, Kanno et al. [2010] reported mesothelioma after abdominal injection of 50 μg of MWCNTs/mouse. Furthermore, peritoneal mesothelioma (with metastases to the pleura) was reported after intrascrotal injection of MWCNTs (1 mg/kg) in a rat model [Sakamoto et al., 2009]. In a recent study in rats (Fischer 344/Brown Norway), mesothelioma was observed after intraperitoneal injection of 1 mg of some types of MWCNT with thin diameters, 4–5 μm lengths, and rigid structures [Nagai et al., 2011].

In another study, no mesothelioma was observed in rats 2 years after intraperitoneal injection of a single dose of either 2 or 20 mg of MWCNTs (short, average length <1 μm) [Muller et al., 2009]. These findings are consistent with the short-term intraperitoneal injection study showing low inflammation response to short CNTs but asbestos-like inflammation and granulomatous lesions on the diaphragm with the longer, straighter structures [Poland et al., 2008]. More recently, Murphy et al. [2011] showed length-dependent CNT retention, inflammation, and fibrosis in the parietal pleura in mice. In summary, CNTs that form fiber-like structures (e.g., $<\sim$3 μm diameter and $>\sim$5–10 μm in length), and are inhaled and deposited in the lungs, may follow the fiber paradigm carcinogenic mode of action.

Disruption of Cell Division

Various fibrous structures have been shown to interfere with cell division. In vitro, thin, short CNTs (either SWCNTs or MWCNTs, ~1–25 nm in diameter and ~500–1,000 nm in length)—suspended by sonication—have been shown to interfere with normal cell division (mitosis), disrupt the distribution of chromosomes, and cause abnormal chromosome numbers (aneuploidy) in the daughter cells at doses below those causing cytotoxicity [Sargent et al., 2009, 2011a,b]. These effects have been observed both in a cultured human cell line (bronchial epithelial cells, BEAS-2B) and in primary human epithelial cells (small airway epithelial cells, SAEC). The cell mitotic spindle damage and aneuploidy were observed 24-hr post-exposure [Sargent et al., 2009, 2011a,b]. It appears that CNTs can interact physically with mitotic spindles components or with proteins involved in chromosome segregation (e.g., actin and tubulin) to cause mitotic dysfunction [Sargent et al., 2009, 2010]. More recently, in an in vitro study using the same human cell types, SWCNT structures were observed in the cell nucleus in physical association with the microtubules and the DNA [Sargent et al., 2011b]. In addition, increased cell proliferation was seen 7 days after exposure to SWCNT, which indicates a greater potential to pass the genetic damage (aneuploidy) to daughter cells. Crocidolite asbestos has also been shown to induce aneuploidy in vitro in human cells [Yegles et al., 1995]. Aneuploidy has been implicated in several types of cancers in humans [Kops et al., 2005; Fukasawa, 2005].

Specific mechanisms of spindle disruption by SWCNTs or MWCNTs include the formation of microtubule/CNT hybrids, which disrupts the normal separation of chromosomes into the dividing cells [Sargent et al., 2009, 2010]. These hybrids can form when the CNT structures are similar in size to the microtubules. Other mechanisms have been shown to involve the formation of carbon bridges joining daughter cells of alveolar macrophages [Mangum et al., 2006] or anaphase bridges between the nuclei, resulting from ''misrepair'' of double-strand DNA breaks [Cveticanin et al., 2010]. Chrysotile asbestos has also been shown to disrupt cell division, but by a different mechanism involving interference with cytokinesis (cell movement) by forming bridges to prevent the normal separation of daughter nuclei [Asakura et al., 2010].

The impact of these short-term in vitro effects was evaluated in a recent experimental model involving primary small airways epithelial cells exposed for 25 weeks to dispersed SWCNTs or MWCNTs. A significant increase in cell proliferation, migration, invasion, and transformation (growth in soft agar) was observed from CNT-treated cells (at a dose as low as 0.02 $\mu g/cm^2$ of cell surface area) compared to controls [Stueckle et al., 2011]. NIOSH is currently investigating the ability of these transformed cells to develop into tumors in vivo by transplanting the CNT-elicited transformed cells into the abdominal cavity of nude mice. Earlier, abnormal nuclei and macrophages without nuclei were observed in mice 28 days after pharyngeal aspiration of 10–40 μg SWCNTs or MWCNTs [Shvedova et al., 2005, 2008; Porter et al., 2010; Hubbs et al., 2011]. These genotoxic events have been shown to occur at quite low mass doses that are relevant to potential occupational exposures (see Section ''CNT Doses Associated With Genotoxicity In Vitro or In Vivo'').

Carbon nanofibers (CNF), SWCNTs, and crocidolite asbestos induced significant increases in micronuclei in vitro in human primary small airways epithelial cells at 12 $\mu g/cm^2$ of cell surface area [Kisin et al., 2011]. Micronuclei are chromosome fragments, either with a centromere (aneugenic) or without a centromere (clastogenic), which can arise due to either chromosome breakage or disruption of mitotic apparatus. MWCNTs have also been shown to induce micronuclei formation in vitro [Muller et al., 2008]. Clastogenic micronuclei, resulting from DNA breaks, have been linked to ROS generation and the iron content in fibers [Shukla et al., 2005]. SWCNTs have been shown to cause DNA damage and activate the same oxidative stress signaling pathways associated with in vitro exposure to asbestos [Pacurari et al., 2011].

In summary, individual CNT structures (thin, short)—which may not be readily detectable by standard

gravimetric or microscopic methods—if deposited in the lungs, may be able to interfere with dividing cells and chromosome distribution through specific mechanisms of actions.

ROLE OF PHYSICAL–CHEMICAL PROPERTIES OF CNT

As discussed above, specific sizes and structures of CNTs have been associated with certain biological effects, including those thought to be involved in carcinogenic pathways. It would seem likely that the types of CNT structures to which workers may be exposed could also show carcinogenic potential. However, workers may be exposed to mixed types of CNT structures, depending on the processes in which they are involved, including CNTs with different wall number (SWCNT or MWCNT), levels of purity (and metal content), surface treatments or functionalization, and degree of agglomeration. CNTs may be mixed into composite materials, where they may become embedded and relatively inaccessible. CNT structures may become dispersed in air by energetic processes (e.g., vortexing, sonication, or blending CNTs) [Maynard et al., 2004; Methner et al., 2010]. CNTs can also be released from cutting or grinding CNT composites, with a few fibers per cubic centimeter of air (higher than the 0.1 - fiber/cm^3 asbestos standard) [Bello et al., 2009], although most of the CNT structures were bound with the composite matrix dust [Bello et al., 2009; Wohlleben et al., 2011]. One study in rats found no observed difference in the acute inflammation of ''degradation products from abraded nanocomposite filler'' either with or without the CNT nanofiller (3 days after intratracheal instillation); both of these materials caused lower inflammation than the CNT nanofiller itself [Wohlleben et al., 2011].

Airborne CNTs that enter the workers' breathing zones and are inhaled and deposited in the respiratory tract could pose a respiratory health hazard. In accordance with the fiber-carcinogenesis paradigm, those CNT structures that are dispersed, thin, long, and biopersistent are likely to pose the greatest risk for lung injury, including interstitial fibrosis [Mercer et al., 2008; Murphy et al., 2011] and cancer [Donaldson et al., 2011]. It is not known to what extent combinations of physical–chemical characteristics might mitigate the carcinogenic potential of CNTs, and whether resultant CNTs would have desired scientific or commercial functionality. However, efforts to design out the toxicity (prevention through design) of engineered nanoparticles, including CNTs, have been proposed [Schulte et al., 2010; Tinkle, 2010; Donaldson et al., 2011]. Research on how to design CNTs to maintain functionality but be less toxic is needed [Allen et al., 2008; Kagan et al., 2010].

