The importance of studying the biodurability of carbon nanotubes

Julia Varet, SAFENANO Toxicologist

September 2011

Carbon nanotubes (CNT) display many unique and valuable properties – mechanical (such as high strength to weight ratio), electrical and thermal - and are therefore of widespread interest for industrial and commercial applications. These include composite materials, field emission displays, electronic circuits, sensors and medical tools for diagnostic and therapeutic purposes. As some of these applications have now been successfully launched onto the marketplace, the requirement to establish an appropriate assessment of potential hazard and risk is paramount.

It has been postulated that the toxicity of fibres is related to fibre length, bio-persistence and dose; a hypothesis known as the 'fibre paradigm' (Donaldson et al. 2006 and Donaldson et al. 2010) (Figure 1). Fibre dimension is important in determining the respirability of the material and its deposition in the respiratory tract. It has been shown that fibre length is also a critical parameter determining its fate in vivo (Donaldson et al. 2010). Indeed, above a certain length, a fibre may not be readily engulfed by cells from the immune system therefore triggering oxidative stress and inflammation, which may result in disease.

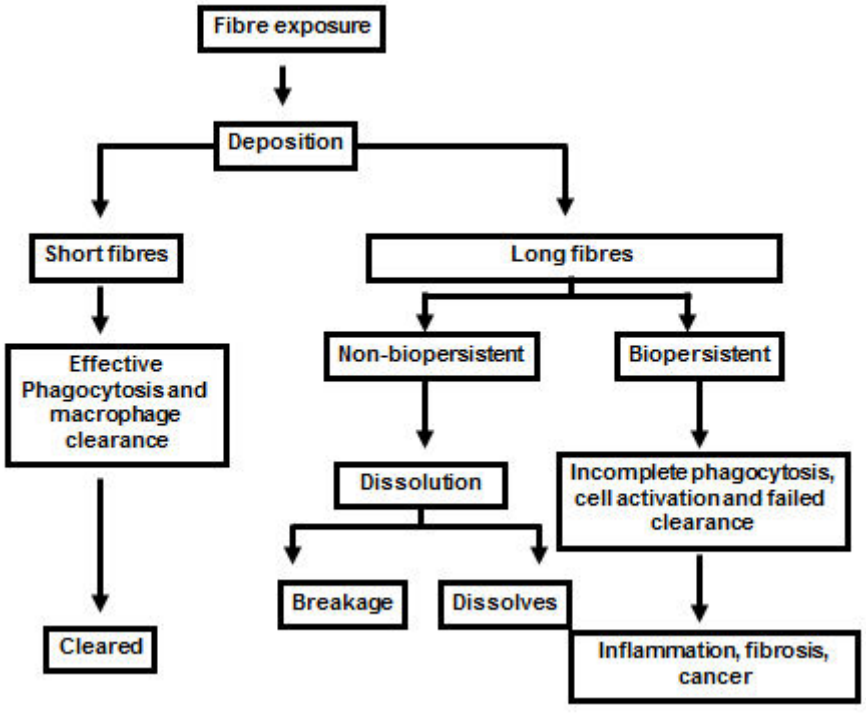

Figure 1: The Fibre Paradigm (adapted from Donaldson et. al. 2006)

The bio-persistence of a long fibre in vivo is also a critical parameter driving pathogenicity. Taking asbestos fibres as an example, chrysotile fibres are long and thin, however they are readily soluble in acidic environments and therefore display low bio-persistence. By contrast, another form of asbestos fibre - namely tremolite - is long and thin and displays strong bio-persistence. Therefore, it has been suggested that the lower pathogenicity of chrysotile fibres compared to tremolite fibres may in part be related to its low bio-persistence in vivo (McDonald

and McDonald 1997). Indeed, the driver of fibre toxicity is the number of long fibres; therefore dissolution of the long fibres also leads to a reduction of their biologically effective dose.

Bio-persistence is an in vivo concept and is defined as the ability of a material to persist in the body by resisting breakage into fragments and/or dissolution in solution and clearance because of their morphology/size. Some information about the bio-persistence of a material can be obtained studying its bio-durability in vitro, as a surrogate for in vivo bio-persistence. The study of material bio-durability involves immersing the material in a biologically relevant fluid such as a physiological solution (e.g. Gamble's solution - a complex salt solution designed to mimic the salt balance of extracellular fluid) and parameters such as pH, enzymatic and oxidative environment can be adjusted in order to mimic the fate of the material under various scenarios. The type of scenarios that researchers try to mimic include, for example, contact with the lung lining fluid for respirable materials or contact with the content of phagolysosomes of scavenging cells, which are cellular compartments dedicated to the digestion of engulfed pathogens and materials. Finally, after incubation, the recovered material is analysed in order to assess its degradation, usually via mass and/or size analysis (Donaldson and Tran 2004). This provides an estimate of how likely a fibre is to persist in the lung under various conditions, which may be intrinsically linked to their ability to cause harm.

