Carbon nanotube toxicity: The smallest biggest debate in medical care

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Abstract: Nanotechnology is one of the most promising technologies of the twenty-first century. These applications are increasing exponentially because of the extremely small size of a large surface area per unit of volume. There is a great diversification in shape and function of manufactured nanoparticles; such as the large variety of polymers, dendrimers, fullerenes, carbon nanotubes, nanoribbons. Regardless of the numerous benefits that are obtained from the use of nanoparticles, one cannot be oblivious to the potential dangers associated with their exposure. Nanoparticles are present in our environment and surroundings from natural sources as well as from anthropogenic sources. Because of the extremely small size they can easily invade the human body through inhalation, ingestion, skin penetration, injections and via medical devices. Due to higher stability, they are foreseen to remain in the body and the environment for the longer periods of time. But unfortunately, limited information is available on their potential adverse effects on the health.

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1. Introduction
Nanotechnology is defined as the creation of functional systems and devices at nano-scale. The material properties changes when the size from micro/macro changes to nano-scale. Since the twenty-first century as our understanding of nanomaterials become deeper and its application in...
both medical and non-medical fields become vast, novel ideas has risen on the use of nanomaterials in the diagnosis, and management of pathological conditions in humans. However our understanding of nanomaterial in the body is lacking; due to its small size, and unpredictable effects we are uncertain of its efficacy and use. One major hurdle in developing and using these nanomaterials in medical application both for diagnostic and treatment approaches is the toxicity of the nanomaterial; and just as any drug or dye given to patients have their own side effects and toxicity levels, nanomaterials could also have their own toxicity. Conflicting results and conclusions on nanotoxicity from different research groups, formulate our aim of this review to discuss the debate on toxicity in carbon nanotubes.

Nanoparticles can enter the human body through several disciplines including skin, ingestion, inhalation, injection, and implantation. Carbon nanotubes are single dimension nanomaterials with two main constituents, single walled, and multi-walled nanotubes (Figure 1). Carbon nanotubes offer a wide range of application due to their unique size, mechanical, optical, high surface area/volume ratio and electronic properties. Scientists have been in a search for toxicity associated with use of carbon nanotube (CNT) on biological cells, tissues, and organs. Many have conducted in vitro studies for studying effect of use of CNT on level of toxicity, but a more realistic approach would be in vivo studies to examine the relationship between use of CNT with toxicity levels in biological organs and systems, as the process of administration, absorption, metabolism, excretion are more complex in vivo.

Nanotoxicology is defined as the study of nano-scale particles, their nature and the mechanism of toxic effects on the living organisms and another biological system, and the quantitative assessment of the severity and rate of recurrence of genotoxic effects of the exposure of the organism (Donaldson, Stone, Tran, Kreyling, & Borm, 2004).

1.1. Structure of CNTs
CNTs are classified into two types; single-walled carbon nanotubes (SWCNTs) and multiple-walled carbon nanotubes (MWCNTs). SWCNTs are in the form of a rolled-up tubular shell of the graphene sheet, made up of benzene type hexagonal rings of carbon atoms. They usually contain ten atoms around the circumference while the thickness is one-atom thick. While, MWCNT, is a stack of
graphene sheets that are rolled up into concentric cylinders. MWCNTs are larger consisting of many single-walled tubes stacked one inside the other.

CNT come from Graphite, which is composed of several sheets of single atom width, in a hexagonal structure in honeycomb crystal lattice known as graphene. Furthermore, single or multiple graphene sheets can be folded into cylindrical structures to give single and multiple walled CNTs.

SWCNT and MWCNT both are hollow cylindrical structures; each with diameter of 0.4–2 nm and 1–3 nm respectively. The MWCNTs can be further divided into two main categories; one has parchments-like structure, and other known as Russian Doll model. Conversely, SWCNTs’ structure are organized in chiral, armchair, helical, and zigzag structures.

