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SINGLE- AND MULTI-WALL CARBON NANOTUBES VERSUS ASBESTOS: ARE THE CARBON NANOTUBES A NEW HEALTH RISK TO HUMANS?

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Carbon nanotubes (CNT), since their discovery, have become one of the most promising nanomaterials in many industrial and biomedical applications. Due to their unique physicochemical properties, interest is growing in the manufacture of CNT-based products and their subsequent marketing. Since their discovery, the prospect of possible undesirable human health effects has been a focus of many scientific studies. Although CNT possess unique physical properties that include (1) nanoscale diameter, (2) a wide length distribution ranging from tens of nanometers to several micrometers, and (3) high aspect ratio, the fibrous-like shape and durability suggest that their toxic properties may be analogous to those observed with other fibrous particles, such as asbestos. The present study provides a summary of published findings on CNT bioactivity, such as the potential of CNT, especially of multi-wall carbon nanotubes (MWCNT), to activate signaling pathways modulating transcription factor activity, induce apoptosis, induce DNA damage, and initiate biological responses. Assessment of risks to human health and adoption of appropriate exposure controls is critical for the safe and successful introduction of CNT -based products for future applications.

Among the several types of engineered nanoparticles, carbon-based nanoparticles are emerging as one of the most promising and revolutionizing nanomaterials for different purposes in many fields, including but not limited to industrial and biomedical applications (Huckzo, 2002). Iijima (1991) described multi-wall carbon nanotubes (MWCNT) consisting of many hollow cylinders of carbon atoms inside one another, as well as single-wall carbon nanotubes (SWCNT) with one layer of carbon atoms. Since then, carbon nanotubes have been extensively studied due to their distinctive properties, and in the last few years their synthesis and industrial applications have increased remarkably. It was reported that the global market for CNT in 2006 was $50.9 million with a growth rate of 73.8% (Olivet, 2007). Holman and Lackner (2006) projected that by 2014 the value of nano-manufactured goods will be $2.6 trillion . Therefore, with increasing industrial production of these nanomaterials and potential widespread distribution in consumer products, considerable concern has been raised in view of their environmental and human health effects (Maynard et al., 2006; Oberdörster et al., 2005; Papp et al., 2008). CNT-associated health hazard risks might occur not only with manufacture/workplace exposure, but also with general exposure from use, degradation, or disposal of commercial products, as well as from direct exposure to biomedical products (Donaldson et al., 2006; Gwinn & Vallyathan, 2006; Stern & McNeil, 2008). Currently, CNT are represented by three major types: SWCNT, MWCNT, and double-wall carbon nanotubes (DWCNT). Of these three types, SWCNT and MWCNT are

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the most studied for their ability to produce biological effects *in vitro* and experimental animal models.

CNT are of great interest to many applications due to their unique physicochemical properties. CNT are 10-fold stronger than steel, 1.2-fold harder than diamond, lightweight, heat resistant, and exhibit unique electrical properties for use in micro-electronics and other nanosize applications (Ebbesen et al., 1996).

In general, CNT length and diameter vary depending on the synthesis procedure. SWCNT are hydrophobic tubes of hexagonic carbon (graphene) with diameters less than 2 nm but with lengths that could be greater than 1 μm (Ajayan & Ebbesen, 1997; Ebbesen et al., 1996). However, due to a tendency to stick together, SWCNT rarely exist as individual nanotubes and mostly as agglomerated "ropes" of nanotubes with diameters that could be as high as 30 nm (Warheit et al., 2004). MWCNT consist of several concentric grapheme tubes with reported mean external diameters that may range from tens of nm to as high as 165 nm, with lengths of several μm or even higher (Bussy et al., 2008; Donaldson et al., 2006; Hou et al., 2003; Motta et al., 2005; Poland et al., 2008; Puretzky et al., 2002).

SWCNT, due to their extraordinary physical and chemical characteristics, are also used in a wide spectrum of applications. Some of the uses include dent-resistant car bodies, earthquake-resistant buildings, stain-resistant textiles, nanowires, semiconductors, transistors, chemical sensors, biomedical imaging, and drug and gene delivery vehicles (Blaise et al., 2008; Gwinn & Vallyathan, 2006). MWCNT are produced for many potential applications in analytical chemistry and other commercial uses (Lee et al., 2006; Mönch et al., 2007; Trojanowicz, 2006).

Although CNT exhibit attractive properties from an engineering perspective, a major concern is the physicochemical durability and apparent biopersistence of CNT in the lung (Lam et al., 2004; Lou et al., 2004; Nel et al., 2006). Furthermore, nanoparticle size, shape, chemistry, charge, surface modifications, and the presence of redox-active metals are some of the parameters that modulate biological activity and biokinetics of nanoparticles. Carbon nanotubes exhibit a high aspect ratio (≥100) along with poor solubility in aqueous media. This has led some in the scientific community to raise health concerns, particularly health concerns linked to past experience with hazardous fibers, especially asbestos (Ajayan & Ebbesen, 1997; Gwinn & Vallyathan, 2006; Lam et al., 2006; Maynard et al., 2006; Muller et al., 2005; Service, 1998). Recent studies suggested the MWCNT may be analogous to asbestos in the induction of mesothelioma, which has been the focus of a few studies (Poland et al., 2008; Takagi et al., 2008).