Carbon nanotubes are High Aspect Ratio Nanomaterials (HARN) and can display various aspects (i.e. straight or entangled), lengths, diameters etc and some fulfil the World Health Organisation (WHO) definition of a fibre (WHO). Therefore, it has been proposed that the pathogenicity of such 'fibre-like' CNT (and more generally HARN) may follow the 'fibre paradigm' (Figure 1; Donaldson et al. 2006). Moreover, several studies have reported the presence of CNT in tissues of animals, even after long post-exposure periods (Ryman-Rassmussen et al. 2009, Pauluhn 2010, Ma-Hock et al. 2009). Hence, due to their fibrous nature, studying the bio-durability of carbon nanotubes is widely considered to be an important component of their hazard assessment and is being actively researched. A number of key research studies on this topic have been published to date and are overviewed below.

Key research studies on CNT bio-durability

One of the first major publications specifically addressing the durability of CNT was published by Allen et al. (2008). In their study, Allen et al. used single-walled carbon nanotube (SWCNT) which had been cleaned of metal contaminants via oxidative acidic treatment, which also induces the formation of carboxylic acid groups. Allen et al. incubated these SWCNT in presence of horseradish peroxidase (HRP), which is a protein which favours the insertion of oxygen onto molecules (using hydrogen peroxide) and can be found in cellular compartments specialised in 'digestion' of compounds, in animal and plant organisms. Over an incubation of 12 weeks, in presence of HRP and hydrogen peroxide, gradual degradation of the SWCNT was observed. The authors therefore suggested that in vivo, naturally occurring peroxidase enzymes (such as myeloperoxidase produced by the inflammatory neutrophils) could be agents involved in the bio-degradability of SWCNT, influencing their bio-persistence in the environment and their pathogenic potential.

Following this work, Liu et al. (2010) investigated the influence of surface chemistry of CNT on their bio-durability. For this purpose, the authors used SWCNT in their pristine (unmodified) form, SWCNT that had been treated with oxidising acids which induced the formation of carboxylic groups on their surface, and a range of other types of functionalised SWCNT. The various CNT were incubated in phagolysosomal simulant fluid which is an acidified physiological buffer with additional hydrogen peroxide to provide an oxidising environment, in order to mimic the phagolysosome, a cellular compartment dedicated to 'digestion' of compounds. SWCNT presenting carboxylic groups on their surface showed signs of degradation over time, supporting the previous findings by Allen et al. (2008). Moreover, the authors observed that this degradation was associated with breaking up and debundling of the SWCNT as well as oxidative attacks of the side walls resulting in release of carbon fragments. In contrast, pristine and other functionalised SWCNT were stable over time. The authors proposed that these results point toward a surface functionalisation-specific degradation mechanism and that understanding relationships between surface functionalisation and durability could be important for the safe development of SWCNT in medical applications.

Kagan et al. (2010) used short-cut carboxylated SWCNT and took a dual approach to studying the bio-degradation of CNT, using both acellular and cellular systems. In the acellular studies, when CNT were incubated with hydrogen peroxide or myeloperoxidase (two natural components of the cellular defence system against pathogens), no signs of degradation were reported. In contrast, when the SWCNT were incubated in presence of both myeloperoxidase and hydrogen peroxide, the CNT were observed to degrade over time. Through further investigations, the authors showed that hypochlorite and radical intermediates of the myeloperoxidase were involved in this degradation process and that the chemical nature of the material surface was of importance in the positioning and further activity of the myeloperoxidase. In the cellular studies, these SWCNT were firstly incubated in the presence of activated neutrophils, which are cells from the immune system dedicated to defend the organism against foreign bodies using an arsenal of oxidative mechanisms. It was shown that these SWCNT could be biodegraded by neutrophils, provided that uptake occurred. Secondly, the same SWCNT were incubated in presence of macrophages, which are cells from the immune system specialised in scavenging of pathogens. However, macrophages were less efficient than neutrophils at degrading the SWCNT and it was suggested that this differential activity was related to their lower content in myeloperoxidase. Altogether these results suggested that if uptake occurs, carboxylated SWCNT could be degraded in vivo by myeloperoxidase expressed in cells such as neutrophils, and to a lesser extent macrophages.

