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Responses to pulmonary exposure to carbon nanotubes

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8.1 Introduction

Nanotechnology is the manipulation of matter on a near-atomic scale to produce nanoparticles with unique physicochemical properties which can be incorporated into new structures, materials, and devices with a wide range of commercial applications. Carbon nanotubes (CNT) discovered in 1991 by Iijima (1), are carbon atoms arranged in a crystalline graphene lattice with a tubular morphology. CNT can be produced as a single tubular structure to form a single-walled carbon nanotube (SWCNT), as a tube within a tube forming a double-walled carbon nanotube (DWCNT), or as multiple tubes within a tube forming a multi-walled carbon nanotube (MWCNT). SWCNT have a diameter of 1–2 nm, while MWCNT can be synthesized with diameters ranging from 10 to 100 nm depending upon the number of encapsulated tubes forming the CNT structure. CNT can range in length from 0.5 to over 20 μm. Therefore, CNT exhibit high aspect ratios and can be classified as man-made fibrous materials.

CNT are resistant to acid or heat treatment, exhibit high tensile strength, possess unique electrical properties, and can be easily functionalized. Therefore, applications as structural materials, in electronics, as heating elements, in the production of conductive fabric, for bone grafting and dental implants, in drug delivery systems, and as non-corrosive coatings on metals are being developed. With the projected increase in the synthesis and commercialization of CNT, human and environmental exposure during production, distribution, use and disposal is anticipated. Maynard *et al.* (2) have reported that vortexing SWCNT results in aerosolization of peak respirable dust levels as high as 53 $\mu g/m^3$. Han *et al.* (3) reported peak aerosolization levels of 210–430 $\mu g/m^3$ of total dust in a MWCNT synthesis laboratory during

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weighing, blending, mixing, and spraying procedures. Peak total particulate levels as high as 320 $\mu g/m^3$ have been reported in MWCNT facilities associated with oven opening, spraying, and sonication (4). Lastly, laboratory procedures, such as weighing or sonication of MWCNT, have been reported to aerosolize 123×10^3 or 42×10^3 particles/l, respectively (5). These studies indicate that worker inhalation of CNT is possible. Therefore, it is critical to determine the bioactivity of CNT and characterize the dose and time dependence of possible adverse health effects of exposure to inform risk assessment and development of prevention strategies.

8.2 Pulmonary response to CNT

8.2.1 SWCNT

The pulmonary effects of exposure of rodents to SWCNT were first reported in 2004. Warheit *et al.* (6) exposed rats by intratracheal instillation to a raw form of SWCNT (0.25–1.25 mg/rat) with the CNT sample containing 30–40% amorphous carbon, 5% nickel, and 5% cobalt. Although suspended in phosphate-buffered saline (PBS) containing 1% Tween 80®, the SWCNT were highly agglomerated. Pulmonary exposure to SWCNT resulted in a rapid but transient inflammatory and injury response as evidenced by increased bronchoalveolar lavage (BAL) levels of neutrophils, lactate dehydrogenase, and protein. Granulomas, mainly in the terminal bronchioles, were reported 1 week post-exposure and persisted through 3 months post-exposure. A 15% mortality within 1 day post-exposure was reported and was due to physical blockage of conducting airways by large SWCNT agglomerates. Lam *et al.* (7) also reported rapid and persistent granulomas following intratracheal instillation of mice to 0.1–0.5 of SWCNT/mouse. They reported no mortality due to SWCNT exposure. Mangum *et al.* (8) exposed rats by pharyngeal aspiration to purified SWCNT (0.5 mg/rat; 2.6% Co and 1.7% Mo) suspended in 1% Pluronic®. They reported no inflammatory responses. However, cell proliferation and platelet-derived growth factor (PDGF) were significantly increased 1 day post-exposure and significant interstitial fibrosis was noted at 21 days post-exposure. They also noted the formation of CNT structures bridging alveolar macrophages. Shvedova *et al.* (9) exposed mice by pharyngeal aspiration to purified SWCNT (10–40 μg/mouse). The suspended SWCNT preparation contained micrometer-sized agglomerates as well as smaller, nanorope structures. They reported rapid and transient inflammation and damage. They also reported granulomatous lesions and interstitial fibrosis within 7 days post-exposure which lasted through the 59-day course of the study. Initiation of the fibrotic response was associated with a peak in transforming growth factor beta (TGF-β) levels in BAL fluid at 7 days post-exposure. Granulomas were associated with the deposition of agglomerates in the terminal bronchioles and proximal alveoli, while interstitial fibrosis was associated with deposition of more dispersed SWCNT structures in the distal alveoli. At