CNT DOSES ASSOCIATED WITH GENOTOXICITY IN VITRO OR IN VIVO

Experimental Systems

Sargent et al. 2011a,b] reported in an in vitro study that 0.024 μg CNT/cm^2 cell surface resulted in significantly increased aneuploidy in human epithelial cells 24 hr after exposure to SWCNTs or MWCNTs (Table II). In the same cell type but with a 25-week exposure to CNTs, Stueckle et al. [2011] reported that 0.02 μg/cm^2 caused transformation of the cells to a cancer phenotype. Thus, to date, ~0.02 μg/cm^2 has been found to be the lowest observed adverse effect level (LOAEL) for in vitro genotoxicity [Sargent et al., 2011b; Stueckle et al., 2011]. In vivo in mice, a similar mass/surface area dose (0.01 μg/cm^2) resulted in K-ras mutations in lung tissue [Shvedova et al., 2008] (Table II). In contrast, a much higher dose (>2 orders of magnitude) of CNTs, SWCNTs, or crocidolite asbestos (12 μg/cm^2) caused chromosomal damage resulting in micronuclei formation [Kisin et al., 2011]. It is interesting that the lowest dose of ~0.02 μg/cm^2 associated with genotoxicity in two of the in vitro studies is similar to the estimated dose of 0.01 μg/cm^2

TABLE II. Experimental Studies In Vitro and In Vivo Showing Genotoxic Responses to Carbon Nanotubes or Nanofibers

Study reference	Carbon nanotube or fiber type	Genotoxic response[a]	Dose (μg/cm^2 cell surface)
In vitro—Human epithelial lung cells			
Stueckle et al. [2011]	SWCNTs, MWCNTs	Cell transformation	0.02
Sargent et al. 2011a,b	SWCNTs, MWCNTs	Mitotic spindle disruption, abnormal chromosome number	0.024
Kisin et al. [2011]	CNFs, SWCNTs	Micronuclei, chromosome abnormality	12
In vivo—Inhalation in mouse			
Shvedova et al. [2008]	SWCNTs	K-ras mutations	0.01[b]

SWCNTs, single-walled carbon nanotube; MWCNTs, multi-walled carbon nanotube; CNFs, carbon nanofiber.
[a]Exposure duration: 25 weeks [Stueckle et al., 2011]; 24 hr [Kisin et al., 2011; Sargent et al., 2011a,b]; 5 hr/day for 4 days and 28-day post-exposure [Shvedova et al., 2008].
[b]Estimated from Shvedova et al. [2008]: 5 μg deposited dose/500 cm^2 mouse lung surface area.

associated with K-ras mutations in the in vivo inhalation study in mice—when dose is expressed in both systems as the mass of CNTs per unit of cell surface area (Table II). K-ras mutations have been reported in both human and mouse lung tumors (although the specific types and incidence differed across species [Jackson et al., 2006]).

Extrapolation to Humans

The airborne size distribution of CNTs estimated as mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) in the in vivo inhalation study in mice is also a relevant measure of workplace airborne exposures [Shvedova et al., 2008]. The MMAD (GSD) of 3.5 μm (2.14) in Shvedova et al. [2008] was estimated from data reported in Baron et al. [2008] and was also used in a NIOSH risk assessment of noncancer lung effects of CNTs [NIOSH, 2010].

To normalize the human-equivalent lung dose of CNT to that in the mouse associated with K-ras mutations (0.01 $\mu g/cm^2$) [Shvedova et al., 2008], the lung dose can be adjusted for the species differences in the average alveolar lung surface area: 1,020,000 cm^2 in humans and 500 cm^2 in mice [Stone et al., 1992]. Thus, a lung dose of 0.01 $\mu g/cm^2$ is equivalent to 5 μg in mice, and 10,200 μg (or 10.2 mg) in humans. To estimate the number of work years to reach that human-equivalent deposited lung dose, additional information needed includes the fraction of airborne particles that deposit in the alveolar region, the volume of air inhaled by a worker per day, the number of workdays per year, and an estimated airborne mass concentration of CNT. The alveolar deposition fraction was estimated to be 0.08 (i.e., 8%), assuming equivalent deposition to that of spherical particles of the same size (MMAD and GSD), using the Yeh and Schum model in the multiple-path particle deposition model (MPPD, version 2.0) [CIIT and RIVM, 2006].[2] Assuming a worker is exposed at the NIOSH draft recommended exposure limit (REL) for CNTs or CNFs of 7 $\mu g/m^3$ (8-hr TWA concentration) [NIOSH, 2010]; breathes 9.6 m^3 air per 8-hr workday [ICRP, 1994]; and works 250 day/year, it would take approximately 8 years to reach the human-equivalent estimated deposited lung dose that was associated with K-ras mutations in mice [Shvedova et al., 2008]. That is: $7.6 \text{ work years} = 10.2 \text{ mg}/[0.007 \text{ mg}/m^3 \times 0.08 \times 9.6 \text{ } m^3/\text{day} \times 250 \text{ day/work year}]$. This calculation of equivalent deposited lung dose assumes spherical-particle behavior in air and does not account for any lung clearance of CNTs.

Uncertainties in Extrapolating Estimated Animal Lung Dose to Humans

There are a number of uncertainties in extrapolating animal data to humans. First, these are estimates of deposited dose only and do not take into account any clearance of CNTs from the lungs or the effect of dose rate. Evidence from animal studies suggests that CNTs may be biopersistent in the lungs, with overloading of lung clearance occurring at lower mass doses of CNT than expected for larger (and denser) respirable particle [Pauluhn, 2010a, 2011]. However, not all CNTs will have the same durability (e.g., due to functionalization) [Osmond-McLeod et al., 2011]. Second, the information on the nature of the CNT exposures in workers is limited, resulting in uncertainty about how physical and chemical properties of workplace airborne CNT exposures compare to the CNTs to which the mice were exposed or which were identified in vitro [Shvedova et al., 2008]. Third, it is not known to the extent to which the CNT agglomerates may become dissociated in the lungs to smaller—and potentially more bioactive—particles, although animal data suggest dispersed CNTs elicit a greater interstitial fibrotic response and can also migrate to the pleural lining of the lungs [Mercer et al., 2009, 2010].

Depending on their aerodynamic size, CNTs may deposit in other regions of the respiratory tract, including in the lung airways (particularly at bifurcations, if fiber-shaped). The lungs have evolved much faster clearance (via the mucociliary escalator) of particles that deposit in the airways (the thoracic region of the lungs), on the order of days (vs. months or years for particles deposited in the human alveolar region). Indeed, MWCNTs deposited on the conducting airways of mice following aspiration exposure were cleared by 1 week post-exposure [Mercer et al., 2011]. Nonetheless, the bronchial epithelial cells lining the airways are a target tissue for the development of lung cancer from exposure to various types of inhaled aerosols (including cigarette smoke and asbestos fibers). Thus, the fraction of airborne particles that deposit in the conducting zone (lung airways) may also be of concern. [As a precaution, workplace air monitoring of the thoracic size fraction (capable of depositing in the lung airways) may also be warranted to determine if there are potential exposures to CNTs capable of depositing in the lung airways.]

