Mounting evidence that carbon nanotubes may be the new asbestos

Executive summary

Carbon nanotubes – thin, hollow cylinders made of carbon atoms – look very much like asbestos. In 2004, the United Kingdom's Royal Society and risk specialists at the world's second largest reinsurance agent Swiss Re warned that once in our lungs, nanotubes may also behave like asbestos. Since then, a series of experiments have demonstrated that when introduced into the lungs of rodents, carbon nanotubes cause inflammation, granuloma development, fibrosis, artery 'plaque' responsible for heart attacks and DNA damage. Two independent studies have shown that carbon nanotubes can also cause the onset of mesothelioma – cancer previously thought to be only associated with asbestos exposure. Unfortunately, despite mounting evidence of the asbestos-like dangers of carbon nanotubes, their commercial use is also growing rapidly – in sports goods, car and aeroplane parts, reinforced plastics and electronics.

To avoid a repeat of the asbestos tragedy, Friends of the Earth is calling for an immediate moratorium on the commercial use of carbon nanotubes and the sale of products that incorporate nanotubes until research can demonstrate whether or not there is any safe level of exposure to them. We are also calling for new nanotechnology-specific regulation to protect human health and the environment, for mandatory labelling of all nanomaterials used in the workplace and in consumer products, and for the public to be given a meaningful role in decision making about nanotechnology governance, policy development and research priorities.

Eminent scientists and risk experts have warned since 2004 that carbon nanotubes could pose asbestos-like risks

In 2004, scientists at the highly regarded United Kingdom's Royal Society and Royal Academy of Engineering (RS/RAE) and risk experts at the world's second largest reinsurance company Swiss Re both warned that because carbon nanotubes share many physical properties with asbestos, they may also present similar health risks.

Swiss Re put it bluntly: "…some nanotubes are similar in size and form to asbestos fibres. The supposition that the potential for harm could be similar would appear to be obvious" (Swiss Re 2004, p42).

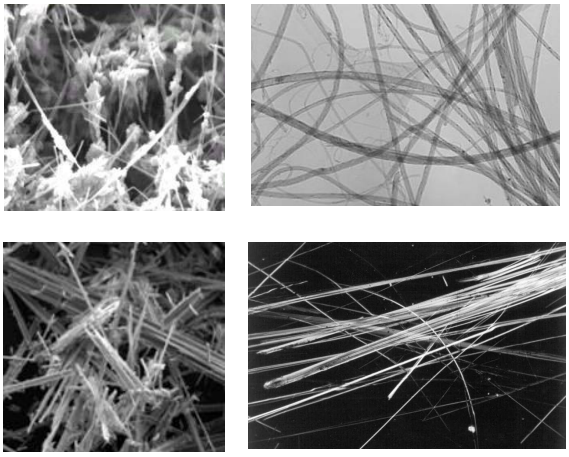

Figure 1: Similarities in physical properties of some carbon nanotubes (top row) and asbestos fibres (bottom row).

The RS/RAE warned that: “Exposure to fibres in industry, in the form of asbestos, is a well-recognised cause of serious illness, including cancer. The toxic properties of such fibres are dependent upon a diameter narrow enough to allow inhalation deep into the lung, a length that prevents their removal by macrophages, resistance to dissolution in tissue fluid, and a surface able to cause oxidative damage... Carbon and other nanotubes have physical characteristics that raise the possibility of similar toxic properties... Such materials require careful toxicological assessment and should be treated with particular caution in laboratories and industry” (RS/RAE 2004, p50).

Carbon nanotubes meet criteria identified by the Royal Society as key indicators of potential for asbestos-like risks

In 2004 the RS/RAE recognised that serious knowledge gaps compromised our ability to predict whether carbon and other nanotubes could pose asbestos-like risks. The RS/RAE recommended that: “Given previous experience with asbestos, we believe that nanotubes deserve special toxicological attention; the types of studies that are required are listed in Box 5.4” (RS/RAE 2004, p43). Appendix 1 evaluates progress made since 2004 in conducting these studies.

Key gaps remain in our understanding of the health risks posed by carbon nanotubes and these require urgent attention. These gaps include: understanding whether airborne nanotubes will reach the lungs in realistic occupational or environmental conditions; potential for long-term inhalation/ exposure at realistic exposure levels to result in mesothelioma and/ or other serious disease; occupational exposure levels likely to be faced by workers across a range of sectors and jobs; role of size, shape and other nanotube properties in affecting the potential for acute toxicity; role of size, shape and other nanotube properties in affecting the potential for fibrosis, cancer and/ or other disease; the role of aggregation, agglomeration, and disaggregation and de-agglomeration in affecting nanotube properties and toxicity; long-term biodurability of nanotubes; and the potential for nanotubes release from products over their life-cycle.

