We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Quality Control and Risk Management of Carbon Nanomaterials

Khalid Parwez and Suman V. Budihal

Abstract

Our atmosphere contains a substantial number of nanoparticles in which some are unintentionally produced, whereas others are intentionally produced engineered nanoparticle. Among all ENPs, the single-walled and multi-walled carbon nanotubes, spherical fullerenes, and dendrimers are attracting attention for biomedical applications, such as biosensor design, drug delivery, tumor therapy, and tissue engineering. Because of the inert nature of pristine carbon nanotubes (CNTs), it needs to be functionalized to make it reactive with other organic and inorganic materials. The functionalization leads to the addition of functional groups, e.g., $\text{C}\equiv\text{O}$, $\text{C}=\text{O}$, $-\text{OH}$, and $-\text{COOH}$, to CNTs, which make them dispersible in solvents and suitable for numerous applications. Functionalized CNTs and their composite need to be tested for biocompatibility before real-time applications. Various toxicity mechanisms have been suggested for CNTs, including interference of transmembrane electron transfer, interruption/penetration of the cell envelope, oxidation of cell elements, and formulation of secondary products such as dissolved heavy metal ions or reactive oxygen species (ROS). Numerous studies have insinuated that well-functionalized CNTs are innoxious to animal cells, while raw CNTs or CNTs without functionalization manifest toxicity to cells at even modest dosage.

Keywords: nanotoxicology, carbon nanotubes, in vitro toxicity, in vivo toxicity

1. Introduction

In the past several years, a significant number of studies have been made to study the toxic effects of carbon nanotubes (CNTs). There are variations in the elucidations of these reports, and they mainly depend on the type of nanomaterials as well as functionalization methods. Properly functionalized carbon nanotubes were shown nontoxic to animals conducted by various groups [1–4], whereas raw carbon nanotubes were shown to be toxic to mice lungs in an in vivo study [5–8]. The latest research revealed that non-functionalized, long MWCNTs might be carcinogenic to mice [9]. Pristine nanotubes are indicated to cause oxidative stress and decrease cell viability [10, 11]; however, there is some sign that leftover catalyst particles also contribute to this effect [12]. The cytotoxicity can be decreased to zero via functionalization with a covalently attached polar functional group [13]. Likewise, the toxicity of noncovalently functionalized carbon nanotubes depends on the variety of the functional group. Cells were viable upon internalization of individually encapsulated DNA-wrapped SWCNT complex [14].

Therefore, the toxicity of carbon nanotube depends on the type of functionalization, aggregation behavior, and the presence of metal catalyst particle leftovers during synthesis.

2. An overview of carbon nanotube research

Manufacturing fundamental elements with great strength to weight ratio using carbon nanotube composite is the contemporary focus of the researchers. One of the likely utilizations of polymer nanocomposite is the CNT-augmented ultrafine fiber via electrospinning [15, 16], which has been known since the 1930s. Today, polymer fibers with nanometer diameter can be produced inexpensively using electrospinning technology. With <100 nm diameter, these fibers are being studied for drug delivery methods, energy storage, and improved functional garments [17–19]. These applications require improved (i) fiber strength, (ii) thermal conductivity, and (iii) electrical conductivity. Incorporating carbon nanotubes (CNTs) within electrospun fibers offers the probability of simultaneously improving all these three properties [20–22]. Xie et al. (2005) reviewed the dispersion and alignment of CNTs in the polymer matrix [23]. They found that the serious challenge is the development of means and ways to promote and increase the dispersion and alignment of CNTs in the matrix. Enhanced dispersion of CNTs in the polymer matrix will foster and extend the applications and developments of polymer/CNT nanocomposites.

Though the optical apprehension techniques are probably the most conventional in biology and life sciences, electrochemical or electronic detection techniques have also been adopted in biosensors/biochips due to their great sensitivity, high specificity, and low cost. Those techniques comprise of voltammetric techniques (cyclic voltammetry and differential pulse voltammetry), chronocoulometry, electrochemical impedance spectroscopy, and electronic detection based on electric field [24]. The sensors developed from CNTs have shown the ability to detect a range of analytes such as particular DNA sequences [25] as cancer biomarkers [26] and larger entities such as viruses [27]. These sensor devices have also been used to monitor enzymatic activities and study the behavior of potential drug molecules [28].

The apprehension of the analytes befalls with great specificity and sensitivity in a rationally precise time. Both SWCNT and MWCNT can be altered and conjugated to a bioactive unit and biological varieties including carbohydrates, amino acids and peptides, nucleic acid, and proteins, for various biological applications. Those biological applications are plausible only because the carbon nanotubes own some anomalous properties like the one-dimensional arrangement, large aspect ratio, outstanding mechanical characteristics, and chemical inertness [29]. The carbohydrate-functionalized carbon nanotubes have previously been used for the identification of pathogenic microorganisms, namely, [30, 31]. In the advancement of energy production and storage, nanotubes exhibit exceptional potential in supercapacitors [32], Li-ion batteries [33], solar cells [34], and fuel cells [35]. Energy applications could become the broadest application realm in the gross application of carbon nanotubes. For the advancement of Li-ion batteries' performance, MnO_2 and LiFePO_4 are being used as a cathode while MWCNTs and graphene as an anode. In the realm of the fuel cell, proton-exchange membrane fuel cells (PEMFCs), CNTs have been widely studied [36].

In a PEMFC, the conversion of chemical energy to electrical energy occurs via a direct electrochemical reaction, and its efficiency is directly dependent on the catalysts used [37]. The catalysts should have high endurance, low cost, and higher activities in oxygen reduction and/or fuel oxidation reaction [38]. Shortly, the most regularly used catalysts in the PEMFCs are metal NPs, mainly Pt and/or Pt-based

alloys, because of high oxygen reduction and/or fuel oxidation reaction due to high Surface area : volume ratio and better Fermi levels for redox reactions [39]. Nonetheless, metal NPs are generally unstable and lose their catalytic activity due to their irreversible aggregation during the electrochemical processes.