Finally, it should also be emphasized that, at this time, cancer has not been observed in any animal model following inhalation of CNTs. However, no chronic exposure studies of CNT exposure in animals have yet been published, and this remains a critical research need in order to reduce the uncertainty about the risk of chronic

[2] Additional model input parameters include: MMAD (GSD) of 3.5 μm (2.14); density of carbon: 2 g/ml; reference worker breathing parameters of 9.6 m^3 air intake/8-hr day (equal to 20 L/min, or 1,143 ml tidal volume and 17.5 breaths/min); oronasal normal augmenter; and inhalability adjustment.

effects, including cancer, from potential long-term exposure in workers. Despite the uncertainties in the experimental animal and cell studies, the relatively low doses at which effects were observed in those studies are relevant to possible workplace exposures and therefore warrant precaution.

WORKPLACE EXPOSURES TO CNTs

Workers are involved with CNTs throughout the nanomaterial lifecycle, from research in laboratories, through start up efforts, manufacture, incorporation of CNTs in products, manipulating and machining CNT-containing products, and finally through disposal, recycling, and end of life [Schulte et al., 2008]. Workers generally have higher exposures than the general population to substances produced for commerce particularly where the material of concern is not the final product but is an active intermediate used in multiple applications to enable or enhance product performance. This is especially likely for CNTs [Bello et al., 2009; NIOSH, 2009a; Wohlleben et al., 2011].

Exposure Measurements

Most studies of workplace air concentrations of CNTs have reported total mass concentrations associated with specific tasks involving production or handling of CNTs [Maynard et al., 2004; Han et al., 2008; Lee et al., 2010; Methner et al., 2010] or particle number concentration [Tsai et al., 2009; Johnson et al., 2010]. However, these mass concentration samples, for the most part, were collected as total mass and could not differentiate between possible CNT structures and other types of particles. Recently, two additional studies of workplace air concentrations have been published which collected mass based samples for elemental carbon, a relatively more specific marker for exposure to CNTs [Birch et al., 2011; Dahm et al., 2011]. At least two published studies have attempted to evaluate airborne fiber concentrations in workplaces producing CNTs or using CNT composite material (Table III), with one reporting a respirable concentration. These studies used NIOSH Method 7400 [NIOSH, 1994] (phase contrast microscopy, PCM, method for asbestos for counting structures >5 μm in length with a 3:1 aspect ratio; with visible limit of detection of approximately 250 nm diameter). Lee et al. [2010] reported observing no fibers in any personal sample, while Bello et al. [2009] reported a few fibers per cm^3 of air based on a short-term sample.

Airborne CNT structures are often agglomerated and do not conform to the 3:1 aspect ratio and thus would not be counted by PCM methods such as NIOSH Method 7400. In addition, structures <250 nm diameter would not be observed by this method, and structures shorter than 5 μm would not be counted with NIOSH Method 7400. Bello et al. [2009] reported the presence of thin, short structures (5–20 nm diameter, <1 μm length) in airborne samples collected during cutting of composite materials and analyzed by transmission electron microscopy (TEM), although the composition of these structures was not reported.

Because of the thin diameter and low effective density of CNTs in air, even a small mass concentration could contain a large number of individual structures and agglomerates. Table IV provides approximate comparisons of the mass and number concentrations for structures of various sizes. These calculations are based on assumed individual structure volume and density. Volume was estimated from the diameter and length, assuming a cylindrical shape of each structure; the density was assumed to be that of carbon ($\sim$2 g/cm^3). The CNT sizes illustrated in Table IV include those reported for SWCNTs and MWCNTs by Sargent et al. 2011a,b] (2–25 nm diameter, 500–1,000 nm length); CNFs by Kisin et al. [2011] (median approximately 100 nm diameter, 50 μm length); mean

TABLE III. Example of Workplace Airborne Particle and Fiber Number Concentrations in Workplaces Producing or Using CNTs

Study reference	Process	Particle number concentration (#/cm^3)	Respirable fiber concentration (#/cm^3)
Lee et al. [2010]	Catalyst preparation	37,000[a]	Not detected[c]
	Opening CVD	11,000[a]	
Bello et al. [2009]	Dry cutting composite		
	CNT-alumina	38,000[b]	1.6[d]
	CNT-carbon	294,000[b]	Not available[e]

[a]Scanning mobility particle sizer (3–685 nm).
[b]Fast mobility particle sizer (5.6–560 nm).
[c]NIOSH [1994] analytical method 7402 and Han et al. [2008] (all fibers and tubes with aspect ratio >3:1).
[d]NIOSH method 7400 (length >5–20 μm and aspect ratio >3:1).
[e]Sampling pump failed.

TABLE IV. Examples of Estimated Equivalent Fiber Number and Mass Concentrations

Fiber dimension (diameter × length; nm)	Fiber number concentration (fiber/cm^3) that is equivalent to 7 μg/m^3	Fiber mass concentration (μg/m^3) that is equivalent to 0.1 fiber/cm^3
2 × 500[a]	2,200,000	0.0000003
25 × 1,000[a]	7,100	0.00098
5 × 188,000[b]	950	0.00074
100 × 50,000[c]	8.9	0.078
29 × 773,000[d]	6.9	0.10
2,110 × 10,000[e]	0.10	7.0

[a]Examples of single-wall carbon nanotube (SWCNT) and multi-wall carbon nanotube (MWCNT) structure sizes reported by Sargent et al. [2009, 2011a,b].
[b]SWCNT mean diameter and length reported by Dahm et al. [2011].
[c]Approximate median dimensions of carbon nanofibers (CNFs) reported by Kisin et al. [2011].
[d]MWCNT mean diameter and length reported by Dahm et al. [2011].
[e]Example of a structure size that would result in a number concentration of 0.1 fiber/cm^3 and a mass concentration of 7 μg/m^3 (NIOSH draft REL for carbon nanotubes, CNTs and CNFs) [NIOSH, 2010].

MWCNT dimensions in a workplace; and an example of a structure size that would result in an equivalent number concentration of 0.1 fiber/cm^3 (>5 μm in length; ≥3:1 aspect ratio) and mass concentration of 7 μg/m^3 (NIOSH draft REL for CNT and CNF) [NIOSH, 2010]. It should be emphasized that these examples may not necessarily represent CNT or CNF airborne number concentrations in the workplace, where structures are often observed to be agglomerated rather than individual structures [Maynard et al., 2004; Methner et al., 2010].

Exposure Measurement Challenges

The physical structures of CNTs present challenges in exposure measurement. Electron micrographs of CNTs collected from a laboratory-generated aerosol are shown in Figure 2 and are representative of the heterogeneous structures typically seen on airborne samples from the workplace. These heterogeneous structures provide challenges to developing instruments and methods to measure exposure to the structures of greatest health concern and to distinguish these structures from background particulate matter.

Sampling instruments exist that are capable of obtaining size-specific information on CNT aerosols [Baron and Willeke, 2005]. However, these instruments provide information on all particles sampled and are not specific to CNTs. Although full characterization often requires analysis by electron microscopy, simple size-selective respirable mass fraction sample collection can be performed in some circumstances. This fraction is capable of depositing in the pulmonary (alveolar) region of the respiratory tract, where pulmonary inflammation and fibrosis can develop in response to particles and fibers that deposit in that region of the lungs (including CNT, as shown in animals, but not in humans to date). However, the collection of workplace respirable mass air samples often includes background ambient particulates including elemental carbon particles (e.g., diesel emissions, seasonal burning of biomass); thus electron microscopy methods are needed to determine what fraction of the mass concentration collected on the filter is the particle of interest (e.g., CNTs).

