



Inhalation toxicity of multi-wall carbon nanotubes in rats exposed for three months

Ma-Hock et. al. - A SAFENANO Commentary

Sheona A K Peters & Rob J Aitken, SAFENANO

In a recent paper published in *Toxicological Sciences*, "Inhalation Toxicity of Multi-wall Carbon Nanotubes in Rats Exposed for Three Months", a team of European researchers have reported the findings of a 90-day inhalation toxicity study of multi-walled carbon nanotubes (MWCNTs) in rats. This study - led by Robert Landsiedel, Head of the Short Term Toxicology Department at [BASF SE](#) in Germany - not only demonstrates the potential for MWCNTs to induce lung toxicity in exposed rats at low concentrations, but also incorporates numerous advances in methodology. The study represents a significant step forward towards generating suitable data to support regulatory risk assessments.

Background

Carbon nanotubes (CNTs) exist in two types: i) single-walled carbon nanotubes (SWCNTs) which consist of a single layer of carbon atoms (single molecule) arranged in a cylinder; and ii) multi-walled carbon nanotubes (MWCNTs) which comprise of multiple stacked single-walled carbon nanotubes with diameters ranging from 2-100 nm. Both SWCNTs and MWCNTs have attracted widespread interest for commercial and industrial applications due to their novel properties, such as high tensile strength, low weight, high electrical and thermal conductivity and unique electronic properties. The potential applications of CNTs are widespread, including: polymer composite materials, transparent conductors, field emission displays, electronic circuits and sensors.

However, despite rapid growth in the manufacturing and use of CNTs, concerns have been raised about their potential adverse effects on both human health and the environment.

Due to structural similarities in terms of their 'needle-like' shape, in combination with their high aspect ratio (ratio of length and width), low solubility and biopersistence, it has been hypothesised that CNTs may exhibit toxic properties similar to those of other fibrous materials such as asbestos.

Indeed, numerous *in vivo* studies have already demonstrated that both SWCNTs and MWCNTs, when instilled into the lungs of rodents, have the potential to cause inflammation, fibrosis (scarring of the lungs) and granuloma (small nodule) formation in the lung tissue (e.g. Lam et al., 2004; Muller et al., 2005, Chou et al., 2008). Long (>20 µm), straight MWCNTs have also been shown to have the potential to cause inflammation and granuloma formation in the mesothelial lining of the pleura, consistent with the pathogenic behaviour of asbestos.



So, what makes this study different?

The majority of in vivo studies conducted to date have involved intratracheal instillation of CNTs, whereby suspensions of CNTs are instilled directly into the lungs of the test species via the trachea. An alternative method which has also been employed is pharyngeal aspiration, involving the application of a small quantity of CNT-suspension to the pharynx at the far back of the rodent's mouth resulting in short-term inhalation. Although these routes are useful in terms of identifying potential hazards and intrinsic substance toxicity, the physiological relevance of such methods is subject to debate and it has been suggested that they may over-estimate the harm caused by genuine inhalation exposure to CNT aerosols. In addition, such methods assume that a certain quantity of particles will reach the lung, based on the concentration employed, and do not take into account deposition within the respiratory tract. Thus, current information on the lung toxicity of CNTs is fragmented and partly inconclusive, and therefore insufficient to inform a proper risk assessment.

In contrast, Landsiedel and co-workers have developed a unique test system to generate aerosols of nanomaterials and, in combination with optimised biological parameters (Ma-Hock et al., 2009), used this to perform an inhalation toxicity study which more accurately represents possible exposure to airborne MWCNTs. Although a few short-term MWCNT inhalation toxicity studies have been conducted before (e.g. Li et al., 2007), this is the first sub-chronic inhalation toxicity study of MWCNTs to be reported. Of additional advantage, this study was undertaken strictly according to [OECD test guidelines](#) (see Box 1) under [Good Laboratory Practice](#) (GLP) conditions, allowing full characterisation of MWCNT toxicity by the inhalation route with a view to providing more robust data to potentially inform a risk assessment for workplaces.

An overview of the study procedure and results

Landsiedel and co-workers generated highly respirable dust aerosol particles of thin MWCNTs (test substance diameter 5-15 nm, length 0.1-10 µm) using a unique brush generator (described in Ma-Hock et al., 2007). Although a high energy process, examinations of the test substance before and after the dust generation procedure confirmed that there was no damage caused to the structure of the tubes by this method and no increase in the level of reactive oxygen species (free radicals involved in oxidative stress, which may lead to cell damage) on the particle surface.

Male and non-pregnant female Wistar rats were allocated randomly to four test groups and then head-nose exposed to the MWCNT aerosol for 90 days (6 hours per day, 5 days per

Box 1: OECD Test Guideline 413

[OECD Test Guideline 413](#) (adopted in 1981) provides guidelines for 90-day subchronic inhalation toxicity studies, whereby groups of experimental animals are exposed for a defined number of hours daily to the test substance in various concentrations (one concentration per group), against a control group of non-exposed animals, for a period of 90 days. Animals are monitored daily for signs of toxicity during the 90 days of exposure, and then necropsied for analysis at the end of the study.

Also included in the document is guidance and recommendations in relation to:

- study preparation;
- selection of experimental animal species, number, sex and housing/feeding conditions;
- inhalation equipment and test conditions (e.g. exposure concentrations, exposure time and observation period);
- experimental procedure;
- physical measurements and monitoring;
- clinical examinations;
- pathology;
- histopathology;
- data and reporting.