This review is focused to highlight the current research on CNT toxicity and the likelihood of CNT to behave like asbestos, although the comparison is limited due to very few studies. The term CNT in this review is used to include SWCNT and MWCNT.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF CNT

CNT are engineered nanosize tubes or fibrous ropes that structurally resemble a rolled up single layer or multiple layers of graphene sheets. There are several methods for CNT production, including deposition of carbon atoms vaporized by electric arc using graphite as the carbon source, laser ablation, and high-pressure carbon monoxide (HiPCO) (Wang et al., 2005). The last two methods produce CNT that can contain 30% or even higher percentages of metal catalysts such as Fe and Ni, by mass. Although, these materials undergo secondary processing for catalyst removal, there are still trace amounts of metals that remain in the nanomaterial, since the metals might be totally or partially encased in carbon, thus making the complete purification difficult without altering nanotubes structure. MWCNT may be fabricated without the use of metals; however, the use of metals in low quantities participates in the alignment of nanotubes. SWCNT and MWCNT produced by these methods contain residual catalytic metals or

other non-nanotube carbon materials (Arepalli et al., 2004; Ebbesen & Ajayan, 1992).

SWCNT are thin fibers that can be more than 1 μm long (Ajayan & Ebbesen, 1997; Lam et al., 2006). Due to Van der Waals forces between carbon atoms, engineered SWCNT do not exist as individual tubes, but rather agglomerate into bundles or ropes of 20 to 50 individual nanotubes that may be several nanometers in diameter (Arepali et al., 2004). In comparison with SWCNT, Van der Waals forces are much weaker for MWCNT, and, as a result, they often exist as single tubes or form a few bundles that microscopically appear as ropes (Yu et al., 2000).

The physical and chemical characteristics of CNT vary due to the use of different production methods and post-synthesis processing by the manufacturers (Lam et al., 2006). Cui et al. (2000) reported that MWCNT purity and physical characteristics show variations from batch to batch even when produced by the same manufacturer.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF ASBESTOS

Asbestos collectively refers to naturally occurring hydrated silicate minerals with fibrous morphology. There are two classes of asbestos: serpentines and amphiboles. The serpentine class includes only one major fiber type, chrysotile, while the amphibole class includes crocidolite, amosite, anthophyllite, tremolite, and actinolite. Both asbestos types consist of fibers of various length and diameters, with lengths ranging from 0.1 to more than 200 μm.In the past, asbestos was extensively used due to its valuable insulating and electrical properties, strength, and heat or chemical resistance. It was used in more than 3000 commercial products, resulting in its wide distribution in public areas and the environment (Manning et al., 2002). The most common pathogenic and carcinogenic asbestos types are amosite, crocidolite, tremolite, and to a lesser degree chrysotile. The critical factors associated with asbestos pathogenicity and carcinogenicity are physical/chemical properties such as biopersistence, pulmonary penetration potential, fiber length/diameter (aspect ratio), and ability to generate reactive oxygen species (ROS) (Shukla et al., 2003b; Vallyathan et al., 1998).

POSSIBLE SIMILARITIES IN THE FIBROUS ASPECT OF CROCIDOLITE AND CNT

Native crocidolite fibers range in length from less than a micrometer to several hundred micrometers. Mining and industrial processes produce fibers of various lengths. Fiber length and diameter are important factors in the pathogenicity of asbestos. Many studies examined the effects of long and short asbestos fibers and found that long fibers are more strongly associated with chronic lung lesions in humans and animal models. Long fibers in animal models were found to induce fibrogenic, inflammatory, and carcinogenic responses more potently than shorter fibers (Davis et al., 1986; Davis & Jones, 1988; Goodglick & Kane, 1996; Harington et al., 1975). Stanton et al. (1981) reported that implantation of any fiber with length of ≥8 μm and diameter of ≤0.25 μm in the pleural cavity was carcinogenic. Stanton and Wrench (1972) also reported that shorter fibers were not harmless, but rather their carcinogenic effect was slower to develop than to long fibers. Similarly, Goodglick and Kane (1990) showed that intraperitoneal (ip) injection of long crocidolite asbestos fibers induced intense inflammatory and cell death responses. Similar administration of short fibers failed to induce the same level of response due to removal of fibers from the peritoneal cavity. Evidence indicated that short fibers would be cytotoxic *in vivo* when fiber clearance is impeded. Stanton et al. (1981) also found that short and long fibers were toxic to macrophages *in vitro*. Another study by Pott et al. (1974) reported that fibers shorter than 10 μm in length induced tumors. Using milled chrysotile that contained few 10-μm-long fibers and 99.8% of the fibers were shorter than 5 μm in length 20% of the animals developed tumors.

A critical factor in asbestos-induced lung diseases, especially mesothelioma, is a prolonged latency period between exposure and disease development. The emerging paradigm for toxicity and pathogenicity of asbestos and synthetic vitreous fibers (SVF) involves dose, durability, and dimension. Whether a similar paradigm will emerge in the case of CNT is yet to be determined (Donaldson et al., 2006). Shvedova et al. (2005) suggested that the mechanism for interstitial fibrotic response to SWCNT differs from the classical mechanism proposed for fibrogenic particles, such as asbestos. Due to a strong tendency to bundle together, SWCNT and MWCNT bundles contain many nanotubes. These structures can be remarkably longer and wider than individual nanotubes. This may have important toxicological consequences. Indeed, the fiber paradigm implies that for a fibrous material to reach the alveolar region of the lung the fibers should have an aerodynamic diameter of less than 3 μm. Furthermore, the fibers to exhibit frustrated phagocytosis and impaired clearance their length needs to approach 20 μm (Craighead et al., 1982; Davis et al., 1986; Donaldson & Tran, 2004; Kane, 1996).

