Increasing evidence indicates low bioaccumulation of carbon nanotubes

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The safe and responsible development of nanoenabled materials requires an assessment of the environmental, health and safety implication of engineered nanomaterials and other emerging technologies. Understanding the environmental fate and bioaccumulation potential of carbon nanotubes is key to advancing the risk evaluation and management process. Our work provides a comprehensive review of this topic and summarizes the current knowledge base to provide an evidence-driven assessment as to bioaccumulation potential and trophic transfer risk across a wide variety of taxa.

Increasing evidence indicates low bioaccumulation of carbon nanotubes

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Abstract

As the production of carbon nanotubes (CNTs) expands, so might the potential for release into the environment. The possibility of bioaccumulation and toxicological effects has prompted research on their fate and potential ecological effects. For many organic chemicals, bioaccumulation properties are associated with lipid-water partitioning properties. However, predictions based on phase partitioning provide a poor fit for nanomaterials. In the absence of data on the bioaccumulation and other properties of CNTs, the Office of Pollution Prevention and Toxics (OPPT) within the US Environmental Protection Agency (EPA) subjects new pre-manufacture submissions for all nanomaterials to a higher-level review. We review the literature on CNT bioaccumulation by plants, invertebrates and non-mammalian vertebrates, summarizing 40 studies to improve the assessment of the potential for bioaccumulation. Because the properties and environmental fate of CNTs may be affected by type (single versus multiwall), functionalization, and dosing technique, the bioaccumulation studies were reviewed with respect to these factors. Absorption into tissues and elimination behaviors across species were also investigated. All of the invertebrate and non-mammalian vertebrate studies showed little to no absorption of the material from the gut tract to other tissues. These findings combined with the lack of biomagnification in the CNT trophic transfer studies conducted to date suggest that the overall risk of trophic transfer is low. Based on the available data, in particular the low levels of absorption of CNTs across epithelial surfaces, CNTs generally appear to form a class that should be designated as a low concern for bioaccumulation.

Introduction

Carbon nanotubes (CNTs) and other carbon-based nanomaterials are major building blocks of nanotechnology [3]. CNTs have been incorporated into diverse products, ranging from lightweight data cables, rechargeable batteries, automotive parts, and sporting goods to boat hulls and water filters [4]. They currently have the highest production volumes among carbonaceous engineered nanomaterials (ENMs) worldwide [7]. As production and use of CNTs grow, so does the potential for their release to the environment and for the exposure of ecological receptors [8, 9]. The prospect of nanomaterial release into the environment and possible bioaccumulation and toxicological effects has prompted research on the fate, transport and effects of these materials on biota. However, the novel or enhanced properties associated with materials that have nanoscale dimensions between 1 nm and 100 nm in at least one dimension, [12] , also creates unique challenges in assessing their likely impact on human health and the environment.

A key component for risk assessment of traditional chemicals includes an evaluation of their persistence, potential for bioaccumulation and potential to cause toxic effects. As governments began articulating concerns about these three properties of chemicals, regulators began placing persistence, bioaccumulation and toxicity (PBT) characteristics into a common regulatory scheme in the identification of chemical hazards e.g., Japan's Chemical Substances Control Law, [13]. Chemicals designated as PBT are priority substances for regulators and environmental managers and may be subject to controls (e.g., limitations on release and toxicity testing).

Bioaccumulation, the second pillar in the PBT framework, occurs when the chemical concentration in an organism exceeds that in its environmental matrix [1, 14]. The propensity for a chemical to accumulate in tissues could increase the probability of transfer up the food chain from prey to predators, thus creating increasingly larger exposures for upper-level predators, including human beings [15]. The potential for bioaccumulation, the B in PBT, represents an assessment of the accumulation of a

chemical from the environment to an organism's tissues [1]. If a chemical has a low persistence in the environment, this would usually end concern regarding its PBT properties. However, given the persistence of CNTs in the environment as will be discussed later, this raises the importance of determining their potential for bioaccumulation within the PBT framework.

This review will focus on non-mammalian organisms and ignores inhalation exposures for terrestrial organisms. Inhalation exposures and the buildup of a chemical in the lungs are important for determining potential toxic effects but accumulation of a chemical in the lungs alone is not an indication of high bioaccumulation potential. Furthermore, biomagnification is an important indicator of bioaccumulation potential and inhalation exposures are not typically connected to the ability of a chemical to biomagnify in a food web. The potential for bioaccumulation (or bioconcentration, see Box 1 for definitions) for many organic chemicals is correlated with phase-distribution properties [16]. Chemicals will redistribute (equilibrate) into the most energetically favorable phase; for hydrophobic organic chemicals this is typically partitioning into another organic phase such as lipid, proteins, or polysaccharides [17]. In contrast, compounds that are hydrophilic tend to have a low potential to bioaccumulate or bioconcentrate and do not readily partition into an organism's tissues [18].

In the 1970s, the concept of bioconcentration as a phenomenon of equilibrium partitioning [19] led to modeling efforts that linked bioconcentration measurements of a chemical to measurements of its partitioning behaviors (the ratio of contaminant concentrations in two phases at equilibrium). In particular, the octanol-water partitioning coefficient (K_{ow}) has been used to categorize and predict the bioconcentration factor of organic chemicals as it frequently reflects a chemical's affinity to partition to lipids within an organism[20, 21]. In general, the hierarchy of evidence for the potential for bioaccumulation or bioconcentration begins with a field measured trophic magnification factor (TMF), followed by field, then laboratory-based biomagnification factors (BMFs), bioaccumulation factors (BAFs), and then laboratory-measured bioconcentration factors (BCF). The lowest tier is a measured or

Box 1. Definitions

Bioaccumulation - Bioaccumulation is the process by which a chemical substance is absorbed by an organism from all routes of exposures as occurs in the natural environment (i.e., dietary and ambient environment sources) and achieves a level that exceeds those in the exposed sources. Bioaccumulation is distinct from bioconcentration because chemical exposure is in the diet and therefore potential biomagnification is included [1, 2].

Bioaccumulation Factor - Ratio of the steady state chemical concentrations in an aquatic water-respiring organism (C_B, g chemical/kg ww) and the water (C_W, g chemical/L) determined from field data in which sampled organisms are exposed to a chemical in the water and in their diet. Thus BAF = C_B /C_W [1]

Bioconcentration- The process by which a chemical substance is absorbed by an organism from the ambient environment only through its respiratory and dermal surfaces, i.e., exposure in the diet is not included (Arnot and Gobas, 2006).

Bioconcentration Factor (BCF) – The ratio of the steady state chemical concentrations in an aquatic water-respiring organism (CB, g chemical/kg ww) and the water (C_W, g chemical/L) determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water (but not in the diet). Thus BCF = C_B /C_W [1].

Biomagnification - Bioaccumulation of a chemical through an ecological food chain by transfer of residues from the diet into body tissues. The tissue concentration increases at each trophic level in the food web when there is efficient uptake and slow elimination [5, 6].

Biomagnification factor (BMF) – The ratio of the steady state chemical concentrations in a water- or air-respiring organism (CB, g chemical/kg ww) and in the diet of the organism (CD, g chemical/kg ww***).*** BMF is determined either in a controlled laboratory experiment in which the test organisms are exposed to chemical in the diet (but not the water or air) or from field data in which sampled organisms are exposed to chemical in air, water, and diet [1].

Biodistribution- Distribution of a chemical within an organism [9, 10].