However as the studies cited in Appendix 1 demonstrate, the majority of the critical preliminary questions the RS/RAE identified regarding the biological behaviour of carbon nanotubes have been answered. The published literature suggests strongly that some forms of nanotubes could pose similar health risks to asbestos and that a wide range of nanotubes cause both localised and system toxic effects. Given early evidence of the potential for a repeat of the asbestos tragedy, there is no acceptable reason for postponing measures to stop the further commercial production and sale of carbon nanotubes until further research can identify whether or not any levels of nanotube exposure can be deemed safe.

A growing number of studies demonstrate that nanotubes can cause asbestos-like disease or acute toxicity

In recent years, evidence has mounted that exposure to carbon nanotubes can cause asbestos-like disease, acute toxicity, accelerated development of artery ‘plaque’ responsible for heart attacks, cell death and DNA damage far from the site of exposure:

- Nanotubes that look like asbestos behave like asbestos: long, multi-walled carbon nanotubes introduced into the mice abdominal cavity caused asbestos-like pathogenicity in a 7 day *in vivo* study (Poland et al. 2008)
- Nanotubes caused more deaths from mesothelioma than did the most potent form of asbestos following their introduction into mice abdominal cavity in a 180 day *in vivo* study (Takagi et al. 2008)
- In instillation *in vivo* studies where sufficient quantities of nanotubes reached the lungs, nanotubes caused inflammation, fibrosis and granulomas (Lam et al. 2004, Muller et al. 2005, Shvedova et al. 2005)
- Comparative *in vivo* study finds intratracheal instillation of multi-walled carbon nanotubes caused inflammation and severe pulmonary damage; inhalation resulted in moderate pathological lesions (Li et al. 2007)
- Reviews of the published literature on carbon nanotubes highlight large persisting knowledge gaps but indicate that SWCNTs and MWCNTs may have the potential to cause severe lung disease and possibly cancer (Donaldson et al. 2007; Lam et al. 2006; Muller et al. 2006;)
- Lung proteins preferentially bound to carbon nanotubes in an *in vitro* study, indicating the potential for damage to lung immune defence mechanisms, increased risk of lung infections and emphysema (Salvador-Morales et al. 2007)
- Nanotubes caused the accelerated development of artery plaque responsible for causing heart attacks and strokes, and damaged DNA in the hearts of test mice in an *in vivo* study (Li et al. 2007)
- Carbon nanotubes were taken up by cell nuclei in an *in vitro* study where they caused dose-dependent cell death (Porter et al. 2007)
- Multi-walled carbon nanotubes localised within skin cells in *in vitro* studies. They caused irritation, impaired protein function and decreased cell viability. The authors warn this could cause skin disease (Monteiro-Riviere et al. 2005; Witzmann and Monteiro-Riviere 2006)

For detailed summaries of these studies see Appendix 2.

There are no suitably sensitive, cost-effective technologies to measure occupational exposure to nanotubes

The absence of suitably sensitive, affordable detection technologies for routine occupational exposure measurement to the full range of carbon nanotubes is extremely concerning (Tantra and Cumpson 2007). Much further research is required to determine whether or not any level of occupational exposure to nanotubes is safe, or whether all levels of long-term exposure present unacceptable risk. But even if we were able to determine and legislate for nanotube-appropriate permissible exposure levels for the workplace, enforcing this will be impossible if the technology doesn't exist for cost-effective, routine measurement of exposure levels in workplaces.

As nanotubes are now used in electronic goods, plastics, car parts, sporting equipment, fuel filters and other products, workers in very diverse industries and positions may face occupational exposure to nanotubes – not just researchers in laboratories. In their recent review of detection technologies for airborne nanotubes, Tantra and Cumpson (2007) observed that: "The need to have a cost effective detection system with high sensitivity and selectivity to CNTs is driven by the increasing evidence of the high level of toxicity of CNTs, particularly exhibited by certain types of nanotubes."