For fibers, high aspect ratio, biopersistence, and the ability to generate ROS appear to be critical factors involved in biological signaling cascades leading to cellular injury, proliferation, and inflammation in the lung and pleura and the development of fibroproliferative diseases (Shukla et al., 2003a). The fiber-like shape and durability have led some scientists to call for studies to investigate the potential pathogenic and carcinogenic potency of CNT (Donaldson et al., 2006; Service, 1998). Service (1998) has gone so far as to warn that CNT might be the next asbestos.

Although CNT have a strong tendency to bundle together, SWCNT and MWCNT aerosols may contain many long, thin nanotube structures. This has led scientists to propose strategies for assessing CNT pathogenicity, which include determination of endpoints that are known to be involved in classical fiber pathogenicity (Donaldson & Tran, 2004; Warheit et al., 1995). Gogotsi (2003) suggested that CNT need to be managed as potentially dangerous materials until more toxicologic, biologic, and immunologic tests are performed.

Whether there is a relationship between CNT length, diameter, and toxicity has not been examined in great detail, and there are few experimental studies that provide scientific evidence for an asbestos-like pathologic response especially to MWCNT (Pacurari et al., 2008b; Poland et al., 2008; Takagi et al., 2008).

DISEASES PRODUCED BY ASBESTOS

Asbestos exposure leads to development of pulmonary fibrosis (asbestosis), bronchogenic lung cancer, malignant mesothelioma, and pleural plaques (Manning et al., 2002; Mossman et al., 1990, 1996a; Vallyathan et al., 1998). Development of bilateral diffuse interstitial pulmonary fibrosis (asbestosis) is the most common clinical disease resulting from exposure to asbestos (American Thoracic Society, 1986). Epidemiologic studies indicated that asbestosis development occurs when there is a exposure to an excessive amount of asbestos. Studies also provided evidence that there is a threshold fiber dose in the range of 25 to 100 fibers/ml/yr (Browne, 1994; Cookson et al., 1986; Mossman & Churg, 1998).

Bronchogenic carcinoma is another well-documented disease produced by asbestos. There is an additive interaction between asbestos and cigarette smoking to induce substantial increase in lung cancer incidence (McDonald, 1990).

Mesothelioma is a rare tumor that affects the mesothelium of the serosal cavities. Pleural mesothelioma is the most common form, but mesothelioma can occur at other sites of the body, including peritoneum, pericardium, and tunica vaginalis of testes and ovaries. Exposure to asbestos is the primary cause that leads to the development of mesothelioma (Craighead & Mossman, 1982). Although asbestos use was reduced in the last decade, the incidence of mesothelioma may increase in Western Europe and the United States until approximately

2020, while in other less industrialized countries where asbestos use is less regulated, the mesothelioma epidemic may be longer (Britton, 2002).

A hallmark of asbestos exposure and mesothelioma development is the latency period between first exposure and diagnosis that can range from 20 to 40 years. This latency period suggests multiple genetic and cellular alterations are necessary for malignant transformation of mesothelial cells (Huncharek, 1995).

FACTORS IMPORTANT IN THE MECHANISM OF TOXICITY, PATHOGENICITY, AND CARCINOGENICITY OF ASBESTOS: ANY SIMILARITIES TO CNT?

The precise molecular mechanisms involved in the development and progression of mesothelioma are unclear, although experimental evidence suggests that the unique biopersistence of amphiboles and their ability to generate an unrelenting increase in ROS are critical mechanisms involved in the genesis of tumor development. The direct penetration of fibers together with the oxidant stress to the mesothelial cells was shown to produce a myriad of cytokines, chemokines, growth factors, and signaling events leading to the activation of transcription factors and early response genes, which are important in cell proliferation and genesis of asbestos-induced mesothelioma (Manning et al., 2002; Mossman et al., 1996a). A comparison of certain physical characteristics and intrinsic ability to generate ROS by crocidolite asbestos, MWCNT, and SWCNT is presented in Table 1.

Biopersistency

Fiber biopersistence is defined as the property of a material to persist and be retained in the lung over time without normal physiological clearance and any changes in the physical or chemical characteristics of the fibers. If the physical and chemical properties are changed during the retention of fibers, their toxicity may be altered. Biopersistence of a fiber depends on the site of deposition and the rate of translocation, clearance, dissolution, and biomodification of the fiber in lung (Donaldson & Tran, 2004; McClellan & Hesterberg, 1994). Persistency of CNT in the lung has been postulated, and there are a few studies suggesting that perhaps CNT may be biopersistent (Lam et al., 2004; Mercer et al., 2008; Muller et al., 2005). Lam et al. (2004) reported the presence of large aggregates of SWCNT in macrophages and in the alveolar space, and to a lesser degree in the interstitium in mice 90 d post intratracheal instillation. Mercer et al. (2008) using two types of SWCNT found that epithelioid macrophages "walled off" agglomerates of SWCNT, while more dispersed structures entered the alveolar interstitium.