Consequently, appropriate methods are obliged to fix and restrict these metal NPs from aggregation, e.g., carbon nanotubes (CNTs) are the most extensively adopted provision in modern development. Though the evolution of PEMFCs is under commercialization process, obstructions including how the CNTs influence the catalytic action of the metal/CNTs and high material cost continue. The evolution of numerous profoundly dynamic catalysts with the economical price for fuel cell commercialization would be one of the notable researches in this domain. Because of the enhanced production and intended use of CNTs in consumer commodities, there is a necessity for evaluation of the implied toxicity of these nanoparticles.

3. Toxicity studies of carbon nanotubes in vivo

In vivo toxicity knowledge impersonated a vital role in risk evaluation. Those techniques can be applied to determine acute toxicity, chronic toxicity, developmental toxicity, genotoxicity, and reproductive toxicity. In vivo study is indispensable in the fields of medicine including cancer therapy. Several animal trials are performed to highlight the possible serious impressions of newly formed medicines and chemical substances on the human. In some trials, researchers attempt to simulate situations concerning humans (e.g., arthritis, cystic fibrosis, and cancer) in animals, to assess the capabilities of new medicines in treatment. To inscribe the potential side effects of CNTs on human health and environment, animal models have been used to investigate the toxicity of CNTs. Non-functionalized CNTs were instilled intratracheally (IT) into animals, exhibited as pulmonary toxicity including inflammation and fibrotic responses due to the collection of raw CNTs in the lung airways [5].

These outcomes suggest that aerosol vulnerability of untreated CNTs in the workplace should be shunned to preserve human health. Notwithstanding, intratracheal instillation of functionalized soluble CNTs has little inference to the toxicology profile. In the latest pilot study, asbestos-like pathogenicity was observed by Poland et al. [9] when the mesothelial lining of the body cavity of mice was exposed to large MWCNTs of 80–160 nm diameter and 10–50 nm length [9]. Yet the assumption of this finding for probable negative effects of CNTs on human health is inadequate. It should be heeded that the MWCNT materials utilized in this research were just sonicated in bovine serum albumin (BSA) without surface functionalization.

Furthermore, no noticeable toxic result was observed for smaller and tinier MWCNTs of 1–20 nm length and 10–14 nm diameter, appreciating that the toxicology characterizations of CNTs may vary between CNTs of varying sizes. It is deserving asserting that functionalized SWCNTs utilized in biomedical research have a length of 50–300 nm and diameter of 1–2 nm, which is completely distinct from the geometry of MWCNTs adopted by Poland et al. [9]. Gambhir and colleague applied covalently and noncovalently PEGylated SWCNTs to investigate the in vivo toxicity [3]. The PEGylated SWCNTs (-3 mg kg^{-1}) were intravenously infused into mice and inspected over 4 months. Systolic blood pressure, total blood counts, and serum chemistry are registered every month. Necropsy and tissue histology analyses were executed at the completion of 4 months. The blood chemistry and histological investigations were normal. Those experiments insinuate that functionalized biocompatible SWCNTs may be secured for in vivo biological reinforcements. An added investigation revealed related outcomes, confirming that PEGylated SWCNTs are gradually eliminated from the body after systemic administration in mouse models, without

manifesting apparent toxicity in the system [40]. Yang et al. acknowledged that SWCNTs dangled in Tween-80 manifested lesser toxicities to the experimented mice at a high dose of -40 mg kg^{-1} , following intravenous inoculation for 3 months [41]. Toxicity may be due to the oxidative stress engendered by SWCNTs assembled in the liver and lungs of mice [42]. The toxicity published was dose-dependent and appeared to be less acceptable at lower doses. A current article by the same group unveiled that covalently PEGylated SWCNTs displayed an ultra-long blood dissemination half-life in rodents. Though the long-term toxicity of altered SWCNTs is still to be investigated, no critical toxicity has been recorded too at a higher dose of 24 mg kg^{-1} .

4. Respiratory toxicity

A guinea pig was inoculated intratracheally with the soot of CNT. Breathing rate, tidal capacity, pulmonary obstruction, bronchoalveolar fluid, and protein content were estimated. The authors admitted that working with soot-carrying CNT was probably not a health jeopardy, but they did not present their pathological investigation [43]. Research in mice is conducted by Lam et al. [5], and they authenticated that SWCNT could be toxic if they entered the lungs; Warheit et al. [6] conveyed a related investigation in rats, reporting the granuloma development apparently due to the collection of CNT. Muller et al. analyzed carbon black, MWCNT, and asbestos influences, implanted in the trachea of rodents. Scholars demonstrated dose-dependent inflammation, and granuloma production, increased considerably with MWCNT than with carbon black than asbestos. The early granulomatous reaction, abnormal acute inflammatory response, and progressive fibrosis were observed upon exposure of SWCNT in mice. Pharyngeal aspiration was used alternately of the intratracheal instillation used in the earlier investigations and rendered aerosolization of fine SWCNT particles. Another contemporary study insinuates shifts in deposition prototype and pulmonary response when SWCNT is uniformly dispersed in the suspension antecedent to pharyngeal aspiration [44]. Current research insinuates MWCNT immigration to the subpleural and associated pleural mononuclear cells and subpleural fibrosis in mice upon inhalation [45] and further admonition, and decent security models are prescribed when manipulating CNT. Research by [46] confirms the earlier reports; they characterized in vitro and in vivo stimulation of collagen deposition, lung fibroblast propagation, and metalloproteinase intensified expression without inflammation when dispersed SWCNT was applied. Following inhalation, the different variety of nanoparticles may enter the central nervous system (CNS) [47] by a method called transcytosis [48]. The investigation unveiled that sniffed gold nanoparticles aggregate in the olfactory tubercle of rats and enter the cerebral cortex, lung, and the distinct organs such as the tongue, esophagus, kidney, spleen, aorta, septum, heart, and blood [49]. Those remarks vindicate that nanoparticles can infiltrate into the CNS via the olfactory venation if they are being in high doses in the air. Those nanoparticles may impact not only on the respiratory tract and neighboring organs but disseminated to remote organs.

