

## Potential Release Pathways, Environmental Fate, And Ecological Risks of Carbon Nanotubes

Elijah J. Petersen,<sup>†,\*</sup> Liwen Zhang,<sup>‡</sup> Nikolai T. Mattison,<sup>§</sup> Denis M. O'Carroll,<sup>§,▲</sup> Andrew J. Whelton,<sup>||</sup> Nasir Uddin,<sup>⊥</sup> Tinh Nguyen,<sup>⊥</sup> Qingguo Huang,<sup>‡</sup> Theodore B. Henry,<sup>#,▽,○</sup> R. David Holbrook,<sup>†</sup> and Kai Loon Chen<sup>◆</sup>

<sup>†</sup>Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

<sup>‡</sup>Department of Crop and Soil Sciences, University of Georgia, Griffin, Georgia 30223, United States

<sup>§</sup>Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario N6A 5B8, Canada

<sup>▲</sup>Water Research Laboratory, School of Civil and Environmental Engineering, University of New South Wales, Manly Vale, NSW, 2093, Australia

<sup>||</sup>Department of Civil Engineering, University of South Alabama, Mobile, Alabama 36688, United States

<sup>⊥</sup>Engineering Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

<sup>#</sup>School of Biomedical and Biological Sciences, University of Plymouth, Plymouth, Devon, United Kingdom

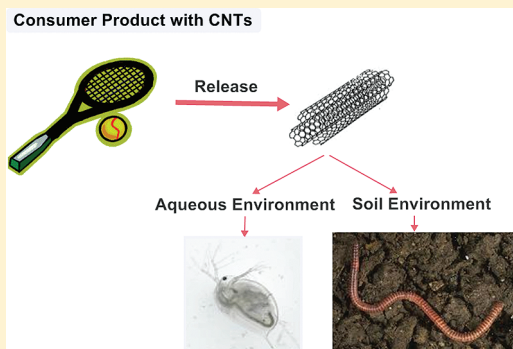
<sup>▽</sup>Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee, United States

<sup>○</sup>Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, Tennessee, United States

<sup>◆</sup>Department of Geography and Environmental Engineering, Johns Hopkins University, Baltimore, Maryland 21218, United States

**S** Supporting Information

**ABSTRACT:** Carbon nanotubes (CNTs) are currently incorporated into various consumer products, and numerous new applications and products containing CNTs are expected in the future. The potential for negative effects caused by CNT release into the environment is a prominent concern and numerous research projects have investigated possible environmental release pathways, fate, and toxicity. However, this expanding body of literature has not yet been systematically reviewed. Our objective is to critically review this literature to identify emerging trends as well as persistent knowledge gaps on these topics. Specifically, we examine the release of CNTs from polymeric products, removal in wastewater treatment systems, transport through surface and subsurface media, aggregation behaviors, interactions with soil and sediment particles, potential transformations and degradation, and their potential ecotoxicity in soil, sediment, and aquatic ecosystems. One major limitation in the current literature is quantifying CNT masses in relevant media (polymers, tissues, soils, and sediments). Important new directions include developing mechanistic models for CNT release from composites and understanding CNT transport in more complex and environmentally realistic systems such as heteroaggregation with natural colloids and transport of nanoparticles in a range of soils.



### INTRODUCTION

Carbon nanotubes (CNTs) comprise a class of engineered nanoparticles (ENPs) composed of extensive  $sp^2$  carbon atoms arranged in fused benzene rings. Their structures give them exceptional electrical, chemical, and physical properties, which are in turn utilized in various applications. For example, polymeric materials containing CNTs (i.e., polymer/CNT composites) are increasingly used in consumer products<sup>2</sup> and for construction,<sup>3</sup> aerospace,<sup>4</sup> medical,<sup>5,6</sup> and other applications. This stems partly from the excellent properties they impart, and because they are easier to manufacture/transport than their traditional counterparts.<sup>7,8</sup> The two main types of CNTs are single-walled CNTs (SWCNTs), which are single-layered graphitic cylinders having diameters

on the order of a few nanometers, and multiwalled CNTs (MWCNTs) which contain between 2 and 30 concentric cylinders with outer diameters commonly between 30 and 50 nm. CNT lengths vary substantially and often range from 100 nanometers to 10 or more micrometers.

There has been considerable research and technical progress regarding applications of CNTs, but far less research on the possible negative consequences after release of these materials

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into the environment. Some studies provide estimated releases of CNTs into various matrices (i.e., air, water, soil, and sediment), and the potential consequent risks in various ecological receptors.<sup>9–11</sup> However, modeling efforts are hindered by the quantity and quality of the data available, and by the limitations of current analytical techniques to detect and quantify CNTs in environmental matrices.

The purpose of this article is to critically review the existing literature on CNT environmental release pathways, environmental fate, transformations, and ecotoxicity. While some reviews have been written on various components of CNT environmental behaviors (e.g., colloidal properties of CNTs in aquatic ecosystems<sup>12,13</sup>), we believe that a comprehensive review is necessary to recognize emerging trends and to identify persistent knowledge gaps on the environmental effects of CNTs. Extensive reviews on chemical sorption to CNTs have been recently published.<sup>14–16</sup> Thus, this topic will not be discussed here.

## ■ CARBON NANOTUBE CHARACTERIZATION AND QUANTIFICATION TECHNIQUES IN ENVIRONMENTAL MATRICES

One of the most critical components of environmental nanotechnology research is thorough ENP characterization of both pristine ENPs and ENPs in environmental matrices. Thorough characterization of starting materials will enhance comparability of results among laboratories, facilitate interpretations of obtained results, and help the development of standardized methods for evaluating CNT environmental behaviors.<sup>17–20</sup> A recent expert panel indicated the following as a priority list for environmental studies of ENPs: size, dissolution, surface area, surface charge, and surface composition/surface chemistry.<sup>21</sup> A thorough discussion of techniques for analyzing the various characteristics of CNTs is beyond the scope of this review article, but relevant characterization and detection techniques and references are provided in Supporting Information (SI) Table S1 (see recent review articles for more information on this topic<sup>13,22,23</sup>). CNT purity is a particularly important consideration as CNT impurities have been shown to cause toxic effects as is described later. CNT characterization can also be a serious challenge in environmental matrices, especially soils and sediments.

CNT quantification in environmentally relevant matrices substantially differs from that of pollutants environmental scientists typically analyze. Unlike organic chemicals that have a defined chemical structure, CNT samples are heterogeneous and typically contain nanotubes with a broad range of diameters and lengths, which limits chromatographic characterization methods. Additionally, CNTs, unlike inorganic pollutants, typically cannot be quantified by measuring the element of interest.

Nevertheless, several techniques are available for quantitative analysis of CNT concentrations in the aqueous phase (see Table 1 for a summary of quantification and identification techniques including their strengths and limitations): UV/vis spectroscopy,<sup>24,25</sup> spectrofluorimetry,<sup>26–28</sup> thermal optical transmittance,<sup>29</sup> and radioactivity measurements for radioactively labeled nanotubes.<sup>30–35</sup> However, these techniques (i.e., UV/vis) may be limited by the presence of natural organic matter (NOM) or other biomolecules, while spectrofluorimetry is limited to detecting individually dispersed semiconducting SWCNTs and is unable to detect MWCNTs, SWCNT aggregates (if they contain metallic SWCNTs), and metallic SWCNTs. Radioactively

labeling CNTs is a promising technique for laboratory experiments, but is not relevant for CNT detection in the natural environment. Nevertheless, the use of radioactive nanotubes may aid in the development of analytical techniques by enabling CNT quantification using radioactivity in addition to the technique being developed. Overall, it is challenging to provide concentration ranges for these techniques (and those for detection in soils, sediments, and organism tissues), because many approaches have only been utilized in a single study, detection limits are often not provided, and there may be significant instrument-to-instrument variability. When detection limits are provided, they are typically substantially higher than the average modeled environmental concentrations.<sup>7</sup> Additionally, it is not straightforward to concentrate CNTs, an approach commonly applied for organic chemicals using solid-phase or liquid–liquid extractions; one potential approach for concentrating CNTs is analytical ultracentrifugation, which has been used to separate and concentrate certain CNTs by species and size.<sup>36</sup> It may also be possible to isolate CNTs from soils and sediments using methods previously designed for hard carbons, but this approach has not yet been tested to our knowledge.