The degree of CNT phagocytosis by macrophages was reported (Lam et al., 2004; Shvedova et al., 2005; Mercer et al., 2008). Lam et al. (2004) and Shvedova et al. (2005) noted that in comparison to carbon black (CB), macrophages containing SWCNT displayed a different behavior within the lung tissue. Agglomerated SWCNT were found on the alveolar septa surrounded by clusters of epitheliod macrophages forming granulomas. Smaller SWCNT structures rapidly entered the alveolar interstitum, while a relatively low percent of SWCNT were found in free alveolar macrophages (Mercer et al., 2008). In contrast, CB-containing macrophages were dispersed in the alveolar space. Dust contained in free macrophages on the alveolar surface was moved upward and cleared into the esophagus, whereas dust in the alveolar interstitium or encased in a granuloma was not easily cleared (McClellan, 1997). Lam et al. (2004) proposed that if a particle is biopersistent, toxic, and irritating, persistent interactions between particles and the cells in the interstitium facilitate the formation of lesions that progress over time. In the case of CNT, Lam et al. (2004) and Shvedova et al. (2005) reported the presence of interstitial fibrotic lesions that progressed over time 90 and 60 days postexposure, respectively. This suggested the biopersistent and toxic nature of CNT. Furthemore, data suggested that CNT are insoluble and most

TABLE 1. Comparison of Physical Characteristics and Biological Responses to CNT and Crocidolite Asbestos Important in Human Diseases Development

Key factors	Crocidolite	MWCNT	SWCNT
Length	+++ (Vallyathan et al., 1992)	++ (Muller et al., 2008; Pacurari et al., 2008b; Poland et al., 2008)	+ (Pacurari et al., 2008a; Warheit et al., 2004; Tian et al., 2006)
Diameter	+++ (Vallyathan et al., 1992)	++ (Muller et al., 2008; Pacurari et al., 2008b; Poland et al., 2008)	+ (Pacurari et al., 2008a; Shvedova et al., 2008; Tian et al., 2006)
Biopersistence	+++ (Macdonald & Kane, 1997)	++ (Muller et al., 2005)	+ (Lam et al., 2004)
Pulmonary clearance	+++ (Macdonald & Kane, 1997)	++ (Muller et al., 2005)	?
Present in alveolar intertitium	+++ (Dodson et al., 1991)	+ Muller et al., 2005	+ (Lam et al., 2004; Mercer et al., 2008; Shvedova et al., 2008)
Translocate to pleura	+++ (Craighead & Kane, 1994; Dodson et a1., 1991)	++ (Hubbs et al., personal communication)	?
Mesothelioma	+++ (Craighead & Kane, 1982;1994; Mossman & Gee, 1989; Suzuki et al., 2005)	+ (Takagi et al., 2008; Poland et al., 2008)	?
Acellular ROS generation	+++ (Vallyathan et al., 1992)	No ROS (Fenoglio et al., 2006; Pacurari et al., 2008a, 2008b)	+ (Kagan et al., 2006; Pacurari et al., 2008a, 2008b)
Cellular ROS generation	+++ (Vallyathan et al., 1992)	+ (Pacurari et al., 2008b; Pulskamp et al., 2007)	++ (Shvedova et al., 2003; Manna et al., 2005; Pacurari et al., 2008a; Sharma et al., 2007)

Note.+ Signs represent particle characteristics and their ability to produce toxic responses. One plus sign + denotes a low biological response or smaller physical size compared to crocidolite. Two plus signs ++ denote moderate biological response or a greater similarity compared to crocidolite. Three plus signs +++presented for crocidolite indicates itseffect is significantly greater than all the biological responses and physical characteristics of SWCNT and MWCNT. The question mark indicates no experimental evidence.

likely are one the most biologically non-degradable man-made materials (Lam et al., 2004).

Muller et al. (2005) noted the persistence of MWCNT in animal lungs 60 d post exposure regardless of the particles' state, ground or unground, respectively. Similarly to SWCNT, MWCNT induced pulmonary granulomas associated with deposition of large agglomerates of SWCNT in the airways. In contrast to unground MWCNT, ground MWCNT were well dispersed in the lung parenchyma. Whether CNT behave similarly to amphibole asbestos in terms of biopersistence remains to be elucidated by studies conducted over longer observation periods.

Penetration and Translocation

Asbestos and refractory ceramic fibers translocation from lung into other tissues, including pleura and peritoneal tissues, was reported (Gelzleichter et al., 1999; Suzuki & Kohyama, 1991; Kohyama & Suzuki, 1991). There are no major studies concerning the ability of CNT to translocate to extrapulmonary sites. However, some scientists suggested that MWCNT might have the potential to translocate or penetrate to the pleura (Donaldson

et al., 2006). In a preliminary study, Hubbs et al. (personal communication) reported that 50% of mice exposed by pharyngeal aspiration to MWCNT had MWCNT-laden macrophages in the pleural lymphatics. Well-dispersed SWCNT were reported to evade phagocytosis and rapidly penetrate the epithelial barrier and enter the interstitium of alveolar walls (Mercer et al., 2008). However, translocation of SWCNT has not yet been demonstrated.

Toxicity and Fibrogenicity of CNT and Potential to Induce Carcinogenicity

Several studies reported the adverse cellular and pulmonary reactions after pharyngeal or intratracheal instillation of SWCNT in rats or mice (Lam et al., 2004; 2006; Mercer et al., 2008; Muller et al., 2005; Shvedova et al., 2005; Warheit et al., 2004). The first *in vivo* study by Lam et al. (2004) showed that a single intratracheal (IT) instillation of SWCNT to mice induced a dose-dependent development of epitheliod granulomas, interstitial inflammation, peribronchial inflammation, and necrosis that progressed into alveolar septa. The effects were more pronounced in animals after 90 d postexposure. Different results were observed when carbon black (CB) or quartz was administered to mice. CB-treated animals were normal, whereas high doses of quartz produced mild inflammation in animals. Lam et al. (2004) concluded that on an equal-weight basis, if CNT reached the lungs, CNT-mediated toxicity would be greater than that of CB or quartz. Shvedova et al. (2005) exposed C57BL/6 female mice to SWCNT by pharyngeal aspiration and found that the animals developed a robust but acute inflammation, persistent interstitial fibrosis, and granulomatous lesions. However, Warheit et al. (2004) using it instillation of SWCNT to rats reported transient inflammation and cell injury, and non-dose-dependent multifocal granulomas that were non-uniform in distribution and did not progress beyond 1 mo postexposure. Warheit et al. (2004) suggested that more research was needed in regard to CNT lung toxicity.