5. Bio-distribution of carbon nanotubes

Knowledge of bio-distribution of CNTs following systemic inoculation inside animals is a pretty serious concern. Numerous investigators investigated in vivo bio-distribution and pharmacokinetic investigations in the preceding several years. Scientist adopted various CNT materials, different surface functionalization methods, and various tracking methodologies. Consequently, they got unsteady and seldom

ambiguous results. Singh et al. and Lacerda et al. utilized radiolabeled (1n-DTPA) SWCNTs and MWCNTs to describe bio-distribution [50, 51]. Exceptionally, following intravenous inoculation of CNTs into mice, no uptake in the reticuloendothelial system (RES) such as the liver and spleen was witnessed. But, quick urinal removal of CNTs was witnessed. More than ninety five percent of CNTs were removed within 3 hours. Those results are comparable to the in vivo response of minute particles yet distinct from that prognosticated of maximum nanoparticles with sizes exceeding the glomerular filtration threshold. To defend their conclusions, the researchers stated that the short diameters of CNTs were eliminated in urine notwithstanding they were large in length. However, this theory is unsettled. For example, for the protein bio-distribution and elimination function of quantum dots (QDs), published by Choi et al., it is observed that the 6 nm maximum size of spherical QDs including coatings was obliged to fast urinal elimination. Nevertheless, the QDs are much shorter than the diameter of SWCNT bundles (10–40 nm) or MWCNTs (20–80 nm) [51] practiced in those bio-distribution investigations. Therefore, the inscribed fast urinal excretion of CNTs requires validation. Various other labs have also assessed the bio-distribution of radiolabeled CNTs in rodents. Wang et al. noticed delayed urinal elimination and weak RES uptake in their primary research. But, consecutive articles by the same association utilizing ^{14}C -taurine-functionalized CNTs recorded steadfast liver accumulation of CNTs following intravenous inoculation [52]. Research carried out by McDevitt et al., utilizing antibody-conjugated radiolabeled CNTs functionalized by 1,3-dipolar cycloaddition, also confirmed delayed urinal excretion and high CNT uptake in the liver and spleen [53]. The bio-distribution investigations of radiolabeled, PEGylated SWCNTs unveiled uptake of SWCNT in RES organs without active clearance [54]. A substantial quantity of CNTs is persisting, even after 15 days. The radiolabel system is a proper technique to identify the bio-distribution of material but may commence to inaccurate outcomes, if excess-free radioisotopes in the radiolabeled CNT specimens are not separated effectively. The free radioisotopes are tiny particles that could be quickly excreted in urine following intravenous inoculation. Moreover, radiolabels could be undeviatingly released from CNTs in vivo and be regularly eliminated in the free form. Consequently, radiolabeling is not an excellent approach to investigate the elimination and long-term predestination of CNTs. The expert has discovered that photoluminescence is the inherent characteristics of CNTs. Cherukuri et al. used single semiconducting SWCNTs which show NIR photoluminescence, to trace nanotubes in rabbits [55]. Without obtaining complete bio-distribution data, the expert could not testify SWCNT photoluminescence signals in every organ besides the liver. Yang et al. carried out the research to comprehend the bio-distribution of ^{13}C -fortified unfunctionalized SWCNTs over a month utilizing isotope ratio mass spectroscopy [56]. The event conferred unusual nanotube uptake in the liver, lung, and spleen without notable elimination within 28 days. Raman spectroscopy has been applied to analyze the long-term predestination of PEGylated nanotubes in rodents. It was reported that most of the PEGylated SWCNTs were assembled in the liver and spleen following intravenous inoculation but gradually eliminated through the biliary pathway toward the feces within months. A low SWCNT Raman signal was also identified in the mouse kidney and bladder. It is unveiled that little portion of SWCNTs with short lengths was eliminated into the urine.

6. Toxicity of carbon nanotubes in vitro

In vitro, toxicological investigations are a highly significant means for nanotoxicology, corresponding to in vivo investigations because of moderate expense, lessening ethical anxieties, and diminishing the number of laboratory animals needed for trial.

7. CNT toxicity investigations in animal cell lines

The subject of carbon nanotube toxicity is still unresolved even in cell culture experiments. Inhibition of HEK 293 cell proliferation following exposure to SWCNTs [10], MWCNTs, inducing cell cycle arrest and increasing apoptosis/necrosis of human skin fibroblasts were examined by different research groups [57]. Nevertheless, it is worth stating that functionalized CNTs were not used in those investigations. Bottini et al. observed T-lymphocyte apoptosis evoked by oxidized MWCNTs [58]. Because simple oxidation, used in these studies, is not enough to disperse carbon nanotubes in saline and cell culture media since it is not a kind of biocompatible functionalization. Sayes et al. indicated that toxicity of CNTs was also dependent on the density of functionalization. Inconsiderable toxicity was observed for those functionalized with the high density of phenyl-SO₃X groups [13]. These results are understandable because CNTs without proper functionalization carry a highly hydrophobic surface. Consequently, they may aggregate in the cell culture medium. The aggregation of CNT channels to binding of several biological species, including proteins, via hydrophobic interactions, provokes cell toxicity. Khalid et al. reported no toxicity of functionalized MWCNT to Saos cell lines up to the tested concentration of 1000 µg/mL [59]. Other factors like surfactants may also play a role in the noted toxicity of CNT in vitro. Extra surfactants, present in the CNT suspensions, are known to be highly toxic to cells [60]. The metal catalyst, used during the synthesis of CNTs, should also be examined as an important factor when the toxicity of carbon nanotubes is analyzed [61]. Furthermore, proper analytical methods must be hired in toxicity analysis to prevent interference of carbon nanotubes with the test reagents [62]. Davoren et al. reported concentration-dependent cytotoxicity of SWCNT on a lung carcinoma cell line (A549) [63]. Another study, led by Sharma, unveiled that SWCNT induced oxidative stress in rat lung cells [64]. Herzog et al. reported the same oxidative stress linked to alterations in primary bronchial epithelial cells and A549 cells, but the study also revealed that the reaction is strongly dependent on the dispersion medium used [65]. Pulskamp used two cells lines (human A549 and rat macrophages NR8383) and tested with CNTs and revealed, as oxidative stress was provoked to these cell lines. However, when purified SWCNT corresponded with commercial CNT, it is unveiled that all the biological consequences are associated with the metal traces. There is a complicated result between WST (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) and MTT (344, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability assays. These dyes depend on the mitochondrial dehydrogenase activity [66]. The modifications can only be described based on associations of CNT with non-soluble formazan crystals in MTT. That is why suitable assay methods and well-characterized materials are the most important requirements for in vitro toxicity assays of carbon nanotubes.