Carbon nanotubes are considered allotropes of carbon with remarkable structural, mechanical, and electronic properties. These properties have enabled these nanomaterials to be used for a number of functions in medicine and pharmacy. In several instances in medicine, these materials have proved to be quite reliable in delivering drugs directly to regions that needed them without undergoing metabolism in the body first. The materials have also been used in the repair and regeneration of tissues, extraction and analysis of drugs and pollutants, and the diagnosis of biosensors among others (He, Pham-Huy, Dramou, & Xiao, 2013). Undoubtedly there has been a continued exposure to CNTs and this has led to the concern that the exposure can result in toxicity. With the development of new technology in the field of nanomaterials, and carbon nanotubes in particular, it is more paramount to ensure that toxicity of these materials is known and thus controlled. Research on the distribution, supply, absorption, and toxicity of the CNTs is fundamental in the drawing safety measures regarding the use of these materials. It has been established by a number of toxicity studies that these CNTs are toxic to the liver and lungs since they accumulate in the reticuloendothelial system (Jackson et al., 2013). There are studies that measure the adverse effects associated with first exposure to a single dose of a substance (Liu, Tabakman, Welscher, & Dai, 2009). These are referred to as acute toxicity studies. There are genotoxicity studies, which investigate how certain substances believed to be toxic interact with genetic material. Repeated-dose toxicity studies are carried out to ascertain if the continued exposure leads to an occurrence of toxicity (Chang, Yang, Liu, & Dong, 2011). There are also studies that are conducted specifically to determine if cancers develop because of exposure. Finally, there are toxicity studies that are conducted to determine the safety of medicines (Fu et al., 2016). Therefore, the results sought in each of these studies serve a different purpose and are thus different. A number of analytical techniques have been employed to measure the level of toxicity in the mouse models during the studies. These include transmission electron microscopy imaging, thermogravimetric analysis, and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry, dynamic light scattering, UV–vis–NIR spectra, Raman spectroscopy, and many more. However, due to the complexity that surrounds the study of carbon nanomaterials metabolism, the analytical techniques are limited in their use evidenced also by the insufficient documentation of pilot studies. One notable study has employed the use of a nanopore-based sensor to investigate the interactions between proteins and CNTs. Here, using molecular dynamics simulations to measure ionic currents in a nanopore, the research was able to report that threading a CNT-protein complex through the pore coupled with its binding capability, allowed the measurement of its toxicity from the resulting electric signals (Luan & Zhou, 2012). The research concluded that the nanopores could be used to measure toxicity even at nano-scale. A number of studies have been carried out on the toxicity of carbon nanotubes. Single walled CNTs and multiple walled CNTs have been used in vivo and in vitro to investigate various phenomena.

Toxicity studies have been used both in vivo and in vitro to investigate the effects of using single walled CNT and multiple walled CNT. Generally, the use of MWCNT has seen more adverse effects being observed in the mouse models as compared to SWCNT. According to Wang et al. (2010), SWCNT can be dispersed effectively in biological media without resulting in cytotoxic effects at given concentrations. The use of surfactants were shown to contribute greatly to this distribution and thus an in vitro approach and the use of lung fibroblasts is a sure way of assessing the potential of SWCNT
with regard to fibroblasts in vivo. In yet other studies, the level of MWCNT toxicity was measured with respect to the relationship between pulmonary fibrosis and different lengths of MWCNT (Chen, Nie, Gao, Yang, & Pu, 2014; Manke, Luanpitpong, Dong, & Wang, 2014). Whereas a surfactant was not employed in this second case, the effects of the MWCNT could be observed such that respiratory exposure to MWCNT resulted in pulmonary fibrosis dependent on length fibroblasts acquired from the epithelial via TGF-β/Smad pathway. The shape and size of MWCNT have also been determined to affect the extent of toxicity with aspects such as cytotoxicity varying according to the size and type of the cells (Haniu et al., 2014). According to Manna et al. (2005), SWCNT used in treatment of keratinocytes can cause oxidative stress and also compromise cell proliferation. This conclusion was arrived at after the results showed that the SWCNT particles activate NF-kappaB depending on the dose and that the mechanism of activation was due to activation of stress-related kinases BY SWCNT particles in the keratinocytes. A comparison of these studies has helped to decipher the effects brought about by the use of SWCNT and MWCNT in medical settings. However, their use is largely dependent on the aim of the treatment procedure since each nanomaterial possesses different properties and can be modified uniquely to fit the procedures (Zhao & Liu, 2012) (Table 1).

2. Concerns about CNT toxicity

2.1. Existing exposures

The adverse effects of nanotoxicity on vulnerable populations, such as neonates, pregnant women, diseased, and aged populations have been overlooked. Investigations show that they suffer more because of variations in physiological structures and functions, and their responses are often more severe. The factors that contribute to intensified toxicity effects include compromised immunity, and the ability of reduced self-repair ability (Li, Zhang, & Yan, 2014).