The most recent study concerning the bio-durability of carbon nanotubes is that of Osmond-McLeod et al. (2011), in which durability of a panel of both single and multi-walled CNT (MWCNT) was investigated. One SWCNT and three different MWCNT as well as asbestos and glass fibre controls were immersed in an acidified physiological solution for up to 24 weeks. The results showed that among this panel, only one MWCNT showed signs of degradation over time as assessed by mass loss and length reduction. The authors emphasised that not all CNT behave the same in terms of durability and that physico-chemical characteristics are important parameters influencing the potential degradability of a material.

Both, Kagan et al. (2010) and Osmond et al. (2011) also performed in vivo studies and showed a reduced inflammatory response in mice exposed to the partially degraded or shortened CNT respectively, as compared to their non-degraded counterparts. This reinforces the importance of bio-durability and its consequences in terms of number of long fibres and therefore dose.

In conclusion...

The latest studies show that CNT, and more generally HARN, can display a large range of shape such as 'fibre-like' or bundles and can also display very distinct and variable bio-durability. Moreover, structural defects, structure (single or multi-walled) and surface functionalisation are important physico-chemical parameters driving the (bio)durability of CNT in vitro and bio-persistence in vivo.

Research into this area is currently of importance, as a better understanding of the (bio)degradation of materials especially for fibrous ones is important for hazard assessment and could allow their safety by design including, for example, the design and production of new medical and pharmaceutical applications.

In conclusion, durability studies are an important aide in rapid screening of potential bio-persistence and in hazard consideration especially of fibrous nanomaterials such as some CNT.

References

Allen BL, Kichambare PD, Gou P, Vlasova II, Kapralov AA, Konduru N, Kagan VE, Star A. 2008. "Biodegradation of single-walled carbon nanotubes through enzymatic catalysis." Nano Lett. vol. 8, no. 11, pp. 3899-903.

Donaldson K, Murphy FA, Duffin R, Poland CA. 2010. "Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma." Part Fibre Toxicol. Vol. 7, no. 5. Review.

Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A. 2006. "Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety." Toxicol Sci., vol. 92, no. 1, pp. 5-22. Review.

Donaldson K, Tran CL. 2004. "An introduction to the short-term toxicology of respirable industrial fibres." Mutat Res., vol. 553, no. 1-2, pp. 5-9. Review.

Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, Volkov Y, Vlasova II, Belikova NA, Yanamala N, Kapralov A, Tyurina YY, Shi J, Kisin ER, Murray AR, Franks J, Stolz D, Gou P, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA. 2010. "Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation." Nat Nanotechnol., vol. 5, no. 5, pp. 354-9.

Liu X, Hurt RH, Kane AB. 2010. "Biodurability of Single-Walled Carbon Nanotubes Depends on Surface Functionalization." Carbon N Y. vol. 48, no. 7, pp. 1961-1969.

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 multiwall carbon nanotubes in rats exposed for 3 months." Toxicol Sci. vol. 112, no.2, pp.468-81.

McDonald JC, McDonald AD.1997. "Chrysotile, tremolite and carcinogenicity." Ann Occup Hyg., vol. 41, no. 6, pp 699-705.

Osmond-McLeod, M.J., Poland, C.A., Murphy, F., Waddington, L., Morris, H., Hawkins, S.C., Clark, S., Aitken, R., McCall, M.J., Donaldson, K. 2011, "Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres" Part Fibre Toxicol, vol. 8, no. 15.

Pauluhn J. 2010. "Multi-walled carbon nanotubes (Baytubes): approach for derivation of occupational exposure limit." Regul Toxicol Pharmacol., vol. 57, no. 1, pp. 78-89.

Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong BA, Dodd DE, Andersen ME, Bonner JC. 2009. "Inhaled carbon nanotubes reach the subpleural tissue in mice," Nat Nanotechnol., vol. 4, no. 11, pp. 747-51.

WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF (1985), Reference methods for measuring airborne man-made mineral fibres (MMMF). World Health Organization, Regional Office for Europe, Copenhagen 1985, pp 16-35. (Environmental health 4).