8. CNT toxicity investigations in bacteria and yeast cells

As an option to animal cell lines, bacteria and yeast can be a relevant model for studying how single-celled microorganisms react to the environmental stressors such as CNTs [67]. Copious toxicity mechanisms have been suggested for CNT including interruption/penetration of the cell envelope, oxidation of cell ingredients, the arrest of transmembrane electron transfer, and generation of secondary products such as reactive oxygen species (ROS) or dissolved heavy metal ions [68]. Toxicity of a CNT is depending on its structure along with its

geometry and surface functionalization. Various researches have shown that adequately functionalized, serum-stable CNTs are innocuous to animal cells, whereas CNTs without functionalization seemed critically toxic to human or animal cell lines at the moderate dosage [2]. The SWCNT displays a potent antimicrobial response for both suspended and deposited bacteria and interrupts the accumulation of bacterial films. The immediate interaction among the SWCNT and bacteria is apparently the central cause to induce cell death [68]. Well-dispersed individual SWCNT is more toxic than agglomerates due to greater physical puncturing of bacterial membranes and impairs the cell integrity [69]. The CNT bacteria interplay is determined by surface functionalization and length of CNT. It may govern the toxic effect also. A negatively charged or neutral SWCNT functionalized with -OH or -COOH aggregates more efficiently with bacteria and diminishes bacteria viability as contrasted to the positively charged SWCNTs functionalized with -NH₂ [70]. Likewise, longer SWCNTs exhibited concentration and time-dependent toxicity to bacteria, whereas short SWCNTs were limited toxically as they aggregate themselves [71]. The purity of SWCNTs may also influence bacterial toxicity. Pure SWCNTs were observed to be less toxic than SWCNTs with higher metal content due to glutathione oxidation following contact [72]. Additionally, greater ionic strength suspensions, such as phosphate buffered saline (PBS) or brain heart infusion broth, also lessen SWCNT toxicity due to decreased intensity of interactions between SWCNT and cells, compared to low ionic strength suspensions (deionized water or saline). Likewise, a film with natural organic matter (NOM) limits SWCNT toxicity, notwithstanding diminished aggregation [73]. Other studies unveiled that SWCNT reduces enzyme activity and microbial biomass at concentration 300 mg kg⁻¹ and above [74]. As it is clear that SWCNT provokes bacterial death, a surface coating with SWCNT would decrease biofilm expansion in both real and industrial settings [75]. The MWCNT appears to be runty toxic to bacteria as contrasted to SWCNT [76]. The decreased toxicity may be due to minor interactions among bacteria and MWCNT. The limited interaction might be due to the greater rigidity and presumably inferior van der Waal's forces at the MWCNT surface. Thin MWCNT with less diameter exhibits greater toxicity to bacteria corresponding to larger ones [77]. When the consequence of the length of MWCNT was estimated, shorter MWCNTs were extra toxic to *Pseudomonas fluorescens* compared to long MWCNT [78]. When MWCNTs are uncapped, debundled, and dispersed in solution, the toxicity to bacteria raised [79]. The purity of CNT has also been vindicated to influence the toxicity in microorganisms. Furthermore, when the toxicity within pristine and purified MWCNT was studied in two bacterial strains (*Escherichia coli* and *Cupriavidus metallidurans*), no variation in toxicity of MWCNT was perceived between the two forms [80]. Heating refinement of CNTs presumably has the inadequate capability to modify the surface corresponding to acid processing, consequently sustaining toxicity of the raw form. However, in both the investigations, gum arabic (GA, 0.25 wt%) was used to suspend CNTs, which might have altered the surface, influencing toxicity. Meanwhile in soil toxicity assay, MWCNT, reduced microbial biomass and enzyme activity at concentration 5000 mg kg⁻¹ [81]. In a separate research, the conidia of the fungi *Paecilomyces fumosoroseus* were incubated for 865 hours with 0.2 mg L⁻¹ raw and/or carboxylated MWCNT. Mycelium growth on solid medium was witnessed following incubation. Association among the fungi and CNTs had no notable effect on germination and biomass production, but the loss of biomass was witnessed following exposure to raw MWCNT for 865 hours [82]. Mechanical impacts of CNT, as observed in bacteria, might have caused the effects.

9. Ecotoxicity of carbon nanotubes

As the production and widespread application of CNTs in industrial and customer products are progressing, the release of this nanomaterial into the environment too will scale up. Many scientific reviews have evaluated the sources, behavior, fate, and the mechanisms of toxicity of carbon nanomaterial. Maximum of these assessments apprehended that additional research is obligatory in the field of nano-ecotoxicology (Table 1).

Organism tested	Types of CNTs	LOEC	EC 50	Mechanism of toxicity	References
<i>Chlorella vulgaris</i>	Pristine CNT	0.053 mg L ⁻¹	1.8 mg L ⁻¹	Oxidative stress, agglomeration and physical interactions	[83]
	MWCNT of diameter 10, 20-40, and 60-100 nm	NA	41.0, 12.7, and 12.4 mg L ⁻¹ , respectively	Oxidative stress, agglomeration and physical interactions	[84]
<i>Pseudokirchneriella subcapitata</i>	Pristine CNT	0.053 mg L ⁻¹	2.5 mg L ⁻¹	Oxidative stress, agglomeration and physical interactions	[83]
	SWCNT	0.25 mg L	NA	Oxidative stress, agglomeration and physical interactions	[85]
<i>Thalassiosira pseudonanas</i>	DWCNTs	0.1 mg L ⁻¹	1.86 mg L ⁻¹	Oxidative stress, agglomeration and physical interactions	[86]
<i>Dunaliella tertiolecta</i>	MWCNT	NA	0.8 mg L ⁻¹	Oxidative stress and photosynthesis inhibition	[87]
<i>Tetrahymena thermophila</i>	SWCNT	1.6 mg L ⁻¹	NA	Physical interactions	[88]
<i>Stylonychia mytilus</i>	Functionalized MWCNT	1 mg L ⁻¹	NA	Physical interactions	[89]
<i>attenuata</i>	SWCNT	1.10 mg L ⁻¹	NA	Physical interactions	[89]
<i>Daphnia magna</i>	SWCNT (60% pure)	NA	1.3 mg L ⁻¹	Physical interactions	[90]
	MWCNT resuspended in NOM	NOEC 20 mg L ⁻¹	NA	No toxicity	[91]
	MWCNT grafted with polyethylenimine	NA	25 mg L ⁻¹	Increased size of the surface coating	[92]
<i>Ceriodaphnia dubia</i>	MWCNT resuspended in NOM	0.25 mg L ⁻¹	NA	Agglomeration	[93]
<i>Danio rerio</i> embryo	SWCNT	120 mg L ⁻¹	NA	Agglomeration	[94]
<i>Oryzias melastigma</i>	DWCNT	10 mg L ⁻¹	NA	Agglomeration	[86]
<i>Xenopus laevis</i> larvae	DWCNT	10 mg L ⁻¹	NA	Physical interactions	[95]
<i>Drosophila melanogaster</i>	SWCNT and MWCNT in 1 g kg ⁻¹ food			No toxicity	[96]
Female Fisher rats	Oral gavage of 0.64 mg kg ⁻¹ SWCNT	NA	NA	Increased levels of oxidative damage to DNA in liver and lung tissue	[97]
Sprague—Dawley rat	1000mg kg ⁻¹ of SWCNT from gestation day 6 to 19	NA	NA	No teratogenicity	[98]