In vitro studies using different cell systems exposed to raw SWCNT (iron [Fe] contaminated) were reported to produce a concentration- and time-dependent rise in intracellular formation of ROS and loss of glutathione level (Shvedova et al., 2003; Kagan et al., 2006; Sharma et al., 2007). ROS generation and cytotoxicity appeared to be due to high levels of Fe contamination in raw SWCNT. A more comprehensive study on CNT toxicity compared the toxic effects of five engineered carbon nanomaterials, including SWCNT and MWCNT, to their physicochemical characteristics and reported that refined SWCNT are most toxic to fibroblasts. Refined SWCNT induced apoptosis/necrosis and affected cell adhesion, while unrefined SWCNT (metal contaminated) induced ROS and oxidative stress (Tian et al., 2006). Based on these results, data suggested that surface chemistry is the variable that best predicts adverse effects of carbon nanomaterials. It was proposed that a mechanism that modifies surface chemistry alters both aggregation and the toxic effect of carbon nanomaterials, indicating that more aggregated unrefined SWCNT and MWCNT are less toxic compared to refined carbon nanomaterials with a larger surface area. Evidence thus indicates that SWCNT exert an intrinsic toxicity to fibroblasts regardless of the presence of transition metals. However, it is worth noting that particle surface area or particle number is a critical factor for bioactivity.

Pacurari et al. (2008a) investigated the effects of raw unpurified SWCNT (metal contaminated) on human mesothelial cells in comparison with crocidolite asbestos containing significant Fe concentration. Raw SWCNT and crocidolite induced ROS, DNA damage, apoptosis/necrosis, and activation of MAPK, and activated the transcription factors activator protein-1 (AP-1) and nuclear factor kappa-B (NF-κB). However, at similar mass concentrations, crocidolite was more potent and produced greater toxicity and molecular activation of several signaling events. Cellular viability, toxicity, and moderate genetic changes were also reported for SWCNT in other cell types, including fibroblast and epidermal cells (Kisin et al., 2007; Muller et al., 2008).

MWCNT have been compared to asbestos (Service, 1998; Harris, 1999; Huczko et al., 2001). Harris (1999) showed that MWCNT, structurally, are similar to asbestos, by comparing transmission electron microscopy (TEM) images of MWCNT to chrysotile asbestos; the particles both appear as long, thin, needle-like fibers. Toxic potential of MWCNT was explored in a few *in vivo* and *in vitro* studies. Muller et al. (2005) administered ground or unground MWCNT by it instillation to rats and found inflammation, granuloma formation, and biopersistence in the lung after 60 d. Muller et al. (2008) also reported genotoxic potential of MWCNT after a single it administration of MWCNT to rats or *in vitro* exposure of rat lung epithelial cells as evidenced by a concentration-dependent increase in micronuclei (MN) formation in type II pneumocytes and in epithelial cells. Another study by Monteiro-Riviere et al. (2005) showed that MWCNT were present within and induced an inflammatory response in human epidermal keratinocytes. Witzmann and Monteiro - Riviere (2006) found that MWCNT altered the levels of proteins with functions such as cell metabolism, cell signaling, stress, and cytoskeletal vesicular trafficking in human epidermal keratinocytes. *In vitro* incubation of T lymphocytes with pristine or oxidized MWCNT resulted in concentration- and time-dependent adverse effects, which were 10-fold lower for pristine MWCNT (Bottini et al., 2006). Pacurari et al. (2008b) investigated the effects of MWCNT containing low levels of Fe on human mesothelial cells in comparison with crocidolite asbestos known to possess a high level of Fe. MWCNT and crocidolite induced DNA damage, apoptosis/necrosis, activation of H2A.X, a variant of histone H2A that is activated following DNA damage, and activation of MAPK and transcription factors AP-1 and NF-κB. MWCNT generated a lower level of ROS. However, at similar mass concentrations, crocidolite was more potent compared to MWCNT.

Whether MWCNT have the potential to induce mesothelioma was first investigated by a Japanese team of scientists (Takagi et al., 2008). Takagi et al. (2008) reported that a high dose (3 mg/mouse) intraperitoneal (ip) injection of MWCNT into p53 heterozygous mice resulted in the development of mesothelioma after 144 d. Data suggested that fibrous or rod-shaped MWCNT behaved like asbestos, promoting a carcinogenic mechanism. The relevance of this study has been questioned due to the use of an extremely high dose of MWCNT (Donaldson et al., 2008; Ichihara et al. 2008). Another study by Poland et al. (2008) showed that abdominal instillation of long MWCNT (50 µg/mouse) resulted in asbestos-like, length-dependent inflammation of the abdominal walls 7 d post exposure. The results of this study prompted Poland et al. (2008) to suggest the need for more research and caution before introducing CNT products into the market. Although the study by Takagi et al. (2008) was received by some scientists with skepticism, others indicated that despite the fact that these studies are preliminary, the results suggest the possibility of an increased risk of cancer development from exposure to long, rigid MWCNT (Kostarelos, 2008; Kane & Hurt, 2008).