No microscopy-based methods have yet been developed for counting CNT structures (although efforts are under way, such as ASTM [2010]). In order to see the thinnest structures, electron microscopy methods are currently the only option. Developing more refined electron microscopy and exploring alternative methods are research needs. Which structures to count depends on the associated health outcome. For asbestos fibers, various counting rules have been developed to quantify exposures to structures of a certain dimension. For example, the current occupational exposure limits (OELs) are based on the air concentration of structures >5 μm in length, and >0.25 μm in diameter (as visible by phase contrast microscopy) [NIOSH, 1994]. Yet, these sizes may represent just a small fraction of the total number of asbestos fibers present in workplace air. For example, Dement et al. [2008] found that only 7–21% of the asbestos fibers in workplace air in a US chrysotile textile facility would be visible by PCM methods. Furthermore, the smallest diameter fibers (<0.25 μm) which were not visible by PCM, were in the size category most predictive of mortality from lung cancer or asbestosis in the textile worker cohort [Stayner et al., 2008]. Longer fibers (especially >10 μm) were also highly predictive of lung cancer mortality (but a trend with increasing length was not as clear with asbestosis mortality) [Stayner et al., 2008].

Thus, it is not clear how one would count CNT fiber-like structures in such heterogeneous configurations (Fig. 2). It would be virtually impossible to determine the number of individual CNT structures within an

agglomerate. These CNT structures often do not have typical fiber dimensions ($\geq$ 3:1 ratio of length:width), and thus they would not be counted under standard fiber counting criteria. Yet, these CNT agglomerated structures are often respirable size (e.g., MMAD $<$ 5–10 μm). The thoracic size fraction may also be of concern, especially for cancers of the bronchial epithelial lining. If so, the sampling size fraction should perhaps not be limited to the respirable size. In addition to specificity (distinction of CNTs from background airborne particles), sensitivity is also an issue; that is, the limit of detection or quantification of currently available sampling and analytical methods for CNTs. Because of the small diameter and low density of individual CNTs, a small mass concentration can be associated with a large number concentration (Table IV), which also has a large surface area (relative to the same mass of larger respirable particles). The mass concentration measurements may indicate a nondetectable mass concentration for CNTs, yet TEM analysis may still show a significant number of airborne CNT structures [Johnson et al., 2010; Methner et al., in press]. However, at this time it is still being recommended by NIOSH [2011] to collect a mass-based sample for elemental carbon by NIOSH Method 5040. By increasing the sample duration and flow rate as well as using a smaller diameter filter, the sensitivity can be increased [Dahm et al., 2011]. As larger quantities of material are put to use in facilities, a mass-based measurement may become much more practical. Ultimately, however, any CNT OEL meant specifically to prevent mesothelioma might have to be based on CNT structure number concentration, with the counting and sizing methodology specified.

RISK MANAGEMENT

Given the evolving state of the scientific evidence concerning the potential carcinogenicity of CNTs, it is appropriate to ask what specific risk management actions are likely needed to protect workers' health—if CNTs are ultimately shown to be carcinogenic. NIOSH and various investigators and organizations disseminated guidance on OELs and exposure controls [The Royal Society, The Royal Academy of Engineering, 2004; SCENIHR, 2006; BAuA, 2007; Nanocyl, 2009; NIOSH, 2009a, 2010; NICNAS, 2010; Nakanishi, 2011].

Exposure Control

The exposures of greatest concern are those situations where the CNTs are unbound, such as generation of dispersed aerosols that may occur through the handling of dry powder forms. Also of concern are any high-energy processes applied to CNT preparation such as mixing or sonication of CNTs in liquids. These processes may occur in research laboratories, start up operations, manufacturing, and during the incorporation of CNT into products (e.g., composites), and possibly during the downstream handling and manipulating of these products (e.g., cutting, drilling) [Bello et al., 2009; Wohlleben et al., 2011]. Because most CNT applications involve creation of a CNT-enabled material, the greatest potential for exposure exists during product fabrication. Otherwise, CNTs are generally expected to be bound in matrices and the extent to which consumers and the environment may have exposures is likely to be less than for workers.

Currently, very few studies have focused on investigating the effectiveness of common types of engineering controls used to reduce workplace exposures [Old and Methner, 2008; Tsai et al., 2010]. Generally, engineering controls and PPE are believed to be effective in reducing exposures, but they have not been assessed across the range of possible exposure concentrations and job tasks [NIOSH, 2009a]. Controls such as fume hoods may not be completely effective in controlling nanoparticle exposures [Tsai et al., 2010]. Exposure control techniques such as source enclosure (i.e., isolating the generation source from the worker) and well-designed local exhaust ventilation (LEV) systems equipped with high-efficiency particulate air (HEPA) filters have been shown to be effective for capturing airborne nanoparticles including CNT and CNF [Old and Methner, 2008; NIOSH, 2009a; Evans et al., 2010]. The need to evaluate controls and PPE will intensify as newer CNT production methods are developed and the number of commercial applications of CNTs increases.

Ultimately, if CNTs are found to pose a carcinogenic hazard, a strong evidence base will be needed to make the case that exposures should be controlled and to convince employers to make the investment to implement appropriate risk management practices. Until the evidence base on the carcinogenicity of CNTs is better developed, precautionary use of controls is essential [Schulte and Salamanca-Buentello, 2007; Schulte et al., 2008]. When more information is obtained, these levels of control can be adjusted to reflect the best available science.

Population at Risk

An impediment to understanding the extent to which workers may be at risk of exposure to CNTs is that little is known of the size of the work population and the extent of the risk. The data on both number of workers exposed and extent of exposure are sparse, and not necessarily reflective of the complete exposure situation. Also lacking is information on the extent to which engineering controls and PPE are being used to reduce exposures. A recent study of US manufacturers operating at small production scale (beyond research and development) found that

there were about 400 workers handling CNT or CNF [Schubauer-Berigan et al., 2011]. This study also estimated this workforce's growth to be 22% annually.

Work Practice Recommendations

Safe handling and exposure control requirements should include the following specific elements:

- Prohibiting the handling of dry powder CNTs except in ventilated exclosures known to be effective for controlling nanoparticles [Tsai et al., 2010].
- Containing tasks in processes that could result in the release of airborne CNTs or CNFs in the workplace.
- Handling of CNT as slurries rather than dry powders where possible.
- Dispensing, weighing, or sonication processes on well-designed ventilated benches or in effective ventilated exposures.
- Conducting spraying applications involving CNTs (or other nanoparticles) at the high level of protection needed for other spray operations, such as in paint booths, with face velocity adequate to contain releases and effective exhaust filtration, and providing workers with respiratory, eye, and skin protection.
- Controlling exposures during the sawing, drilling, and cutting of CNT composite material, for example, using well-designed LEV, to prevent adverse effects of the mixed dust.
- Evaluating the workplace for other potential hazards in the production and use of CNTs—such as elevated PAHs and carbon monoxide [Evans et al., 2010] and metal catalysts—which may present additional health hazards within facilities producing CNTs.
- Performing initial and periodic area and personal exposure monitoring to confirm the effectiveness of engineering controls.
- Instituting respiratory protection training and establish conditions for use (e.g., based on NIOSH respirator selection logic, including consideration of assigned protection factors for respirator selection [NIOSH, 2005]).
- Starting worker health and safety training programs that include communicating the current knowledge about the health hazards of CNTs and the work practices and procedures that are to be performed to reduce the risk of exposures and adverse health effects to CNTs.

Exposure Limits for CNTs

Additionally, to ensure implementation of exposure controls with proper effectiveness, quantitative guidance is needed in the form of OELs. NIOSH [2010] issued a draft REL for CNTs and CNFs based on the estimated risk of noncancer adverse lung effects (inflammation and fibrosis) for working lifetime exposure at the upper limit of quantification (LOQ; 7 $\mu g/m^3$) of the NIOSH sampling and analytical method for elemental carbon [NIOSH, 2010]. Other proposed OELs for specific types of CNTs have ranged from approximately 2 to 50 $\mu g/m^3$ [Ma-Hock et al., 2009; Nanocyl, 2009; Pauluhn, 2010b; Nakanishi 2011]. These OELs provide benchmarks for exposure controls, but the increasing data on the carcinogenic potential of certain types of CNTs provides sufficient evidence to take additional steps to reduce exposure further.