Uptake -that part of the bioaccumulation/bioconcentration process(es) involving the movement of a chemical from the external environment into an organism, either through direct exposure to a contaminated medium and/or by consumption of food containing the chemical [9, 11].

estimated octanol-water partition coefficient (K_{ow}). Bioconcentration measurements for dissolved chemicals may have included the total organism mass including the contents of the gut, and for highly bioaccumulated chemicals, distribution into systemic circulation and accumulation in specific tissues were assumed. Nanomaterials that do not penetrate the epithelial surfaces, such as the gut tract, require removal of the gut or inclusion of a depuration period to distinguish between nanomaterials that

have been ingested or uptaken and those that have been absorbed across epithelial tissues and entered into systemic circulation in the organisms; in the nanomaterial bioaccumulation literature, it is common to use the term uptake to refer to nanomaterials that have entered the organism and remain in the gut tract while this term is more typically used for other chemicals to reflect those that have passed through epithelial surfaces and into systemic distribution in the organism. Even if a bioaccumulation study only exposes the organism to nanomaterials suspended in water (i.e. BCF type study), filter feeders like *Daphnia* may show accumulation of the material in their intestines because they ingest them. Typical lipid normalization approaches may also not be appropriate as a given nanomaterial will not necessarily associate with lipids.

The earliest use of the PBT concept was by Japan and other jurisdictions then adopted this usage [22, 23]. In the US, the association of persistence, bioaccumulation and toxicity was set out in the Resources Conservation and Recovery Act (RCRA) of 1976 (42 U.S.C. §6921). By the late 1980s, the OPPT had established working categories for chemicals, including one for PBT compounds [24]. In the 1990s, under the RCRA's Waste Minimization Action Plan, EPA developed a scoring system for human and ecological risk potential based on PBT characteristics [25]. That system placed substances in different categories corresponding to a low, medium and high value for each assessment factor (P, B, and T) giving the substances a ranking from 1 to 3 (where 3 indicates high concern). As mentioned in the previous paragraph, a good correlation between K_{ow} and BCF has been found for many nonionic organic molecules [26]. Models based on this relationship have been built into EPA's Estimation Program Interface (EPI) Suite™ software, a widely used tool for predicting physico-chemical properties and environmental fate of chemicals in the absence of measured data. Such models provide the basis for many of the initial assessments by the New Chemical Review Program (NCRP) within OPPT of the potential for bioaccumulation or bioconcentration for chemicals where test data are not available.

OPPT later refined its approach to include a formal consideration of PBT under the Toxic Release Inventory (TRI) program established under the Emergency Planning and Community Right-to-Know Act (EPCRA, 42 U.S.C. §11001 et seq. (1986). This approach was adopted by OPPT as part of its management of new chemicals under the Toxic Substances Control Act [24]. The 1999 Federal notice also outlined a tiered test strategy OPPT believed necessary for a PBT chemical evaluation. This information is provided here to provide context for the process that would typically be used for chemical submissions. The PBT policy takes into account factors such as magnitude of releases, results of physicochemical and potential ecotoxicological testing, and structure-activity relationship (SAR) prediction [24]. Evaluation factors for the potential for bioaccumulation or bioconcentration include experimental determination of Log K_{ow} (tier 1) and experimental determination of a fish BCF for tier 2. The European Union (EU) regulates chemicals under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and employs similar evaluation methods for standard industrial chemicals.

While the use of partitioning models to estimate the potential for bioaccumulation or bioconcentration has been available for traditional organic chemicals for decades, this framework is not considered valid for determining how CNTs or other nanomaterials would behave in food webs given the substantial differences in the partitioning behaviors between nanomaterials and organic chemicals. Unlike dissolved organic chemicals, nanomaterial dispersions are colloidal suspensions, requiring energy input to become suspended throughout another phase [27].Therefore, ratios of nanoparticle concentrations in two phases violate the fundamental description of an equilibrium partitioning coefficient. Despite their use in the regulatory framework for organic chemicals, current test guidelines for estimating bioaccumulation (e.g., BCF) using partitioning coefficients are not appropriate to measure the bioconcentration of chemicals that do not reach equilibrium among phases such as nanomaterials (e.g., the organism tissue and water, see Handy et al. [28, 29]).

Nanomaterials also behave differently than traditional low solubility organic chemicals that are challenging to test in traditional assays for measuring the potential for bioaccumulation. Exposing a test organism to a steady dose of a low solubility organic chemicals can be difficult due to challenges in solubilizing the chemical in media and measuring the concentration of the test substance during the study. However, for the most part, these low solubility organic molecules are still expected to partition to lipids. In contrast, it is not clear that nanomaterials, such as CNTs, will partition to lipids or that equilibrium behavior will be responsible for determining their fate in an organism. This arises from the instability of CNTs in water and the slow and not well understood mechanism for CNTs passing through epithelial surfaces which often leads to concentrations in the organism tissues outside of the gut tract being below the instrument detection limits.

There is evidence that CNTs will persist in the environment. Hydrolysis is not expected to be a significant environmental degradation pathway for CNTs [30]. Photodegradation of CNTs has been shown to transform CNTs by changing their surface chemistry [31] or causing a loss of fluorescence when hydrogen peroxide was also present [32, 33], but complete degradation has not been confirmed. Neither pure fungal cultures of white rot fungi, *Trametes versicolor*, nor environmental microbial communities degraded radioactively-labeled SWCNTs after a six-month incubation period [34].

Studies testing the enzymatic and microbial degradation of radioactively-labeled multiwall carbon nanotubes (MWCNTs) also showed minimal degradation except when a specific microbial grouping was used [35, 36]. The results with radioactively-labeled CNTs contrast with enzymatic studies on non-radioactive CNTs which showed quicker degradation [37]. However, the experimental conditions in the enzymatic studies did not reflect environmentally relevant concentrations of these enzymes and the CNTs in the study were pre-treated with acid to introduce additional defect sites that should increase the ability of the CNTs to degrade. Thus, these results may not be directly applicable to determining environmental persistence values. Overall, the reported data suggests a half-life of CNTs in

environmental systems (soil, sediment, water) greater than 6 months [9, 38]. The current persistence scale (P1, P2 and P3) in OPPT is generally based on these guideposts: environmental half-lives lower than 2 months (P1), between 2 and 6 months (P2) and greater than 6 months (P3). Therefore, CNTs would be considered P3 (i.e., high potential for environmental persistence).

Field measurements of CNTs, which have only recently entered commerce, are not yet available. A modeled average CNT surface water concentration in Europe was estimated to be 0.0035 ng/L [39], a concentration below the detection limit of all currently available analytical methods [40]. However, greater concentrations could be present in the environment at release locations. OPPT does not generally permit environmental releases of CNTs. As a result of these factors, there are no direct measurements of the bioaccumulation behavior of CNTs in the environment to evaluate.

To assess the potential of CNTs to bioaccumulate or bioconcentrate, we summarize the literature on CNT bioaccumulation and bioconcentration by invertebrates and non-mammalian vertebrates, and discuss how these measurements were made as well as their implications for assessing the placement of CNTs in the bioaccumulation component of a PBT framework. Because the physicochemical behavior of CNTs may be affected by type (single versus multiwall), surface modifications (functionalizations), and exposure conditions, the bioaccumulation studies were reviewed with respect to these factors. In addition, we investigated the extent of CNT absorption across epithelial tissues and retention of CNTs among species. Other key topics such as general findings on the potential toxicity of CNTs to ecological receptors and humans [41-43] or the potential for carbon nanotubes to modify the bioaccumulation of co-contaminants [44-47] were not systematically reviewed in this study.

Method

We identified recent publications (2005 to 2016) that reviewed single, double-walled and multiwall carbon nanotube bioaccumulation and ecotoxicity as the starting point for our summary [8, 9, 48,

[49]. We reviewed the bioaccumulation behaviors reported in these studies and extracted information on factors that might affect bioaccumulation (detection method, functionalization, exposure concentration, test taxa). We searched the Web of Science to update the article list from the reviews using a range of search terms including, for example, "nanotube" AND "bioaccumulation," to identify manuscripts published up to August 2016.