MOLECULAR MECHANISMS IMPLICATED IN THE PATHOGENICITY AND CARCINOGENICITY OF ASBESTOS AND CORRELATION WITH CNT

The molecular mechanisms involved in asbestos-induced diseases are complex. Numerous studies in animal models and cell cultures have provided an understanding of the effects of asbestos on cells with respect to biological interactions that produce persistent ROS generation, resulting in the release of pro-inflammatory cytokines and chemokines, DNA damage, phosphorylation of MAPK, activation of transcription factors, and induction of early response genes. These cascades of events lead to cell proliferation and culminate in fibrogenesis and carcinogenesis. ROS generation by "frustrated" phagocytosis is considered to be a major factor involved in the sustained cellular signaling (Mossman et al., 1996a; Manning

et al., 2002). The redox reactions are multifaceted, being delivered from fiber generation of ROS due to the large amounts of Fe present in asbestos fibers and cell-derived ROS produced by sustained "frustrated" phagocytosis. Evidence reported in the literature suggests that CNT may be biopersistent, be fibrous, and induce the formation of ROS, characteristics that may induce biologic reactions that subsequently promote the development of human diseases similar to those produced by asbestos.

REACTIVE OXYGEN SPECIES (ROS) AND OXIDATIVE STRESS

Cell signaling by asbestos fibers is suggested to occur through the generation of oxidants, including reactive oxygen /nitrogen species (ROS/RNS), either through their surface redox potential or through the interaction and uptake of fibers by cells (Ramos-Nino et al., 2002). Transfection of the manganese-containing superoxide dismutase gene (Mn-SOD), free radicals scavengers, or Fe chelators prevented asbestos-induced cytotoxicity of hamster tracheal epithelial cells (Mossman et al., 1996b). The production of oxidants by alveolar macrophages and other cell types during the respiratory burst and enhanced by "frustrated" phagocytosis of asbestos fibers contributes to the initiation of an inflammatory cascade of events leading to cell signaling and activation of interconnected cellular mechanisms favoring proliferation and malignant transformation (Cerutti, 1985; Hansen & Mossman, 1987; Manning et al., 2002).

Unpurified SWCNT containing high levels of Fe was shown to induce the generation of ROS and oxidative stress in cellular system models (Kagan et al., 2006; Manna et al., 2005; Monteiro-Riviere et al., 2005; Pacurari et al., 2008a; Sharma et al., 2007; Shvedova et al., 2003). Shvedova et al. (2003) found that exposure of human keratinocytes to SWCNT containing 30% Fe by mass resulted in formation of peroxidative products and antioxidant depletion. Sharma et al. (2007) exposed rat epithelial cells to SWCNT and reported a decrease in the levels of superoxide dismutase enzymes (SOD-1 and SOD-2), but the use of rotenone, a mitochondrial inhibitor, exerted no marked effects on ROS species, whereas the use of glutathione, *N*-acetylcysteine (NAC), and vitamin C decreased the levels of ROS. Pacurari et al. (2008a, 2008b) demonstrated that human mesothelial cells exposed to SWCNT or MWCNT induced generation of ROS to a lesser degree compared to crocidolite. SWCNT used by Pacurari et al. (2008a) contained only negligible amounts redox active Fe but contained nickel and yttrium in greater concentrations. MWCNT used by Pacurari et al. (2008b) also contained only a low concentration of redox-active Fe and other metal contaminants. Both CNT types generated ROS and persistent interactions with mesothelial cells resulted in molecular alterations similar to those induced by crocidolite asbestos but to a lesser degree.

ACTIVATION OF MITOGEN-ACTIVATED KINASES (MAPK)

The activation and transactivation of many transcription factors regulated by MAPK pathways is crucial in promoting cell proliferation and carcinogenesis. Interaction of cells with asbestos fibers results in the activation of numerous signaling cascades involving MAPK, NF-κB, and AP-1 (Karin, 1995; Shukla et al., 2003a). Asbestos fibers activate ERK1/2 that is necessary for cell proliferation (Scapoli et al., 2004). Goldberg et al. (1997) found that in mesothelial cells exposed to asbestos fibers, apoptosis and cell proliferation occur in a dynamic balance. The ERK 1/2 pathway is stimulated predominantly by several growth factors and oxidative stress. ERK pathway plays a central role in cell proliferation. In mice, inhalation exposure to asbestos produced a marked phosphorylation of ERK1/2 within pulmonary epithelial cells, and mice developed fibrotic lesions after 14–20 d of asbestos inhalation (Robledo et al., 2000). The activation of ERK cascade in mesothelial cells by asbestos is due to auto-phosphorylation of the EGF receptor, an event that may initiate cell signaling cascades important for asbestos-induced cell proliferation and mesothelioma development (Pache et al., 1998).

Among the MAPK family members, p38 and ERK 1 /2 were reported to be activated by SWCNT and MWCNT in normal human and malignant mesothelial cells in a concentration- and time-dependent manner (Pacurari et al. 2008a; 2008b). Another study by Manna et al. (2005) reported activation of ERK1/2 by SWCNT in a concentration-dependent manner in human keratinocytes. The potential contribution of p38 and ERK1/2 in transactivation of transcription factors AP-1 and NF-κB was postulated to be significant (Manna et al., 2005; Pacurari et al., 2008a, 2008b).