In soils, sediments, or organism tissues, radioactively labeled nanotubes,<sup>30–35,37,38</sup> coherent anti-Stokes Raman scattering,<sup>39</sup> spectrofluorimetry,<sup>27,40</sup> photothermal/photoacoustic imaging,<sup>41</sup> and chemothermal oxidation at 375 °C (CTO-375)<sup>42</sup> have been used to quantify CNT concentrations (see Table 1 for additional details). However, no technique has yet demonstrated detection of the full range of CNTs (i.e., aggregates, SWCNTs, and MWCNTs) at environmentally relevant concentrations without radiolabeling. This limitation must be overcome to enable adequate assessment of environmental samples contaminated with CNTs. One promising approach is field-flow fractionation (FFF) to isolate CNTs from biological, soil, or sediment matrices, as has been accomplished for other nanoparticles (see<sup>43,44</sup> for recent reviews). FFF has been previously used to assess CNT size and for CNT purification.<sup>36,45</sup> It is likely that FFF in combination with another analytical technique can be used to detect CNTs at low concentrations by removing interferences from the biological, soil, or sediment matrix.

## ■ RELEASE PATHWAYS OF CARBON NANOTUBES INTO THE NATURAL ENVIRONMENT

**Release during the Polymer Nanocomposite Life Cycle.** As indicated in the introduction, polymer/CNT based nanocomposites are commercially available and increasing numbers of consumer products applications are expected. While other types of CNT-containing products are expected in future years such as for biomedical applications, they are not yet commercially available to our knowledge and thus this section will focus solely on polymer/CNT nanocomposites. Worldwide CNT production values have been estimated by Mueller and Nowak<sup>11</sup> to range from 350 tons/year to 500 tons/year, with other pertinent CNT production estimates being summarized by Gottschalk et al.<sup>9,10</sup> While CNT releases during their production and the manufacturing of CNT-incorporated products have been demonstrated,<sup>46</sup> and, therefore, represent potential CNT release pathways into the environment, the focus of this section is to examine the fate and release of CNTs during the full life cycle (i.e., during service and after disposal) of polymer/CNT nanocomposites. During use and disposal, polymer/CNT nanocomposites will be exposed to a wide range of harsh environmental

**Table 1. Selected Techniques for Identifying or Quantifying Carbon Nanotubes in Environmentally Relevant Matrices**

technique <sup>a</sup>	mode of operation	strengths	limitations	references
radioactive labeling	measures beta emissions from carbon-14	detects CNPs in any matrix, quantitative, can test degradation or transformation	high cost, safety issues, limited availability	30–32,37,38
spectrofluorimetry	detects absorption and emission of photons in near-IR range	low detection limit, can examine biodistribution using a microscope	only tests individually dispersed semiconducting SWCNTs	26,27,40
Raman spectroscopy	detects characteristic CNT Raman peaks	allows for detection of CNTs in tissues	qualitative	143,144
thermal optical transmittance	combusts aqueous sample and measures mass loss at different temperatures	quantitative, can be used in presence of DOM	uncommon equipment, aqueous phase only	29
light microscopy	visually identifies large CNP aggregates	readily available in many environmental laboratories	qualitative, nonspecific, only works for very large aggregates	31,71,143,144,169
transmission electron microscopy (TEM)	investigates absorption or scattering of an electron beam through a sample	high resolution, can “fingerprint” CNPs using electron energy loss spectroscopy (EELS)	aqueous samples typically require drying, identification with EELS requires uncommon expertise, challenging sample preparation for tissues, may be challenging to locate CNTs in samples	140,143,178
UV/vis absorbance	measures absorbance of aqueous sample at different wavelengths	readily available in many environmental laboratories	potential interference from other sample components	24,25
coherent anti-Stokes Raman scattering	achieves contrast from different vibrational frequencies within a sample	identifies nanoparticles in tissues without additional sample processing	not specific to particle type, uncommon equipment	39
chemothermal oxidation at 375 °C (CTO-375)	removes organic and inorganic carbon to isolate black carbons and then tests C concentration	quantitative measurement of nanotube concentrations in soils	low accuracy, only very high CNT concentrations were tested	42
two-photon excitation spectroscopy	excites sample with photons and then measures fluorescence	detects individual nanotubes in tissues	equipment is uncommon	145
scanning electron microscopy (SEM)	measures backscattered electrons off of a sample	investigates surface of organisms or cross sections, faster and more readily available than TEM	qualitative, lower sample resolution compared to TEM	72,146
photothermal/photoacoustic imaging	measures conversion of absorbed laser energy to heat or acoustic signals	detects CNT clusters in tissues spatially using a light microscope setup	equipment is uncommon, thin sections required, qualitative	41

<sup>a</sup> Modified version reprinted with permission from ref 22

conditions (e.g., ultraviolet radiation, biological environments, and ozone) that are likely to cause the release of CNTs from the polymer matrices. These release pathways pose a serious concern from material performance and environmental health and safety standpoints.

Potential CNT release mechanisms during the CNT/polymer nanocomposite lifecycle include biodegradation, mechanical (abrasion, scratching, sanding), washing, diffusion, matrix degradation (photo-, thermo-, hydrolytic), and incineration. As such,

some fraction of CNTs incorporated in the polymer matrix will be released to the environment. For example, during use of a CNT/polymer composite framed tennis racket, some CNTs may be released whenever the racket frame is scratched by abrasion on the court, or after disposal in a landfill or by incineration. In a landfill, the matrix may undergo hydrolytic degradation and, to a lesser extent, photodegradation, but release to the environment will likely be limited by the landfill lining. The expected fate of CNTs during incineration will be discussed in the next section.

ENP release from polymer nanocomposites by mechanical mechanisms has been studied for polymer films containing ZnO and Fe<sub>2</sub>O<sub>3</sub><sup>47,48</sup> and TiO<sub>2</sub>,<sup>49</sup> but essentially no work has been conducted on CNT release from polymer nanocomposites by mechanical action. Nanocomposite washing and rain falling onto nanocomposite surfaces are also likely environmental release routes for loosely bound CNTs on the polymer surface, but no studies were found in the literature on this topic. Likewise, CNT diffusion of CNTs through nanocomposites and into the environment is another release mechanism that has received little attention.

Most common polymers are known to undergo some form of degradation when exposed to radiation, moisture, temperature, or to a combination thereof.<sup>50</sup> Loss of the polymer matrix by degradation likely exposes CNTs on the composite surface and potentially releases them into the environment. In addition, matrix degradation will cause chain scissions and cross-linking in the polymer, which results in an increase in brittleness and cracking of the nanocomposites. This will enhance the likelihood of CNT release. For that reason, the release and fate of CNTs by matrix degradation in exposed environments has received some attention. Armentano et al.<sup>51</sup> have reported *in vitro* degradation and fates of CNTs in poly(DL-Lactide-co-glycolide) (PLGA) composites made with unfunctionalized SWCNTs and carboxylated SWCNTs (SWCNTs-COOH). SWCNTs-COOH increased the dimensional stability of the matrix, but did not change its degradation kinetics or mechanism (hydrolysis). For unfunctionalized SWCNTs, the matrix degradation produced large pores and SWCNT bundles on the composite surface. However, the presence of SWCNTs-COOH accelerated the hydrolytic degradation of the matrix and mass loss of the nanocomposite, which resulted in release of SWCNTs into the medium. Using magnetic resonance imaging (MRI), van der Zande et al.<sup>52</sup> observed the release of CNTs resulting from hydrolysis of (PLGA)/gadolinium-doped SWCNTs composite scaffold during *in vivo* experiments. Although these two studies clearly showed evidence of SWCNT release into the biological environment, they lack quantitative data to estimate the release rate and to support release modeling. Both release mechanism and mathematical models that sufficiently account for release controlling factors (e.g., pH, temperature, water diffusion in polymer, etc.) are sorely needed for assessing long-term release of CNTs from biodegradable nanocomposites.