Carbon nanotubes (CNT) and in particular SWCNTs and MWCNTs have been used extensively commercially due to their desirable mechanical, magnetic, and electrical properties (He et al., 2013). SWCNT and MWCNT have been widely used in biological settings and this has increased the potential of exposure to nanomaterials from both a human and environmental perspective. Moreover, just like for the materials mentioned at the onset, this potential has sanctioned the use of toxicological research to analyze possible adverse effects on the health of humans and the environment (Madani, Mandel, & Seifalian, 2013).

Table 1. Current studies carried out; each study describe its methodology, aims, and results, and whether single walled CNT (SWCNT) or multiple walled CNT (MWCNT) were used as a substrate in study

| Title of study                                                                 | Method (in vivo or in vitro) | Aims/results                                                                 | Toxicity measured/determined |
|-------------------------------------------------------------------------------|-----------------------------|-------------------------------------------------------------------------------|------------------------------|
| Dispersion of single-walled carbon nanotubes by a natural lung surfactant for pulmonary in vitro and in vivo toxicity studies | Both in vitro and in vitro  | The study aimed to create a simple and rapid method of nanoparticle dispersion using a natural lung surfactant and to evaluate the effect of dispersion status of SWCNT on cytotoxicity and fibrogenicity in vivo and in vitro | SWCNT                       |
| 2010                           | 2010 Wang et al. (2010)     |                                                                               | MWCNT                       |
| Epithelial–mesenchymal transition involved in pulmonary fibrosis induced by multi-walled carbon nanotubes via TGF-beta/Smad signaling pathway | In vivo and in vitro        | Studies the relationship between pulmonary fibrosis and different lengths of MWCNT, both short and long | MWCNT                       |
| 2014                           | 2014 Chen et al. (2014)     |                                                                               | MWCNT used as substrate     |
2.2. In vivo evidence of toxicity

Some studies have pointed out that the toxicity of the nanoparticles is associated with their physical properties, like the size distribution and surface area reactivity because it can help in the distribution and deposition of particles (Kim et al., 2010). In a study, the pulmonary toxicity of SWNT in mice was investigated. The histopathology studies of the lungs indicated that the single intratracheal instillation of SWCNT dispersion triggered the epithelial granulomas and interstitial inflammation, which then developed to peribronchial inflammation and necrosis (Lam, James, McCluskey, & Hunter, 2004).

In another study SWCNT dispersed in phosphate buffer saline with the aid of 1% Tween 80, when exposed by intratracheal instillation at 5 mg/kg lead to a 15% mortality rate. It was concluded that the SWCNT agglomeration in the airways was the primary cause of death (Warheit et al., 2004).

In a recent study, to understand the dose-dependence and time-course of pulmonary responses, mice were exposed to the pharyngeal aspiration of the purified pristine SWCNT. It produced acute inflammation, progressive fibrosis, the formation of granulomas and an increase in protein levels was verified (Shvedova et al., 2008) (Figure 2).

2.3. Malignancy by chronic exposure to SWCNT

SWCNT is one of the major forms of engineered carbon nanotubes. The cytotoxicity and fibrogenicity of SWCNT and MWCNT indicate that the effects of SWCNT are more toxic (Hu et al., 2010; Mercer et al., 2011; Wang et al., 2011). Long-term occupational exposure is known to induce some irreversible malignant transformations and alter the pathways of lung epithelial cells that are related to...
cancer (Wang, Sun, Bao, Liu, & An, 2011; Wang et al., 2014). The fundamental mechanisms of SWCNT tumorigenesis are not clear; however the chronic exposure of mesothelial cells induced an aggressive phenotype (Lohcharoenkal et al., 2013).

It is evident from the previous studies that some solid tumors like the brain, colon, breast, bone marrow, and lung contain cancer stem cells CSC which are the main driving force of tumor initiation and progression (Christgen et al., 2010; Ho, Ng, Lam, & Hung, 2007). While the latest research presented the evidence on the supporting role of CSC in SWCNT tumorigenesis, due to the chronic exposure of SWCNTs (Luanpitpong, Wang, Castranova, & Rojanasakul, 2014).