ACTIVATION OF TRANSCRIPTION FACTORS AP-1 AND NF-κB

MAPK, transcription factors AP-1 and NF-κB, and the early response genes regulated by these pathways are induced by ROS generated after asbestos exposure (Janssen et al., 1995; Mossman et al., 1997; Simeonova & Luster, 1996; Su & Karin, 1996). Transcription factor AP-1 is a dimer comprised of members of homo- and heterodimers of the early-response protoocongenes of the Jun and Fos proteins. NF-κB complexes are dimers of proteins of the Rel family (Beauerle et al., 1988). AP-1 and NK-κB are redox-sensitive transcription factors and regulate the expression of genes mediating inflammation, proliferation, apoptosis, and cellular transformation (Angel & Karin, 1991; Janssen et al., 1995). Several lines of evidence indicate that inducible transcription factors, AP-1 and NF-κB, participate in oncogenic transformation and carcinogenesis (Baldwin, 1996; Schutte et al., 1989).

Exposure of rat pleural mesothelial cells or hamster tracheal epithelial cells to crocidolite asbestos resulted in concentration-dependent activation of AP-1 and persistent expression of *c-fos* and *c-jun* genes (Heintz et al., 1993). A persistent, time-dependent activation of AP-1 was noted 3 d following a single dose of crocidolite asbestos in the lung, and increased activation in bronchial tissues was reported in a transgenic mouse model (Ding et al., 1999). These results suggested a model where asbestos-induced carcinogenesis involves chronic stimulation of cell proliferation through the activation of an early-response genes pathway. BéruBé et al. (1996a; 1996b) also reported cell proliferation, apoptosis, and histopathologic changes in rat lungs and mesothelial cells after exposure to asbestos. Crocidolite asbestos was reported to increase NF-κB activation in a protracted and concentration-related manner in hamster tracheal epithelial, lung, and pleural mesothelial cells (Janssen et al., 1995, 1997). Faux and Howden (1997) treated lung rat fibroblast cells with asbestos and found activation of NF-κB and AP-1 that was mediated through ROS-induced lipid peroxidation and arachidonic acid metabolism. The presence of vitamin E decreased ROS-dependent transactivation of AP-1 and NF-κB to control levels. This NF-κB activation was also shown to be important in the increased expression of the pro-inflammatory cytokine interleukin (IL)-8 in human pulmonary epithelial cells (Simeonova & Luster, 1996). Furthermore, an NF-κB-dependent rise in cytokine expression was reported to play a key role in asbestos-induced lung cancer and mesothelioma development (Mossman & Churg, 1998).

Several studies showed that SWCNT and MWCNT exhibit the potential to activate AP-1 and NF-κB in several cell types (Chou et al., 2008; Manna et al., 2005; Pacurari et al., 2008a,

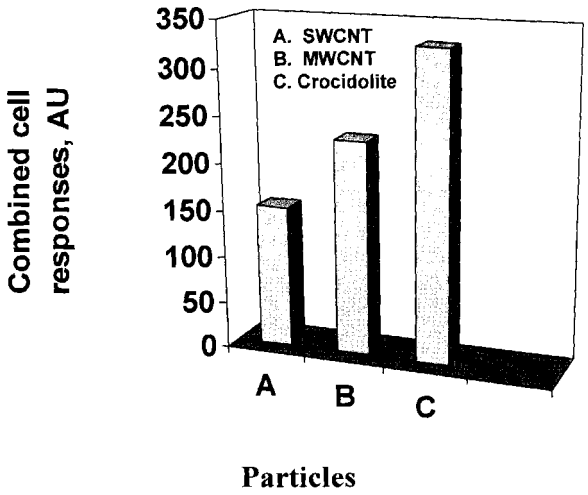

FIGURE 1. A comparative model for the combined toxic, biologic, and molecular responses of mesothelial cells to CNT and crodidolite compiled from the studies by Pacurari et al. 2008a; 2008b.