General findings

CNTs have been detected and quantified in environmental matrices and organisms using a broad range of analytical techniques, including fluorescence spectroscopy, Raman spectroscopy, electron microscopy, elemental analysis of the metallic impurities in the CNTs, thermal methods, and radiolabeling [9, 40, 50, 51]. Qualitative measurements (e.g., electron microscopy) do not determine the mass or concentration of CNTs but instead only determine their presence or absence, and thus the preferred methods for measuring biodistribution of CNTs are quantitative ones that determines the mass of CNT in organs. While there have been few quantitative CNT biodistribution measurements in organism tissues, numerous qualitative measurements have revealed no absorption of CNTs across the gut tract wall after uptake from the environment in either lower vertebrates or invertebrates [8, 48, 52-55]. In addition, quantitative measurements of total CNT body burden in organisms have consistently revealed limited bioaccumulation or bioconcentration [8, 56]. In general, uptake (from the environment) is rapidly followed by elimination of CNTs since the presence of food dramatically increases the egestion of significant proportions of ingested CNTs, particularly for aquatic invertebrates [53]. In a study investigating bioaccumulation in benthic marine organisms, near-infrared spectroscopy was used quantify body burdens of marine taxa (amphipods and mysids) after exposure to SWCNT in sediment and/or food matrices but found no bioaccumulation (measured BAF < 1) [52]. Another study observed no appreciable bioaccumulation in any biotic compartments in a wetland mesocosm spiked with SWCNTs in the water column [57]. A summary of studies of CNT bioaccumulation or bioconcentration is provided in Table 1.

In addition, studies that have evaluated trophic transfer of SWCNTs using carbon-14 labeled CNTs or near infrared fluorescence either in a mesocosm or a marine benthic food web have shown SWCNTs may be bioavailable for uptake but were rapidly eliminated to below the detection limit during depuration experiments [52, 57, 58]. The limits of quantification for NIRF for plants, biofilms, and fish were reported to be 1140 ng/g, 250 ng/g, and 780 ng/g (based on wet mass), respectively; these values were determined by concentrations giving analytical signals 3 x blank measurements [57]. Overall, measurements of CNT bioaccumulation using orthogonal techniques have given similar results thus indicating that the results were unlikely to be a result of a bias specific to one of the techniques.

As previously mentioned, there are substantial limitations with using equilibrium-based bioaccumulation methodologies (i.e., the correlation of log K_{ow} values to BCF and BAF values) with CNTs since they will not follow the lipid partitioning behavior that is the basis for modeling BCF or BAF using a K_{ow}. There is a lack of data on CNTs to apply criteria recommended by the Pellston Workshop experts for identifying bioaccumulation: e.g.,information from field studies, laboratory experimentation, food web modeling, structure-property relationships and molecular computation [1]. In addition to reporting the determination of each study of the potential for bioaccumulation or bioconcentration, we also we compared research findings using these metrics (BCFs, BAFs) to enable the most straightforward comparison to regulatory thresholds for the bioaccumulation and bioconcentration determinations for traditional chemicals (Table 1). Across the taxa studied, almost all of the estimates of CNT bioaccumulation are below common regulatory thresholds for designating a chemical as a concern for bioaccumulation. For example, OPPT's New Chemical and TRI programs have adopted two thresholds: BCF> 1000 (B2) and BCF ≥5000 (B3) [24] for characterizing a chemical. Taxa for which BCF ≥ 5000 have been estimated include *Daphnia* [53] and the chlorophyte *Desmodesmus* [59]. From the perspective of the total body burden, the data suggests that there are substantially different bioaccumulation behaviors for CNTs for these species compared to others (e.g., fish, earthworms). However, CNT movement across

the gut lining and into the internal tissues has rarely been documented in any organism; evidence of absorption across epithelial tissues in environmentally relevant species exists only for *Drosophila* [10], but the estimated quantities were small (10^{-8} of total dose). Importantly, studies that investigated bioaccumulation using dietary exposure (e.g., [10, 34]) or aqueous exposure (e.g., [11, 60]) both indicated similar low levels of CNT absorption across the gut lining. During the studies investigating aqueous exposures, the extent of CNT settling was often quantified[60, 61]. Agglomeration of the CNTs and settling out of the water phase was not observed in many studies (e.g., [61]) and is not believed to be the cause of the finding that absorption of low bioaccumulation.

Studies on bioaccumulation in marine bivalve tissues have found no evidence for bioaccumulation/bioconcentration [62], or possible absorption across epithelial surfaces only at high exposure concentrations [63]. CNTs were detected in the mantle of mussels but occurred potentially as a result of direct surface association of the mantle to the suspended CNTs in the test media as opposed to absorption across epithelial surfaces [63]. The small number of reported BCF values for CNTs represent uptake into the gut lumen but revealed little to no absorption across the gut tract and into other tissues. When depuration with feeding occurs, there is often a rapid decrease in the gut tract concentration with the CNT concentration often below the detection limit; for example, *Daphnia* exposed to a concentration of 25 µg/L of oxidized or polyethyleneimine functionalized MWCNTs for 24 h nearly fully eliminated (89 % to 99 % of the initial body burden) the MWCNTs after being fed algae for 48 h e.g., [61]. Thus, it may be more appropriate to use data from depurated organisms when assessing the bioaccumulation/bioconcentration of nanomaterials, because high BCF values may be predominately or solely a function of high concentrations in the gut tract when filter feeders are used in studies where estimates of whole body are made without purging gut contents prior to analysis.

Discussion

Quantifying bioaccumulation

Overall, quantification of CNTs in environmental matrices such as organism tissues, soils, and sediments is challenging because of the difficulties of distinguishing CNTs from the largely carbonaceous background of soils and sediments [9]. Analytical approaches used for hydrophobic organic chemicals are generally not applicable because CNTs samples are often heterogeneous, with varying lengths and diameters and therefore cannot be quantified by chromatographic techniques [9]. In addition, many quantification techniques require extraction of CNTs from these matrices prior to quantitative measurements and these extraction methods are still largely being developed [64].

The most commonly used approach for quantifying CNT concentrations in complex environmental media (e.g., soils and sediments) and the tissues of ecological receptors to date is through the use of radiolabeled CNTs [34, 44, 55, 65-67]. This approach provides unequivocal quantification of the CNTs and avoids potential artifacts encountered when using other measurements of CNT bioaccumulation such as microscopic techniques including SEM and TEM [68]. While there may be limitations with some quantitative methods with regard to potential artifacts or insufficient detection limits, overall, the quantification methods are considered sufficiently robust that the bioaccumulation findings from these studies are reliable [9]. In other words, the findings described in the previous section are unlikely to result from method-specific artifacts or insufficient limits of detection to determine if, for example, BCF values were greater or less than 1000, the criterion needed to determine if CNTs should be in the B2 or B3 category as discussed above. In addition, recent pioneering advances in near infrared fluorescence microscopy methods [10] and recently utilized to assess biodistribution in fish [69] allow for detection of individual unagglomerated SWCNTs yet still did not show absorption through the gut tract and into other tissues. In addition, similar results have been observed when bioaccumulation studies were conducted in the same or different laboratories using orthogonal techniques (e.g., infrared fluorescence methods and radioactive labeling [52] or the microwave method [70] and radioactive labeling [54,

[56]). Lastly, similar findings have been observed in studies investigating the bioaccumulation of radioactively labeled few layer graphene for several of the same ecological receptors [71, 72].