The fact that technologies capable of assessing occupational exposure to carbon nanotubes are lagging so far behind nanotubes' commercial development is extremely concerning. Tantra and Cumpson cautioned that whereas some detection technologies have been demonstrated in the academic literature, these have not yet been investigated for their suitability in a realistic workplace context. They also warn that there are no 'perfect' detection techniques that are sensitive to a wide range of nanotubes and also cost-effective. "Out of all techniques, Raman spectroscopy has the most potential. Nonetheless, the method is not without its limitations and may only prove suitable if the instrument provides multiple laser lines and that detection only revolves around single/double walled CNTs" (Tantra and Cumpson 2007, p261). That is, even the technology these authors identified as the most promising may not be able to reliably detect wider multi-walled carbon nanotubes.

Conclusion

Preliminary studies demonstrate that some forms of carbon nanotubes can cause the development of mesothelioma. Many studies show that a wide range of nanotubes can cause asbestos-like pathogenicity if sufficient quantities reach the lungs, in addition to localised and/ or systemic toxicity. Significant knowledge gaps persist that require urgent research. However, preliminary studies have already answered in the affirmative most of the questions identified in 2004 by the United Kingdom's Royal Society as key to indicating the potential for nanotubes to cause asbestos-like harm. This warrants a strongly precautionary approach to the commercial use of nanotubes while further toxicological research is conducted, until regulations are established to protect the health of workers and the public, and improved detection technologies for routine occupational exposure measurement are developed.

To avoid a repeat of the asbestos tragedy, Friends of the Earth Australia is calling for an immediate moratorium on the commercial use of carbon nanotubes and the sale of products that incorporate nanotubes until research can demonstrate whether or not there is any safe level of exposure to them. Given the evidence of vastly greater health risks of carbon nanotubes, it is completely unacceptable that permissible occupational exposure levels to carbon nanotubes and materials safety data sheets provided to workers should remain based on synthetic graphite.

Before any further commercial use of carbon nanotubes, we are calling for new nanotechnology-specific regulation to protect workers, the public and the environment. This must include nano-specific safety assessments for nanotubes and all other manufactured nanomaterials, requiring full physico-chemical characterisation and a comprehensive range of safety tests. Metrics used must also be appropriate to nanomaterials (ie particle surface area and number of particles rather than mass). New permissible exposure levels must clearly be enforceable. We emphasise that this necessitates the development of cost-effective, reliable technologies for routine occupational exposure measurement before commercial production of nanotubes can proceed.

Carbon nanotubes must be subject to safety assessment as both nanomaterials and as fibres. MWCNTs that are demonstrated to cause serious health harm may measure up to 200nm in diameter. As key mechanisms for harm associated with MWCNT and other particles in this size range appear to be oxidative stress, inflammation and protein

interactions commonly associated with particles <100nm in size, we call for all particles and materials measuring up to 300nm in size to be subject to nano-specific safety assessment and metrics (see Friends of the Earth Australia 2008). Given that the other key mechanism for nanotube-related harm appears to be their behaviour as respirable, persistent high aspect ratio fibres, we also support calls for their toxicity to be assessed alongside asbestos and other fibres as part of a unified strategy.

In recognition of the public's 'right to know', we are calling for mandatory labelling of all nanomaterials used in the workplace and in consumer products. Finally, given the predicted large-scale social, economic and environmental implications of nanotechnology's development more generally, we are calling for the public to be given a meaningful role in decision making about nanotechnology governance, policy development and research priorities.

Appendix 1: Evaluation of progress in assessing likely health risks of carbon nanotubes as identified by UK Royal Society & Royal Academy of Engineering (2004, Box 5.4)