2.4. **Inflammatory cytotoxicity following exposure to CNT**

The size and the composition of a nanomaterial play a distinct and variable role in the cellular response, which is related to the physiological function of the cell (Sohaebuddin, Thevenot, Baker, Eaton, & Tang, 2010). The carbon nanotubes are reported to penetrate the lung and remain in the tissues through the mechanisms that are analogous to some other fibrous particles like asbestos (Di Giorgio et al., 2011). Backscatter scanning electron microscopy has shown that the macrophages present in our body take up nanofibers via frustrated phagocytosis (Schinwald & Donaldson, 2012).

Some researchers have indicated that some types of CNT were cytotoxic to; different lung epithelial cell lines, human astrocyte D384 cells (Coccini et al., 2010), lung cells and T4 lymphocytes (Hu et al., 2010). Oxidative stress seems to be the primary cause of the behind the side effects of CNT because it induces inflammation by the activation of the transcription factors of oxidative stress-responsive (Aschberger et al., 2010). In a study, five times higher than control levels of intracellular ROS production was seen in MWCNT-treated RTL-W1 cells (Simon, Maletz, Hollert, Schäffer, & Maes, 2014).

2.5. **Gene-toxicity and inflammatory response**

There are some in vitro studies that demonstrate the cytotoxic and genotoxic potential (Cavallo, Ursini, & Iavicoli, 2012; Lindberg et al., 2013; Ursini et al., 2012) and inflammatory effects of carbon nanotubes (Haniu et al., 2011; He et al., 2011; Tsukahara & Haniu, 2011). A recent study compared the in vitro cytotoxic, genotoxic, and inflammatory effects of commercial pristine and COOH-functionalized MWCNTs in two respiratory cell lines of humans; lung alveolar epithelial cells (A549) and bronchial epithelial cells (BEAS-2B) (Ursini et al., 2014). It showed that thinner MWCNTs-COOH induced higher levels of cytotoxicity than the thicker pristine MWCNTs. Pristine MWCNTs had higher genotoxicity in A549 cells, indicating that they had the capability to reach the nucleus. Only the MWCNTs-COOH induced an inflammatory response. Some other studies also had the same results (Al-Jamal et al., 2011; Fenoglio et al., 2011).

2.6. **Studies that measured carbon nanotube toxicity in organisms**

Toxicity studies of carbon nanotubes are still at their early stage to evaluate the occupational safety concerns, preliminary toxicity studies of CNTs were performed. A number of controversial in vitro toxicity studies on different types of CNTs have been carried out. However, the differences in the results have been attributed to various factors, like length, concentration, type of functional group, duration and method of exposure. These discrepancies seem to arise primarily due to different experimental protocols.

Table 2 shows dose related response of studies that have been conducted on small organisms and their associated nanotoxicities.

The reduction in size to the nano-scale increases the surface area ratio of the materials and thus the potential to cause damage also increases, a phenomenon that was not possible while they are in larger forms (Heister, Brunner, Dieckmann, Jurewicz, & Dalton, 2013). The toxic effect has been suggested to be attributed to their properties such as the electronic properties with respect to their
bulk (Erdely, Dahm, Chen, & Zeidler-Erdely, 2013). Table 3 highlights adverse effects of single walled CNT in relation to the adverse effects that may arise from the extensive use of MWCNT.

### 2.7. Exposure time and clearance rates

According to the preliminary studies, the CNTs released into environmental compartments, are persistent bioavailable to different organisms, and therefore, there is a possibility of CNTs to accumulate in the food chain in a previous study. Maynard et al. (2004) found glove deposits of SWCNTs that were estimated to be between 0.2 and 6 mg per hand and concluded that large SWCNT had the tendency to become and remain airborne for long periods. This may cause dermal exposure and health risks.

Horseradish peroxidase1, a plant-derived enzyme, can catalyze the biodegradation of SWCNT. While hypo-chlorite and reactive radical intermediates of the human neutrophil enzyme, myeloperoxidase, catalyze in vitro degradation of SWCNT, in neutrophils and to a lesser degree in macrophages. However, it does not lead to an inflammatory response when aspirated into mice lungs (Kagan et al., 2010).