equivalent mass lung burdens, nano carbon black failed to cause any significant pulmonary responses. Therefore, persistent granulomas and interstitial fibrosis were viewed as CNT-specific responses. Shvedova *et al.* (10) reported the pulmonary response of mice to inhalation of SWCNT (5 mg/m^3, 5hr/day, 4 days). Qualitatively, short-term inhalation of mice produced pulmonary responses similar to bolus exposure by pharyngeal aspiration, i.e. transient inflammation and damage but persistent granulomas and interstitial fibrosis. The development and progression of fibrosis in response to pulmonary exposure to SWCNT appears to involve production of reactive oxygen species, since SWCNT-induced fibrosis is enhanced in mice on a vitamin E–deficient diet and is decreased in NADPH oxidase knockout mice (11, 12).

8.2.2 MWCNT

Muller *et al.* (13) exposed rats by intratracheal instillation to MWCNT (0.5–5 mg/rat) suspended in 1% Tween 80®. They reported inflammation, granulomas, and fibrosis with the unground MWCNT (6 μm length) being more potent than ground MWCNT (0.7 μm length). At 60 days post-exposure, 81% of the unground MWCNT were uncleared compared to 36% for the short MWCNT. Exposure of rats by intratracheal instillation of purified MWCNT (0.25–1.75 mg/rat) suspended in 1% Tween 80® resulted in a rapid but transient inflammatory response and persistent alveolar wall thickening (14). Ma-Hock *et al.* (15) reported pulmonary responses of rats to inhalation of MWCNT (0.1–2.5 mg/m^3, 6 hours/day, 5 days/week, 13 weeks; resultant burden 47–1170 μg/rat). The aerosolized MWCNT were well dispersed. Pulmonary responses included in lung weight, neutrophilic inflammation, and granulomatous inflammation. No fibrosis was reported. However, the authors did not use a specific collagen strain for the histopathologic analysis, so fibrosis may have been underscored. Kobayashi *et al.* (16) exposed rats by intratracheal instillation to a well-dispersed suspension of MWCNT (10–250 μg/rat). They reported transient inflammation and damage and a granulomatous response. They found no fibrosis but failed to use a collagen stain for histopathology. In contrast, Porter *et al.* (17) reported a rapid and persistent fibrotic response in mice after aspiration of a well-dispersed suspension of purified MWCNT (10–80 μg/mouse), using Sirius red staining for collagen. They also reported transient inflammation and damage with persistent granulomas at sites of agglomerate deposition. Likewise, Aiso *et al.* (18) reported transient inflammation and damage and persistent granulomas and alveolar wall fibrosis in rats after intratracheal instillation of MWCNT (40–160 μg/rat).

8.2.3 Comparison of pulmonary responses to SWCNT and MWCNT

Although studies used different modes of exposure (aspiration, intratracheal instillation, or inhalation) and different species (mice or rats), there is striking coherence

among these studies. In general, studies with both SWCNT and MWCNT report qualitatively similar pulmonary responses, described as follows:

- a rapid but transient inflammation and lung injury response
- a granulomatous response of rapid onset which is persistent at deposition sites of CNT agglomerates
- a rapid and persistent/progressive interstitial fibrotic response.

Such a similar set of pulmonary responses to CNT exposure has allowed risk assessment across available animal studies using granulomatous inflammation or interstitial fibrosis as the pulmonary endpoint of health significance (19). A first step in this process was to determine the benchmark dose which resulted in a 10% risk of obtaining an adverse pulmonary response in a given rodent study. Benchmark lung burdens were then normalized to alveolar epithelial surface area (0.05 m^2/lung for mice, 0.4 m^2/lung for rats, and 102 m^2/lung for humans) to allow cross-species comparisons (20). This allowed estimation of the lung burden in workers which would result in a 10% risk of developing granulomas or interstitial fibrosis. Lastly, the National Institute for Occupational Safety and Health (NIOSH) in the United States is using such analyses to calculate workplace exposure levels which would result in the benchmark lung burden in a CNT worker over a working lifetime (8 hous/day, 5 days/week, 50 weeks/year, 45 years). Table 8.1 gives benchmark exposure limits calculated from three SWCNT and three MWCNT studies. NIOSH

Table 8.1 *Calculation of benchmark workplace levels for human exposure.*[a,b,c]

Study	Exposure	CNT	Benchmark exposure level (mg/m^3)
Lam *et al.*, 2004 (7)	Intratracheal (mice)	SWCNT	10.0
Shvedova *et al.*, 2005 (9)	Aspiration (mice)	SWCNT	1.80
Shvedova *et al.*, 2008a (10)	Inhalation (4 days) (mice)	SWCNT	0.11
Muller *et al.*, 2005 (13)	Intratracheal (rat)	MWCNT	18.0
Porter *et al.*, 2010 (17)	Aspiration (mice)	MWCNT	0.61
Ma-Hock *et al.*, 2009 (15)	Inhalation (13 weeks) (rat)	MWCNT	0.50