Key questions regarding likely health risks of nanotubes identified by RS/RAE	Answer	Comments/ relevant information
Occupational hygiene study of production and use/disposal to determine the sizes and concentrations of fibres likely to be present in the workplace.	Likely workplace concentrations of nanotubes remains unknown	Only one occupational hygiene study has been published which looked at exposure at NASA, Rice University and a commercial facility (Maynard et al. 2004). The study found low exposure levels, but as the production facilities were only producing several grams of carbon nanotubes per day, the authors observed that "under large-scale manufacturing conditions, the potential for significant worker exposure may exist" (Shvedova et al. 2005, p707).
Are fibres longer than about 15µm (preventing their removal by macrophages)?	Yes	Some commercially available nanotubes are longer than 15µm/ 15,000nm (eg samples used by Poland et al. 2008, Takagi et al. 2008). However studies show that even nanotubes less than 15µm in length can be persistent and toxic (Donaldson et al. 2004, Muller et al. 2005, Shvedova et al. 2005).
Can fibres reach the part of the lung responsible for gas-exchange (ie are they narrower than 3µm)?	Yes	Single walled carbon nanotubes are typically less than several nm in diameter; multi-walled carbon nanotubes are typically 10-200nm/ 0.01-0.2µm wide (Donaldson et al. 2006).
Are fibres durable (an indication that they might persist in the lung)?	Yes	See below
Do fibres kill cells, provoke inflammation and release free radicals?	Yes	Several studies have shown that nanotubes provoke inflammation (Lam et al. 2004, Witzmann and Monterio-Riviere 2006, Muller et al. 2005). Shvedova et al. (2005) found dose-dependent biomarkers of inflammation, oxidative stress and cytotoxicity in lavaged fluid from SWCNT exposed mice. Monteiro-Riviere et al. (2005) found that purified MWCNT produced pro-inflammatory cytokine and decreased cell viability of human epidermal keratinocytes in a time- and dose-dependent manner. Porter et al. (2007) visualised uptake of SWCNT into the cytoplasm and nuclei of human cells, where they caused dose-dependent cell death.
What is the effect of removal of metals on their toxicity?	Nanotubes remain toxic	Purified carbon nanotubes cause inflammation, granulomas and fibrosis in the lungs of test rodents (Lam et al. 2004, Shvedova et al. 2005), demonstrating that metal impurities were not responsible for the lesions and that SWCNTs are intrinsically toxic.
Do fibres persist in rat lung following	Yes, *in vivo* studies show	Muller et al. (2005) found that MWCNT were persistent in pulmonary lesions 60 days after intratracheal

inhalation or instillation?	CNTs persisting in rodent lungs	instillation; 81.2% of 5.9µm MWCNT persisted, 36% of 0.7µm persisted. Lam et al. (2004) found that 90 days after intratracheal instillation, lung granulomas contained particle-laden macrophages and SWCNT particles. Shvedova et al. (2005) found SWCNT were persistent in lungs 60 days after pharyngeal aspiration. Lam et al. (2006, p209) note that: "CNTs are totally insoluble and probably one of the most biologically non-degradable man-made materials".
Do fibres cause an inflammatory response following inhalation or instillation?	Yes	Pharyngeal aspiration of SWCNTs caused lung inflammation and lesions and dose-dependent oxidative damage to mitochondrial DNA in the hearts of test mice (Li et al. 2005, 2006). Muller et al. (2005) found that MWCNT were highly fibrogenic and inflammogenic, roughly equivalent to a chrysotile asbestos control. Shvedova et al. (2005) found that SWCNT induces a "robust acute inflammatory reaction". In a 24 day study, Li et al. (2007) compared intratracheal instillation with inhalation exposure of mice to MWCNTs. Intratracheal exposure caused inflammation and severe destruction of the alveolar netting, inhalation exposure resulted in development of only "moderate pathological lesions".
Do fibres cause fibrosis and/or cancer after long term inhalation?	Unknown	Only one short term inhalation study has been conducted. The longest instillation study is 90 days. Given the possible differences in effects following instillation vs inhalation exposure of nanotubes, more inhalation studies and studies of greater length are required in order to investigate effects of more realistic exposure (Warheit et al. 2006). Lam et al. (2004) found that 90 days after intratracheal instillation, 3 different types of SWCNTs caused severe granulomas and fibrous lesions. 60 days after intratracheal instillation, Muller et al. (2005) found MWCNT caused fibrosis. 60 days after pharyngeal aspiration of SWCNT Shvedova et al. (2005) found the onset of interstitial fibrosis.
Do fibres cause mesothelioma (a cancer) after injection into rat pleura/ peritoneum (membranes of the lung and abdominal cavity, respectively)?	Yes	Poland et al. (2008) found that 7 days after injection of a moderate MWCNT dose into the peritoneum of mice, long MWCNTs caused inflammation, granulomas and the onset of mesothelioma; short and tangled MWCNTs did not. Takagi et al. (2008) found that 180 days after injection of a high dose of MWCNTs into the periotoneum of one group of mice, and crocidolite (blue asbestos) into the periotoneum of another group, mice exposed to MWCNTs suffered greater deaths by mesothelioma than those exposed to asbestos.