Abbreviations: LOEC: Least observable effect concentration, EC 50: Effective concentration 50, NOEC: No observed effect concentration, NOM: Natural organic matter.

Table 1. Summary of the studies related to eco-toxicity of the CNTs on different organisms [82–98].

10. Conclusions

Toxicity of carbon nanomaterials is an essential concern in the modern world for the scientific community, environmentalists, and governments. The chances of exposure to the environment are more like the application of carbon nanomaterial which is increasing every day. Some research shows the different toxicity patterns for the materials when it is exposed to living cells *in vitro* or *in vivo*, whereas other studies say that the adequately functionalized bearing carboxylic or hydroxyl group and serum-stable CNTs are safe for living cells. I want to conclude my discussion by highlighting the factors involved in toxicity and the toxicity mechanism. The toxicity of the nanomaterials depends on many factors including functionalization, catalyst, size, shape, dimensions, dispersion, and methods used for detecting toxicity. The pristine carbon nanotubes are more damaging to the cells than the functionalized one. The covalently functionalized CNTs are more compatible for the cells than non-covalent functionalization. The catalyst used during the production of the nanotubes like platinum or iron also contributes to the toxicity of the cells. Hence it is imperative to differentiate the toxicity of carbon nanotubes and catalyst. Dispersion in the high ionic strength solvent like PBS makes the CNTs more compatible with living cells compared to the less ionic strength solvent like deionized water. Hence it is always recommended to prepare the solution in PBS or other high ionic strength solvents for better compatibility and less toxicity. The short and broken CNTs with a small diameter are observed to be damaging to bacterial cells because of physical puncturing. *In vivo* studies help us to understand the acute toxicity, chronic toxicity, developmental toxicity, genotoxicity, and reproductive toxicity of CNTs in laboratory animals. No critical acute toxicity, chronic toxicity, developmental toxicity, genotoxicity, and reproductive toxicity are observed following intravenous or intratracheal instillation of CNTs. Adequately functionalized CNTs are biocompatible and promptly eliminated through urine or biliary pathway following intravenous inoculation. Pharmacokinetic studies of CNTs show very less or no uptake of CNTs to the reticuloendothelial systems including the liver, lung, and spleen. Various mechanisms are also listed to study the toxicity of the CNTs to the living cells which includes oxidation of cell components, arrest of electron transport chain, reactive oxygen species, and physical puncturing of the cell. Further studies need to be conducted in the field of eco-toxicity of CNTs and validation of the toxicological data for the safety of aquatic and aerial animals. These studies shall help the public regulatory organization to frame a rule for ensuring the safety of this modern engineered nanoparticle.

IntechOpen

Author details

Khalid Parwez^{1*} and Suman V. Budihal²

1 Department of Clinical Laboratory Science, Faculty of Applied Medical Science, Shaqra University (SU), Al-Dawadmi, Kingdom of Saudi Arabia

2 Department of Physiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

*Address all correspondence to: drkhalidpm@gmail.com

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Shi Kam NW, Jessop TC, Wender PA, Dai H. Nanotube molecular transporters: Internalization of carbon nanotube–protein conjugates into mammalian cells. *Journal of the American Chemical Society*. 2004;**126**(22):6850-6851
- [2] Dumortier H, Lacotte S, Pastorin G, Marega R, Wu W, Bonifazi D, et al. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. *Nano Letters*. 2006;**6**(7):1522-1528
- [3] Schipper ML, Nakayama-Ratchford N, Davis CR, Kam NW, Chu P, Liu Z, et al. A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nature Nanotechnology*. 2008;**3**(4):216
- [4] Wu P, Chen X, Hu N, Tam UC, Blixt O, Zettl A, et al. Biocompatible carbon nanotubes generated by functionalization with glycodendrimers. *Angewandte Chemie*. 2008;**120**(27):5100-5103
- [5] Lam CW, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological Sciences*. 2004;**77**(1):126-134
- [6] Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicological Sciences*. 2004;**77**(1):117-125
- [7] Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, et al. Unusual inflammatory and fibrogenic pulmonary responses to single walled carbon nanotubes in mice. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2005;**289**(5):698-708
- [8] Muller J, Huaux F, Moreau N, Misson P, Heilier JF, Delos M, et al. Respiratory toxicity of multi-wall carbon nanotubes. *Toxicology and Applied Pharmacology*. 2005;**207**(3):221-231
- [9] Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*. 2008;**3**(7):423
- [10] Cui D, Tian F, Ozkan CS, Wang M, Gao H. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicology Letters*. 2005;**155**(1):73-85
- [11] Manna SK, Sarkar S, Barr J, Wise K, Barrera EV, Jejelowo O, et al. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor- κ B in human keratinocytes. *Nano Letters*. 2005;**5**(9):1676-1684
- [12] Kagan VE, Tyurina YY, Tyurin VA, Konduru NV, Potapovich AI, Osipov AN, et al. Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: Role of iron. *Toxicology Letters*. 2006;**165**(1):88-100
- [13] Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, Beach JM, et al. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. *Toxicology Letters*. 2006;**161**(2):135-142
- [14] Kam NW, O'Connell M, Wisdom JA, Dai H. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;**102**(33):11600-11605
- [15] Khalid P, Hussain MA, Suman VB, Arun AB. Toxicology of carbon