### Table 2. Summary of the studies related to CNTs toxicity in different organisms

| Organism tested          | Types of CNTs            | LOEC       | EC 50       | Mechanism of toxicity                                                                 |
|--------------------------|--------------------------|------------|-------------|--------------------------------------------------------------------------------------|
| Chlorella vulgaris       | Pristine CNT             | 0.053 mg L⁻¹ | 1.8 mg L⁻¹ | Oxidative stress, agglomeration and physical interactions (Schwab et al., 2011)        |
|                          | MWCNT of diameter 10, 20–40 nm, and 60–100 nm | NA         | 41.0, 12.7, and 12.4 mg L⁻¹, respectively | Oxidative stress, agglomeration and physical interactions (Long, Ji, Yang, Lin, & Wu, 2012) |
| Pseudokirchneriella subcapitata | Pristine CNT            | 0.053 mg L⁻¹ | 2.5 mg L⁻¹ | Oxidative stress, agglomeration and physical interactions (Schwab et al., 2011)        |
|                          | SWCNT                    | 0.25 mg L⁻¹ | NA          | Oxidative stress, agglomeration and physical interactions (Youn et al., 2012)           |
| Dunaliella tertiolecta   | MWCNT                    | NA         | 0.8 mg L⁻¹ | Oxidative stress and photosynthesis inhibition (Wei et al., 2010)                       |
| Daphnia magna            | MWCNT grafted with polyethyleneimine | NA         | 25 mg L⁻¹ | Increased size of the surface Coating (Khalid, Hussain, Suman, & Arun, 2016)            |
| Ceriodaphnia dubia       | MWCNT resuspended in NOM | 0.25 mg L⁻¹ | NA          | Agglomeration (Li & Huang, 2011)                                                      |
| Xenopus leavis larvae    | DWCNT                    | 10 mg L⁻¹  | NA          | Physical interactions (Bourdil et al., 2013)                                           |
| Sprague-Dawley rat       | 1,000 mg kg⁻¹ of SWCNT from gestation day 6–19 | NA         | NA          | No teratogenicity (Lim et al., 2011)                                                   |

Notes: LOEC: least observable effect concentration; EC 50: effective concentration 50; NOEC: no observed effect concentration; NOM: natural organic matter.

### Table 3. Adverse effects of single walled CNT in relation to the adverse effects that may arise from the extensive use of MWCNT

| Single walled (SW) CNT | Multiple walled (MW) CNT |
|------------------------|--------------------------|
| Induces malignancy in stem cells by chronic exposure to CNTs | Differences in cytotoxicity, genotoxic, and inflammatory response of bronchial and alveolar human lung epithelial cells |
| Inflammatory response of immortalised and primary human lung epithelial cells | Severe fibrotic peritoneal adhesions, fibrotic peritoneal thickening, and a high incidence of macroscopic peritoneal tumors (Chen et al., 2016) |
| In vitro Inhibition of HEK 293 cell proliferation (Herzog et al., 2009) | In vitro Induce cell cycle arrest and increase apoptosis/ necrosis of human skin fibroblasts (Liu et al., 2009) |
The needle-like fibre shape of CNT is comparable to asbestos, raising the fear that it may lead to mesothelioma, which is caused by exposure to asbestos. In a study, by exposing the mesothelial lining of mice to long MWCNT resulted in asbestos-like, pathogenic behaviour, including inflammation and granulomas formation.

Lung effects are quite significant and form the basis for the current recommended exposure limits. The exposure limits or exposure concentrations over an 8-h TWA (time weighted average) are given for a 45 year period in which the CNTs are 10% more likely to cause adverse lung effects. For minimal lung effects, the maximum likelihood estimate is given as 0.5–4 μg/m³. The recommended exposure limit for a 45-year period is given as 1 μg/m³ (8-h TWA) (Herzog et al., 2009). The CNTs usually deposit in the liver, spleen, or lungs after they have served their purpose from where they are expelled gradually out of the body through the renal excretion route. Continued accumulation of CNTs in the body can lead to granulomatous inflammation or alveolar septal thickening (Zhao & Liu, 2012). The CNTs stay in the body or organs such as blood for a brief periods between 0 and 28 h with many studies reporting a complete elimination from the body after an hour or two post injection (Singh, Pantarotto, & Lacerda, 2006). However, in another study, it was determined that these CNTs can remain in the liver and spleen for over three months (Yang, Luo, Zhou, & Wang, 2012). The clearance rates are measured by monitoring the concentration of the CNTs in the renal path excrement.