If the potential risk for respiratory cancer is linked to the dimensional characteristics of CNTs, then an OEL based on the tube count and size/unit volume of air may be an appropriate exposure metric, rather than the mass-based standard described above based on fibrosis [Donaldson et al., 2010]. However, given the incomplete toxicological information on the relationship between CNT size and effect, and the technical issues associated with the absence of analytical criteria for sizing and counting CNT structure and agglomerates, the airborne mass/unit volume of air may be the most feasible interim OEL metric. However, because even low mass concentration can involve very large numbers of CNT structures, the OEL could correspond to a very low mass concentration. In addition, a reliable method is needed to distinguish CNTs as elemental carbon from other types of elemental carbon (e.g., diesel soot, carbon black) that might be present as background contaminants. An electron microscopy count-based method, in addition to a mass-based method, may be needed to ensure that workers are not exposed to airborne CNTs, including at concentrations below those detectable by mass-based methods.

Secondary Prevention Measures

Given the uncertainty about the cancer risks to current and future CNT workers, there is a need, in addition to implementing precautionary controls, to monitor and study the health of the workforce. This will involve efforts in three areas. First, to be effective in assessing health effects, in risk communication, and in ongoing research, exposure registries should be developed for all workers with potential exposure to CNTs [Schulte et al., 2011]. Exposure registries have a long history in public and occupational health. They allow for identifying and following workers potentially at risk of disease for purposes of risk communication, medical monitoring, and inclusion in epidemiological studies. Second, medical surveillance of all CNT workers should be established [NIOSH, 2009b; Trout and Schulte, 2010; Schulte and Trout, 2011]. Guidance for medical surveillance of workers involved with CNTs has

been proposed and includes initial and periodic evaluations of the respiratory system [NIOSH, 2010]. Third, various epidemiologic studies (prospective and cross-sectional) should be developed [Schubauer-Berigan et al., 2011]. Because there has not been a long history of exposure to CNTs, it is likely that there has been neither adequate latency nor enough persons exposed to detect cancer through epidemiological studies. However, cross-sectional studies involving biomarkers and prospective studies could be developed (possibly including biomarkers of mutations, such as K-ras), chromosomal damage (e.g., micronuclei), or cell transformation (cell phenotype). In order to ensure the largest-possible population base, all three of these efforts should be international; coordination and cooperation will be essential to make sure that data are collected using the same metrics and methods.

DISCUSSION AND REFLECTION

The questions that arise from society and occupational safety and health practitioners and decision makers involve what actions are to be taken now, with the incomplete evidence base on the carcinogenicity of CNTs. More specifically, are current and future exposed workers at an increased risk of cancer from exposure to CNTs? The importance of this question is magnified by the very long latency period of mesothelioma [Brody, 1997] meaning workers exposed today may not develop cancer for 20–40 years. If significant exposures and risks are suspected, what should be done? Should certain types of CNTs be banned or restricted like many asbestos-containing products are in some countries, or could controls be put in place to protect workers so that society could obtain the benefits of CNTs? Clearly this is a complex issue because there are many types of SWCNTs and MWCNTs, and functionalized variants.

Although this commentary concerns the possible carcinogenicity of CNTs, it is not clear that cancer is necessarily the most sensitive or significant health endpoint of CNT exposures. There is also concern for pulmonary fibrosis, inflammation, and cardiovascular effects [NICNAS, 2010; NIOSH, 2010; Castranova and Mercer, 2011; Hubbs et al., 2011]. Based on evaluation of the animal data of pulmonary inflammation and fibrosis following exposure to CNTs, NIOSH [2010] estimated a $>10\%$ estimated risk over a working lifetime of early-stage fibrotic lung effects in workers at mass concentrations below the LOQ of the current measurement method (NIOSH Method 5040). Thus, more sensitive methods are needed to detect any exposures occurring below the LOQ. Electron microscopy-based methods are considered most sensitive, but they also have limitations [ASTM, 2009].

The rallying cry by many government, business, and labor interests with regard to nanotechnology is that "we should do it right" [Florini et al., 2008; Hansen et al., 2008; Van Zijverden and Sips, 2009]. What does doing it right mean? First and foremost, it means instituting the necessary exposure controls to protect workers in the event that the studies suggesting carcinogenicity of CNTs are confirmed in further testing. It means taking advantage of the opportunity for prevention through design [Schulte et al., 2008; Tinkle, 2010; Donaldson et al., 2010]. It also means evaluating the uses of this material, whether these products can be produced and used safely, including transport and disposal. Of particular concern are the small operations that produce small batches in research and development, and also during scale-up. Such small facilities may not have adequate expertise in exposure control technology, and outside resources may be needed to provide effective occupational safety and health guidance.

In the evolution of human civilizations, learning from the history and not repeating it has been a key guiding principle. Society can learn from how asbestos was inappropriately considered and not make the same mistake with CNTs. It is possible to safely realize the benefits of CNTs, but it will require rigorous and timely actions. The time to act is now.

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REFERENCES

Allen BL, Kichambare PD, Gou P, Vlasova II, Kapralov AA, Konduru N, Kagan VE, Star A. 2008. Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. Nano Lett 8(11): 3899–3903.

American Society for Testing and Materials International (ASTM). 2009. Standard test method for airborne asbestos concentration in ambient and indoor atmospheres as determined by transmission electron microscopy direct transfer (TEM). ASTM D6281-09. West Conshohocken, PA: ASTM.

American Society for Testing and Materials International (ASTM). 2010. New test methods for the analysis of air samples for carbon nanotubes by transmission election microscope. ASTM WK28561. West Conshohocken, PA: ASTM.

Asakura M, Sasaki T, Sugiyama T, Takaya M, Koda S, Nagano K, Arito H, Fukushima S. 2010. Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with chrysotile A fibers. J Occup Health 52(3):155–166.

Baron PA, Willeke K. 2005. Aerosol measurement, principles, techniques, and applications, 2nd edition. Hoboken, NJ: Wiley Interscience.

Baron PA, Deye GJ, Chen BT, Schwegler-Berry DE, Shvedova AA, Castranova V. 2008. Aerosolization of single-walled carbon nanotubes for an inhalation study. Inhal Toxicol 20(80):751–760.

Bellmann B, Muhle H, Creutzenberg O, Dasenbrock C, Kilpper R, MacKenzie JC, Morrow P, Mermelstein R. 1991. Lung clearance and retention of toner, utilizing a tracer technique, during chronic inhalation exposure in rats. Fundam Appl Toxicol 17(2):300–313.

Bello D, Wardle BL, Yamamoto N, de Villoria RG, Garcia EJ, Hart AJ, Ahn K, Ellenbecker MJ, Hallock M. 2009. Exposure to nanoscale particles and fibers during machining of hybrid advanced composites containing carbon nanotubes. J Nanopart Res 11(1):231–249.

Birch E, Ku BK, Evans D, Ruda-Eberenz T. 2011. Exposure and emissions monitoring during carbon nanofiber production-Part I: Elemental carbon and iron-soot aerosols. Ann Occup Hyg 55(9):1037–1047.

Brody AR. 1997. Asbestos. In: Sipes GA, McQueen CA, Gandolfi AJ, editors, Roth RA, volume editor. Comprehensive toxicology; Volume 8; Toxicology of the respiratory system. New York, NY: Elsevier Science Ltd. pp. 393–413.