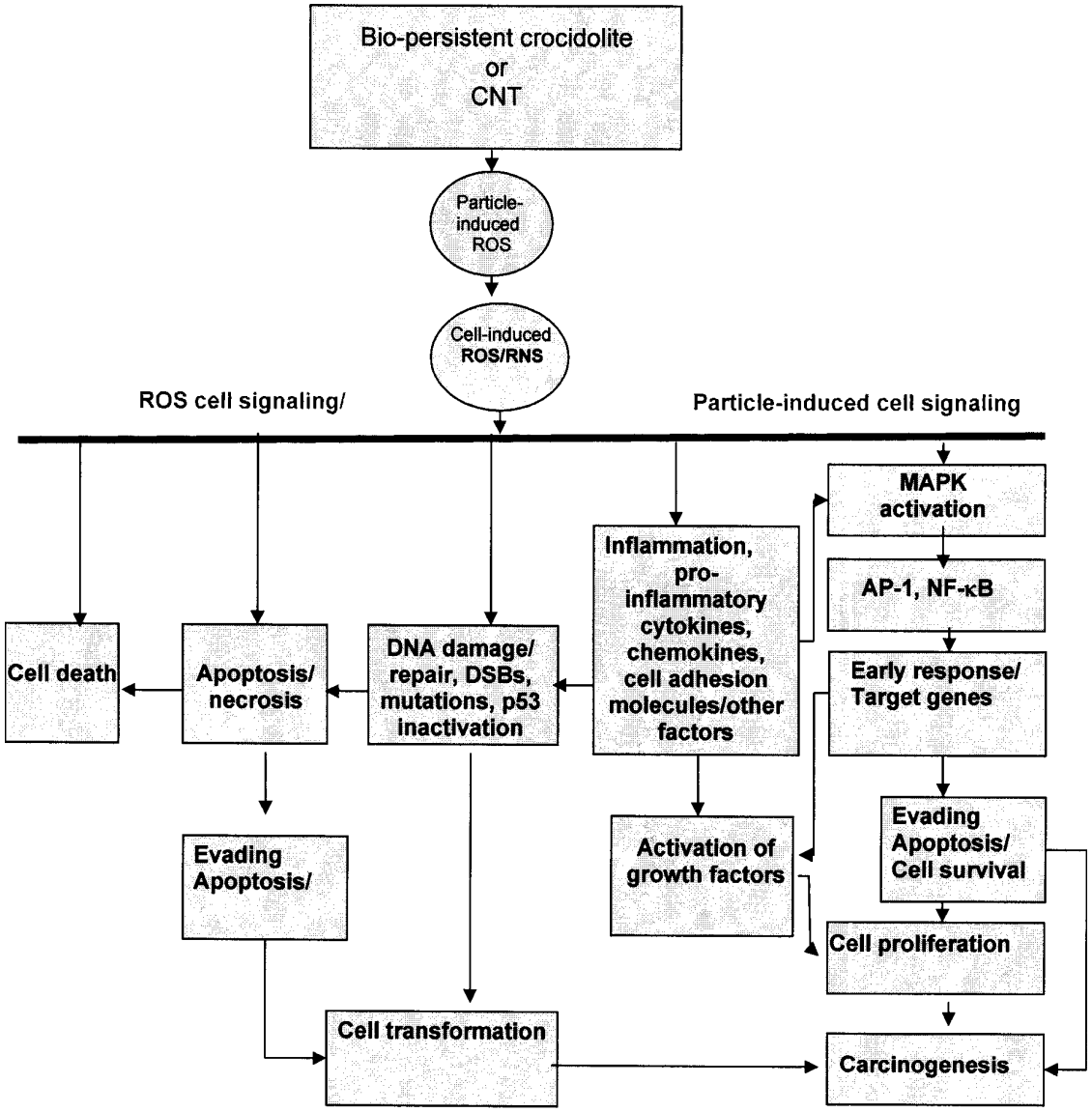

FIGURE 2. Hypothetical schema for asbestos or CNT-induced molecular changes and diseases development. Abreviations: AP-1, activator protein-1; NF-kB, nuclear factor kappa-B; DSBs, DNA strand breaks; MAPK, mitogen activated kinase; p53, protein 53.

2008b). Manna et al. (2005) demonstrated transactivation of NF-κB in a concentration-dependent manner in human keratinocytes by SWCNT. Studies by Pacurari et al. (2008a, 2008b) reported time-dependent NF-κB and AP-1 transactivation by SWCNT and MWCNT in human mesothelial cells. The possibility of cross-talk between lung and systemic circulation following CNT exposure was noted by Erdely et al. (2008). Erdely et al. (2008) exposed C57BL/6 mice to SWCNT and MWCNT by pharyngeal aspiration and found a signature of genes to be upregulated in lung, whole blood, and aorta. Among the genes in the signature, cFos mRNA levels in whole blood were increased 2.7-fold by MWCNT at 4 h postexposure.

SIMILARITIES OF ASBESTOS- AND CNT -INDUCED MOLECULAR CHANGES

The molecular mechanisms critical to the development of fibro-proliferative and

carcinogenic responses produced by asbestos were shown to be cascades of signaling pathways activated by the intrinsic ability of asbestos to generate ROS and cellular reactions promoted by the persistent generation of ROS and the biopersistent nature of asbestos. This, in turn, induces and stimulates key molecular events leading to the activation of transcription factors and early response genes involved in cell proliferation, apoptosis, inflammation, and DNA damage (Craighead & Kane, 1996; Kamp & Weitzman, 1999; Kane, 1996; Mossman & Churg, 1998). There are several studies suggesting asbestos-like behavior by MWCNT. In an *in vitro* report by Pacurari et al. (2008b), MWCNT particles with an average length of 8.19 μm induced several important molecular responses, such as DNA damage, apoptosis, activation of H2AX, PARP MAPK, and AP-1 and NF-κB transcription factors and a similar manner as those caused by crocidolite asbestos of 10 μm length in normal human mesothelial cells. In this study, the activation of molecular events by MWCNT was found to occur to a lesser degree compared to asbestos. Poland et al. (2008) reported that abdominal instillation of long MWCNT particles (>15 μm) exhibited asbestos-like inflammation of the abdominal wall and granulomas formation while short MWCNT did not. Takagi et al. (2008) also reported asbestos-like pathogenicity of MWCNT abdominally administered intraperitoneally to p53 heterozygous. Recently, Hubbs et al. (personal communication) reported that inhalation of MWCNT by C57BL/6J mice resulted in foci of pleural lymphatic inflammation in 37% of the mice. Therefore, based on our studies and of others, it was postulated that the fibrous characteristics of MWCNT, their durability and their ability to ROS at low levels in cellular systems may contribute to the initiation and progression of asbestos-like pathological responses.

Comparison of cell responses produced by SWCNT, MWCNT, and asbestos using similar mass doses in mesothelial cells clearly indicated that asbestos was most toxic while SWCNT was least (Figure 1). Based upon these studies, a hypothetical scheme of asbestos and CNT-induced molecular alterations associated with human lung diseases is presented in Figure 2.

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