Appendix 2: Detailed summaries of some key studies

The following section provides some detailed summaries of a sample of the published literature on carbon nanotubes toxicology. It is far from comprehensive. In recent years there has been a dramatic growth in the published literature investigating the toxicity of carbon nanotubes. For a more comprehensive review of the literature see Donaldson et al. (2006), Jain et al. (2007), Lam et al. (2006), Muller et al. (2006), Oberdörster et al. (2007) or Warheit (2006).

Injection of nanotubes into rodent body cavities show nanotubes behave like asbestos and can cause mesothelioma

In order to evaluate whether or not nanotubes have the potential to cause mesothelioma, the RS/RAE recommended conducting experiments involving injection of nanotubes into the membrane linings of mice lungs or abdominal cavities (RS/RAE 2004). Injection does not simulate realistic exposure scenarios as studies directly introduce nanotubes into the mesothelial lining rather than requiring nanotubes to be inhaled and then transported into the lining. Although nanomaterials are known to be translocated widely throughout the body, there are no data yet on the capacity for carbon nanotubes to be transported into the lung lining (Donaldson et al. 2006). Nonetheless, injection studies are one valuable component of the testing regime required to investigate the potential for carbon nanotubes to show asbestos-like pathogenicity. The two injection studies carried out to date demonstrate that carbon nanotubes which look like asbestos cause asbestos-like pathogenicity. Takagi et al. (2008) further showed that in an 180 day study carbon nanotubes cause fatal mesothelioma in test mice.

Nanotubes that look like asbestos behave like asbestos: Injection of nanotubes into mice abdominal cavity caused asbestos-like pathogenicity in 7 days

A team of researchers from 3 Scottish universities, the Institute of Occupational Medicine (UK) and the US Woodrow Wilson International Center for Scholars (Poland et al. 2008) injected a low dose of multi-walled carbon nanotubes (MWCNTs) into the abdominal cavities of mice. The researchers found that long MWCNTs - nanotubes that look most like asbestos - showed “asbestos-like, length-dependent, pathogenic behaviour”. This included inflammation and development of granulomas and giant cells “that were qualitatively and quantitatively similar to the foreign body inflammatory response caused by long asbestos”. Of most concern, Poland et al. found that long MWCNTs caused asbestos-like pathogenicity within the short 7 day period of their study. They found that short, tangled MWCNTs did not mimic the behaviour of long asbestos fibres. The authors observed successful clearance of these shorter MWCNTs by macrophages (scavenger cells), which explains the absence of an inflammatory effect. However the small number of animals in the study (n=3 for each treatment of short MWCNTs), the single injection of MWCNTs and the short time period suggest that further study of short MWCNT behaviour in the mesothelial lining would be useful. Furthermore, these results do not mean that short MWCNTs are safe. Poland et al. write that “short CNTs may be pathogenic by virtue of being particles, and that would not have been detected in the assays used here, which are sensitive only to asbestos/fibre-type effects”.

The authors concluded that: “Our results suggest the need for further research and great caution before introducing such products [containing carbon nanotubes] into the market if long-term harm is to be avoided” (Poland et al. 2008).

Nanotubes caused more deaths from mesothelioma than did the most potent asbestos following their injection into mice abdominal cavity in 180 day study

A group of researchers from the Japanese National Institute of Health Sciences and the Tokyo Metropolitan Institute of Public Health (Takagi et al. 2008) injected a high dose of MWCNTs into the abdominal cavity of mice and compared the effects of this with mice injected with the most potent form of asbestos (crocodolite) over a 180 day period. A third group was treated with carbon fullerenes as a control. The researchers used MWCNTs where 72.5% of the sample had a length <5µm, and 27.5% of the sample had a length 5-20µm; the length that Poland et al. (2008) referred to as short and intermediate. MWCNT treated mice showed moderate to severe fibrotic abdominal adhesion (abnormal binding of two tissues as a result of inflammation or damage), abnormal multi-nucleated giant cells and a high incidence of large (2.7 x 1.5cm) tumours; the mice treated with asbestos showed the same symptoms, but to a lesser extent. MWCNT and asbestos-laden cells were also found in the liver and the lymph nodes. The mice treated with fullerenes showed abdominal lesions, but no abdominal adhesion or tumour development. Deaths as a result of mesothelioma were greatest in the MWCNT treated mice, followed by the asbestos-treated mice; no fullerene-treated mice developed mesothelioma. It should be emphasised that the lack of asbestos-like pathogenicity from fullerenes does not mean that they are safe – as with Poland et al. (2008), this study is sensitive only to identification of fibre-related as opposed to particle-related toxic damage.