Castranova V. 2000. From coal mine dust to quartz: Mechanisms of pulmonary pathogenicity. Inhal Toxicol 12(Suppl. 3):7–14.

Castranova V, Mercer RR. 2011. Responses to pulmonary exposure to carbon nanotubes. In: Donaldson K, Duffin R, Bonner J, Pol C, editors. The nanotoxicology of carbon nanotubes. Cambridge UK: Cambridge University Press.

Centers for Health Research (CIIT) and National Institute for Public Health and the Environment (RIVM). 2006. Multiple-path particle dosimetry (MPPD, version 2.0): A model for human and rat airway particle dosimetry. Research Triangle Park, NC: CIIT and The Netherlands: RIVM.

Christenson EM, Anseth KS, van den Beucken JJ, Chan CK, Ercan B, Jansen JA, Laurencin CT, Li W-J, Murugan R, Nair LS, Ramakrishna S, Tuan RS, Webster TJ, Mikos AG. 2007. Nanobiomaterial applications in orthopedics. J Orthop Res 25(1):11–25.

Cveticanin J, Joksic G, Leskovac A, Petrovic S, Sobot AV, Neskovic O. 2010. Using carbon nanotubes to induce micronuclei and double strand breaks of the DNA in human cells. Nanotechnology 21(1): 015102.

Dahm M, Yencken MS, Schubauer-Berigan MK. 2011. Exposure control strategies in the carbonaceous nanomaterial industry. J Occup Environ Med 53(6 Suppl):S68–S73.

Davis JM, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T. 1986. The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and injection. Br J Exp Pathol 67:415–430.

Dement JM, Kuempel ED, Zumwalde RD, Smith RJ, Stayner LT, Loomis D. 2008. Development of a fibre size-specific job-exposure matrix for airborne asbestos fibres. Occup Environ Med 65(9):605–612.

Donaldson K. 2009. The inhalation toxicology of p-aramid fibrils. Crit Rev Toxicol 39(6):487–500.

Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, Stone V. 2005. Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure. Part Fibre Toxicol 2:10.

Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A. 2006. Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol Sci 92(1):5–22.

Donaldson K, Murphy FA, Duffin R, Poland C. 2010. Asbestos, carbon nanotubes, and the pleural mesothelium: A review of the hypothesis regarding the role of long fibre retention in the parietal pleura inflammation and mesothelioma. Part Fibre Toxicol 7:1–17.

Donaldson K, Murphy F, Schinwald A, Duffin R, Poland CA. 2011. Identifying the pulmonary hazard of high aspect ratio nanoparticles to enable their safety-by-design. Nanomedicine (Lond) 6(1):143–156.

Driscoll KE. 1996. Role of inflammation in the development of rat lung tumors in response to chronic particle exposure. In: Mauderly JL, McCunney RJ, editors. Particle overload in the rat lung and lung cancer, implications for human risk assessment. Proceedings of the Massachusetts Institute of Technology Conference. Washington, DC: Taylor and Francis. pp. 139–153.

Driscoll KE, Deyo LC, Carter JM, Howard BW, Hassenbein DG, Bertram TA. 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis 18(2):423–430.

Elder A, Gelein R, Finkelstein JN, Driscoll KE, Harkema J, Oberdörster G. 2005. Effects of subchronically inhaled carbon black in three species. I. Retention kinetics, lung inflammation, and histopathology. Toxicol Sci 88(2):614–629.

Evans DE, Ku BK, Birch ME, Dunn KH. 2010. Aerosol monitoring during carbon nanofiber production: Mobile direct-reading sampling. Ann Occup Hyg 54(5):514–531.

Federal Institute for Occupational Safety and Health (BAuA) and German Chemical Industry Association (VCI). 2007. Guidance for handling and use of nanomaterials at the workplace. Berlin/ Dortmund/Frankfurt, Germany: BAuA and VCI.

Florini K, Walsh S, Balbus JM, Denison R. 2008. Nanotechnology: Getting it right the first time. J Clean Prod 16:1018–1020.

Fukasawa, 2005. Centrosome amplification, chromosome instability and cancer development. Cancer Lett 230:6–19.

Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, Lee SB, Ji JH, Cho MH, Yu IJ. 2008. Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. Inhal Toxicol 22:269–381.

Hansen SF, Maynard A, Braun A, Tickner JA. 2008. Late lessons from early warnings of nanotechnology. Nat Nanotechnol 3:444–477.

Hubbs AF, Mercer RR, Benkovic SA, Harkema J, Sriram K, Schwegler-Berry D, Goravanahally MP, Nurkiewicz TR, Castranova V, Sargent LM. 2011. Nanotoxicology—A pathologist's perspective. Toxicol Pathol 39(2):301–324.

Ichihara G, Castranova V, Tanioka A, Miyazawa K. 2008. Induction of mesothelioma in p53+/− mouse by intraperitoneal application of multi-walled carbon nanotube (Letter to the editor). J Toxicol Sci 33:381–382.

International Commission on Radiological Protection (ICRP). 1994. Human respiratory tract model for radiological protection. In: Smith H, editor. Annals of the ICRP. ICRP Publication No. 66. Tarrytown, New York: ICRP.

International Organization for Standardization (ISO). 2008. Nanotechnologies: Terminology and definitions for nano-object—nanoparticle, nanofibre and nanoplate. ISO/TS 27687:2008. Geneva, Switzerland: ISO.

Invernizzi N. 2011. Nanotechnology between the lab and the shop floor: What are the effects on labor? J Nanopart Res 13(6):2244–2268.

Jackson MA, Lea I, Rashid A, Peddada SD, Dunnick JK. 2006. Genetic alterations in cancer knowledge system: analysis of gene mutations in mouse and human liver and lung tumors. ToxicolSci 90:400–418.

Jaurand M-CF, Renier A, Daubriac J. 2009. Mesothelioma: Do asbestos and carbon nanotubes pose the same health risk? Part Fibre Toxicol 12:6.

Johnson DR, Methner MM, Kennedy AJ, Steevens JA. 2010. Potential for occupational exposure to engineered carbon-based nanomaterials in environmental laboratory studies. Environ Health Perspect 118(1):49–54.

Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, Volkov Y, Vlasova II, Belikova NA, Yanamala N, Kapralov A, Tyurina YY, Shi J, Kisin ER, Murray AR, Franks J, Stolz D, Gou P, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA. 2010. Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. Nat Nanotechnol 5:354–359.

Kanno J, Takagi A, Nishimura T, Hirose A. 2010. Mesothelioma induction by micrometer-sized multi-walled carbon nanotube intraperitoneally injected to heterozygous mice. Toxicologist 114: A1397.

Kisin E, Murray AR, Sargent L, Lowry D, Chirila M, Siegrist KJ, Schwegler-Berry D, Leonard S, Castranova V, Fadeel B, Kagan VE, Shvedova AA. 2011. Genotoxicity of carbon nanofibers: Are they potentially more or less dangerous than carbon nanotubes or asbestos? Toxicol Appl Pharmacol 252(1):1–10.

Kops GJ, Weaver BA, Cleveland DW. 2005. On the road to cancer: Aneuploidy and the mitotic checkpoint. Nat Rev Cancer 5:773–785.

Lee JH, Lee SB, Bae GN, Jeon KS, Yoon JU, Ji JH, Sung JH, Lee BG, Lee JH, Yang JS, Kim HY, Kang CS, Yu IJ. 2010. Exposure assessment of carbon nanotube manufacturing workplaces. Inhal Toxicol 22(5):369–381.