[a] Rodent benchmark lung burdens were calculated as the CNT burden which would result in a 10% risk of a significant granulomatous inflammatory or interstitial fibrotic response.
[b] Benchmark lung burdens were normalized across species as deposited CNT/alveolar epithelial surface area.
[c] Benchmark exposure level was calculated as lung burden = air level × ventilation × duration × deposition fraction, assuming worker ventilation = 9.6 m3/day; duration = 8 hours/day, 5 days/week, 50 weeks/year, 45 years; deposition fraction = 10%.

has used this information to propose a recommended exposure limit for CNT of 7 $\mu g/m^3$, which is the current limit of detection for airborne CNT using elemental carbon as the detection method (21).

Although pulmonary responses to SWCNT and to MWCNT are qualitatively similar, resulting in transient inflammation but persistent granulomatous lesions and fibrosis, quantitative differences in pulmonary responses have been reported. In mice exposed to CNT by pharyngeal aspiration (10 μg/mouse), SWCNT caused a greater inflammatory response than MWCNT at 1 day post-exposure, as shown in Table 8.2 (9, 17, 22). Morphometric analyses indicate that well-dispersed SWCNT are not well recognized by alveolar macrophages (only 10% of the alveolar burden being within alveolar macrophages), while 90% of dispersed SWCNT structures rapidly cross alveolar epithelial cells and enter the interstitium (22). In contrast, 70% of MWCNT in the respiratory zone enter alveolar macrophages and 8% migrate into the alveolar septa (23, 24). This difference in pulmonary fate of SWCNT versus MWCNT is shown in Figure 8.1. As a result, well-dispersed SWCNT are more

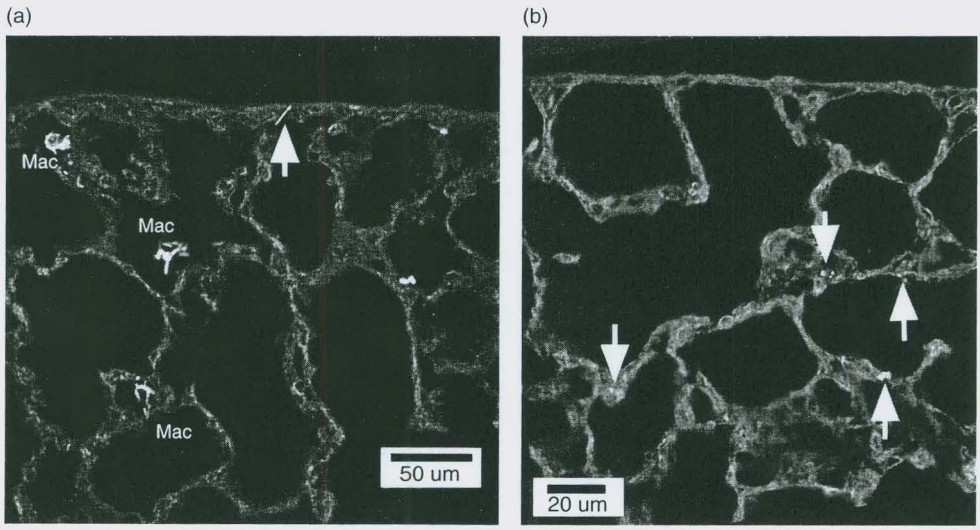

Figure 8.1 Enhanced darkfield image of CNT-exposed lungs. Panel (a) shows the general distribution of MWCNT in the lungs 7 days after aspiration (40 μg dose) with the section oriented with the pleural space running along the top. CNTs scatter light with high efficiency and thus produce the bright white structures in these enhanced darkfield images, while nuclei and other tissues produce a significantly duller image. Arrow points to an individual MWCNT in subpleural tissue. Alveolar macrophages (Mac) are foci for MWCNT. However, a few submicron MWCNT structures can be found in the alveolar interstitium throughout the section. Panel (b) gives a comparison image from a mouse lung exposed to a highly dispersed preparation of SWCNT (aspiration 10 μg dose, 7 days). In the case of dispersed SWCNT, the majority of CNT structures are rapidly incorporated into the alveolar interstitium (arrows).

potent in causing interstitial fibrosis on an equal mass lung burden basis than MWCNT (22, 24). This results in a lower calculated benchmark exposure level for SWCNT than for MWCNT (Table 8.1), using data from the Shvedova *et al.* (10) and Porter *et al.* (17) studies, respectively.