The authors conclude by emphasising the need for hazard identification studies to take place before wide-spread commercial use of nanotubes “so that harmful exposure can be prevented before it happens. In this way, manufacturers can produce safer products without risking themselves and the consumers by waiting for the full chronic toxicology studies including carcinogenicity studies to be finished after their initial (less safe) products are widely marketed” (Takagi et al. 2008, p145).

Instillation exposure studies show that carbon nanotubes can cause inflammation, fibrosis and development of granulomas

Several peer-reviewed studies have demonstrated the potential for exposure to carbon nanotubes to result in asbestos-like disease in rats and mice. These studies have generally used either intratracheal instillation or pharyngeal aspiration to expose rodents to carbon nanotubes. These methods rely on introducing a bolus (ball) of carbon nanotubes into back of the animals’ throat, from where the nanotubes are inhaled.

Instillation experiments have been criticised for not replicating realistic exposure scenarios and potentially overestimating the harm associated with inhalation exposure (Donaldson et al. 2006; Warheit et al. 2006; Oberdörster et al. 2007). The one published study comparing inhalation with instillation exposure to nanotubes has found instillation exposure does cause greater harm than inhalation exposure, although

inhalation also resulted in the development of moderate lesions (Li et al. 2007). Despite the clear desirability of studies using realistic exposure scenarios, instillation studies do reveal the intrinsic toxicity of carbon nanotubes (Lam et al. 2006). They demonstrate that if sufficient quantities of carbon nanotubes reach the lungs, they cause inflammation, fibrosis and the development of granulomas.

Intratracheal exposure of mice to single walled carbon nanotubes induced granulomas and inflammation

Researchers from NASA and the University of Texas (Lam et al. 2004) exposed mice to a single intratracheal dose of SWCNT (with some contaminants) and sacrificed the animals after 7 and 90 days. They found the SWCNT resulted in dose-dependent, persistent epithelioid granulomas and in some cases also interstitial inflammation. The lesions were more pronounced in the 90 day group, where the lungs of some animals also revealed "peribronchial inflammation and necrosis that had extended into the alveolar septa".

Lam et al. used carbon black and high-dose quartz dust as controls, and concluded that: "…if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures". They also warned that: "…if workers were chronically exposed to respirable NT [carbon nanotubes] dust at a fraction of the PEL [permissible exposure limit] concentration for synthetic graphite, they would likely develop serious lung lesions".

Exposure of mice to single walled carbon nanotubes by pharyngeal aspiration resulted in the early onset of granuloma development and fibrosis

Researchers from the United States National Institute for Occupational Safety and Health and NASA exposed mice to a single intratracheal inhalation dose of single walled carbon nanotubes (SWCNT; Shvedova et al. 2005). They used purified (contaminant-free) SWCNT at levels that proportionately reflected the existing permissible exposure limit for graphite particles (there are no exposure limits set for carbon nanomaterials). They sacrificed the animals after 1, 3, 7, 28, and 60 days. Exposure to the SWCNT resulted in inflammation, reduced pulmonary function, granuloma development and the early onset of fibrosis. SWCNT were more toxic than comparable quantities (by weight) of ultra-fine carbon black or silica dust.

The authors concluded that: "results of this study suggest that if workers are exposed to respirable SWCNT [single-walled carbon nanotubes] particles at the current PEL [permissible exposure limit] (for graphite particles), they may be at risk of developing some lung lesions" (Shvedova et al. 2005, p703).

Intratracheal exposure of rats to single walled carbon nanotubes induced granulomas and inflammation

Researchers from three Belgian universities (Muller et al. 2005) used intratracheal instillation to expose rats to both intact and ground multi-walled carbon nanotubes (MWCNTs) for 60 days. Intact MWCNT agglomerated in the airways and induced granulomas and surrounding alveolitis. Ground MWCNT were better dispersed in the lung parenchyma and also induced inflammation and fibrotic responses (granulomas).

MWCNT persisted in the lung over the two months of the study period; 80% of the intact MWCNT and 40% of the ground MWCNTs were still in the animals' lungs after 60 days. The authors concluded that: "MWCNTs are potentially toxic to humans".