Li X, Gao H, Uo M, Sato Y, Akasaka T, Abe S, Feng Q, Cui F, Watari F. 2009. Maturation of osteoblast-like SaoS2 induced by carbon nanotubes. Biomed Mater 4:015005. DOI: 10.1088/1748-6041/4/1/015005.

Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, Ravenzwaay B, Landsiedel R. 2009. Inhalation toxicity of multi-wall carbon nanotubes in rats exposed for 3 months. Toxicol Sci 112(2):468–481.

Mangum JB, Turpin EA, Antao-Menezes A, Cesta MF, Bermudez E, Bonner JC. 2006. Single-walled carbon nanotube (SWCNT)-induced interstitial fibrosis in the lungs of rats is associated with increased levels of PDGF mRNA and the formation of unique intercellular carbon structures that bridge alveolar macrophages in situ. Part Fibre Toxicol 3:15.

Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V. 2004. Exposure to carbon nanotube material: Aerosol release during the handling of unrefined single-walled carbon nanotube material. J Toxicol Environ Health 67(1):87–107.

Maynard A. 2008. Carbon nanotubes: The new asbestos? Not if we act fast. The 2010 Science blog. http://2020science.org/2008/05/21/8521-carbon-nanotubes-the-new-asbestos-not-if-we-act-fast

Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, Shvedova AA, Castranova V. 2008. Alteration 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 294:L87–L97.

Mercer RR, Scabilloni JF, Wang L, Battelli LA, Castranova V. 2009. Use of labeled single walled carbon nanotubes to study translocation from the lungs. The Toxicologist 108:A2192.

Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Schwegler-Berry D, Castranova V, Porter DW. 2010. Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes. Part Fibre Toxicol 7:28.

Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Friend S, Castranova V, Porter DW. 2011. Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. Part Fibre Toxicol 8:21.

Methner M, Hodson L, Dames A, Geraci C. 2010. Nanoparticle emission assessment technique (NEAT) for the identification and measurement of potential inhalation exposure to engineered nanomaterials—Part B: Results from 12 field studies. J Occup Environ Hyg 7(3):163–176.

Methner M, Crawford C, Geraci C. [in press]. Evaluation of the potential airborne release of carbon nanofibers during the preparation, grinding, and cutting of epoxy-based nanocomposite material. J Occup Environ Hygiene.

Morrow PE. 1988. Possible mechanisms to explain dust overloading of the lungs. Fund Appl Toxicol 10(3):369–384.

Muller J, Decordier I, Hoet PH, Lombaert N, Thomassen L, Huaux F, Lison D, Kirsch-Volders M. 2008. Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells. Carcinogenesis 2(2):427–433.

Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D. 2009. Absence of carcinogenic response to multiwall carbon in a 2-year bioassay in the peritoneal cavity of the rat. Toxicol Sci 110(2):442–447.

Murphy FA, Poland Ca, Duffin R, Al-Jamal KT, Ali-Boucetta H, Nunes A, Byrne F, Prina-Mello A, Volkov Y, Shouping L, Mather SJ, Bianco A, Prato M, MacNee W, Wallace WA, Kostarelos K, Donaldson K. 2011. Length-dependent retention of carbon nanotubes in the pleural space of mice initiates sustained inflammation and progressive fibrosis on the parietal pleura. Am J Pathol 178(6):2587–2600.

Nagai H, Okazaki Y, Chew SH, Misawa N, Yamashita Y, Akatsuka S, Ishihara T, Yamashita K, Yoshikawa Y, Yasui H, Jiang L, Ohara H, Takahashi T, Ichihara G, Kostarelos K, Miyata Y, Shinohara H, Toyokuni S. 2011. Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. Proc Natl Acad Sci USA 108 (49):E1330–E1338.

Nakanishi J. 2011. Risk assessment of manufactured nanomaterials: ''Approaches''—Overview of Approaches and Results. Final report issued on August 17, 2011. NEDO project (P06041) ''Research and Development of Nanoparticle Characterization Methods.''

Nanocyl. 2009. Responsible care and nanomaterials case study nanocyl. Presentation at European Responsible Care Conference, Prague, 21–23 October, 2009. Sambreville, Belgium: Nanocyl. http://www.cefic.org/Documents/ResponsibleCare/04_Nanocyl.pdf.

National Industrial Chemicals Notification, Assessment Scheme (NICNAS). 2010. Human health hazard assessment,classification of carbon nanotubes. Australian Government Department of Health, Aging. Surry Hill, NSW, Australia: NICNAS.

National Institute for Occupational Safety and Health (NIOSH). 1994. NIOSH Manual of Analytical Methods. Method II 7400-Asbestos and other fibers by PCM. DHHS, NIOSH, Publication No 94-113.

National Institute for Occupational Safety and Health (NIOSH). 2005. NIOSH respirator selection logic. Cincinnati, OH: U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. NIOSH, DHHS, Publication No. 2005-100.

National Institute for Occupational Safety and Health (NIOSH). 2009a. Approaches to safe nanotechnology: Managing the health and safety concerns with engineered nanomaterials. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for

Occupational Safety and Health. NIOSH, DHHS, Publication No. 2009-25.

National Institute for Occupational Safety and Health (NIOSH). 2009b. Current intelligence bulletin 60: Interim guidance for medical screening and hazard surveillance for workers potentially exposed to engineered nanoparticles. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. NIOSH, DHHS, Publication No. 2009-116.

National Institute for Occupational Safety and Health (NIOSH). 2010. Occupational exposure to carbon nanotubes and nanofibers. Draft for public comment. Current intelligence bulletin, NIOSH Docket Number: NIOSH 161-A.

National Institute for Occupational Safety and Health (NIOSH). 2011. Current intelligence bulletin 63. Occupational exposure to titanium dioxide. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. NIOSH, DHHS, Publication No. 2011-160.

Oberdörster G, Ferin J, Lehnert BE. 1994. Correlation between particle-size, in-vivo particle persistence, and lung injury. Environ Health Perspect 102(Suppl. 5):173–179.

Old L, Methner M. 2008. Effectiveness of local exhaust ventilation (LEV) in controlling engineered nanomaterial emissions during reactor cleanout operations. J Occup Environ Hyg 5(6):D63–D69.

Osmond-McLeod MJ, Poland CA, Murphy F, Waddington L, Morris H, Hawkins SC, Clark S, Aitken R, McCall M, Donaldson K. 2011. Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. Part Fibre Toxicol 8:15.

Pacurari M, Schwegler-Berry D, Friend S, Leonard SS, Mercer RR, Vallyathan V, Castranova V. 2011. Raw single wall carbon nanotube-induced toxic effects in human bronchial epithelial cells: Comparison to asbestos. J Toxicol Environ Chem 93(5):1045–1072.

Pauluhn J. 2010a. Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: Toxic effects are determined by density of agglomerate structures, not fibrillar structures. Toxicol Sci 113(1):226–242.

Pauluhn J. 2010b. Multi-walled carbon nanotubes (Baytubes®): Approach for derivation of occupational exposure limit. Regul Toxicol Pharmacol 57(1):78–89.

Pauluhn J. 2011. Poorly soluble particulates: Searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation. Toxicology 279(1–3):176–188.

Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A. 2008. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathology in a pilot study. Nat Nanotechnol 3:423–428.

Porter DW, Hubbs AF, Mercer RR, Wu N, Wolfarth MG, Sriram K, Leonard S, Battelli LA, Schwegler-Berry D, Friend S, Andrew M, Chen BT, Tsuruoka S, Endo M, Castranova V. 2010. Mouse pulmonary dose- and timecourse-response induced by exposure to multiwalled carbon nanotubes. Toxicology 269:136–147.

Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong BA, Dodd DE, Andersen ME, Bonner JC. 2009. Inhaled carbon nanotubes reach the subpleural tissue in mice. Nat Nanotechnol 4:747–751.

Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, Hirose A, Nishimura T, Ohashi N, Ogata A. 2009. Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. J Toxicol Sci 34(1):65–76.

Sanchez VC, Pietruska JR, Miselis NR, Hurt RW, Kane AB. 2009. Biopersistence and potential adverse impacts of fibrous nanomaterials: What have we learned from asbestos? Wiley Interdiscip Rev Nanomed Nanobiotechnol 1(5):511–529.

Sargent LM, Shvedova AA, Hubbs AF, Salisbury JL, Benkovic SA, Kashon ML, Lowry DT, Murray AR, Kisin ER, Friend S, McKinstry KT, Battelli L, Reynolds SH. 2009. Induction of aneuploidy by single-walled carbon nanotubes. Environ Mol Mutagen 50(8):708–717.

Sargent L, Reynolds SH, Castranova V. 2010. Potential pulmonary effects of engineered carbon nanotubes: In vitro genotoxic effects. Nanotoxicology 4:396–408.

Sargent LM, Reynolds SH, Hubbs AF, Benkovic SA, Lowry DT, Kashon ML, Siegrist KJ, Mastovich J, Sturgeon JL, Bunker KL, Dinu CZ. 2011a. Understanding carbon nanotube genotoxicity. Toxicologist 120:A59.

Sargent LM, Hubbs AF, Young SH, Kashon ML, Dinu CZ, Salisbury JL, Benkovic SA, Lowry DT, Murray AR, Kisin ER, Siegrist KJ, Battelli L, Mastovich J, Sturgeon JL, Bunker KL, Shvedova AA, Reynolds SH. 2011b. Single-walled carbon nanotube-induced mitotic disruption. Mutat Res. Dec 8. [Epub ahead of print].

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2006. Request for a Scientific Opinion on the Appropriateness of Existing Methodologies to Assess the Potential Risks Associated with Engineered and Adventitious Nanotechnologies (SCENIHR/OO2/05). Brussels: SCENIHR.

Schubauer-Berigan MK, Dahm MM, Yencken MS. 2011. Engineered carbonaceous nanomaterials manufacturers in the United States, workforce size, characteristics and feasibility of epidemiologic studies. J Occup Environ Med 53(6 Suppl):S62–S67.

Schins RPF, Knaapen AM. 2007. Genotoxicity of poorly soluble particles. Inhal Toxicol 19(Suppl. 1):189–198.

Schulte PA, Salamanca-Buentello F. 2007. Ethical and scientific issues of nanotechnology in the workplace. Env Health Persp 115(1):5–12.

Schulte P, Geraci C, Zumwalde R, Hoover M, Kuempel E. 2008. Occupational risk management of engineered nanoparticles. J Occup Environ Hyg 5(4):239–249.

Schulte P, Geraci C, Hodson L, Zumwalde R, Castranova V, Kuempel E, Methner M, Hoover M, Murashov V. 2010. Nanotechnologies and nanomaterials in the occupational setting. Ital J Occup Environ Hyg 1(2):63–68.

Schulte PA, Mundt DJ, Nasterlack M, Mulloy K, Mundt KA. 2011. Exposure registries: Overview and utility for nanomaterial workers. J Occup Environ Med 53(6 Suppl):S42–S47.

Schulte PA, Trout DB. 2011. Nanomaterials and worker health: Medical surveillance, exposure registries, and epidemiologic research. J Occup Environ Med 53(6 Suppl):S3–S7.

Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. 2005. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free Radical Biol 34(9):1117–1129.

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, Ramsey 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(5):L698–L708.

Shvedova AA, Kisin E, Murray AR, Johnson VJ, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Keohavong P, Sussman N, Jin J, Stone S, Chen BT, Deye G, Maynard A, Castranova V, Baron PA, Kagan VE. 2008. Inhalation versus aspiration of single walled carbon

nanotubes in C57BL/6 mice: Inflammation, fibrosis, oxidative stress and mutagenesis. Am J Physiol: Lung Cell Mol Physiol 295(4): L552–L565.

Stanton MF. 1973. Some etiological considerations of fibre carcinogenesis. In: Bogovski P, Gilson JC, Timbrell V, Wagner JC, editors. Biological effects of asbestos. Lyon, France: International Agency for Research on Cancer (IARC). pp. 289–294.

Stayner LT, Kuempel E, Gilbert S, Hein M, Dement J. 2008. An epidemiologic study of the role of chrysotile asbestos fiber dimensions in determining respiratory disease risk in exposed workers. Occup Environ Med 65(9):613–619.

Stone KC, Mercer RR, Freeman BA, Chang LY, Crapo JD. 1992. Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissue. Am Rev Respir Dis 146(2):454–456.

Stueckle TA, Mishra A, Derk R, Rojanasakul Y, Castranova V, Wang L. 2011. In vitro assessment of chronic SWCNT and MWCNT exposure to lung epithelium. Toxicologist 120:A1182.

Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N. 2008. Induction of mesothelioma in p53+/− mouse by intraperitoneal application of multi-walled carbon nanotube. J Toxicol Sci 33:105–116.

The Royal Society, The Royal Academy of Engineering. 2004. Nanoscience and nanotechnologies. London: The Royal Society and The Royal Academy of Engineering. www.nanotec.org.uk/finalReport.htm

Tinkle SS. 2010. Maximizing safe design of engineered nanomaterials: The NIH and NIEHS research perspective. Advance review. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2(1):88–98.

Tran CL, Buchanan D, Cullen RT, Searl A, Jones AD, Donaldson K. 2000. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhal Toxicol 12(12):1113–1126.

Trout DB, Schulte PA. 2010. Medical surveillance, exposure registries, and epidemiologic research for workers exposed to nanomaterials. Toxicology 269(2–3):128–135.

Tsai S, Huang RF, Ellenbecker MJ. 2010. Airborne nanoparticle exposures while using conventional, constant velocity and air-curtain isolated hoods. Ann Occup Hyg 54(1):78–87.

Tsai S, Hofmann M, Hallock M, Ada E, Kong J, Ellenbecker M. 2009. Characterization and evaluation of nanoparticle release during the synthesis of single-walled and multi-walled carbon nanotubes by chemical vapor deposition. Env Sci Technol 43(15):6017–6023.

Van Zijverden M, Sips AJAM, editors. 2009. Nanotechnology in perspective. Risks to man and the environment RIVM Report 601785003/2009. Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM).

Wang L, Mercer RR, Rojanasakul Y, Qiu A, Lu Y, Scabilloni JF, Wu N, Castranova V. 2010a. Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts. J Toxicol Environ Health A 73(5):410–422.

Wang X, Xia T, Ntim SA, Ji Z, George S, Meng H, Zhang H, Castranova V, Mitra S, Nel AE. 2010b. Quantitative techniques for assessing and controlling the dispersion and biological effects of multiwalled carbon nanotubes in mammalian tissue culture cells. ACS Nano 4:7241–7252.

Wohlleben W, Brill S, Meier MW, Mertler M, Cox G, Hirth S, von Vacano Strauss V, Treumann S, Wiench K, Ma-Hock L, Landsiedel R. 2011. On the lifecycle of nanocomposites; comparing released fragments and their in-vivo hazards from three release mechanisms and four nanocomposites. Small 7(16):2384–2395.

Yegles M, Janson X, Dong HY, Renier A, Jaurand MC. 1995. Role of fibre characteristics on cytotoxicity and induction of anaphase/telophase aberrations in rat pleural mesothelial cells in vitro. Correlations with in vivo animal findings. Carcinogenesis 16(11):2751–2758.