A draft proposal for a [revised Test Guideline 413](#) was released for public comment last year and is currently under consideration.



week, for 13 weeks; a total of 65 exposures) at concentrations of 0 (control), 0.1, 0.5 or 2.5 mg/m³. The over pressure in the inhalation chamber was maintained to prevent dilution of the aerosol with laboratory air and, using scattered light photometers, aerosol concentrations in the chambers were continuously monitored throughout exposure.

Characterisation of samples of the test atmosphere using a Scanning Mobility Particle Sizer (SMPS) indicated that good dispersion of particles had been achieved, with most particles in the nanometre size range in terms of particle number (median particle diameter ~60 nm). However, according to mass-based measurements using a cascade impactor the aerosol was dominated by larger-sized clumps of particles with mass median aerodynamic diameters (MMAD) of between 0.7-2.0 µm. It is recognised that SMPS is a somewhat limited method for measuring CNTs, due to their unusual shape and physical properties, which the authors do acknowledge, recommending it be used for monitoring rather than quantitative measurements.

Clinical examinations of the test species during the 90-day study revealed no clinical signs of adverse health effects (e.g. no changes in food consumption, body weight, motor activity etc.) and there were no premature deaths. Following the final inhalation exposure, numerous clinical chemistry, haematology and pathology examinations were undertaken. With regards systemic toxicity, no adverse effects were observed following exposure at any of the test concentrations. However, at concentrations of 0.5 and 2.5 mg/m³, necropsy revealed grey lung discoloration and concentration-dependent lesions in the lung and lymph nodes (primarily inflammation and multifocal granuloma formation).

Even at the lowest concentration of 0.1 mg/m³ single granulomas were observed and thus, although only sub-clinical effects, this meant that a No Observed Adverse Effect Concentration (NOAEC) for MWCNTs could not be established. The lowest observed adverse effect level (LOAEC) for MWCNTs established in this study is therefore 0.1 mg/m³.

Conclusions

The study results clearly indicate the potential for MWCNTs to induce lung toxicity following inhalation exposure at what, in mass terms, are low concentrations. There is some uncertainty as to whether the researchers succeeded in generating an aerosol in the nanometre-size range, and dustiness measurements of the test substance (using standardised drop methods in a dust box) indicated its dust-forming potential to be relatively low. However, toxic effects were still observed, indicating the inherent toxicity of the substance and the potential health hazard posed by exposure to even very low concentrations of MWCNTs.

"This study is part of our commitment to identifying and subsequently eliminating the potential health risks posed by nanomaterials, and serves to highlight the need for safety research to identify concerns associated with the production and use of new nanomaterials," Robert Landsiedel commented to SAFENANO. "The outcomes of this study demonstrate that OECD test guideline study protocols are suitable for nanomaterial testing if their specific properties are taken into account within, for example, the generation and characterisation of aerosols from the MWCNTs. This study was performed in collaboration with our MWCNT supplier, with both parties benefiting from the results. We maintain that the safe handling and use of nanomaterials is possible, but only if appropriate safety measures are employed and, where necessary, certain uses are avoided."



Rob Aitken, SAFENANO's Director, concludes that:

"This is clearly an important study showing that MWCNT bundles of size 0.7-2 μm can cause pulmonary adverse effects in addition to the pleural effects according to the long fibre paradigm as observed by Poland et al. Whether the workers will be exposed to dusts in concentration or in the form used in this study remains to be seen. However, it reinforces the need to handle all CNTs with extreme care."

Please click here for free access to the journal article abstract from [Toxicological Sciences](#).

To find out more about published results from the BASF Safety Research on Nanomaterials group, [click here to visit their website](#).

Title paper: Ma-hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, van Ravenzwaay B, Landsiedel R. (2009) Inhalation toxicity of multi-wall carbon nanotubes in rats exposed for 3 months. *Toxicol. Sci.* (in press). [Advance access available online](#).

References:

Chou C-C, Hsiao H-Y, Hong Q-S, Chen C-H, Peng Y-W, Chen H-W, Yang P-C. (2008). [Single-walled carbon nanotubes can induce pulmonary injury in mouse model](#). *Nano Lett*; 8(2): 437-445.

Lam C-W, James JT, McCluskey R, Hunter RL. (2004). [Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation](#). *Toxicol. Sci.*; 77(1): 126-134.

Li J-G, Li W-X, Xu J-Y, Cai X-Q, Liu R-L, Li Y-L, Zhao Q-F, Li Q-N. (2007). [Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation](#). *Environ. Toxicol.*; 22: 415-421.

Ma-Hock L, Burkhardt S, Strauss V, Gamer A, Wiench K, van Ravenzwaay B, Landsiedel R. (2009). [Development of a short-term inhalation test in rats using nano-titanium dioxide as a model substance](#). *Inhal Toxicol*, 21:102-118.

Ma-Hock L, Gamer AO, Landsiedel R, Leibold E, Frechen T, Sens B, Linsenbuehler M, van Ravenzwaay B. (2007). [Generation and characterization of test atmospheres with nanomaterials](#). *Inhal Toxicol.*, 19(10): 833-848.

Muller J, Huaux F, Moreau N, Mission P, Heillier JF, Delos M, et al. (2005). [Respiratory toxicity of multi-wall carbon nanotubes](#). *Toxicol. Appl. Pharmacol.*; 207: 221-231.

Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. (2008). [Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study](#). *Nature Nanotech*; 3: 423-428.