Chapter

Quality Control and Risk Management of Carbon Nanomaterials

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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., C||O, C—O, —OH, and —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 SWNCT 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-iodopheny1)-3-(4-nitropheny1)-5-(2,4-disulfopheny1)-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 innoxious 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 -NH_2 [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
Chlorella vulgaris	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]
Pseudokirchneriella subcapitata	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]
Thalassiosira pseudonanas	DWCNTs	0.1 mg L^{-1}	1.86 mg L^{-1}	Oxidative stress, agglomeration and physical interactions	[86]
Dunaliella tertiolecta	MWCNT	NA	0.8 mg L^{-1}	Oxidative stress and photosynthesis inhibition	[87]
Tetrahymena thermophila	SWCNT	1.6 mg L^{-1}	NA	Physical interactions	[88]
Stylonychia mytilus	Functionalized MWCNT	1 mg L^{-1}	NA	Physical interactions	[89]
attenuata	SWCNT	1.10 mg L^{-1}	*Hydra* NA	Physical interactions	[89]
Daphnia magna	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]
Ceriodaphnia . dubia	MWCNT resuspended in NOM	0.25 mg L- 1	NA	Agglomeration	[93]
Danio rerio embryo	SWCNT	120 mg L^{-1}	NA	Agglomeration	[94]
Oryzias melastigma	DWCNT	10 mg L^{-1}	NA	Agglomeration	[86]
Xenopus leavis larvae	DWCNT	10 mg L^{-1}	NA	Physical interactions	[95]
Drosophila melanogaster	SWCNT and MWCNT in 1 g kg^{-1} food			No toxicity	[96]
Female Fisher rats	Oral gavage of 0.64 mg ka^{1} SWCNT	NA	NA	Increased levels of oxidative damage to DNA in liver and lung tissue	[97]
Sprague—Dawleyrat	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.

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