Limitation of current bioaccumulation concept to CNTs

The behavior of CNTs and some other ENMs does not fit classical concept of bioaccumulation, which assumes membrane passage [73] and accumulation into lipid phases [74]. Unlike organic chemicals, nanomaterials do not reach thermodynamic equilibrium among the phases during octanol-water distribution measurements [27, 75], although accumulation at the interface of the phases has been observed [76]. Thus, predictions based on phase partitioning behaviors like log K_{ow} provide a poor fit for CNTs in their as-produced form, which are generally not stable long-term in aqueous dispersions without additional dispersants or surfactants (e.g., sodium deoxycholate). CNT stability in solvents is often limited, compared to many organic chemicals. However, it is possible that nanomaterials may associate with more cellular compartments of an organism than just the lipid layers if absorption through the gut tract occurs**.**

CNTs do not readily pass through the membranes lining the gut lumen and where detected, the quantity of absorbed material is extremely low [10]. Because CNT absorption across the gut has rarely been observed, predators consuming exposed animals will be exposed to CNTs predominately or only in the gut tract of the prey organisms. Given that depuration of CNTs has been observed in feeding studies, the concentration of CNTs in the exposed prey organisms would depend on the feeding conditions and whether the organisms are consistently exposed to CNTs or during limited intervals. Moreover, the predators are also unlikely to have absorption of the CNTs through their GI lining, thus indicating a low probability of biomagnification.

We identified no studies that have documented the absorption of CNTs through the gut tract in daphnids even when electron microscopy was used [11, 77]. However, absorption of nanomaterials by

daphnids into tissues other than the gut tract has been observed for quantum dots [78], carboxylated polystyrene beads [79], and silver nanowires [80]. It is important to point out that a larger number of studies have not identified absorption of nanomaterials through the GI tract and into other tissues by daphnids: quantum dots [81], fullerenes [82], and gold nanoparticles [83, 84]. It is currently unclear why absorption into systemic circulation is observed in some studies but not others for tests with similar nanomaterials (e.g., quantum dots). This finding could be a result of differences in the nanoparticles themselves (size, charge, surface coating), test organism (e.g., *Daphnia* age and health), the method used to assess bioaccumulation and associated potential biases, and the method used to conduct the bioaccumulation experiments. Additional research is needed to investigate this topic.

With the broad range of potential surface functionalizations being explored for CNT applications, it is important to also consider whether CNT surface characteristics would influence their potential for bioaccumulation or bioconcentration. The bioaccumulation and bioconcentration studies conducted with CNTs with varying surface chemistries have not yet shown distinctly different bioaccumulation results (Table 2), but only a limited number of studies on this topic have been conducted [85]. In a biodistribution study investigating *D. magna* biodistribution of four types of functionalized SWCNTs (i.e., hydroxylated, silicon dioxide, poly aminobenzenesulfonic acid, and polyethylene glycol coated) after mixing with natural organic matter (NOM), none of the functionalized CNTs showed detectable absorption through the gut tract using transmission electron microscopy (TEM) [11]. Once CNTs are released into the environment, it is likely that they will be covered with NOM regardless of the initial surface functionalization based on the strong adsorption capacity of CNTs for NOM [86]. Thus, bioaccumulation behaviors of CNTs with varying functionalizations will likely be similar to each other in the natural environment in that they will likely be coated with NOM. Continued advances in approaches to quantify CNTs (e.g., near infrared fluorescence spectroscopy, microwave methods) will facilitate the collection of additional bioaccumulation data to explore any potential effects from

different CNT functionalizations on bioaccumulation. Prospective studies should consider investigating if differences in bioaccumulation from these functionalizations persist in the presence of NOM, which would represent more realistic environmental conditions. In the studies conducted in a mesocosm[57] or with CNTs wrapped with NOM (e.g., [11, 77, 60]), similar bioaccumulation findings were observed as compared to studies without NOM, namely a lack of absorption across the gut tract, and thus NOM is not expected to change CNT's bioaccumulation behaviors.

Implications for current risk assessment paradigm

Designating a chemical as bioaccumulative has important regulatory implications. For example, the EU's REACH guidelines give consideration to waiving certain tests for compounds with low potential to bioaccumulate and/or low potential to cross biological membranes to reduce animal testing [87]. OPPT currently considers CNTs a category that may present a potential concern for bioaccumulation due to a lack of data on which to assess their environmental risk [88]. As a replacement for the traditional framework that views the buildup of a chemical in the lipids of fish as the indication that a chemical is bioaccumulative, an alternative framework for nanomaterials would assess first if any material is being absorbed from the gut to other tissues. A growing body of work finds a low potential for bioaccumulation for CNTs due to the absence of material being absorbed across the gut tract. The findings of bioaccumulation studies are robust across multiple organisms and multiple quantification methods, and the lines of evidence show a lack of CNT transport across epithelial layers at detectable concentrations.

While research on CNT trophic transfer potential has mainly been limited to SWCNTs, biomagnification was not identified in aquatic systems [52, 57]. In one study on the trophic transfer of MWCNTs from bacteria to protozoa, the BMF was below 1 (ranging from 0.01 to 0.04) for all conditions tested [89, 90]. There are also some studies which have demonstrated the capacity for metal-based ENMs

such as gold (Au), cerium dioxide, lanthanum oxide and titanium oxide nanoparticles to be transferred along a food chain [91-97]. While one study observed BMF values up to 11.6 when hornworms (*Manduca sexta*) ingested leaves of tomato plants that had accumulated AuNPs [96], in most studies, biomagnification was also not observed (i.e., BMF <1) [92, 94, 95, 97].

Using the traditional measures of bioaccumulation and bioconcentration for CNTs without significant caveats may be misleading [29, 48]. The lack of absorption into organism tissues is a significant difference between the bioaccumulation behavior of CNTs and dissolved chemicals. Given that exposure concentrations investigated generally exceed modelled environmental concentrations of CNTs by several orders of magnitude and the lack of biomagnification in the studies conducted to date, these findings suggest that the overall potential for trophic transfer should also be considered low.

Overall, we recommend that classes of ENMs be investigated on a case by case basis with regard to their potential for bioaccumulation and bioconcentration. This is consistent with scientific recommendations [98, 99] and the current United States national policy position on avoiding sweeping generalizations on nano-enabled products [100]. Based on the literature review and analysis conducted in this paper, CNTs appear to be a group of substances that should be designated low or no concern for bioaccumulation.

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Table 1. Summary of qualitative and quantitative carbon nanotube bioaccumulation results for single-walled carbon nanotubes (SWCNT), double-walled (DWCNT), and multiwall carbon nanotubes (MWCNT) and surface functionalized CNTs.

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
DWCNT	ND[b]	0.7 nm to 2.2 nm	Acid-purified	Ramen spectroscopy; scanning electron microscopy (SEM)	Aqueous medium (distilled tap water with nutritive salts).	10 mg/L to 500 mg/L	12 days	Amphibian	*Xenopus laevis*		Masses of CNT accumulated on gills and gut tract of the tadpoles.	101
DWCNT	ND	1.2 nm to 3.2 nm	Acid-purified	Field Emission Gun SEM/ High resolution Transmission Electron Microscopy (TEM)	Aqueous medium (distilled tap water with nutritive salts).	1 mg/L to 1000 mg/L	12 days	Amphibian	*Ambystoma mexicanum*		Ingested CNTs accumulated in the gut even at lowest exposures tested.	
MWCNT	< 1 µm	5 nm to 20 nm	None	Carbon-14 labeling,TEM	Aqueous medium	1 mg/L	24 h to 72 h	Algae	*Desmodesmus subspicatus*	5000 (BCF)	Most material agglomerated around cells, but single CNTs were detected in the cytoplasm. Large amounts of CNTs detached from the cells after moving them to water without CNTs.	59
MWCNT	5 µm to 15 µm	10-20 nm	None or Acid-purified	TEM	Sand substrate in aqueous medium	10^3 mg/L	14 days	Amphipod	*Hyalella azteca*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell membranes. No significant removal on depuration.	102
MWCNT	2 µm	20 nm to 70 nm	None	TEM	Substrate of gauze layers moistened with MWCNT solution	0 mg/L to (1 x 10^3) mg/L	10 days	Angiosperm	*Onobrychis arenaria*		Qualitative demonstration in plant seedlings of the translocation of MWCNTs from the roots via stems to leaves.	103
MWCNT	0.5 µm	36.5 nm ± 12.7 nm	Acid-purified, 14C-labeled	SEM; AMS (accelerator mass spectrometry);	In growth medium	0.01 mg/L, 1 mg/L		Bacteria	*Pseudomonas aeruginosa*		MWCNTs identified in endosperm.	90