Among the mechanisms through which natural conditions can impact polymers, exposure to ultraviolet (UV) light is known as the most detrimental.<sup>50</sup> Under solar radiation, most polymers undergo photo-oxidation, which is responsible for polymer chain scission, material loss, and reduced mechanical properties. Effects of UV irradiation on the fate of CNTs in polymer composites have been investigated by Nguyen and his co-workers.<sup>53–55</sup> These researchers exposed epoxy composites containing a 0.72% MWCNTs (by mass) to a UV radiation source having an intensity of 480 W/m<sup>2</sup> in the 295–400 nm wavelength for a period of up to 9 months. Although the intensity of the UV source used in these studies is about 20 times that of the sun, the wavelength range is similar to that of the sun's spectrum at the ground level. Therefore, except for the accelerating effect, nanocomposite degradation behavior is expected to be similar for polymer/CNT composites used outdoors. Surface morphology, chemical degradation, and mass loss were measured with exposure time. To provide data for release of CNTs during

exposure, they used a special holder to collect and analyze particles falling off the sample surface with exposure time. The epoxy matrix of the CNT composite was found to readily undergo photodegradation, although at a lesser rate than the unfilled polymer. Photodegradation resulted in the gradual accumulation of a dense, entangled CNT layer on the composite surface (SI Figure S1), but no evidence of CNT release after a 9-month exposure was observed. Similar entanglement networks of MWCNTs and SWCNTs have been observed for burned residues of polymer CNT composites.<sup>56</sup> The gradual accumulation of a large amount of CNTs at the composite surface during UV exposure is similar to that observed for polyurethane graphene oxide (GO)<sup>57</sup> and epoxy nanosilica<sup>55</sup> composites exposed to the same UV conditions. The thickness of the CNT layer on the composite surface was about 350 nm ± 50 nm after UV irradiation for 45 days, and remained essentially unchanged between 45 day and 9 month exposure periods, indicating that the CNTs on the sample surface effectively screened the UV radiation and reduced degradation of the polymer layer underneath. Such entangled structures of CNTs on the surface were probably responsible for minimizing/preventing their release to the environment. This is in contrast to the same epoxy composite containing nanosilica, where spherical SiO<sub>2</sub> nanoparticles were clearly released to the environment.<sup>55</sup> Based on spectroscopic and microscopic evidence, Nguyen et al.<sup>43</sup> proposed a conceptual model for the accumulation of a large amount of entangled MWCNTs on polymer/MWCNT composites surface during their exposure to UV radiation. Based on this model, the matrix layer on and near the nanocomposite surface was photodegraded and removed by the UV radiation. The matrix removal resulted in an accumulation of a dense, entangled layer of MWCNTs on the nanocomposite surface, which showed little evidence of release to the environment and protected the epoxy polymer layer underneath from further damage by UV radiation. This model should be applicable to all polymer/CNT composites containing high aspect ratio, fiber-like nanoparticles exposed to outdoor environments, because most polymers used for composites are susceptible to UV radiation. Although MWCNTs were not observed to be released in this study, natural events such as rain, wind, and snow may enhance their likelihood of release. The presence of a CNT layer on the surface of UV-exposed nanocomposites may also pose potential risk for workers that come into contact with these surfaces. Therefore, appropriate measures should be taken to minimize the potential harm this problem may cause, for example, using UV-stabilized paint to cover the CNT composite surface or incorporating effective UV stabilizers to retard degradation of the polymer surface.

Although these studies provided credible data about the fate of MWCNTs in epoxy composites exposed to UV radiation and such data is needed for effective risk assessment, there are many unanswered questions. For example, what is the rate of MWCNT accumulation on the composite surface? Is this rate the same for all polymers used in composites given that photodegradation is polymer dependent? Also, how is this rate affected by other natural elements, such as relative humidity, temperature, and ozone? Further research that includes these environmental factors will help to provide an accurate risk assessment of CNTs in polymer composites used outdoors. Another shortcoming of these studies is the lack of quantitative data and models for CNT accumulation on composite surfaces. In order to determine and



predict the rate of CNT accumulation, a method needs to be developed to quantify the MWCNTs masses formed on the nanocomposite surface during UV irradiation.

**Release Due to Fire.** There are several ongoing research efforts directed at elucidating the potential hazards associated with ENP release from nanocomposites when they are burned either in the process of incineration or in accidental fires. Much of this work has focused on the release of carbon nanofibers (CNFs) rather than CNTs. While CNFs are similar to CNTs, they are typically larger (having diameters ranging from about 50 to 150 nm and can be up to 100  $\mu\text{m}$  in length) and they are comprised of multiple nested cones (as opposed to continuous tube(s)) such that their surfaces exhibit steps where the tube walls have been terminated. Advantages of using CNFs are that they are cheaper and easier to disperse in polymers than CNTs, and that CNF release can be monitored by optical microscopy, rather than only by electron or atomic force microscopy. Although the difference in size may affect dispersion and transport properties, the expectation is that CNFs act similar to large CNTs with respect to their release in fires.

To assess ENP release during burning, specimens of polyurethane foams (PUFs) (with and without CNFs) were burned using the procedure specified by NFPA 270 (or equivalently, ISO 5659-2).<sup>58,59</sup> Particulate emissions and nonvolatilized particulates (char) were collected, suspended in deionized water by sonication, and analyzed using optical microscopy. While CNFs were visible in the char, no CNFs could be identified in the particle emissions (see SI Figure S2). Additional experiments were performed on PUF foams that were coated with CNFs using the layer-by-layer assembly process.<sup>60</sup> Specimens were burned in a cone calorimeter,<sup>61</sup> and particulates in the smoke collected in the exhaust port. Micrographs obtained using scanning tunneling microscopy (at magnifications between 10 000 and 100 000) also failed to reveal the presence of any CNFs in the fire smoke. These observations suggest that the CNFs, which are present in both the foam and nonvolatilized char, are either destroyed in the flames or entrapped in the CNF network that comprises the char.

Release of MWCNTs during combustion in an oven was observed by conducting transmission electron microscopy (TEM) on aerosolized emissions from nanocomposite waste material.<sup>62</sup> However, the oven temperature (less than 1000 °C) was lower than those used in the CNF experiments, which were greater than 1200 °C. Thus, the CNTs/CNFs present in nanocomposite materials that have been disposed of as municipal waste would most likely not be aerosolized during incineration because incineration facilities are designed to ensure that off-gases and aerosolized particulates have long residence times at high temperatures ((1000 to 1100) °C). However, incinerator ash may contain high ENP concentrations.

The possibility that ENPs might be released during mechanical agitation of burnt char was also investigated.<sup>63</sup> After agitating chars from CNF-containing PUF using a mechanical vibrator, the particle count measurements obtained by sampling the air above the char (using a condensation particle counter) revealed that the maximum peak count was an order of magnitude larger than the background signal, suggesting that significant amounts of submicrometer particles were released. Spectroscopic measurements of the chars indicated that they consisted of only about a 50% mass fraction of CNFs. Thus, it was speculated that the observed particle counts are probably CNF bundles partially encapsulated by a thin layer of charred PUF. Additional research

in this area should include a comprehensive characterization of the size and surface area distributions of the nanoparticles released from these chars. Another important topic is to compare the combustion particulate emissions and chars of composites with different types of SWCNTs and MWCNTs to those containing CNFs. Such information would provide guidance in the development of test methods and standards for the assessment of exposure hazards after nanocomposite exposure to fire.

**Removal in Wastewater Treatment Plants.** There is a general lack of knowledge regarding the fate and transport of any ENP within engineered systems, including wastewater treatment facilities. This deficiency, however, appears to be more prominent for CNTs than for other types of ENPs. Phase distribution measurements involving activated sludge have been made for nanoparticles synthesized from silver, copper, fullerenes, and titanium, cerium, and silicon oxides<sup>64–69</sup> but not, to our knowledge, with any type of CNT. Although the relevant literature is quite limited, a thorough review of what has been reported with respect to CNTs and activated sludge systems may prove useful in helping to develop future research plans.