Comparative study finds intratracheal instillation of multi-walled carbon nanotubes caused inflammation and severe pulmonary damage; inhalation resulted in moderate pathological lesions

In a 24 day study, researchers from the Chinese Academy of Sciences and Ningbo University (Li et al. 2007) compared the effects on mice of exposure to MWCNTs via a single intratracheal instillation with inhalation exposure over 8 to 24 days. Exposure via the single intratracheal instillation resulted in inflammation of the bronchial lining and severe destruction of the alveolar netting. Exposure via repeated inhalation of aerosolised MWCNTs resulted in lesser damage, which the authors described as "moderate pathological lesions". Li et al. suggested that the lesser damage caused by exposure to aerosolized MWCNTs could be caused by: non-respirable MWCNTs particles not being taken into the mice's bodies, slow exposure occurring over a relatively long period of time, and the smaller size of MWCNT aggregates in the aerosol compared to the instillation. This experiment suggests that instillation experiments may over-estimate the harm caused by MWCNT exposure, although the inhalation treatment still resulted in moderate pathological lesions.

Reviews of the published literature on carbon nanotubes toxicity conclude that SWCNTs and MWCNTs have the potential to cause severe lung disease and possibly cancer

A review of the published literature by Belgian researchers (Muller et al. 2006) concluded that: "Overall, the available studies indicate that, if they reach the lung, CNTs (SWCNTs and MWCNTs) have the potential to cause severe inflammatory and fibrotic reactions. While recognizing their limitations, these data suggest that if workers were exposed to respirable CNTs, they may be at risk of developing serious lung diseases".

"… When considering the possible adverse health effects of inhaled particles, it seems important for future studies to address the possible carcinogenic potential of CNTs, especially as these particles have proved to elicit an inflammatory reaction (alveolitis) which is considered as a source of genotoxic lesions that may be the forerunners of lung cancer. The fibrotic activity of CNTs is a second reason for seriously considering the carcinogenic potential of these particles because we know that lung fibrosis and malignant pneumoconioses are associated with an increased risk of lung cancer."

In their "Review of Carbon Nanotube Toxicity and Assessment of Potential Occupational and Environmental Risks", researchers from NASA and the University of Texas Medical School concluded that there is strong evidence that carbon nanotubes present serious health hazards (Lam et al. 2006).

"The animal studies of CNT pulmonary toxicity collectively showed that CNTs are capable of inducing inflammation, epitheliallioid granulomas, fibrosis, and biomechanical toxicity changes in the lungs that might impair pulmonary functions… Cell culture studies also showed that CNTs were cytotoxic. These results indicate that, if CNT particulates reach the lung in sufficient quantities, they will produce a toxic response (Lam et al. 2006, p210).

In their review of the carbon nanotube toxicological literature, Donaldson et al. (2006) sought to "to set out the toxicological paradigms applicable to the toxicity of inhaled CNT, building on the toxicological database on nanoparticles (NP) and fibers". The researchers from the University of Edinburgh and the Institute of Occupational Medicine concluded that carbon nanotubes present potential health risks both as respirable fibres and as nanoparticles that cause oxidative stress and inflammation:

"Such peer-reviewed literature as is currently available suggests that CNT may have toxic effects beyond those anticipated for their mass exposure. For example, they have more adverse effects than the same mass of NP carbon and quartz, a commonly used yardstick of harmful particles... However, it should be noted that there is, as yet, no definitive inhalation study available that would avoid the potential for artifactual effects due to large mats and aggregates forming during instillation exposure procedures. CNT may have their effects through oxidative stress and inflammation (Fig. 6), and this is borne out by some published toxicology studies. Additionally, the studies so far suggest that they may have an unexpected ability to cause granuloma formation and fibrogenesis. The NP toxicology paradigm also emphasizes the potential for NP to translocate from their portal of entry to other tissues, and this should remain a potential target in CNT research" (Donaldson et al. 2006, p18).

Toxicity studies show nanotubes can accelerate development of artery 'plaque' that causes heart attacks, impair immune system function, damage DNA and cause cell death

Intratracheal exposure to single walled carbon nanotubes caused development of artery 'plaque' that causes heart attacks, DNA damage in hearts of test mice

Nanomaterials can be translocated through the body, presenting the risk of damage far from the site of exposure. The link between exposure to air pollution, especially containing particles measuring <100nm, and the onset of cardiovascular disease has been suggested by both experimental and epidemiologic studies. Researchers from the United States National Institute of Occupational Safety and Health (NIOSH; Li et al. 2007b) found that in an *in vivo* study, addition to lung toxicity, intratracheal exposure to SWCNTs caused the development of artery plaque (atheroma) responsible for heart attacks and strokes, and damaged mitochondrial DNA in the hearts of test mice. They observed that: "respiratory exposure to high concentrations of mostly agglomerated SWCNTs provokes not only pulmonary toxicity but vascular effects related to mitochondrial oxidative modifications and accelerated atheroma formation" (Li et al. 2007b, p382). Atheroma - the accumulation and swelling in artery walls of 'plaque' made of lesions, lipids and fibrous tissue - is the root cause of cardiovascular diseases such as angina, heart attack, stroke and peripheral vascular disease.

