

**Thesis abstract submitted by Matthew S. P. Boyles for the award of Doctor of Philosophy, September 2012. Thesis title: "The toxicity and potential pathogenicity of high-performance engineered multi-walled carbon nanotubes"**

The potential health consequences of carbon nanotube (CNT) exposure is often compared to asbestos and other fibre like materials due to their similar high aspect ratio and potential biopersistence; both are key in driving fibre toxicity and pathogenicity. With similar characteristics CNT are hypothesised to induce similar toxicity, and potentially similar pathogenicity. It is important to test this hypothesis in order to inform safe methods for production, handling and disposal of CNT.

The aim for this research was to employ a range of biological techniques to ascertain the cytotoxicity of different multi-walled (MW)CNT that are morphologically and compositionally distinct, and comparing these samples to toxicologically relevant materials such as asbestos and carbon black nanoparticles. The MWCNT used were either supplied by an industrial source, or were produced using controlled growth methods to allow investigation into certain size ranges, catalytic iron content, and sample purity (crystallinity).

Using cell free, *in vitro* and *in vivo* techniques for oxidative stress assessment, an early generation of ROS was found in response to entangled MWCNT, with greater observed responses to both short and long, straight MWCNT found as exposure times progressed. Also most prominent at the later exposure periods, substantial and significant cell death was observed in MM6 and J774A.1 cells in response to MWCNT samples, measured through reduced cellular viability and LDH release. The level of cell death induced by MWCNT was not matched by cell exposures to reference materials. Numerous markers of a pro-inflammatory responses and markers indicative of tissue damage and angiogenesis were assessed *in vitro* using MM6 and J774.A1 cell. Both cell types were found to secrete significantly elevated levels of MCP-1, TNF- $\alpha$ , TGF- $\beta$  and VEGF in response to MWCNT. Although not as high as the CNT, LFA was also found to stimulate pronounced pro-inflammatory conditions, when compared to the other reference materials. Numerous techniques were employed to assess the ability

of immortalised and primary cells to phagocytose particles. Frustrated phagocytosis was observed in response to the longer particles (both CNT and asbestos) and to agglomerates formed of shorter CNT. This frustrated phagocytosis induced by the long MWCNT samples was found to translate to an exaggerated respiratory burst, and a dysfunction and inhibition in the ability of cells to phagocytose fluorescently labelled E. coli.

Taking all of the results of this study into consideration it was clear that the MWCNT samples tested display a greater toxicity than the reference materials in this panel. Above all, differences in the responses to the five MWCNT samples were considered to be induced by either a long individual length, or large agglomerate formation, and therefore the effects attributed to a high aspect ratio and frustrated phagocytosis. However, at times there was an inference that a high bioavailable iron content or high level of sample purity may intensify cellular response to MWCNT. The findings here, and throughout the current literature, demonstrate that CNT are certainly capable of inducing pathogenesis, but biological responses vary with differences in CNT morphology and composition.