Another difference between pulmonary responses to SWCNT and to MWCNT is the ability to enter the intrapleural space. Both SWCNT and MWCNT have been reported in the subpleural tissue of the lung (22, 25). However, strong evidence for penetration of the visceral pleura and translocation to the intrapleural space (Figure 8.2) has been reported only for MWCNT (23).

Table 8.2 *Inflammatory potency of SWCNT versus MWCNT.*[a,b]

Exposure	PMN	References
SWCNT	$2.72 \pm 0.14 \times 10^5$	Shvedova *et al.*, 2005 (9)
SWCNT	$2.30 \pm 0.55 \times 10^5$	Mercer *et al.*, 2008 (22)
MWCNT	$1.33 \pm 0.46 \times 10^5$	Porter *et al.*, 2010 (17)

[a] Mice were exposed by pharyngeal aspiration to 10 μg of CNT.
[b] Bronchoalveolar lavage was performed 1 day post-exposure and the number of polymorphonuclear neutrophilic leukocytes (PMN) in the lavage fluid was determined as an indicator of pulmonary inflammation.

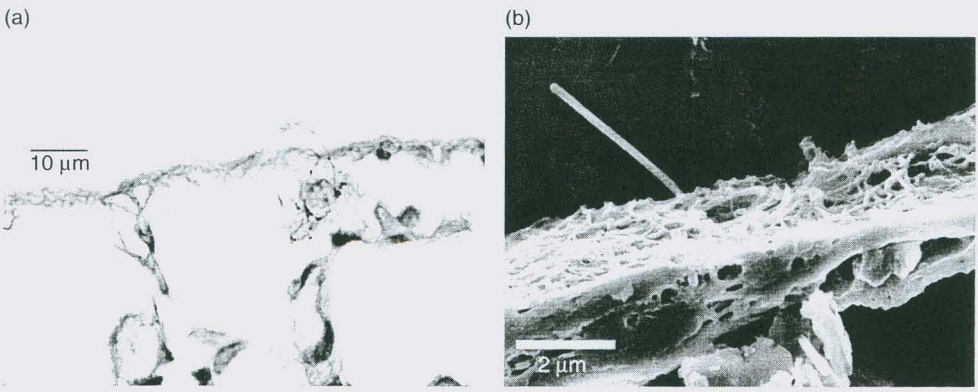

Figure 8.2 Penetration of the visceral pleura by MWCNT. Mice were exposed to well-dispersed MWCNT by pharyngeal aspiration. (a) A light micrograph of Sirius red-stained lung tissue 28 days after exposure to 40 μg MWCNT. Note a MWCNT leaving a macrophage and entering the intrapleural space. (b) Field-emission electron micrograph showing a MWCNT (50 nm × 5 μm) entering the intrapleural space (Figure 8.2(b) used with permission from Mercer *et al.* (23)).

8.2.4 Mechanism for CNT-induced pulmonary fibrosis

Both SWCNT and MWCNT have been reported to enter the alveolar septa, as in shown in Figure 8.3, and induce interstitial fibrosis (22, 17). Classic fibrogenic particles, such as crystalline silica and asbestos, cause persistent inflammation and lung injury, which results in parenchymal damage and scarring, i.e. fibrosis. Bronchoalveolar markers of inflammation and damage increase within 1–3 days after exposure to SWCNT or MWCNT, but decline toward control levels after 1 week post-exposure (9, 10, 17). Therefore, CNT-induced interstitial fibrosis persists and progresses over several months post-exposure in the absence of persistent pulmonary inflammation (9, 22, 17). Therefore, CNT are acting by a different mechanism than crystalline silica or asbestos to cause fibrosis. *In vitro* studies with lung fibroblasts indicate that SWCNT and MWCNT can directly enhance fibroblast proliferation and collagen production (26, 27, 28). Dispersed MWCNT also induce release of the fibrogenic factor TGF-β from lung epithelial cells in culture (28). Therefore, CNT appear to rapidly enter the alveolar interstitium (Figure 8.3) and lay down a substrate upon which fibroblast proliferate, leading to interstitial fibrosis. A similar scaffolding effect on osteoblast proliferation has led to the use of CNT in bone grafting and dental implants (29, 30).