Unlike the natural environment, wastewater treatment facilities have only one method of receiving contaminants, by accepting influent wastewater. The Nowack group has published an interesting and helpful series of environmental modeling papers<sup>9–11</sup> that may be useful in understanding how CNTs may originate at the wastewater treatment facility. As most CNTs in consumer products are either bound to polymers or incorporated into sealed devices<sup>11</sup> and therefore more likely to be disposed of in a landfill or by incineration, the fraction of CNTs that do reach a wastewater treatment facility are thought to be comparatively small.<sup>9</sup> It is currently believed that the majority of CNTs released into a sewer system will have originated from the tailoring, finishing, use, and degradation of CNT-containing textiles<sup>70</sup> or in their use during research and development.<sup>10</sup> These authors, however, readily admit that their current predictions may no longer be valid should, for example, new CNT applications be developed that would change the assumptions behind their current CNT mass flow calculations. For example, the authors have recently incorporated CNT removal at wastewater treatment facilities into their environmental exposure models,<sup>9,10</sup> a process not included in their original simulations.<sup>11</sup> Such maturation in environmental model development highlights the need for future field measurements and validation assessments to provide a more complete and detailed description of the efficiency of removal systems.

Once CNTs reach wastewater treatment facilities, there are two main issues that need to be addressed: (1) how well will the CNTs be removed from the aqueous phase (CNT removal efficiency)? and (2) are influent CNTs toxic to the diverse bacterial community responsible for both pollutant and contaminant removal? Neither CNT removal efficiency nor cytotoxicity applied to, for example, an activated sludge microbial community, is well documented. In the environmental modeling simulations, Gottschalk et al.<sup>9,10</sup> assumed CNT removal efficiencies in wastewater treatment facilities of 96.3% and 99.7% based on natural organic matter (NOM)-induced CNT stabilization data provided by Hyung<sup>29</sup> and Kennedy,<sup>71</sup> respectively. Using a simulated coagulation-flocculation-sedimentation process, Holbrook et al.<sup>72</sup> reported average MWCNT removals ranging between 10% and 85%, and which were dependent upon influent CNT concentrations as well as coagulant type and dosage. And although Kiser et al.<sup>66</sup> did not specifically use CNTs, their suite

of ENPs (fullerenes, fullerols, silver, titanium dioxide, and silver dioxide nanoparticles) cover at least a portion of the expected range of CNT surface charge and functional group compositions. They concluded that surface chemistry will play a major role in ENP removal, with nonfunctionalized ENPs being more effectively removed than functionalized ENPs. Interestingly, Kiser et al.<sup>66</sup> achieved ENP removal efficiencies approaching those used in Gottschalk et al.<sup>9,10</sup> for only silver nanoparticles, an observation that may be caused by the comparatively low biosolid sorbent concentrations. The two key parameters of improving confidence in ENP environmental exposure modeling results are a better understanding of the quantities of raw sewage that bypass treatment facilities (for example, during storm events) and ENP removal efficiencies in wastewater treatment facilities.<sup>10</sup> Similar to other pollutants and contaminants, wastewater treatment facilities are expected to play a prominent role in protecting receiving streams from (and hence limiting environmental exposure to) ENPs.

CNT-induced cytotoxicity to the diverse bacterial community found at wastewater treatment facilities has only been recently reported. Using monocultures and rotating biological contactor effluent, Kang et al.<sup>73</sup> determined that both MWCNT- and SWCNT-impregnated filters resulted in cell inactivation, which, in turn, was contingent upon cell-to-filter contact and exposure time. Yin et al.<sup>74</sup> reported that SWCNT surface charge, soluble chemical oxygen demand removal, solids concentration, and sludge volume index were affected in a batch activated sludge system upon SWCNT addition. Luongo and Zhang<sup>75</sup> demonstrated a dose-dependent relationship between MWCNT concentration and respiration inhibition, with sheared mixed liquor demonstrating a greater degree of inhibition compared to unshredded mixed liquor. These authors suggest that the extracellular polymeric substances (EPS) associated with biological floc offer protection from the CNTs. Using the same experimental conditions as Yin et al.,<sup>74</sup> Goyal et al.<sup>76</sup> concluded that bacterial communities were impacted differently following SWCNT dosing compared to SWCNT-associated impurities, such as amorphous carbon and residual metal catalysts. Lastly, the ability of ciliated protozoa to ingest and digest bacteria, an important mechanism in activated sludge processes for producing a robust biological system, was negatively impacted by high concentrations of SWCNTs.<sup>77</sup>

Although realistic environmental exposure concentrations were not used in any of the above works (dosed CNT concentrations range from the low mg/L to low g/L levels; expected influent CNT concentrations range from high ng/L to low  $\mu\text{g/L}$ ), there are at least two consistent observations. First, physical contact between CNTs and bacteria appear to be required for cytotoxicity to occur.<sup>73,75</sup> This suggests that CNT-induced cytotoxicity may be limited to bacteria located on the periphery of the biological floc; organisms found internally or protected by a layer of EPS may therefore be somewhat protected from CNTs. Second, the presence and type of EPS may strongly influence the removal efficiency and impact of ENPs on an activated sludge system.<sup>66,75</sup> EPS content is a function of organic loading and sludge retention time and therefore will certainly vary widely among different facilities. One important component of future research on CNT removal efficiency and cytotoxicity in activated sludge systems is to thoroughly measure and characterize biological floc properties, such as the extrapolymeric substances composition, microbial diversity and activity, and floc architecture. These properties will likely profoundly influence observed

CNT behaviors and effects, and are thus necessary to enable comparisons among studies.

## ■ ENVIRONMENTAL TRANSPORT

Once CNTs are released into the environment through any of the release pathways described above, their mobility and colloidal stability are expected to control their bioavailability and impact on the environment. In recent years, several studies have been conducted to investigate the transport and retention of CNTs in porous media in order to better understand their mobility in subsurface environments. In addition, the influence of solution chemistry on the colloidal stability of CNTs has been examined by monitoring the aggregation behavior of CNTs. The effects of NOM on the transport and colloidal stability of CNTs and their interactions with soils and sediments have also been investigated. The following sections will critically review the relevant literature on such topics and highlight future research directions.

**Mobility in the Subsurface Environment.** Particle migration through porous media, typically with all dimensions much larger than 100 nm, has been extensively studied in the filtration literature, and can be described mathematically using liquid and solid phase mass balance equations:<sup>78</sup>

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{n} \frac{\partial S}{\partial t} + v \frac{\partial C}{\partial x} - v\alpha_1 \frac{\partial^2 C}{\partial x^2} = 0$$

$$\frac{\rho_b}{n} \frac{\partial S}{\partial t} - kC + \frac{\rho_b k_{\text{det}}}{n} S = 0$$

where  $C$  is the concentration of particles in the aqueous phase,  $t$  is time,  $\rho_b$  is bulk density of the solid phase,  $n$  is porosity,  $S$  is the mass of particles attached to the solid phase,  $v$  is the pore water velocity,  $x$  is the spatial dimension,  $k_{\text{det}}$  is the detachment rate constant,  $\alpha_1$  is the longitudinal dispersivity and  $k$  is the deposition rate constant. These equations are typically used as a starting point for the prediction of nanoparticle, and specifically to this review, CNT transport in porous media.

While deposition of all manufactured nanoparticles on porous media was recently reviewed,<sup>12</sup> this section focuses solely on the limited number of laboratory studies related to CNTs.<sup>79–86</sup> Nevertheless, some of the general trends identified in that review may also be relevant here, namely the substantial variability among ENP deposition studies for a single ENP and limitations related to experimental methods. As discussed below, there are significant differences among the types of CNTs studied, including the stabilization technique, and the experimental conditions tested, factors that hinder direct comparisons among studies and help explain the varying results observed.

