

## **Ranking Carbon-based Nanomaterials using Cytotoxicity to Minimize Public Health Risks**

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### **Abstract**

Carbon-based nanomaterials (CNMs) have generated a great interest in different sectors as they are stable, have limited reactivity, wide surface area, and are strong antioxidants. This study involves the ranking of CNMs and its derivatives on the basis of cellular toxicity as they are small enough to have the potential to enter the body and the detrimental effects reported are similar to those associated with asbestos. Literature review of toxicity studies was conducted to understand the effects of functionalization, size, solvents, light, length, etc. on cytotoxic effects of CNMs. Cytotoxicity of different CNMs with considerable differences in structure has also been compared. The information is utilized to prioritize CNMs used for different applications using cytotoxic studies. This analysis helps in identifying CNMs with high cellular toxicity, needing immediate attention for monitoring and regulation purposes.

**Keywords:** Carbon-based nanomaterials; exposures; public health; ranking; emerging contaminants.

### **1. Introduction**

Engineered nanomaterials (ENMs) have been reported to be present in environment (Kumar, 2012; Sundaram and Kumar, 2012; Chawla and Kumar, 2013). Among carbon-based nanomaterials (CNMs), fullerenes and carbon nanotubes (CNTs) appear to be widely used and might pose health risks during their exposures from inhalation,

ingestion, and absorption through the skin pathways. There is a need for ranking these ENMs for monitoring and regulation purposes (Kumar, 2012) in order to protect public health. The objective of this paper was to prioritize CNMs based on their toxicities to cell (i.e., using cytotoxicity information), useful information in decision-making process for determining CNMs types to be monitored and regulated.

## 2. Methodology

We have reviewed the cytotoxic studies of CNMs and discussed the factors affecting the cytotoxicity using information available from literature reports. This information was used to rank CNMs based on their cytotoxicity information and existing knowledge gaps were also identified.

## 3. Results and Discussion

### 3.1 Review of cytotoxic studies of CNMs

Cytotoxic studies of many CNMs like carbon black, carbon nano fibre, nano diamond, fullerenes, single walled carbon nano tubes, multi walled carbon nanotubes etc. have been reported in literature. Jia et al., 2005 studied the cytotoxicity of guinea pig alveolar macrophage and reported that cytotoxicity of SWCNTs was higher than MWCNTs and no cytotoxicity was seen for fullerenes (C60). It has been reported that SWCNTs considerably impaired phagocytosis of macrophages at dose of 0.38 µg/ml, while MWCNTs and C60 induced injury only at a high dose of 3.06 µg/ml. Fiorito et al, 2006 predicted no cytotoxicity for C60 and SWCNT for murine macrophage cell line: J 774 in concentration range 15-60 µg/mL. Magrez et al, 2006 found that lung tumor cells exposed to CBs, CNFs, and MWCNTs (0.02 µg/ml) for 2 days shown toxicity in the order of CB >CNFs >MWCNTs and discussed that MWCNTs, with maximum aspect ratio may have least number of dangling bonds. Schrand et al, 2007 found that nanodiamonds were more biocompatible than carbon black, MWCNTs, and SWCNTs, respectively, in two different cell types (neuroblastoma and alveolar macrophage). Sayes et al, (2004) showed that cytotoxicity to human liver carcinoma cells was inversely related to the solubility of fullerene derivatives, largely as a consequence of the reduced ability to generate oxygen free radicals that are the cause of cytotoxic effects via lipid peroxidation. Cui et al, 2005 tested SWCNTs on human embryo kidney cells (HEK293) and reported that they inhibit the proliferation of these cells by inducing cell apoptosis and decreasing cellular adhesive ability. We have tried to identify the factors affecting the toxicity and discussed in following section:

*Variation in size and shape:* Shape and size of MWCNTs affect the cytotoxicity due to change in agglomeration and dispersion behavior. Wang et al, 2009 reported that MWCNTs with smaller diameters showed less cytotoxicity. These results suggest that the cytotoxicity of MWCNTs was strongly affected by their size, purity, and surface conditions. Sato et al, 2005 investigated the activation of the human acute monocytic leukemia cell line THP-1 in vitro and the response in subcutaneous tissue in

vivo to CNTs of different lengths 9220nm and 850 nm). Results indicated that the degree of inflammation around 825-CNTs was stronger than that around 220-CNTs since macrophages could envelop 220-CNTs more readily than 825-CNTs. In another study by Fonseca et al, 2012 including cellular toxicity using macrophages from the J774 cell line; cytotoxicity was related to the length and COOH content.