The authors conclude that: "Taken together, the findings are of sufficient significance to warrant further studies to evaluate the systemic effects of SWCNTs under inhalation exposure paradigms more likely to occur in the workplace or environment, such as low-level chronic inhalation exposure" (Li et al. 2007b, p382).

Lung proteins preferentially bound to carbon nanotubes, indicating potential for damage to immune system, increased risk of lung infections and emphysema

Researchers from Oxford University and the Université Paul Sabatier suggest that the preferential binding of lung proteins to carbon nanotubes could impair immune system function, even increasing vulnerability to emphysema (Salvador-Morales et al. 2007). Surfactant proteins A and D (SP-A and SP-D) are secreted by airway epithelial cells in the lung. These proteins play an important role in immune system function and are a first-line defence against infection within the lung. Mice lacking these proteins show greater risk of infection and emphysema. This *in vitro* study found that SP-A and SP-D selectively bound to double walled carbon nanotubes (MWCNTs) in the presence of Ca^{2+}-ions. The binding was variable between batches of nanotubes, leading the authors to suggest that it was likely to be mediated by surface impurities or chemical modifications of the nanotubes.

The authors conclude that: "The results suggest that long-term exposure to inhalation of nanotubes has the potential to enhance susceptibility to infection and emphysema" (Salvador-Morales et al. 2007, p608).

Carbon nanotubes entered cell nuclei and caused dose-dependent cell death

The potential for carbon nanotubes and other nanomaterials to be taken up into individual cells has been touted as offering new options for pharmaceutical delivery. However the unique bioavailability of nanomaterials also presents serious new toxicity risks for non-medical nanomaterial exposure. SWCNTs measure only 0.6-3.5nm in diameter, much smaller than the pores in the membrane of cell nuclei, enabling them to translocate across it. In an *in vitro* experiment, researchers from the University of Cambridge and Daresbury Laboratory (Porter et al. 2007) have visualised the uptake of SWCNT into the cytoplasm of human immune cells where they localised within the cell nucleus. Porter et al. (2007, p716) note that: "Uptake to these sites implies that they [SWCNTs] may interact with intracellular proteins, organelles and DNA, which would greatly enhance their toxic potential". Porter et al. observed significant cell mortality after 4 days that was dose-dependent. Regions of higher SWCNT density were correlated with a marked increased in cell mortality.

Multi-walled carbon nanotubes localised within skin cells, caused irritation, impaired protein function, decreased cell viability, could cause skin disease

Monteiro-Riviere et al. (2005) from North Carolina State University tested MWCNT for cellular toxicity. The group used human epidermal keratinocytes (major cell types of the outer layer of our skin) in an *in vitro* study to mimic potential skin exposure. However they caution that their study cannot provide information about realistic occupational risks of nanotube skin exposure as keratinocyte cultures do not have the protective outer barrier seen with intact skin. The study found that MWCNTs caused time and dose-dependent release of a pro-inflammatory cytokine (signalling protein) and decreased cell viability. They observed that the MWCNTs were "capable of both localizing within and initiating an irritation response in a target epithelial cell that composes a primary route of occupational exposure for manufactured nanotubes" (Monteiro-Riviere et al. 2005, p377).

In a subsequent *in vitro* study Witzmann and Monteiro-Riviere (2006) investigated the effect of MWCNT exposure on the proteins of the keratinocytes. They found that after 24 hours of exposure the expression of 36 proteins had been altered; after 48 hours 106 were altered. The authors also observed decreased cell viability, although they weren't able to establish whether this was because the keratinocytes had stopped growing or started dying in response to MWCNT exposure, or both. They concluded that the alterations in protein expression, the observed irritation and decreased cell viability were evidence of the MWCNT's "injurious nature". "If one hypothesizes that the responses observed here also occur in vivo, epidermal MWCNT exposure creates the potential for chronic inflammation and injury which, in turn, may contribute to the development or progression of disease states in the skin" (Witzmann and Monteiro-Riviere 2006, p167).

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