3. Designs of new strategies to reduce levels of toxicity in CNT

By reviewing the literature, we noticed that different pretreatment methods of CNTs are used, with no set criteria for physical properties and chemical content. One of the solutions to this discrepancy is to develop tools to characterize for measuring the relevant characteristics of CNTs, such as; diameter, length, dose, surface chemistry, surface area, etc.

Major concern regarding the use of CNT is raised and new strategies to overcome the delay in clearance and side effects are studied.

The possible use of CNT in clinical practice needs safety evaluations and confirmation of the absence of CNT immune-mediated adverse effects. Different studies were carried on CNT immune effects without any final conclusion about immune safety for methods of administration. Few studies were looked after new strategies to reduce the toxicity of CNT. CNTs seem to activate complement cascade that in the other hand can attack body tissues. Overcome the complement activation in various tissues of the body could be achieved by modifying better surface and functionalizing with complement inhibitors (Lettiero, Andersen, Hunter, & Moghimi, 2012; Moghimi, Peer, & Langer, 2011).

Many studies showed that MWCNTs can induce inflammation, fibrosis, angiogenesis and cytotoxicity to macrophages. These data approve that inflammation, fibrosis and angiogenesis can be triggered by MWCNTs due to its length, iron content or its crystal structure (Boyles et al., 2015). Nanoparticle carbon black and asbestos are less toxic when compared with MWCNT. More studies are needed to look at new features for carbon nanotubes and to be conducted in vitro for better result.

On the other hand, no toxicity had been observed in SWCNTs in mice over period of three months (Liu et al., 2008). In one of the studies shows that higher molecular weight PEG chain attachment to CNTs has no toxicity and removed safely from the body (Yang et al., 2008). Yang et al. (2011) noted that PEGylated CNTs has lower RES uptake, more circulating time and reduced deposition in liver and spleen.

Pondman et al. (2015) reported new novel methods to overcome the activation of classical inflammatory pathway which will lead to reduce inflammation and toxicity of CNTs by coating CNTs with recombinant globular heads. Coated CNTs lack the collagen region of human C1q that will help escaping phagocytosis (Kouser et al., 2015; Pondman et al., 2015). Further studies are needed to examine this method in drug delivery.
Silva et al. (2014) studied the two different methods of administration (instillation vs. inhalation) and their effect on immune system with consideration of CNTs (dose, time, and physicochemical characteristics). The study found that Instillation method induces inflammation in the 1st day and not after 21 days but the CNTs persist in the lung. Whereas inhalation methods showed no inflammation in the 1st day and there was inflammation in day 21. The study also showed that original MWCNTs cause more inflammation than purified or functionalized MWCNTs so each MWNCT produced different effects with different pulmonary responses (Silva et al., 2014). Significant renal clearance with decrease in RES uptake were demonstrated in chemically functionalized SWCNT while Perstine SWCNTs have shown to accumulate in the liver and significant RES uptake. Choosing the right form of SWCNTS is another strategy to reduce toxicity (Cherukuri et al., 2006).

The maximal residence time for PEGylated CNTs is a 21 h whereas 7 h in covalently functionalized CNTs. The route of excretion of noncovalent is through the hepatobiliary system but the covalent is through the kidney. They are both through the shielding strategy. The shielding strategy provide stealth agent. One of the important obstacles needs to overcome is the early degradation of the CNTs before it reaches its target. New novel evidence emerged by adding catalytic enzymes such as (horseradish peroxidase) that will allow the degradation of carboxylated CNTs in acidic environments in the presence of hydrogen peroxide which in turn required longer time for degradation. The degraded CNTs showed no inflammatory reaction in mice lungs (Allen et al., 2008; Otsuka, Nagasaki, & Kataoka, 2003).

Furthermore, the conclusions should not be based on a single biological assay rather correlating measurements from multiple assays should be taken to strengthen the observations.

4. Conclusion

CNTs may be safe for human use. In vivo studies have been informative in demonstrating that different methods of administration result in different pathologies. Whilst in vitro studies have been useful in identifying the determinants of CNT toxicity, drawing clear conclusions from the literature is sometimes made difficult by the inconsistency between studies. There is need for greater standardization in the field and a consensus on appropriate ways to measure nanotoxicity can greatly minimize CNT toxicity and represent promising progress towards clinical use. Modification and characterization of CNTs are not consistent, and the methodology can also be problematic. In light of the issues above, more than one measurement techniques are needed to assess CNT toxicity.

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