The studies of Lecoanet et al.<sup>81</sup> and Tian et al.<sup>84</sup> found that dodecylbenzene sulfonic acid, sodium salt (SDBS) stabilized SWCNTs were quite mobile in porous media systems. Lecoanet et al.<sup>81</sup> reported mobility indices (i.e., the distance required to achieve a decrease in the CNT concentration to 0.1% of the initial concentration) of 10 m through porous media comprising silica glass beads. These findings suggest that SWCNTs specifically modified to enhance hydrophilicity can be very mobile in porous media systems. In a subsequent study by Lecoanet and Wiesner,<sup>82</sup> it was observed that pore water velocity significantly impacted SWCNT transport with breakthrough occurring sooner, when compared to the conservative tracer, at the lower pore velocity investigated (34.6 m/d) in comparison to the higher pore velocity (121.0 m/d). At both velocities SWCNTs reached

steady state effluent concentrations sooner than the conservative tracer (NaCl). They attributed this behavior to pore exclusion, where the SWCNTs only flowed through larger pores. These water velocities are significantly larger than typically found in groundwater aquifers, and additional work is necessary to assess CNT mobility under representative subsurface velocities. Lecoanet and Wiesner<sup>82</sup> also observed that the breakthrough curve dipped at the higher pore water velocity and attributed this behavior to site ripening (i.e., increase in retention with increased deposition of particles<sup>87</sup>). However, no other transport studies using CNTs have suggested that site ripening is important. Tian et al.<sup>86</sup> showed that SDBS coated SWCNTs were highly mobile in both saturated and unsaturated porous media consisting of quartz sand of varying collector grain size. Their findings suggest that SDBS-coated SWCNTs show very little affinity for the porous media, and SWCNT breakthrough can be described and predicted using traditional colloid filtration theory. In contrast to the findings of Lecoanet and Weisner<sup>82</sup> and Tian et al.,<sup>84</sup> Jaisi et al.<sup>80</sup> found that carboxyl functionalized SWCNTs showed a much greater affinity for the porous media used in their study (quartz sand). They reported travel distances 2 orders of magnitude lower than Lecoanet and Weisner.<sup>82</sup> Differences in these findings may be due, in part, to the different stabilization techniques employed (i.e., SDBS coating can produce electrostatic interactions while CNT functionalizing produces electrostatic repulsion only). Jaisi et al.<sup>80</sup> suggested that transport of SWCNTs through quartz sand was in qualitative agreement with traditional Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (i.e., deposition of SWCNTs increased with increasing ionic strength) and that straining (i.e., physical removal of particles between two or more collectors<sup>87</sup>) may be an important removal mechanism at low ionic strengths. Results from columns containing divalent ions ( $\text{Ca}^{2+}$ ) indicated SWCNT deposition occurred in the primary minimum, while SWCNT release from the collector surface following elution with deionized water indicated some SWCNT deposition in the secondary minimum.<sup>80</sup>

In a later study, Jaisi and Elimelech<sup>79</sup> found that SWCNT breakthrough occurred before the bromide tracer and attributed this behavior to size exclusion (large CNTs are unable to enter smaller pores), which is similar to what was reported by Lecoanet et al.<sup>81</sup> Unlike their previous study,<sup>80</sup> Jaisi and Elimelech<sup>79</sup> used a natural soil containing a significant clay fraction (29%) with very small pores (22  $\mu\text{m}$ ). Jaisi and Elimelech<sup>79</sup> suggest that straining was a dominant removal mechanism of SWCNTs in the natural soil column due to high SWCNT removal (80%) at low ionic strengths. Their work indicated that nonspherical nanoparticles may be removed from the aqueous phase due to nontraditional removal mechanisms (e.g., hydrodynamic factors and nonphysiochemical removal mechanisms such as straining).

The only study investigating CNT transport in unsaturated porous media found that retention of SDBS dispersed CNTs was only observed at very low soil moisture contents (<0.10) with very little physical trapping of SWCNTs at higher moisture contents and in the saturated porous media experiments.<sup>86</sup> In addition, CNTs were shown to have no affinity for the air–water interface using bubble experiments. Experiments in this study were conducted at relatively high pore water velocities (i.e., greater than 7 m/d) using a CNT stabilization technique that is known to yield mobile CNT dispersions. Further work is therefore necessary to explore the impact of stabilization technique on CNT transport in unsaturated systems as well as the impact of lower pore water velocities on transport. Different stabilization

techniques may yield dispersions that have different affinities for the air–water interface.

Limited work has been conducted on MWCNT transport.<sup>83,85</sup> Liu et al.<sup>83</sup> found that above a pore velocity of 4 m/d, acid-treated MWCNTs were very mobile in sand packed column experiments. However, significant MWCNT retention was observed at lower pore water velocities (i.e., 0.42 m/d). MWCNTs were more mobile in glass bead packed column experiments conducted at 0.42 m/d. They proposed that the roughness and angularity of quartz sand, in comparison to the glass beads, increased the number of MWCNT deposition sites. Their experiments were complemented by a modeling component that suggested that along with traditional colloid filtration theory removal mechanisms, the inclusion of a site blocking term was necessary to predict observed MWCNT transport behavior. The fitted transport parameters from the Liu et al.<sup>83</sup> study were used to assess the transport of MWCNTs at the field scale in a numerical modeling study.<sup>88</sup> They investigated a number of system parameters, including composition of the porous media, a range of pore velocities, and MWCNT transport properties, and suggested that MWCNTs would be mobile at the field scale under conditions that would be expected in the field. Mobility at the field scale was a strong function of the site blocking term (i.e., porous media has a limited capacity for deposition of CNTs ( $S_{\text{max}}$ )). Very limited work has been completed investigating the factors that control this site blocking term in CNT systems. Given its strong influence on CNT transport, further work is required to determine the factors that control  $S_{\text{max}}$ .

In summary, future research in the area of CNT transport in porous media should incorporate more of the subsurface system's complexities to expand on the limited conditions investigated thus far. For example, studies should include a wider range of porous media grain size, mineralogy (e.g., collectors which are not quartz), aqueous chemistry (including DOM), and natural soils (including clays, silts, and peats). Additionally, how functionalization and surface properties of CNTs and nanotube size (length and diameter) impact their transport are critical areas for future research.

**Interactions with Soils and Sediments.** The association of CNTs with solid phases is one of the most important processes governing the distribution of CNTs between water and soil and sediments, but is not yet well understood. One type of soil organic matter (Canadian peat) has been found to sorb acid-treated MWCNTs with sodium concentrations of 4 or 40 mmol/L, whereas the presence of peat did not increase the removal of CNTs from the solid phase without sodium.<sup>34</sup> The sodium ions screened the surface charge of soil organic material and CNTs, thus facilitating interactions between them. Dissolved organic matter (DOM) released from peat was found to stabilize MWCNTs and dramatically reduce MWCNT settling even when additional cations were added.<sup>34</sup> Removal of MWCNTs from the aqueous phase in the presence of peat was not affected by changing the pH from 4.0 to 8.0, which suggests that the surface charge of peat and DOM-wrapped MWCNTs did not change substantially in this pH range. Overall, these results suggest that in hard water or seawater, MWCNTs would be more readily sorbed by sediments, whereas in aquatic systems with high concentrations of DOM, MWCNTs will tend to stay suspended in the water column. In a separate study with MWCNTs modified with polyethyleneimine (PEI) to give them positive, negative, or neutral surface charges, association of these nanotubes with three soils was tested in a deionized water solution.<sup>35</sup>



The surface coatings made the interaction patterns more non-linear, but there were rarely statistically significant differences in the Freundlich coefficients among the soils. While that study only tested DI water, additional research is needed to investigate the effect of adding cations to the aqueous solution on CNT interactions with soils and also to investigate the extent to which there are differences between SWCNTs and MWCNTs. The fate of CNTs in sediments is another related topic recently investigated by experimental modeling.<sup>89</sup> CNT concentrations in sediments were predicted to be several orders of magnitude lower than those of black carbon nanoparticles mainly as a result of the low source concentrations of CNTs. As the mechanisms underlying interactions between CNTs and soil and sediment particles are further elucidated, this information can be used to inform these types of modeling efforts to provide more accurate predictions for the expected behaviors.

**Aggregation and Colloidal Stability.** The fate and transport of CNTs in aquatic systems are greatly dependent on their colloidal stability.<sup>72,90–92</sup> The aggregation of CNTs will influence their ability to remain suspended in solution and thus their mobility and bioavailability in natural aquatic systems. The aggregation of CNTs will also control their sedimentation rates. A brief discussion on sedimentation experiments conducted using CNTs can be found in Chen et al.<sup>13</sup> Furthermore, the aggregation state of CNTs is expected to impact their cytotoxicity, which has been extensively investigated using bacteria by Kang and co-workers,<sup>93–95</sup> as well as their uptake and bioaccumulation by higher organisms.