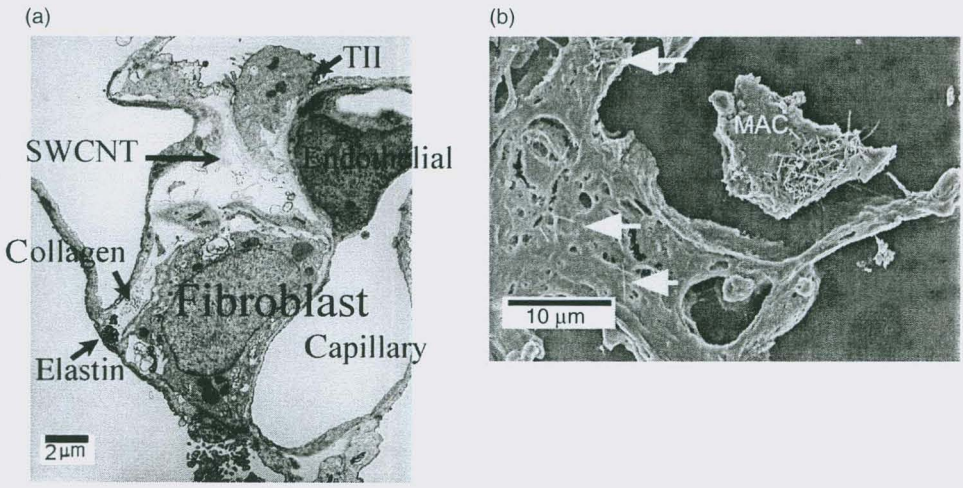

Figure 8.3 CNT within the alveolar septa. Mice were exposed to SWCNT or MWCNT by pharyngeal aspiration. (a) An electron micrograph of alveolar wall 3 days after aspiration of SWCNT, showing nanotubes in the alveolar interstitium. (b) A field-emission scanning electron micrograph of an alveolar wall 28 days after aspiration of MWCNT. Arrows indicate nanotubes in the alveolar interstitium. A MWCNT-loaded alveolar macrophage (MAC) is present in the neighboring airspace.

Table 8.3 *Inflammatory potential of raw versus purified SWCNT.*[a,b]

	PMN (fold increase)
Raw SWCNT (30% Fe)	51 ± 10
Purified SWCNT (0.3% Fe)	45 ± 1

[a] Mice were exposed to 10 μg of raw or purified SWCNT.
[b] At 1 day post-exposure, inflammatory response was determined by measuring polymorphonuclear neutrophilic leukocytes (PMN) in bronchoalveolar lavage fluid.

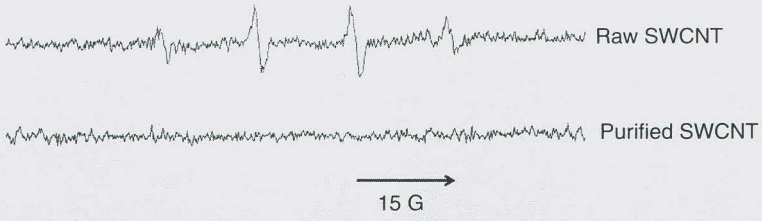

Figure 8.4 Acellular generation of hydroxyl radicals by CNT. In the presence of hydrogen peroxide, raw SWCNT generate hydroxyl radicals while purified SWCNT do not.

8.2.5 Comparison of raw versus purified CNT

As synthesized, raw CNT contain as much as 30% catalytic metals. Catalytic metals, such as iron, can generate hydroxyl radicals in the presence of hydrogen peroxide or cells (31). These catalytic metals can be removed by acid treatment or by high temperature to yield purified CNT with low metal content. As shown in Figure 8.4, removal of catalytic metals abolishes the ability of SWCNT (MWCNT not shown) to generate hydroxyl radicals *in vitro*. The *in vitro* cytotoxicity of CNT has been linked to oxidant injury (32). However, *in vivo* the pulmonary bioactivity of SWCNT does not appear to be affected by the presence or absence of catalytic metals. Lam *et al.* (7) compared the pulmonary response of mice to intratracheal instillation of raw (containing 25% metal catalyst) versus purified (≈ 2% iron) SWCNT and found the granulomatous reaction was not dependent on metal contamination. Likewise, the acute inflammatory reaction of mice after aspiration of raw (30% iron) versus purified (1% iron) SWCNT was not affected by metal content, as shown in Table 8.3 (9, 10).

8.2.6 Effect of functionalization of CNT

CNT are highly hydrophobic. Functionalization of MWCNT with COOH or OH makes these CNT highly water-soluble. Han *et al.* (33) exposed mice by aspiration

to carboxylic- and hydroxyl-functionalized MWCNT (20–40 μg/mouse). These functionalized MWCNT were well dispersed in PBS. Like other reports, transient inflammation, cytokine production, and lung injury were found. However, these authors did not compare the bioactivity of functionalized versus non-functionalized CNTs. Wang *et al.* (28) have reported that carboxylation of MWCNT partially decreased the ability of CNT to stimulate fibroblast proliferation *in vitro*. In addition, COOH-functionalized MWCNT have been reported to cause a significantly lower inflammatory response in mouse lungs at 1 and 7 days post-aspiration than unmodified MWCNT (34).