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
				LSC								
MWCNT	5 µm to 15 µm	10 nm to 20 nm	None/Acid-purified	TEM		10^3 mg/L	14 days	Dipterid	*Chironomus dilutus*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell membranes. No significant removal on depuration.	102
MWCNT	ND	ND	None	Field Emission Scanning Electron microscopy	Added to larval food gel	100 mg/kg 10^3 mg/kg	4 days	Dipterid	*Drosophila melanogaster*		Nanomaterials observed as dark concentrations in tissues of hatched adults. Nanomaterials consumed by the larvae were assimilated into the developing fly and sequestered into the tissue	104
MWCNT	0.2 µm to 1 µm	ND	None	Carbon-14 labeling	Aqueous medium with/without organic matter	1 mg/L	7 days	Fish	*Danio rerio*	16 (wet) and 73 (dry) (BCF)	MWCNTs mainly accumulated in the gut, but large relative amounts of radioactivity were also detected in gills, skin, and muscle samples of briefly exposed fish (3 h). MWCNTs were largely eliminated via the digestive tract. In the presence of DOC, 10-fold decrease in uptake after 48 h. No distribution to the liver, the gonads, and the brain was observed. Low amounts of radioactivity were detected in the blood of fish exposed for more than 1 week.	60

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
MWCNT	0.5 µm to 2 µm	10 nm to 20 nm	None	Raman microscopy	Suspended in Pluronic F-108	50-200 mg/L	5 days post fertilization	Fish (embryo)	*Danio rerio*		Accumulation in embryos were exposure concentration-dependent.	[42]
MWCNT	0.5 µm to 2 µm	10 nm to 20 nm	Carboxylated	Raman microscopy	Suspended in Pluronic F-108	50-200 mg/L	5 days post fertilization	Fish (embryo)	*Danio rerio*		Accumulation in embryos were exposure concentration-dependent.	[42]
MWCNT	0.05 to 2.0 µm	20 nm to 30 nm	Amine and carboxylate functionalization	TEM	Hydroponically	10mg/L to 50 mg/L	18 days	Legume (soy bean) and monocot (corn)	*Glycine max* and *Zee mays*		MWCNTs accumulated in the xylem and phloem cells and within intracellular sites.	[105]
MWCNT	0.05 µm to 2.0 µm	20 nm to 30 nm	Carboxylate-functionalized	TEM	Hydroponic solution	10 mg/L to 50 mg/L	18 days	Legume (Soy bean) and monocot (corn)	*Glycine max and Z. mays*		MWCNT accumulated in xylem and phloem and intracellular sites. Stems had lower levels of MWCNTs. Functionalization did not affect uptake and translocation.	[105]
MWCNT		15nm to 40 nm	HCL-purified and carboxylated	TEM; Raman spectroscopy	In growth medium	100 mg/L	11 days	Legume (soy bean),	*G. max.*		Aggregates of MWCNTs detected inside the endosperm of exposed seeds.	[106]
MWCNT		15nm to 40 nm	HCL-purified and carboxylated	TEM; Raman spectroscopy	Deposited through air spray on seed	25-100 mg/L	24 h	Monocot (corn and barley) Legume (soy bean), barley	*Z. mays, Hordeum vulgare and G. max.*		Varied-size clusters of MWCNTs detected inside the endosperm of exposed seeds.	[106]
MWCNT	0.5 µm to 2 µm	40 nm to 70 nm		Raman spectroscopy	In germination medium	2.5mg/L to 800 mg/L	14 days	Monocot (rice)	*Oryza sativa*		The uptake of MWNTs at concentrations of 20 mg/L to800 mg/L was found to be insignificant, with some aggregates appearing in the vascular system and almost none in the plant tissue.	[107]

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factor[a]	Results	Reference
MWCNT	2.65 µm ±1.55 µm	10 nm to 150 nm	MWCNT stabilized in gum Arabic and humic acids	Radioimaging, TEM, Raman spectroscopy	in hydroponic media	50 mg/L	7 days	Monocot (wheat) and rosid (rapeseed)	*Triticum aestivum*; *Brassica napus*	Transfer Factor[c] $4.739 \times 10^{-6} \pm 1.126 \times 10^{-6}$ for wheat; $4.739 \times 10^{-6} \pm 1.126 \times 10^{-6}$ dispersed in gum Arabic (GA). In humic acid (HA), $1.113 \times 10^{-6} \pm 0.066 \times 10^{-6}$. For rapeseed- $1.699 \times 10^{-6} \pm 0.694 \times 10^{-6}$ in GA and $0.830 \times 10^{-6} \pm 0.276 \times 10^{-6}$	Radioimaging qualitatively demonstrated uptake of MWCNT by plant roots and translocated to leaves.	108
MWCNT	386 nm to 407 nm	30 nm to 70 nm	HCL purified or acid oxidized MWCNT	Carbon-14 labeling	Sediment	0.037 mg/g	14 days	Oligochaete	*Lumbriculus variegatus*	BAF for acid-purified CNT in peat-amended sediment =0.39 (± 0.08) and non-amended sediment= 0.67 (± 0.026)	Oxidizing the MWCNT had no effect on BAF.	75
MWCNT	386 nm to 407 nm[4]	30 nm to 70 nm	HCl purified	Carbon-14 labeling	Sediment	(3.7×10^2) mg/kg	28 days	Oligochaete	*Lumbriculus variegatus*	0.40 ± 0.1 (BAF)	Bioaccumulation factors and order of magnitude lower than PAHs. Almost complete depuration after 3 days in CNT- free sediment or water.	55
MWCNT	386 nm to 407 nm[d]	30 nm to 70 nm	HCl purified	Carbon-14 labeling	Soil	30 mg/kg and (3×10^2) mg/kg	14 days	Oligochaete	*Eisenia foetida.*	0.023± 0.01, 0.014± 0.003, 0.016 ± 0.001 (BAF)	MWCNTs into the tissues of E. foetida is minimal in comparison to that of a representative PAH counterpart, pyrene.	54