Pristine CNTs are known to be highly hydrophobic. One of the most common strategies to enhance the dispersibility of CNTs in aqueous solutions is to covalently attach charged or hydrophilic functional groups on the CNT sidewalls and tube ends. Some methods widely used to modify the surface chemistry of CNTs include high-power ultrasonication and chemical treatment with strong oxidants or acids as is discussed in a subsequent section. Another common strategy is to employ surfactants to enhance the stability of CNTs through either electrostatic or steric (electrosteric) repulsion.

Although the origin of CNT surface charge is still not completely understood, evidence from recent studies has shown that the aggregation behavior of functionalized CNTs is in qualitative agreement with the classic DLVO theory.<sup>96,97</sup> Saleh et al.<sup>98</sup> performed time-resolved dynamic light scattering (DLS) measurements in order to capture the aggregation kinetics of MWCNTs, which had been dispersed in water using an ultrasonication probe. By performing measurements over a wide range of monovalent (NaCl) and divalent (CaCl<sub>2</sub> and MgCl<sub>2</sub>) electrolyte concentrations, the authors obtained inverse stability profiles comprising both the reaction- and diffusion-limited regimes, which implied that the aggregation kinetics of the functionalized MWCNTs were controlled by electrostatic and van der Waals interactions. Saleh et al.<sup>99</sup> also observed that the aggregation kinetics of MWCNTs decreased with increasing solution pH, which they attributed to the dissociation of functional groups on the surface of the nanotubes.

Recently, several systematic studies have been conducted to investigate the influence of surface oxidation on the aggregation kinetics of CNTs. Smith et al.<sup>100</sup> prepared a suite of MWCNTs with varying degrees of surface oxidation and characterized the distribution of oxygen-containing functional groups, specifically the carbonyl, carboxyl, and hydroxyl groups, of the MWCNTs using X-ray photoelectron spectroscopy (XPS) in conjunction

with vapor phase chemical derivatization. By performing time-resolved DLS measurements, the authors found that the critical coagulation concentrations (CCCs) of the oxidized MWCNTs in NaCl solutions were linearly correlated to the degree of MWCNT oxidation. As the degree of surface oxidation of the MWCNTs increases, the NaCl concentration required to completely screen the surface charge of the MWCNTs increases correspondingly. Interestingly, Yi and Chen<sup>101</sup> investigated the aggregation behavior of two MWCNTs with different degrees of surface oxidation in the presence of CaCl<sub>2</sub> electrolytes and their results showed that the colloidal stability of both MWCNTs was very similar over a wide range of the divalent electrolyte concentration. The authors hypothesized that the unexpected similarity in the colloidal stability of the MWCNTs could be related to the difference in the spatial distribution of carboxyl groups on the nanotubes, which may result in calcium having dissimilar affinities to form complexes with the carboxyl groups for both MWCNTs.

Because naturally occurring colloids and nanoparticles are ubiquitous in the environment, it is more likely that CNTs will undergo heteroaggregation than homoaggregation when they are released into natural aquatic systems.<sup>13</sup> Thus, more research is required to better understand the influence of CNT physicochemical properties and those of naturally occurring colloids, as well as solution chemistry, on heteroaggregation kinetics and structures of the heteroaggregates. In a study investigating the interaction between clay minerals (kaolinite and montmorillonite) and surfactant (SDBS, CTAB, and Triton X 100) stabilized MWCNT suspensions, clay minerals reduced the stability of MWCNTs through competitive adsorption of surfactants, thus reducing the surfactants' stabilizing effect, and through the formation of bridges between clay mineral and MWCNTs by surfactants.<sup>102</sup> The degree to which the presence of clay particles will cause MWCNT removal from the aqueous phase depends on surfactant properties and the sorption capability of clay minerals. Additionally, natural organic matter (NOM) is almost always present in aquatic systems and has been shown to influence the colloidal stability of CNTs.<sup>29,71,90–92,98,103,104</sup> For example, the colloidal stability of MWCNTs was observed to strongly correlate with the concentration of tannic acid in the solution,<sup>91</sup> and MWCNTs can be stabilized in natural waters containing high concentrations of DOM.<sup>92</sup> Both humic acid and polysaccharides can result in the enhancement of colloidal stability of SWCNTs, likely due to steric repulsion impacted by the adsorbed macromolecules.<sup>98</sup> Additional research is needed to yield a better understanding of the adsorption of the key components of NOM on CNTs and their subsequent effects on CNT stability.

## ■ TRANSFORMATIONS IN THE ENVIRONMENT

Possible CNT transformations in natural or engineered systems can change the properties of CNTs and consequently affect their mobility and bioavailability. However, CNT transformations under natural conditions have not yet been fully investigated. There are, however, investigations regarding CNT reactions in chemistry and chemical engineering studies, and this information suggests likely routes of CNT transformation in the natural environment. In general, there are two types of transformations that can occur to CNTs: covalent reactions and biodegradation.

**Covalent Reactions.** The graphene structure of CNTs, although inert in general, is still favorable to covalent addition



to some extent. The fullerene-like end-caps and the defects on sidewalls are expected to be sites susceptible to oxidation.<sup>105,106</sup> Curvature-induced pyramidalization is the main cause of fullerene end-cap reactivity.<sup>105,107,108</sup> In general, smaller diameters are associated with larger curvatures and consequently more reactivity. Pyramidalization of CNT sidewalls are analogous to, but much weaker than, that of fullerenes, while the misalignment of the pi-orbitals between adjacent pairs of conjugated carbon atoms would also contribute to CNT sidewall reactivity.<sup>109</sup> However, the sidewall structure of CNTs is still relatively inert to oxidation, except for the defect sites such as pentagon-heptagon pairs called Stone-Wales defects,  $sp^3$ -hybridized defects, and vacancies in the nanotube lattice.<sup>110</sup> A synchronous or consecutive attack on unsaturated bonds of  $C=C$  on the sidewalls by electrophilic addition is another mechanism of CNT surface reactions.<sup>109</sup>

Chemical oxidation of CNTs requires strong oxidative forces, which are unlikely to frequently occur naturally in the environment, but photooxidation is possible. When exposed to the sunlight or to lamps that emit light only within the solar spectrum, carboxylated SWCNT solution produced reactive oxygen species (ROS) such as  $^1O_2$ ,  $O_2^{\cdot-}$ , and  $^{\cdot}OH$ ,<sup>111</sup> and these radicals can oxidize CNTs and modify their surfaces. Some oxidants commonly used in wastewater such as ozone,<sup>112,113</sup> Fenton's reagents,<sup>114–116</sup> and Photophenton reagents<sup>117</sup> may impact nanotubes released into the environment through this pathway. Ultrasonication<sup>118,119</sup> is also known to oxidize and shorten CNTs in the process of producing CNT dispersions, and this possibility should be considered when this dispersion process is used. Common results of these oxidative treatments are to open the end-cap and introduce oxygen-containing surface functional groups, such as carboxyl, hydroxyl, carbonyl, and ester groups, on either the ends or the sidewalls of CNTs,<sup>106,120–123</sup> an outcome similar to the degradation of black carbon.<sup>124</sup> Oxidative treatments of fullerene-like caps and graphene layers may also generate oxidized polycyclic aromatic substances, which are like fulvic acids, and which remained adsorbed on MWCNT surface in acidic and neutral solutions.<sup>121</sup> These adsorbed organic materials helped stabilize CNTs in water.