8.2.7 Bioactivity of agglomerated versus well-dispersed CNT

Well-dispersed CNT have been shown to exhibit greater bioactivity than agglomerated CNT *in vitro*. Wang *et al.* (26) have demonstrated that SWCNT dispersed in diluted alveolar lining fluid were effective in enhancing proliferation and collagen production by cultured fibroblasts, while SWCNT agglomerates were ineffective. Similarly, Wang *et al.* (28) reported that MWCNT were effective in inducing TGF-β production from bronchial epithelial cells and proliferation of fibroblasts *in vitro* only when well-dispersed using an artificial diluted alveolar lining fluid. Mercer *et al.* (22) gold-labeled poorly-dispersed and well-dispersed preparations of SWCNT prior to aspiration by mice (Figure 8.5). They demonstrated that poorly-dispersed SWCNT agglomerates deposited in the terminal bronchioles and proximal alveoli where they induce granulomatous lesions. In contrast, well-dispersed SWCNT structures deposited in the distal alveoli and rapidly migrated into the alveolar septa where they induce progressive interstitial fibrosis. In addition, transient inflammation and persistent interstitial fibrosis were four-fold greater on an equal mass burden basis for well-dispersed SWCNT than for poorly-dispersed SWCNT. Shvedova *et al.* (10) also reported an increased pulmonary response to a more dispersed preparation of SWCNT. Aerosolization of dry SWCNT resulted in smaller structures (count mode aerodynamic diameter (CMAD) ≈ 220 nm) than suspension of SWCNT for aspiration. Quantitatively, the dispersed SWCNT aerosol was four-fold more potent in causing inflammation and fibrosis after inhalation than aspiration of less-dispersed SWCNT (10). This increased pulmonary bioactivity of well-dispersed versus agglomerated SWCNT is summarized in Table 8.4. Pauluhn (35) exposed rats by inhalation (0.1–6 mg/m^3, 6 hours/day, 5 days/week, 13 weeks) to Baytube® MWCNT. Aerosolized structures were large, compact agglomerates with a mass median aerodynamic diameter (MMAD) ≈ 3 μm. Sub-chronic inhalation resulted in persistent inflammation, lung damage, granulomas, alveolar wall thickening, and a small increase in interstitial collagen staining. In general, the interstitial fibrotic response in the Pauluhn study was less than that reported after