*Effect of functionalization:* It has been reported in many studies that functionalization of CNMs, carbon nanotubes and fullerenes plays a very important role in deciding the cytotoxicity. Most of the studies support that after functionalization cytotoxicity of carbon nanotubes and fullerenes decreases up to considerable extent. However different functional groups increase the biocompatibility of nanomaterials to different extent. Sayes et al, 2004 discussed that Increase of water soluble functional group on C60 decreased cytotoxicity (HepG2, HDF). Isakovic et al, 2006 in a cytotoxic study (L929, C6, U251) for C60 (0.01-1 µg/ml), C60(OH)<sub>n</sub> (10-1,000 µg/mL) that LC50 for functionalized fullerene is more than unfunctionalized one (LC50 of C60: 0.25 µg/mL LC50 of C60(OH)<sub>n</sub>: 800-1000 µg/ml). Sayes et al, 2005 studied human dermal fibroblasts incubated with unfunctionalized, surfactant stabilized SWCNT and functionalized water dispersible SWCNTs with different groups., phenyl-SO<sub>3</sub>H, phenyl-SO<sub>3</sub>Na, phenyl-(COOH)<sub>2</sub> and found functionalized one more biocompatible compared to other. Cells incubated with SWCNT-COOH were considerably toxic at concentrations 10 to 200 µg/ml, but SWCNT-phenyl-SO<sub>3</sub>H was not toxic at concentrations up to 2 mg/ml. these studies indicate the effect of functionalization on toxicity of CNMs and need to be considered during ranking process.

*Percentage of catalyst residue/solvent:* The commonly used technique for manufacturing of SWCNT is catalytic disproportionation of gaseous carbon molecules supported on catalytic iron particles (Bladh et al, 2000). The catalyst residues also have remarkable effect on cytotoxicity. Garibaldi et al, 2006 reported that cardiac muscle cells incubated for up to 3 days with highly purified SWCNTs (200ug/ml) were little modified in shape due to SWCNT binding to the cell membranes with cell viability remaining >90%. The effect of the residual metals in SWCNTs was also reported by Pulskamp et al, 2007. Kagan et al, 2006 concluded that the presence of iron in SWCNT affects the redox-dependent responses of macrophages. Panessa-Warren et al, 2008 also compared human lung epithelial cell responses and reported less cytotoxicity for metal containing particles. All cell types are different, and may respond differently to specific metals. Cytotoxicity has also been related to residual solvent used. Kovoichich et al. (2009) studied C60, Cytotoxicity (RAW264.7) 5 µM and reported that THF treated C60, C60 (OH) 18 generated cytotoxicity is related to the residual THF.

*Estimation methods/Cell type/Exposure time and Dose:* Wörle-Knirsch et al, 2006 reported that the MTT assay of A549 showed a decrease in cell viability upto 40% after adding SWCNTs, but no viability decrease was observed by WST-1 assay. Herzog et al, 2007 reported that EC50 (50% reduction concentration in cell viability) of SWCNTs for A549 was higher than 400µg/mL, which is tens of times higher than those

for human bronchial epithelial cells (BEAS-2B) and HaCaT. However, the incorporation of hydrochloric acid purified MWCNTs (>96 wt% purity) into polysulfone thin films was found to show good biocompatibility with both human osteoblasts and fibroblasts for up to 7 days (Chlopek et al. 2006). Yamawaki et al, 2006 reported Cytotoxicity >100 µg/mL (human vascular endothelial cell) for modified fullerenes C60(OH)<sub>24</sub>. However in another study (Cytotoxicity CHO, MDCK) by Han et al, 2008 reported the LD50 of C60 33mg/l. Cytotoxic responses are found to increase with respect to increase in concentration of dose as well as time of exposure.

### **3.2 Ranking of CNMs on the basis of cytotoxic studies**

A general trend for cytotoxicity as per reviewed studies follows the single-walled carbon nanotubes > multiwalled carbon nanotubes > carbon black powders/Fullerenes > Nano diamonds. Macrophages found to be more sensitive as per cytotoxicity results. If we compare functionalized materials with non-functionalized one; type of functionality decides the cytotoxic behavior. In general, cytotoxicity decreases after modification and purification of nanomaterial. Few studies have also reported that the purification could contribute to CNT toxicity. Some studies have also found that purified or acid-treated CNTs had greater toxic effect than unpurified ones (Coccini et al, 2010; Vittorio et al, 2009). The residual solvents have also their effects in case of fullerenes. The main factors affecting the actual results are material specific. The sample characterization and standardized estimations methods are one of the mandatory requirements in order to predict the safer alternatives for different applications.

## **4. Conclusion**

Cytotoxicity of CNMs was found to vary as per the nature of material and cell lines under consideration. The observed general trend for cytotoxicity of CNMs was found as: single-walled carbon nanotubes > multiwalled carbon nanotubes > carbon black powders/Fullerenes > Nano diamonds. Macrophages were found to be more sensitive to the cytotoxicity of these CNMs. However, the cytotoxicity to CNMs could be tuned by functionalizing them with different surface groups. There is a need for conducting elaborative and systematic cytotoxic studies for aiding in ranking proposes. The tendency of nanomaterial for cellular damage (oxidative stress), physical damage or dissolution and bioaccumulation at cell surface should be explored material wise with defined size and purity for each cell type. The subsequent step in prioritizing CNMs which involves incorporation of criteria, such as occurrence, toxicity, removal in treatment to rank CNMs based on human health-based rank (Kumar, 2012) is underway.

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