**Biodegradation.** One useful comparison for the biodegradation of CNTs in the environment is black carbons, which usually contains similar structures to those of CNTs and are also chemically inert.<sup>125,126</sup> The degradation efficiency or half-life of black carbon varies significantly according to different soil types, aeration, and black carbon composition.<sup>125,127,128</sup> For example, in well-aerated tropical soil environments, the degradation half-life of oxidation-resistant elemental carbon can be less than 50 years.<sup>128</sup> In contrast, the turnover time in a Russian steppe soil ranges from 182 to 541 years.<sup>125</sup> However, black carbon has been identified in sediments that was 65 million year old.<sup>127</sup> It remains unclear whether black carbon is oxidized completely to  $CO_2$ , reduced to a sufficiently small particle size, or changed into a chemical form which is more susceptible to oxidation attacks. Nevertheless, black carbon mineralization has been shown.<sup>127,129</sup>

Recent studies indicate that carboxylated SWCNTs can be transformed via mediation by typical soil enzymes, such as horseradish peroxidase, but pristine SWCNTs were not impacted by these enzymes.<sup>130,131</sup> During such reactions, SWCNT lengths were shortened, carboxyl groups were added to SWCNT surfaces, and  $CO_2$  was produced.<sup>131</sup> Recently, the same research group has found that the neutrophil myeloperoxidase, a peroxidase generated inside human cells, can degrade SWCNTs and

the resulting nanotubes do not generate an inflammatory response when aspirated into mice lungs.<sup>132</sup> Degradation of SWCNTs was also found with a phagolysosomal stimulant, a membrane-enclosed organelle which forms when a phagosome fuses with a lysosome.<sup>133</sup> This phagolysosomal stimulant degraded carboxylated SWCNTs in a 90 day period, leading to length reduction and accumulation of ultrafine solid carbonaceous debris. Unmodified, ozone-treated, and aryl-sulfonated nanotubes did not show degradation under the same conditions.<sup>133</sup> Fullerenes, another carbon nanoparticle that has a similar structure to CNTs but is more reactive, were also enzymatically degraded. Using  $^{13}C$ -labeling, Schreiner et al.<sup>134</sup> found fullerol, the hydroxylated derivative of  $C_{60}$ , can be mineralized to  $CO_2$  in the presence of white rot fungi after 32 weeks of decay, indicating another possible organism that may degrade CNTs.

These results indicate the ability of naturally occurring enzymes or organelles to degrade SWCNTs thereby diminishing their potential environmental risks if the nanotubes are exposed to such enzymes in the natural environment. However, quantitative data is required to estimate to what extent the SWCNT can be degraded by various enzymes during specific time intervals under various environmentally relevant conditions. Moreover, the impacts of these enzymes on MWCNTs have not yet been investigated.

## ■ ECOTOXICITY OF CARBON NANOTUBES

After CNT release and distribution within various environmental compartments, organisms will be exposed and unpredictable effects may occur. These unique properties of ENPs have challenged common assumptions in ecotoxicology and led to the emergence of the subdiscipline “nanoecotoxicology”.<sup>135</sup> While some studies have investigated the potential impact of CNTs on the bioavailability of cocontaminants,<sup>30,136,137</sup> this review will focus on the risks posed by the CNTs themselves. Additionally, there is extensive research on the possible impacts of CNTs to humans such as through inhalation,<sup>138,139</sup> but this review will focus on the potential ecological risks.

**Carbon Nanotube Uptake, Elimination, And Biodistribution Behaviors.** The ecotoxicity of CNTs has been explored in recent years using organisms that inhabit terrestrial, sediment, or aquatic habitats. While toxic effects have been observed, there does not appear to be appreciable absorption of CNTs across epithelial membranes as summarized in Table 2.<sup>30–32,37–39,140</sup> Despite MWCNT surface modification to enhance their bioavailability by altering octanol–water distribution behaviors, increased bioaccumulation was not observed for modified MWCNTs in the oligochaete *Lumbriculus variegatus* or earthworm *Eisenia fetida*.<sup>32</sup> For soil and sediment dwelling organisms, sorption of CNTs to soil particles could impede absorption in organisms, but a similar lack of uptake was found in aquatic organisms. A study with aquatic organism *Daphnia magna* found a large mass of MWCNTs in the gut,<sup>31</sup> but TEM examination provided no evidence of absorption of the MWCNTs by *D. magna* in a separate study,<sup>140</sup> a result similar to the lack of absorption previously observed for *D. magna* and *L. variegatus* exposed to fullerenes.<sup>141,142</sup> Fruit flies (*Drosophila melanogaster*) exposed to SWCNT-spiked food (10 mg/kg) did not have appreciable absorption into tissues outside of the GI tract (only  $10^{-8}$  of the total dose).<sup>40</sup> While CNTs have been detected in the intestine by microscopy,<sup>71,143,144</sup> absorption of CNTs across epithelial membranes appears to be negligible in all

Table 2. Summary of Studies of Uptake, Elimination, And Biodistribution of CNTs in Ecological Receptors

reference	type of CNT	detection method	organism(s) tested	findings
Cañas et al. 2008 <sup>146</sup>	SWCNTs	scanning electron microscopy	<i>Cucumis sativus</i> (cucumber)	after exposure for 48 h, nanotubes were found on the root surfaces but absorption into the roots was not observed
Edgington et al. 2010 <sup>140</sup>	MWCNTs	transmission electron microscopy	<i>Daphnia magna</i>	MWCNTs were observed in the gut of <i>D. magna</i> but nanotube uptake into the microvilli was not observed
Ferguson et al. 2008 <sup>30</sup>	SWCNTs	radioactive carbon nanotubes	estuarine invertebrates <i>Amphiascus tenuiremis</i> and <i>Streblospio benedicti</i>	SWCNTs were not detected in the organism after a depuration period the same SWCNT concentration was measured in the organism pellets as in the sediment indicating that CNT uptake and elimination had occurred
Galloway et al. 2010 <sup>39</sup>	SWCNTs	coherent anti-Stokes Raman scattering microscopy and transmission electron microscopy	polychaete worm <i>Arenicola marina</i> (lugworm)	both techniques indicated that SWCNTs did not cross the epithelial membrane in significant quantities SWCNTs were eliminated from the gut lumen with sediment during starvation
Ghafari et al. 2008 <sup>77</sup>	SWCNTs	phase-contrast microscopy	protozoan <i>Tetrahymena thermophila</i>	SWCNT internalization and elimination were observed
Kennedy et al. 2008 <sup>71</sup>	MWCNTs	light microscopy	<i>Ceriodaphnia dubia</i>	MWCNTs were observed in the guts and algae was necessary for elimination
Khodakovskaya et al. 2009 <sup>148</sup>	MWCNTs	Raman spectroscopy and transmission electron microscopy	tomatoes	uptake of MWCNTs into tomato plant seeds was observed, but they were not detectable in grown plant tissues (roots, leaves, or stems) using Raman spectroscopy
Khodakovskaya et al. 2011 <sup>41</sup>	MWCNTs	photoacoustic and photothermal methods and Raman spectroscopy	tomatoes	uptake into the plant roots, leaves, and fruits was observed for MWCNTs added to growth medium uptake into the various tissues including fruits was also observed when MWCNTs were added to a commercial soil mix
Leeuw et al. 2007 <sup>40</sup>	SWCNTs	near-infrared fluorescence	<i>Drosophila melanogaster</i> (fruit flies)	SWCNT biodistribution was observed, but the fraction of the ingested nanotubes absorbed was $10^{-8}$ with the vast majority of the nanotubes being excreted
Lin et al. 2009 <sup>179</sup>	MWCNTs	light and scanning electron microscopy	<i>Oryza sativa</i> (rice)	MWCNTs were observed in the vascular system but very rarely in the plant tissues
Liu et al. 2009 <sup>180</sup>	MWCNTs	light and scanning electron microscopy	<i>Drosophila melanogaster</i> (fruit flies)	MWCNTs spiked to food were ingested by larvae and visible in the larvae and developing flies