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
MWCNT	407 nm	30 nm to 70 nm	Polyethyleneimine (PEI) coating w/ negative, positive, or neutral surface charges	Carbon-14 labeling	Sediment	($5x 10^2$) mg/kg	28 days	Oligochaete	*Eisenia fetida*	0.03 (BAF)	No substantial absorption of carbon nanotubes having PEI surface modifications. The PEI-grafted MWCNTs had higher BAF values compared to the nonmodified MWCNTs, but standard deviations were consistently large, hindering definitive conclusions about relative uptake rates.	56
MWCNT	10 µm 20 µm	30 nm to 50 nm	None	Microwave method	Soil	($3 x 10^3$) mg/kg	14 days	Oligochaete	*Eisenia fetida*	0.015± 0.004 (BAF)	Low potential to bioaccumulate; minimal uptake and ready elimination on depuration.	70
MWCNT	5 µm to 15 µm	10 nm to20 nm	None/Acid-purified	TEM	Water exposure but sand was provided as a substrate	($1x 10^3$) mg/L	14 days	Oligochaete	*Lumbriculus variegatus*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell membranes.	102
MWCNT	10 µm to30 µm	10 nm to 30 nm	Hydroxylated and carboxylated MWCNTs	TEM	48 h waterborne exposure to 32 mg/L to 120.2 mg CNT/L in water with or without algae as food	32 mg/L to 120.2 mg/L	48 h	Planktonic crustacea	*Ceriodaphnia dubia*		Qualitative demonstration of MWCNT retention in gut at all concentrations.	98
MWCNT	407 nm	30 nm to 70 nm	Acid-oxidized	Carbon-14 labeling	waterborne exposure	0.04 mg/L, 0.1 mg/L and 0.4 mg/L	48 h	Planktonic crustacea	*Daphnia magna*	360000 ± 40000, 440000 ± 190000, and 350000 ± 80000 (BCF)	Minimal depuration w/o feeding, however the fraction released rises 50 % to 85 % depurated with feeding.	53
MWCNT	407 nm	30 nm to 70 nm	Polyethyleneimine (PEI) coating w/ negative, positive, or neutral surface charges	Carbon-14 labeling	in artificial freshwater	0.025 mg/L, 0.25 mg/L	48 h	Planktonic crustacea	*Daphnia magna*	6000 to 46000 (BCF)	Surface coating did not substantially affect accumulation or elimination rate	61

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
MWCNT	ND	10 nm to 70 nm	Ozone - treated	TEM, XRD	Aqueous medium	10 mg/L	24 h	Planktonic crustacea	*Ceriodaphnia dubia*		Nanoparticle accumulation in brood chamber and digestive tract. CNTs Largely eliminated during depuration.	[109]
MWCNT	ND	ND	Bisphosphonic acid	TEM, Raman spectroscopy	Filtered pond water medium	0.1 mg/L to 200 mg/L	5 days	Protozoa	*Stylonychia mytilus*		MWCNTs exclusively localized to the mitochondria of the cells.	[110]
MWCNT	0.5 µm	36.5 nm ± 12.7 nm	Acid-purified, 14C-labeled	SEM; AMS LSC	In growth medium	0.3 mg/L, 1 mg/L	22 h	Protozoa	*Tetrahymena thermophila*	2900 ± 800 L/kg (at 0.3mg/L MWCNT exposure) 1200 ± 800 L/kg (at 1 mg/L, BCF)	BCF estimates were highest after 2 h (35,000 ± 16000). MWCNT accumulated in the protozoan did not biomagnify, based on estimated BMFs (<1).	[90]
MWCNT	0.5 µm	36.5 nm ± 12.7 nm	Acid-purified, 14C-labeled	SEM; AMS	Bacteria exposed to 0.01 mg/L MWCNTs	0.004 mg/L to protozoans	22 h	Protozoa	*Tetrahymena thermophila*	790 ± 200 (BCF)	BCF estimates were highest after 16 h (2200 ± 900). MWCNT accumulated in the protozoan did not biomagnify, based on estimated BMFs (<1).	[90]
MWCNT	0.5 µm	36.5 nm ± 12.7 nm	Acid-purified, 14C-labeled	SEM, LSC	Bacteria exposed to 1 mg/L MWCNTs	0.3 mg/L to protozoans	22 h	Protozoa	*Tetrahymena thermophila*	790 ± 300 (BCF)	BCF estimates were highest after 16 h (5700 ± 3000). MWCNT accumulated in the protozoan did not biomagnify, based on estimated BMFs (<1).	[90]
MWCNT	NS	10 nm	Acid-purified, carboxylated	TEM and Raman spectroscopy	In aqueous solutions	1, 10 mg/L	16 days	Rosid (mustard)	*Brassica juncea*		MWCNTs permeated into roots of intact plants.	[111]
MWCNT	0.1 µm to 0.5 µm	6 nm to 9 nm		TEM	In nutrient solution	10- 60 mg/L	7 days	Rosid (broccoli)	*Brassica Oleracea*		MCNTs were taken up in the roots localized in cell vacuole, intercellular space and cytoplasm. No MWCNTs were detected in plant leaves.	[112]
MWCNT	0.1 µm to 0.5 µm	6 nm to 9 nm		TEM	In nutrient solution, with 12mM NaCl to create salt-stressed conditions	10 mg/L	7 days	Rosid (broccoli)	*Brassica Oleracea*		Saline-stressed plants showed a higher accumulation of isolated MWCNTs than non-saline treated plants.	[112]

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
MWCNT	1 µm to 10 µm	100 nm to 200 nm	None; helical morphology	TEM and Raman spectroscopy	In tobacco callus growth medium	50 mg/L	24 h (seed) 10 days (seedling)	Solanid (tomato)	*Lycospersicon esculentum*		MWCNTs identified in endosperm.	[113]
MWCNT	1 µm to 12 µm	13 nm to 18 nm	Carboxylate-functionalized, long morphology	TEM and Raman spectroscopy	In tobacco callus growth medium	50 mg/L	24 hours (seed) 10 days (seedling)	Solanid (tomato)	*Lycospersicon esculentum*		Black aggregates of MWCNTs identified in endosperm.	[113]
MWCNT	0.5 µm to 2 µm	20nm to 30 nm	Carboxylate-functionalized, short morphology	TEM and Raman spectroscopy	In tobacco callus growth medium	50 mg/L	24 hours (seed) 10 days (seedling)	Solanid (tomato)	*Lycospersicon esculentum*		MWCNTs identified in endosperm.	[113]
MWCNT	0.05 µm to 2.0 µm	20 nm to 30 nm	None	TEM	Hydroponic solution	10 mg/L to 50 mg/L	18 days	Soybeans and corn	*Glycine max and Z. mays*		MWCNT accumulated in xylem and phloem and intracellular sites. Stems had lower levels of MWCNTs.	[105]
MWCNT	0.05 µm to 2.0 µm	20 nm to 30 nm	Amine-functionalized	TEM	Hydroponic solution	10 mg/L to 50 mg/L	18 days	Soybeans and corn	*Glycine max and Z. mays*		MWCNT accumulated in xylem and phloem and intracellular sites. Stems had lower levels of MWCNTs. Functionalization did not affect uptake and translocation.	[105]
MWCNT	10 µm to 30 µm	20 nm to 30 nm	None	Microwave-induced heating (MIH); multi-angle light scattering; Raman spectroscopy; TEM	Soil	3 mg/kg and 2933 mg/kg	14 weeks	Wheat and corn	*Triticum spp., Z. mays*		Levels of MWCNT taken up could not be fully quantified since they were below the limit of quantification. TEM imaging of root cross sections was not conclusive. Two estimates of translocation of MWCNT into plants were above the limits of detection (0.15 % and 9.8 %).	[114]

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
SWCNT	5 µm to 15 µm	2 nm	None/Acid-purified	TEM	In waer	($1x10^3$) mg/L	14 days	Amphipod	*Hyalella azteca*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell membranes. No significant removal on depuration.	102
SWCNT	ND	1.22 nm to 1.96 nm	None	Fluorimetry and stable isotope analysis	Filtered seawater with phytoplankton feed	1 mg/L to 3 mg/L	28 days	Bivalve mollusc (marine)	*Mytilus galloprovincialis*		CNT accumulated in biodeposit (feces and pseudofeces). Metal residues associated with CNTS were detected in Visceral, mantle and gill tissue ((0.04 ± 0.02) mg/g to (1.04 ± 0.1) mg CNTs/g tissue.	63
SWCNT	ND		None	Near-infrared Fluorescence spectroscopy (NIRF)	SWCNT-spiked algal (*isochrysis galbana*) food.	100 mg/ kg, (1×10^3) mg/ kg SWCNT-amended algae	14 days	Bivalve mollusc (marine)	*Mercenaria mercenaria*		No evidence marine bivalves) fed marine algae *(Isochrysis galbana*) exposed to SWCNT accumulated SWCNT, or that the bivalve served as a vector for SWCNT to polychaetes consuming the bivalves.	62
SWCNT	500 nm to 1.5 µm	4 nm to 5 nm	Carboxylated SWCNT in marine sediment	Carbon-14 labeling	marine sediment	(3.64×10^2) mg/kg	14 days	Copepod	*Amphiascus tenuiremis*		No detectable bioaccumulation after depuration.	44
SWCNT	5 µm to 15 µm	2 nm	None/Acid-purified	TEM	In water	(1×10^3) mg/L	14 days	Dipterid	*Chironomus dilutus*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell	102