- nanotubes—A review. International Journal of Applied Engineering Research. 2016;**11**(1):148-157
- [16] Formhals A. Process and apparatus for preparing artificial threads. US patent. 2 October 1934;**1**(975): 504
- [17] Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced by electrospinning. Nanotechnology. 1996;**7**(3):216
- [18] Kenawy ER, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders EH, et al. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. Journal of Controlled Release. 2002;**81**(1-2):57-64
- [19] Laxminarayana K, Jalili N. Functional nanotube-based textiles: Pathway to next generation fabrics with enhanced sensing capabilities. Textile Research Journal. 2005;**75**(9):670-680
- [20] Ko F, Gogotsi Y, Ali A, Naguib N, Ye H, Yang GL, et al. Electrospinning of continuous carbon nanotube-filled nanofiber yarns. Advanced Materials. 2003;**15**(14):1161-1165
- [21] Dror Y, Salalha W, Khalfin RL, Cohen Y, Yarin AL, Zussman E. Carbon nanotubes embedded in oriented polymer nanofibers by electrospinning. Langmuir. 2003;**19**(17):7012-7020
- [22] Lim JY, Lee CK, Kim SJ, Kim IY, Kim SI. Controlled nanofiber composed of multi-wall carbon nanotube/poly(ethylene oxide). Journal of Macromolecular Science, Part A: Pure and Applied Chemistry. 2006;**43**(4-5):785-796
- [23] Xie XL, Mai YW, Zhou XP. Dispersion and alignment of carbon nanotubes in polymer matrix: A review. Materials Science & Engineering R: Reports. 2005;**49**(4):89-112
- [24] Cai H, Cao X, Jiang Y, He P, Fang Y. Carbon nanotube-enhanced electrochemical DNA biosensor for DNA hybridization detection. Analytical and Bioanalytical Chemistry. 2003;**375**(2):287-293
- [25] Tu X, Manohar S, Jagota A, Zheng M. DNA sequence motifs for structure-specific recognition and separation of carbon nanotubes. Nature. 2009;**460**(7252):250
- [26] Thakare VS, Das M, Jain AK, Patil S, Jain S. Carbon nanotubes in cancer theragnosis. Nanomedicine. 2010;**5**(8):1277-1301
- [27] Patolsky F, Zheng G, Hayden O, Lakadamyali M, Zhuang X, Lieber CM. Electrical detection of single viruses. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(39):14017-14022
- [28] Prato M, Kostarelos K, Bianco A. Functionalized carbon nanotubes in drug design and discovery. Accounts of Chemical Research. 2007;**41**(1):60-68
- [29] Endo M, Strano MS, Ajayan PM. Potential applications of carbon nanotubes. In: Carbon Nanotubes. Berlin, Heidelberg: Springer; 2007. pp. 13-62
- [30] Elkin T, Jiang X, Taylor S, Lin Y, Gu L, Yang H, et al. Immuno-carbon nanotubes and recognition of pathogens. ChemBioChem. 2005;**6**(4):640-643
- [31] Wang H, Gu L, Lin Y, Lu F, Mezziani MJ, Luo PG, et al. Unique aggregation of anthrax (*Bacillus anthracis*) spores by sugar-coated single-walled carbon nanotubes. Journal of the American Chemical Society. 2006;**128**(41):13364-13365
- [32] Kim YJ, Kim YA, Chino T, Suezaki H, Endo M, Dresselhaus MS. Chemically

modified multiwalled carbon nanotubes as an additive for supercapacitors. *Small*. 2006;**2**(3):339-345

[33] Leroux F, Metenier K, Gautier S, Frackowiak E, Bonnamy S, Beguin F. Electrochemical insertion of lithium in catalytic multi-walled carbon nanotubes. *Journal of Power Sources*. 1999;**81**:317-322

[34] Landi BJ, Castro SL, Ruf HJ, Evans CM, Bailey SG, Raffaele RP. CdSe quantum dot-single wall carbon nanotube complexes for polymeric solar cells. *Solar Energy Materials and Solar Cells*. 2005;**87**(1-4):733-746

[35] Wang C, Waje M, Wang X, Tang JM, Haddon RC, Yan Y. Proton exchange membrane fuel cells with carbon nanotube based electrodes. *Nano Letters*. 2004;**4**(2):345-348

[36] Kamavaram V, Veedu V, Kannan AM. Synthesis and characterization of platinum nanoparticles on in situ grown carbon nanotubes based carbon paper for proton exchange membrane fuel cell cathode. *Journal of Power Sources*. 2009;**188**(1):51-56

[37] Wang X, Waje M, Yan Y. CNT-based electrodes with high efficiency for PEMFCs. *Electrochemical and Solid-State Letters*. 2005;**8**(1):A42-A44

[38] Kundu S, Nagaiah TC, Xia W, Wang Y, Dommele SV, Bitter JH, et al. Electrocatalytic activity and stability of nitrogen-containing carbon nanotubes in the oxygen reduction reaction. *The Journal of Physical Chemistry C*. 2009;**113**(32):14302-14310

[39] Li W, Liang C, Qiu J, Zhou W, Han H, Wei Z, et al. Carbon nanotubes as support for cathode catalyst of a direct methanol fuel cell. *Carbon*. 2002;**40**(5):787-790

[40] Liu Z, Davis C, Cai W, He L, Chen X, Dai H. Circulation and long-term

fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;**105**(5):1410-1415

[41] Yang ST, Wang X, Jia G, Gu Y, Wang T, Nie H, et al. Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice. *Toxicology Letters*. 2008;**181**(3):182-189

[42] Yang ST, Fernando KS, Liu JH, Wang J, Sun HF, Liu Y, et al. Covalently PEGylated carbon nanotubes with stealth character in vivo. *Small*. 2008;**4**(7):940-944

[43] Huczko A, Lange H, Calko E, Grubek-Jaworska H, Droszcz P. Physiological testing of carbon nanotubes: Are they asbestos-like? *Fullerene Science and Technology*. 2001;**9**(2):251-254