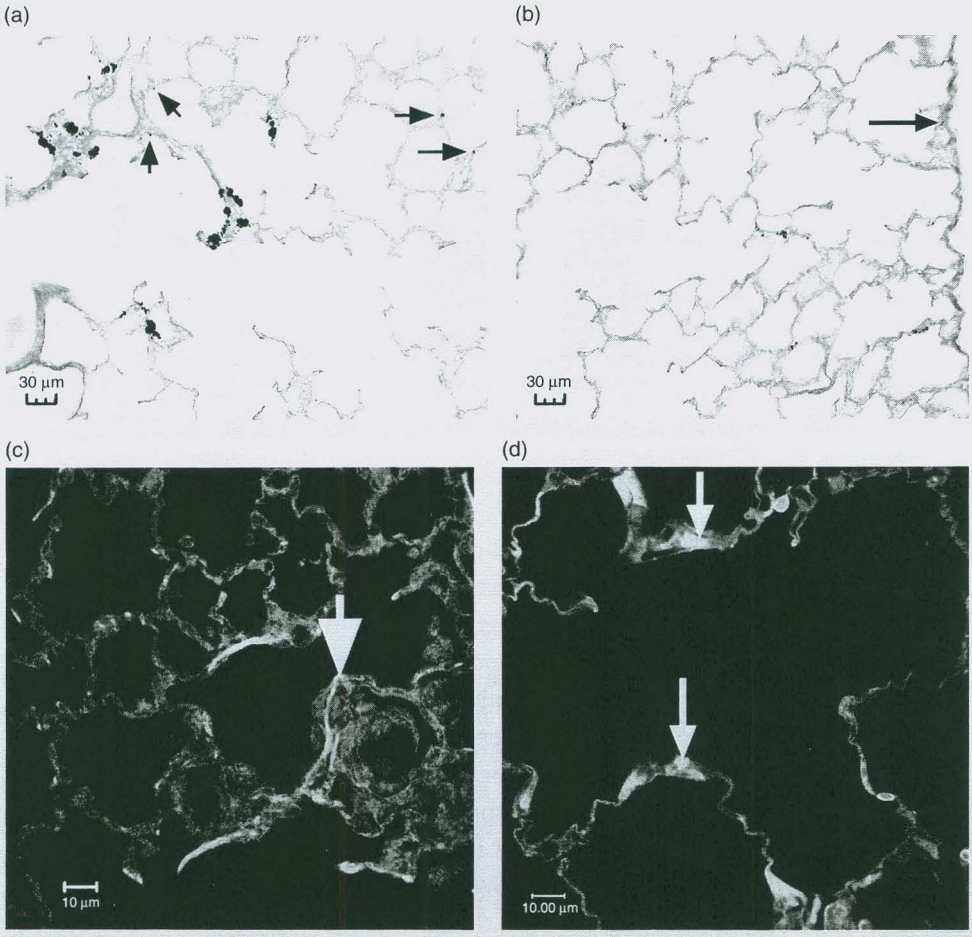

Figure 8.5 Pulmonary distribution and response after aspiration of gold-labeled SWCNT in mice. (a) Deposition of poorly-dispersed SWCNT agglomerates in the terminal bronchioles and proximal alveoli results in granulomatous lesions (Figure 8.5(c)). Arrows indicate deposition of small SWCNT structures in the distal alveolar walls. (b) Well-dispersed SWCNT within alveolar septa of the distal lung and resulting interstitial fibrosis (Figure 8.5(d)). (c), (d) Arrows in confocal micrographs indicate collagen stained by Lucifer yellow. Figures from Mercer *et al.* (22) with permission from the American Physiological Society.

aspiration of well-dispersed MWCNT preparation (17). It should be noted that individual MWCNT were observed within the alveolar septa in the Porter study (17), while no individual MWCNT were reported in the Pauluhn study (35). Because of the non-fibrous structure of these MWCNT agglomerates in the Pauluhn study, responses were attributed to volumetric overload (36).

Table 8.4 *Comparison of pulmonary responses to inhalation versus aspiration of raw SWCNT by mice. Superscripts indicate days post-exposure.*

	Inhalation (lung burden 5 μg/mouse)	Aspiration (10 μg/mouse)
PMN (fold increase)[1]	136 ± 20	51 ± 10
BAL protein (↑ from control)[1]	68 ± 3%	35 ± 3%
BAL TGF–β (fold increase)[7]	7.9 ± 7%	2.0 ± 0.1
Lung collagen (↑ from control)[7]	127 ± 7%	53 ± 1%

8.2.8 Comparison of inhalation versus bolus exposure to CNT

Shvedova *et al.* (10) compared the biological response resulting from a bolus aspiration and a 4-day inhalation of SWCNT. Both exposures resulted in qualitatively similar transient inflammation and damage and rapid but persistent fibrosis. However, on an equal mass burden basis, inhalation causes inflammatory and fibrotic responses that were four-fold greater than bolus exposure of mice by aspiration (Table 8.4). This difference in potency was most likely due to differences in the structure size distribution of the inhaled versus the aspirated SWCNT, with inhalation of dry SWCNT having smaller structures (CMAD ≈ 220 mm) than the fluid-suspended SWCNT sample used for aspiration. When mice aspirated a well-dispersed SWCNT preparation, bolus aspiration produced a quantitatively similar degree of fibrosis as inhalation (22, 10). Li *et al.* (37) also compared the pulmonary response of mice exposed to purified MWCNT by intratracheal instillation (50 μg/mouse bolus dose) with inhalation (32.6 mg/m^3, 6 hours/day, 5–15 days). The MWCNT aerosolized from dry material were much less agglomerated than when suspended in 1% Tween 80®. Intratracheal instillation resulted in granulomas with some alveolar wall thickening, while inhalation resulted predominately in alveolar wall thickening and cell proliferation. Lastly, Wolfarth *et al.* (38) compared the degree of pulmonary inflammation and damage 1 day after aspiration of a well-dispersed MWCNT preparation to responses 24 hours after a 4-day inhalation exposure, which resulted in the same lung burden (Figure 8.6). Results indicate that the degree of pulmonary response to a bolus versus a short-term inhalation exposure was not significantly different.

8.3 Systemic responses to pulmonary exposure to CNT

Li *et al.* (39) reported that multiple aspirations of SWCNT (20 μg/mouse, every 2 weeks, for 2 months) in Apo E −/− mice caused a 71% increase in aortic plaques.