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
											membranes. No significant removal on depuration.	
SWCNT	ND		None	NIRF	Paste containing SWCNTs containing SWCNTs in BSA buffer in yeast paste	25 mg/L	4 days to 5 days	Dipterid	*D.melanogaster*		Only a tiny fraction (10^{-8}) of these SWCNTs become incorporated into tissues. after traversing the gut wall, nanotubes in the hemolymph accumulate in the dorsal vessel as a result of its pumping action.	[10]
SWCNT	ND	ND	None	Field Emission Scanning Electron microscopy	Added to larval food gel	100 mg/kg 10^3 mg/kg	4 days	Dipterid	*Drosophila melanogaster*		Nanomaterials observed as dark concentrations in tissues of hatched adults. Nanomaterials consumed by the larvae were assimilated into the developing fly and sequestered into the tissue	[104]
SWCNT	ND	0.7 nm to 1.3 nm	acid-purified and carboxylated	NIRF and carbon-14 labeling	Added SWCNTS to food source	10 mg/kg and 100 ^{14}C mg-SWCNT/kg dried algae + / or sediment	28 days	Estuarine amphipod	*Leptocheirus plumulosus*	0.013 ± 0.002 to 0.068 ± 0.016 (nondepurated); 0.0040 ± 0.0008-to0.0074 ± 0.0012 (depurated) (BAF)	Nondepurated organisms exposed to SWCNT amended sediment and algae had significantly elevated body burden. Uptake via sediment was more critical for accumulation than uptake via algae for amphipods. After 24 h depuration, only the highest SWCNT-amended sediment and algae treatment showed significantly increased body burden compared to background.	[52]

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
SWCNT	1.5 µm	0.78 nm	None	NIRF	Added to water column of experimental wetland mesocosm	2.5 mg/L	10 months	Fish	*Gambusia holbrooki*		CNT length and diameter are product-specified. No bioaccumulation in aquatic vertebrates (fish) or plants was identified (below NIRF detection limits).	57
SWCNT	5 µm to 30 µm	1.1 nm	None	TEM	In aqueous solution (with SDS solvent)	0.1 mg/L to 0.5 mg/L	10 days	Fish	*Oncorhynchus mykiss*		Stress-induced drinking caused SWCNT ingestion and accumulation of CNT in gut tract. SWCNT's also precipitated on gill mucosa.	115
SWCNT	1.5 µm	0.8 nm	None	NIRF	Pelleted fish fish food amended with SWCNT in gum arabic solution	50 mg SWCNTs/kg food	96 h	Fish	*Pimephales promelas*		SWCNTs among the intestinal lumen contents but no apparent association with intestinal epithelia or underlying tissue .	116
SWCNT	1.5 µm	0.8 nm	None	NIRF	force fed SWCNT in gum arabic solution	Dosed at 0.01 ml of 426 mg/L SWCNT for 7 gavages	7 days	Fish	*Pimephales promelas*		NIRF images showed strong SWCNT-derived fluorescence signals in whole fish and excised intestinal tissues. Fluorescence was not detected in tissues other than intestines, indicating that no appreciable intestinal absorption occurred.	69
SWCNT	0.5 µm to 3 µm	1.4 nm	None	Raman microscopy	Suspended in Pluronic F-108	50 mg/L to 200 mg/L	5 days post fertilization	Fish (embryo)	*Danio rerio*		Accumulation in embryos were exposure concentration-dependent.	42
SWCNT	0.5 µm to 3 µm	1.4 nm	Carboxylated	Raman microscopy	Suspended in Pluronic F-108	50 mg/L to 200 mg/L	5 days post fertilization	Fish (embryo)	*Danio rerio*		Accumulation in embryos were exposure concentration-dependent.	42
SWCNT	ND	0.7 nm to 1.3 nm	acid-purified and carboxylated None	NIRF and carbon-14 labeling	Dietary inclusion Sediment	10 mg SWNT/kg dry sediment, 10 mg SWNT/L algae	7 days	Marine amphipod	*Ampelisca abdita*		SWCNT detected in nondepurated amphipods exposed to amended food items (algae). SWCNT not detected in nondepurated	52

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
						(*Cyclotella spp.*)or 10 mg SWNT/kg brine Shrimp (*Artemia spp)*					amphipods in treatment with sediment alone.	
SWCNT	ND	ND	None	NIRF	SWCNT suspension in gum arabic added to marine sediment. SWCNT-spiked algae fed to *Mercenaria* bivalve which was feds to the polychaete	Worms exposed in 10 mg SWCNT/ kg dry sediment and 100 and 1000 mg SWCNT/ Kg SWCNT-amended prey	14 days	Marine polychaete	*Nereis virens*		No accumulation of SWCNT observed.	62
SWCNT	5 µm to 30 µm	1 nm to 4 nm	None	TEM and microwave method	In amended soil	10 mg/kg, 100 mg/kg	40 days	Monocot (corn)	*Z. mays*		SWCNTs taken up into roots most concentrations between 0 mg/kg and 24 mg/kg. Translocation to leaves and stems between 2 mg/kg and 10 mg/kg.	85
SWCNT	5 µm to 30 µm	1 nm to 4 nm	Hydroxyl-functionalized	TEM and microwave method	In amended soil	10 mg/kg, 100 mg/kg	40 days	Monocot (corn)	*Z. mays*		SWCNTs taken up into roots most concentrations between 0 mg/kg and 24 mg/kg. Translocation to leaves and stems between 2 mg/kg and 10 mg/kg. Uptake was not dependent on functionalization.	85
SWCNT	5 µm to 30 µm	1 nm to 4 nm	Surfactant stabilized	TEM and microwave method	In amended soil	10 mg/kg, 100 mg/kg	40 days	Monocot (corn)	*Z. mays*		SWCNTs taken up into roots most concentrations between 0 mg/kg and 24 mg/kg. Translocation to leaves and stems between 2 mg/kg and 10 mg/kg.	85