[44] Mercer RR, Scabilloni JF, Wang L, Kisin ER, Murray AR, Schwegler-Berry D, et al. Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single walled carbon nanotubes in a mouse model. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2008;**294**(1):87-97

[45] Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, et al. Inhaled carbon nanotubes reach the subpleural tissue in mice. *Nature Nanotechnology*. 2009;**4**(11):747

[46] Wang L, Mercer RR, Rojanasakul Y, Qiu A, Lu Y, Scabilloni JF, et al. Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts. *Journal of Toxicology and Environmental Health, Part A*. 2010;**73**(5-6):410-422

- [47] Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environmental Health Perspectives*. 2006;**114**(8):1172-1178
- [48] Zensi A, Begley D, Pontikis C, Legros C, Mihoreanu L, Wagner S, et al. Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones. *Journal of Controlled Release: Official Journal of the Controlled Release Society*. 2009;**137**(1):78-86
- [49] Yu LE, Lanry Yung LY, Ong CN, Tan YL, Suresh Balasubramaniam K, Hartono D, et al. Translocation and effects of gold nanoparticles after inhalation exposure in rats. *Nanotoxicology*. 2007;**1**(3):235-242
- [50] Singh R, Pantarotto D, Lacerda L, Pastorin G, Klumpp C, Prato M, et al. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(9):3357-3362
- [51] Lacerda L, Soundararajan A, Singh R, Pastorin G, Al-Jamal KT, Turton J, et al. Dynamic imaging of functionalized multi-walled carbon nanotube systemic circulation and urinary excretion. *Advanced Materials*. 2008;**20**(2):225-230
- [52] Deng XY, Yang ST, Nie HY, Wang HF, Liu YF. A generally adoptable radiotracing method for tracking carbon nanotubes in animals. *Nanotechnology*. 2008;**19**(7):75101
- [53] McDevitt MR, Chattopadhyay D, Kappel BJ, Jaggi JS, Schiffman SR, Antczak C, et al. Tumor targeting with antibody functionalized, radiolabeled carbon nanotubes. *Journal of Nuclear Medicine*. 2007;**48**:1180-1189
- [54] Liu Z, Cai WB, He LN, Nakayama N, Chen K, Sun XM, et al. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nature Nanotechnology*. 2007;**2**(1):47-52
- [55] Cherukuri P, Gannon CJ, Leeuw TK, Schmidt HK, Smalley RE, Curley SA, et al. Mammalian pharmacokinetics of carbon nanotubes using intrinsic near infrared fluorescence. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(50):18882-18886
- [56] Yang ST, Guo W, Lin Y, Deng XY, Wang HF, Sun HF, et al. Biodistribution of pristine single-walled carbon nanotubes in vivo. *Journal of Physical Chemistry C*. 2007;**111**(48):17761-17764
- [57] Ding LH, Stilwell J, Zhang TT, Elboudwarej O, Jiang HJ, Selegue JP, et al. Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. *Nano Letters*. 2005;**5**(12):2448-2464
- [58] Bottini M, Bruckner S, Nika K, Bottini N, Bellucci S, Magrini A, et al. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicology Letters*. 2006;**160**(2):121-126
- [59] Khalid P, Hussain MA, Rekha PD, Arun AB. Carbon nanotube-reinforced hydroxyapatite composite and their interaction with human osteoblast in vitro. *Human & Experimental Toxicology*. 2014;**33**(9):897-990
- [60] Dong L, Joseph KL, Witkowski CM, Craig MM. Cytotoxicity of single-walled carbon nanotubes suspended in various surfactants. *Nanotechnology*. 2008;**19**(25):255702
- [61] Plata DL, Gschwend PM, Reddy CM. Industrially synthesized single walled carbon nanotubes: Compositional data for users, environmental risk assessments and

source apportionment. *Nanotechnology*. 2008;**19**(18):185706

[62] Casey A, Herzog E, Davoren M, Lyng FM, Byrne HJ, Chambers G. Spectroscopic analysis confirms the interactions between single walled carbon nanotubes and various dyes commonly used to assess cytotoxicity. *Carbon*. 2007;**45**(7):1425-1432

[63] Davoren M, Herzog E, Casey A, Cottineau B, Chambers G, Byrne HJ, et al. In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicology In Vitro*. 2007;**21**(3):438-448

[64] Sharma CS, Sarkar S, Periyakaruppan A, Barr J, Wise K, Thomas R, et al. Single walled carbon nanotubes induces oxidative stress in rat lung epithelial cells. *Journal of Nanoscience and Nanotechnology*. 2007;**7**(7):2466-2472

[65] Herzog E, Casey A, Lyng FM, Chambers G, Byrne HJ, Davoren M. A new approach to the toxicity testing of carbon-based nanomaterials—The clonogenic assay. *Toxicology Letters*. 2007;**174**(1-3):49-60

[66] Pulskamp K, Diabate S, Krug HF. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicology Letters*. 2007;**168**(1):58-74

[67] Boor KJ. Bacterial stress responses: What doesn't kill them can make them stronger. *PLoS Biology*. 2006;**4**(1):e23

[68] Kang S, Pinault M, Pfefferle LD, Elimelech M. Single walled carbon nanotubes exhibit strong antimicrobial activity. *Langmuir*. 2007;**23**(17):8670-8673

[69] Liu S, Wei L, Hao L, Fang N, Chang MW, Xu R, et al. Sharper and faster nano darts kill more bacteria: A study of antibacterial activity of

individually dispersed pristine single-walled carbon nanotube. *ACS Nano*. 2009;**3**(12):3891-3902

[70] Arias LR, Yang L. Inactivation of bacterial pathogens by carbon nanotubes in suspensions. *Langmuir*. 2009;**25**(5):3003-3012

[71] Yang C, Mamouni J, Tang Y, Yang L. Antimicrobial activity of single walled carbon nanotubes length effect. *Langmuir*. 2010;**26**(20):16013-16019

[72] Vecitis CD, Zodrow KR, Kang S, Elimelech M. Electronic structure dependent bacterial cytotoxicity of single walled carbon nanotubes. *ACS Nano*. 2010;**4**(9):5471-5479