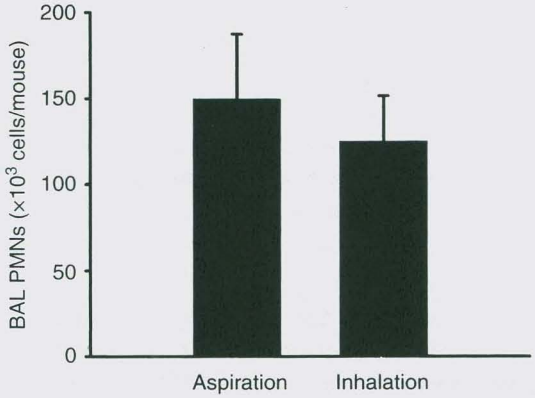

Figure 8.6 Acute inflammatory response to aspiration versus inhalation of MWCNT. Mice were exposed to a 10 μg lung burden of well-dispersed MWCNT by aspiration or by inhalation (10 mg/m^3, 5 hours/day, 4 days). At 1 day post-exposure, inflammation was determined by counting the polymorphonuclear neutrophilic leukocytes (PMN) in bronchoalveolar lavage fluid. There was no significant difference in the response to aspiration versus short-term inhalation.

Inhalation of MWCNT (26 mg/m^3 for 5 hours; lung burden of 22 μg) results in a 92% depression of the responsiveness of coronary arterioles to dilators 24 hours post-exposure (40). Furthermore, pharyngeal aspiration of MWCNT (80 μg/mouse) results in induction of mRNA for certain inflammatory mediators and markers of blood/brain barrier damage in the olfactory bulb, frontal cortex, midbrain, and hippocampus 24 hours post-exposure (41). Several possible mechanisms have been put forward to explain systemic responses to pulmonary exposure to CNT:

- **Translocation of CNT to systemic sites**: Translocation of intraperitoneally instilled MWCNT from the abdominal cavity to the lung has been reported (42). However, thus far there is no evidence that the systemic effects reported above are associated with translocation of CNT from the lung to the affected tissue. Indeed, aspirated gold-labeled SWCNT were not found in any systemic organ 2 weeks post-exposure (43).
- **Systemic inflammation**: Pulmonary exposure to particles causes localized inflammation at the sites of particle deposition in the alveoli. Erdely *et al.* (44) reported that aspiration of SWCNT or MWCNT (40 μg/mouse) induced a small but significant increase in blood neutrophils and mRNA expression and protein levels for certain inflammatory markers in the blood at 4 hours post-exposure, but not at later times. Such pulmonary CNT exposure also significantly elevated gene expression for mediators, such as Hif–3α and S100a, in the heart and aorta at 4 hours post-exposure. Evidence also exists that pulmonary exposure to particles alters systemic microvascular function by potentiating polymorphonuclear neutrophilic leukocytes (PMN) as they flow through pulmonary capillaries in close proximity to affected alveoli. These potentiated blood PMN adhere to microvessel

walls and release reactive species which scavenge NO produced by endothelial cells (45, 46). Therefore, less dilator-induced NO diffuses to vascular smooth muscle, resulting in less dilation.

- **Neurogenic signals**: Although data for CNT are not yet available, pulmonary exposure to ultrafine TiO_2 has been reported to stimulate sensory neurons in the lung as indicated by an increase in Substance P (a sensory neurotransmitter) levels in the nodose ganglion 24 hours after exposure (47). Furthermore, inhibition of sympathetic input to systemic arterioles reverses the decreased responsiveness of the microvasculature to dilators after pulmonary exposure to ultrafine TiO_2 (48). Therefore, it is proposed that particle-induced airway irritation stimulates airway sensory neurons which send a signal to the brain, causing mediator responses in the brain. This neurogenic signal is then transmitted to the cardiovascular system.

8.4 Summary

In general, pulmonary exposure of rats or mice by intratracheal instillation, pharyngeal aspiration, or inhalation of SWCNT or MWCNT results in transient inflammation and lung damage. Granulomatous lesions and interstitial fibrosis, which are of rapid onset and persistent in nature, have also been a common report. The presence (raw) or absence (purified) of catalytic metals does not appear to greatly affect pulmonary response. The degree of agglomeration does affect deposition site and response. Large agglomerates tend to deposit at the terminal bronchioles and proximal alveoli and induce a granulomatous response, while more dispersed structures can deposit in the distal alveoli and cause interstitial fibrosis. The lung may be exposed to more dispersed CNT structures by inhalation than by exposure to a suspension of CNT. When this is the case, the response to short-term inhalation is often greater than that for the same lung burden given as a bolus dose. Pulmonary exposure to CNT has also been shown to cause changes in cardiovascular and central nervous system function. Mechanisms, such as inflammatory signals or neurogenic pathways, causing these systemic responses to pulmonary CNT exposure are under investigation. In light of the pulmonary and systemic responses reported in rodent studies, it appears prudent to minimize worker exposure to CNT. Normal industrial hygiene practices, i.e. containment, ventilation, and personal protective equipment, appear effective in controlling exposure (3, 49).

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