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
SWCNT	ND	0.7 nm to 1.3 nm	None	NIRF and carbon-14 labeling	Sediment	0.01 mg/kg	7 days	mysid	*Americamysis bahia*		SWCNT not detected in either depurated or nondepurated mysids	[52]
SWCNT	ND	1 nm to 2nm	HCl purified	Carbon-14 labeling	Sediment	30 mg/kg dry sediment	28 days	Oligochaete	*Lumbriculus variegatus*	0.28 ± 0.03 (BSAF)	Bioaccumulation factors an order of magnitude lower than PAH. Almost complete depuration after 3 days in CNT- free environment (sediment and/or water).	[55]
SWCNT	ND	1 nm to 2 nm	HCl purified	Carbon-14 labeling	Soil	30 mg/kg	14 days 100 mg/kg	Oligochaete	*Eisenia foetida.*	BAF: 0.0061 ± 0.002 (Chelsea soil) 0.022 ± 0.003 (Ypsilanti soil) 0.0078 ± 0.005 (Chelsea soil)	Low levels of uptake; most nanotubes in soil mass remaining in the worm's gut after depuration.	[54]
SWCNT	ND	ND	Acid-purified	Fluorescence spectroscopy	Sediment	50 mg/g and 250 mg/g	7 days	Oligochaete	*Lumbriculus variegatus*	0.0021 ± 0.0011 (BAF)	Detected the presence of labeled carbon nanotubes in worms exposed for 1 week to CNT-laden sediment.	[117]
SWCNT	5 µm to 15 µm	2 nm	None/Acid-purified	TEM	Water exposure but sand was provided as a substrate	(1×10^3) mg/L	14 days	Oligochaete	*Lumbriculus variegatus*		Qualitative determination of the presence of CNTs in the gut as well as on the outer surface of the test organisms, although no evidence of penetration of CNTs through cell membranes.	[102]
SWCNT	ND	1.2 nm	Phospholipid (lysophophatidyl-choline or LPC)	Micro-Raman	Waterborne exposure	2.5 mg/L	96 h	Planktonic crustacea	*Daphnia magna*		Qualitative demonstration that *D. magna* were able to ingest solubilized LPC-SWCNTs and egest precipitated SWCNTs.	[118]

Type	Length	Diameter	Functionalization	Detection method	Exposure	Conc.	Duration	Taxon	Species	Factora	Results	Reference
SWCNT	500 nm to 1.5 µm	4 nm to 5 nm	Carboxylated SWCNT	Carbon-14 labeling	Marine sediment	(3.64×10^2) mg/kg	14 days	Polychaete	*Streblospio benedicti*		No detectable bioaccumulation after depuration.	[44]
SWCNT	0.5 µm to 2.0 µm	1 nm to 2 nm	SWCNT dispersed with surfactant	coherent anti-Stokes Raman (CARS) scattering microscopy	Sediment exposure	3 mg/kg to 30 mg/kg	10 days	Polychaete	*Arenicola marina*		Qualitative; bioaccumulation in worm exposed to SWCNT-amended sediment is minimal.	[119]
SWCNT	2 nm to 10 nm	< 500 nm	Acid oxidized SWCNT	Atomic force microscopy and SEM	In Osterhout's medium.	0 mg/L to 0.0172 mg/L	72 h	Protozoa	*Tetrahymena thermophila*		SWCNT internalization and subsequent egestion were observed.	[120]

[a] Bioaccumulation, bioconcentration and biota sediment accumulation factors (BAF, BCF and BSAF resp.) as reported by the studies referenced. The reporting BAF, BSAF, and BCF values in the tables is not meant to indicate that these coefficients should be interpreted similarly to values of these coefficients for organic chemicals. The limitations of using these factors for CNTs as described in the text (e.g., lack of steady-state, accumulation in the gut tract instead of systemic circulation, lack of absorption across the gut tract) are relevant for the values indicated in this table.

[b] "ND" indicates "Not determined".

[c] Transfer Factor (TF)= CNT content in leaves/CNT content in exposure suspension.

[d] This data was provided in a later paper

Table 2. Summary of bioaccumulation trends (range, functionalization and CNT type) across taxa.

	Taxon						
Parameter	Daphnids	Soil invertebrates Oligochaetes	Protozoans /Ciliates	*Drosophila*	Benthic and sediment-dwelling (aquatic and marine) invertebrates	Fish	Amphibians
Range (BCF, BAF, or BSAF)	SWCNT: Not est. MWCNT: *D. magna*, 360000 ± 40000, 440000 ± 190000, and 350000 ± 80000 (BCF)[53], 6000-46,000[61]	SWCNT: *E. foetida*, 0.0061 to 0.022 (BAF) [54] MWCNT: *E. foetida* 0.014 to 0.023 (BAF average 0.02± 0.006)[54]; 0.03[56], 0.015 ± 0.004 [70] (BAF)	SWCNT: Not est. MWCNT: Not est.	SWCNT: Not est. MWCNT: Not est.	SWCNT: *L. plumulosus,* 0.013 ± 0.002 to 0.068 ± 0.016 (nondepurated), 0.0040 ± 0.0008 to 0.0074 ± 0.0012 (depurated, BAF [52] ; *L. Variegatus*, 0.0021 ± 0.0011 (BAF) [117], 0.28 ± 0.03(BSAF) [48], MWCNT: *L. variegatus*, 0.39 (± 0.08 to 0.67 (± 0.026)[55, 75].	SWCNT: Not est. MWCNT: *D. rerio*, 73 (BCF dry mass), 16 (BCF wet mass)[60]	SWCNT: Not est. MWCNT: Not est.
Effect of functionalization	SWCNT: No comparative studies MWCNT: Surface coating did not affect accumulation or elimination rates by *D. magna* [61].	SWCNT: No comparative studies MWCNT: No increase in bioaccumulation with increased concentration of oxygen functional groups by *E. foetida*; surface coating did not affect accumulation or elimination rates [56].	SWCNT: No comparative studies. MWCNT: No comparative studies.	SWCNT: No comparative studies MWCNT: No comparative studies.	SWCNT: No differences between varied functionalized CNTs on *L. variegatus* reported[102]. MWCNT: No increase in bioaccumulation by *L. variegatus* with increased concentration of oxygen functional groups [75] .	SWCNT: No comparative studies MWCNT: No studies.	SWCNT: No comparative studies. MWCNT: No comparative studies.
Absorption of CNTs across	SWCNT: Absorption from	SWCNT: No studies MWCNT: Almost	SWCNT: Internalization	SWCNT: Only a very small	SWCNT: Absorption from gut tract to	SWCNT: Present in gut lumen of	SWCNT: No studies

	Taxon						
Parameter	Daphnids	Soil invertebrates Oligochaetes	Protozoans /Ciliates	*Drosophila*	Benthic and sediment-dwelling (aquatic and marine) invertebrates	Fish	Amphibians
epithelial cells	gut tract to other tissue not detected by *D. magna*[118]. MWCNT: Ingested material by *D. magna* largely eliminated on depuration. Absorption from gut tract to other tissue not detected[61, 77].	complete elimination during depuration[56, 70].	and subsequent egestion were observed[120]. MWCNT: Exclusive localization into the mitochondria of the cells[110].	fraction of the quantity ingested became incorporated into organs of the larvae[10]. MWCNT: Not studied.	other tissues not shown; depurated *L, variegatus* worms had very little SWCNT in their tissue[55, 102, 117]. No accumulation found in *Mercenaria mercenaria* [34], accumulation in visceral, mantle and gill tissues in *Mytilus galloprovincialis* [63]; MWCNT: Almost complete elimination on depuration in *L. variegatus* after 72 h[55, 102].	*Pimephales promelas*, no appreciable uptake through the intestinal epithelium [116]. MWCNT: Largely eliminated via the digestive tract with very little detected in the blood and muscle tissue [60].	DWCNT: Present in gut lumen of *Ambystoma mexicanum* and *Xenopus laevis*[101, 121].
SWCNT versus MWCNT	No comparative studies.	No differences found in accumulation behaviors between SWCNT and MWCNT for *E. foetida* [54] .	No comparative studies.	Investigated MWCNT and SWCNT on *D. melanogaster* but no quantitative comparison made on uptake[104] .	No absorption across the gut for either type of CNT in amphipod *H. azteca* and dipterid *C. dilutus* [102]. No differences found in accumulation behaviors between SWCNT and MWCNT for *L. variegatus* [55] .	No comparative studies.	No comparative studies.

		Taxon					
Parameter	Daphnids	Soil invertebrates Oligochaetes	Protozoans /Ciliates	*Drosophila*	Benthic and sediment-dwelling (aquatic and marine) invertebrates	Fish	Amphibians