[73] Kang S, Mauter MS, Elimelech M. Microbial cytotoxicity of carbon based nanomaterials: Implications for river water and wastewater effluent. *Environmental Science & Technology*. 2009;**43**(7):2648-2653

[74] Jin L, Son Y, Yoon TK, Kang YJ, Kim W, Chung H. High concentrations of single walled carbon nanotubes lower soil enzyme activity and microbial biomass. *Ecotoxicology and Environmental Safety*. 2013;**88**:9-15

[75] Rodrigues DF, Elimelech M. Toxic effects of single walled carbon nanotubes in the development of *E. coli* biofilm. *Environmental Science & Technology*. 2010;**44**(12):4583-4589

[76] Kang S, Mauter MS, Elimelech M. Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity. *Environmental Science & Technology*. 2008;**42**(19):7528-7534

[77] Zheng H, Liu L, Lu Y, Long Y, Wang L, Ho KP. Rapid determination of nanotoxicity using luminous bacteria. *Analytical Sciences*. 2010;**26**(1):125-128

[78] Riding MJ, Martin FL, Trevisan J, Llabjani V, Patel II, Jones KC, et al.

Concentration dependent effects of carbon nanoparticles in gram negative bacteria determined by infrared spectroscopy with multivariate analysis. *Environmental Pollution*. 2012;**163**:226-234

[79] Kang S, Herzberg M, Rodrigues DF, Elimelech M. Antibacterial effects of carbon nanotubes: Size does matter. *Langmuir*. 2008;**24**(13):6409-6413

[80] Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C, et al. Size composition and shape dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. *Environmental Science & Technology*. 2009;**43**(21):8423-8429

[81] Chung H, Son Y, Yoon TK, Kim S, Kim W. The effect of multi walled carbon nanotubes on soil microbial activity, Ecotoxicology and Environmental Safety, 2011;**74**(4):569-575

[82] Gorczyca A, Kaspruwicz MJ, Lemek T. Physiological effect of multi walled carbon nanotubes (MWCNTs) on conidia of the entomopathogenic fungus, *Paecilomyces fumosoroseus* Deuteromycotina: Hyphomycetes. *Journal of Environmental Science and Health, Part A*. 2009;**44**(14):1592-1597

[83] Schwab F, Bucheli TD, Lukhele LP, Magrez A, Nowack B, Sigg L, et al. Are carbon nanotube effects on green algae caused by shading and agglomeration? *Environmental Science & Technology*. 2011;**45**(14):6136-6144

[84] Long Z, Ji J, Yang K, Lin D, Wu F. Systematic and quantitative investigation of the mechanism of carbon nanotubes' toxicity toward algae. *Environmental Science & Technology*. 2012;**46**(15):8458-8466

[85] Youn S, Wang R, Gao J, Hovespyan A, Ziegler KJ, Bonzongo JC, et al. Mitigation of the impact of single-walled carbon nanotubes

on a freshwater green algae: *Pseudokirchneriella subcapitata*. *Nanotoxicology*. 2011;**6**(2):161-172

[86] Kwok KW, Leung KM, Flahaut E, Cheng J, Cheng SH. Chronic toxicity of double walled carbon nanotubes to three marine organisms: Influence of different dispersion methods. *Nanomedicine*. 2010;**5**(6):951-961

[87] Wei LP, Thakkar M, Chen YH, Ntim SA, Mitra S, Zheng XY. Cytotoxicity effects of water dispersible oxidized multiwalled carbon nanotubes on marine alga, *Dunaliella tertiolecta*. *Aquatic Toxicology*. 2010;**100**(2):194-201

[88] Ghafari P, St-Denis CH, Power ME, Jin X, Tsou V, Mandal HS, et al. Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. *Nature Nanotechnology*. 2008;**3**(6):347-351

[89] Zhu Y, Zhao Q, Li Y, Cai X, Li W. The interaction and toxicity of multi walled carbon nanotubes with *Stylonychia mytilus*. *Journal of Nanoscience and Nanotechnology*. 2006;**6**(5):1357-1364

[90] Zhu X, Zhu L, Chen Y, Tian S. Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *Journal of Nanoparticle Research*. 2009;**11**(1):67-75

[91] Kim KT, Edgington AJ, Klaine SJ, Cho JW, Kim SD. Influence of multi-walled carbon nanotubes dispersed in natural organic matter on speciation and bioavailability of copper. *Environmental Science & Technology*. 2009;**43**(23):8979-8984

[92] Petersen EJ, Pinto RA, Mai DJ, Landrum PF, Weber WJ Jr. Influence of polyethyleneimine graftings of multi walled carbon nanotubes on their accumulation and elimination by and toxicity to *Daphnia magna*.

Environmental Science & Technology.
2011;**45**(3):1133-1138

[93] Li M, Huang CP. The responses of *Ceriodaphnia dubia* toward multi-walled carbon nanotubes: Effect of physical–chemical treatment. Carbon. 2011;**49**(5):1672-1679

[94] Cheng J, Flahaut E, Cheng SH. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. Environmental Toxicology and Chemistry. 2007;**26**(4):708-716

[95] Bourdiol F, Mouchet F, Perrault A, Fourquaux I, Datas L, Gancet C, et al. Biocompatible polymer-assisted dispersion of multi walled carbon nanotubes in water, application to the investigation of their ecotoxicity using *Xenopus laevis* amphibian larvae. Carbon. 2013;**54**:175-191

[96] Leeuw TK, Reith RM, Simonette RA, Harden ME, Cherukuri P, Tsyboulski DA, et al. Single-walled carbon nanotubes in the intact organism: Near-TR imaging and biocompatibility studies in *Drosophila*. Nano Letters. 2007;**7**(9):2650-2654

[97] Aschberger K, Johnston HJ, Stone V, Aitken RJ, Hankin SM, Peters SA, et al. Review of carbon nanotubes toxicity and exposure appraisal of human health risk assessment based on open literature. Critical Reviews in Toxicology. 2010;**40**(9):759-790

[98] Lim JH, Kim SH, Lee IC, Moon C, Kim SH, Shin DH, et al. Evaluation of maternal toxicity in rats exposed to multi-wall carbon nanotubes during pregnancy. Environmental Health and Toxicology. 2011;**26**:e2011006