Table 2. Continued

reference	type of CNT	detection method	organism(s) tested	findings
Mouchet et al. 2008 <sup>143</sup>	MWCNTs	Raman spectroscopy, transmission electron microscopy, and light microscopy	amphibian <i>Xenopus laevis</i> larvae	MWCNTs were identified in the gut lumen
Mouchet et al. 2010 <sup>172</sup>	MWCNTs	Raman spectroscopy, transmission electron microscopy, and light microscopy	amphibian <i>Xenopus laevis</i> larvae	it was challenging to identify MWCNTs in the tissues using TEM, and Raman spectroscopy was suggested as the more realistic technique Raman analysis identified MWCNTs and DWCNTs in the gut lumen but not in the intestinal cells MWCNTs were also not detected in the blood or liver using Raman spectroscopy
Mouchet et al. 2011 <sup>171</sup>	MWCNTs	Raman spectroscopy	amphibian <i>Xenopus laevis</i> larvae	MWCNTs were detected in the lumen but not in the blood and did not pass across the intestinal barrier
Petersen et al. 2008 <sup>37</sup>	SWCNTs and MWCNTs	radioactive carbon nanotubes	earthworm <i>Eisenia fetida</i>	SWCNT and MWCNT masses in the organisms during a 28 day exposure period were always less than an estimated value for a nonbioaccumulating chemical nanotubes detected in the organism were indicated to be from soil not yet purged from the organism guts a steady elimination process was not observed and instead nanotube concentrations varied randomly during a 7 day elimination period
Petersen et al. 2008 <sup>38</sup>	SWCNTs and MWCNTs	radioactive carbon nanotubes	oligochaete <i>Lumbriculus variegatus</i>	CNT uptake and elimination behaviors indicated that nanotubes measured in the organisms were from sediment not yet purged from the organism guts complete elimination from the worms was observed after depuration for 2 days in clean sediment but elimination was slower for organisms in only water
Petersen et al. 2009 <sup>31</sup>	MWCNTs	radioactive carbon nanotubes	<i>Daphnia magna</i>	MWCNT concentrations in the organisms reached a maximum of 6.4% of the organism dry weight elimination was not observed in clean water or filtered lake water which contained natural organic matter feeding with algae was necessary for elimination changing the apparent octanol–water distribution behavior did not impact the uptake or elimination behaviors of MWCNTs
Petersen et al. 2010 <sup>32</sup>	MWCNTs	radioactive carbon nanotubes	<i>Eisenia fetida</i> and <i>Lumbriculus variegatus</i>	surface coatings giving the MWCNTs different surface charges did not appear to change their accumulation or elimination rates
Petersen et al. 2011 <sup>33</sup>	MWCNTs	radioactive carbon nanotubes	<i>Daphnia magna</i>	



Table 2. Continued

reference	type of CNT	detection method	organism(s) tested	findings
Petersen et al. 2011 <sup>35</sup>	MWCNTs	radioactive carbon nanotubes	<i>Eisenia fetida</i>	algae was necessary for MWCNT elimination surface coatings giving the MWCNTs different surface charges did not appear to change their accumulation behaviors MWCNTs elimination during a 9 day period could be modeled using an exponential decay model
Roberts et al. 2007 <sup>144</sup>	SWCNTs	Raman spectroscopy	<i>Daphnia magna</i>	SWCNTs were identified in the organisms and in black precipitates egested from the organisms
Tan et al. 2009 <sup>147</sup>	MWCNTs	transmission electron microscopy	rice cell line <i>Oriza sativa</i> L. (CS928)	MWCNTs were associated with the cell wall but intracellular uptake was not observed
Templeton et al. 2006 <sup>169</sup>	SWCNTs	confocal laser scanning microscopy	Estuarine copepod <i>Amphiscus tenuiremis</i>	SWCNT clusters were identified in the organism and in the feces
Wild and Jones 2009 <sup>145</sup>	MWCNTs	two-photon excitation microscopy	wheat	MWCNTs were associated with and pierced root cells but complete internalization was not observed

organisms examined. CNTs are generally readily eliminated by soil and sediment organisms,<sup>30,32,35,38,39</sup> but a food source is often necessary for gut clearance by aquatic organisms.<sup>31,33,71</sup>

Investigations with terrestrial plants exposed to CNTs have been conducted. MWCNTs pierced root cell walls of wheat plants but were not observed to completely enter the cells.<sup>145</sup> Likewise, SWCNTs were not observed in root cells of plants examined by scanning electron microscopy (SEM),<sup>146</sup> or in suspended rice cells exposed to MWCNTs and examined with TEM.<sup>147</sup> Raman spectroscopy showed uptake of MWCNTs into tomato plant seeds, but MWCNTs were not subsequently detected in mature plant tissues (roots, leaves, or stems).<sup>148</sup> Similarly, NOM-coated MWCNTs were microscopically observed within the vascular system of rice plants, but they were almost never observed in the plant tissues.<sup>149</sup> Conversely, photoacoustic and photothermal methods recently showed absorption of MWCNTs into tomato plant leaves and crops.<sup>41</sup> Thus, plant uptake may have been limited in previous studies by the analytical techniques available, or there may be variations in uptake among the plant species or for different types of CNTs.

**Ecotoxicological Effects in Soils and Sediments.** Without absorption of CNTs across epithelial membranes, toxic effects observed can only be attributed to effects exerted at surfaces of organisms. Minimal ecotoxicological effects have typically been observed in exposures in soils and sediments (see Table 3 for a summary). High concentrations of MWCNTs (0.3 g/kg) and SWCNTs (0.03 g/kg) spiked to sediments did not decrease the number of *L. variegatus* after 28 days compared to control sediments.<sup>38</sup> Similarly, SWCNT-spiked sediment (0.03 g/kg) did not impact lugworm burrowing behavior, feeding rates, or DNA damage as measured by the comet assay.<sup>39</sup> However, extremely high MWCNT concentrations of 300 g/kg and 30 g/kg decreased survival for *Hyallela azteca* and *Leptocheirus plumulosus*, respectively.<sup>150</sup> Reproduction of earthworms (*Eisenia veneta*) was decreased at a food concentration above 37 mg double-walled CNT (DWCNT)/kg food, while survival and hatchability were not impacted at concentrations up to 495 mg DWCNT/kg food.<sup>151</sup> Likewise no effects were observed on the earthworm lipid content or dry mass after exposure to concentrations up to 3 g/kg for SWCNTs and MWCNTs in two soils.<sup>136</sup> Comparison of the LC50 (i.e., concentration predicted to be lethal to 50% of the organisms) values for MWCNTs, activated carbon, and carbon black spiked to sediments for amphipods *L. plumulosus* and *H. azteca* indicated that MWCNTs had the lowest toxicity.<sup>71</sup> Lower toxicity of MWCNT than activated carbon, an amendment that is being widely considered for treatment of contaminated sediments,<sup>152–154</sup> indicates that toxicity of MWCNTs in sediments may be of minimal concern. Moreover, MWCNTs added to sediments at concentrations up to 2 g/kg did not impact the biodiversity of a benthic macroinvertebrate community and actually lead to a significantly higher number of organisms.<sup>155</sup> Similarly, MWCNTs in soil significantly lowered microbial biomass carbon and nitrogen and lowered enzymatic activity at 5 g/kg, but a soil concentration of 0.5 g/kg typically did not cause statistically significant effects.<sup>156</sup>

Research on effects of CNT exposure in plants conducted under hydroponic conditions (i.e., without soil) has generated inconsistent results (see recent review articles for summaries of all nanoparticle studies with plants<sup>157,158</sup>). MWCNT treatment did not impact plant germination for any of the plant species tested at an MWCNT solution concentration of 2 g/L.<sup>159</sup> Other studies showed decreases in root elongation for some plant

Table 3. Summary of Studies on Ecotoxicity of CNTs in Soils and Sediments

reference <sup>a</sup>	type of CNTs	organism(s) tested	end points and duration	findings
Chung et al. 2011 <sup>156</sup>	MWCNTs	soil microorganism communities	microbial biomass (20 days), activities of extracellular enzymes (30 min, 1 day, 4 days, 11 days)	concentrations of 5 mg/g impacted all end points, but significant effects were rarely observed at concentrations of 0.5 mg/g and lower
Galloway et al. 2010 <sup>39</sup>	SWCNTs	polychaete worm <i>Arenicola marina</i> (lugworm)	mortality, DNA damage, cytotoxicity of coelomic cells, feeding behavior (10 days for all end points)	no impact on any end point at SWCNT sediment concentrations up to 0.03 mg/g
Kennedy et al. 2008 <sup>71</sup>	MWCNTs	amphipod <i>Leptocheirus plumulosus</i>	mortality (10 days)	increased mortality not observed until a concentration of 99 mg/g sediment the LCS0 value was 68 mg/g, which was significant greater than those for activated carbon and black carbon increased mortality not observed until a concentration of 264 mg/g sediment LCS0 value was >264 mg/g sediment LCS0 value was significantly greater than those for activated carbon and black carbon
Kennedy et al. 2009 <sup>150</sup>	MWCNTs	amphipod <i>Hyalella azteca</i>	mortality (10 days)	30 and 200 mg/g concentrations caused significantly increased mortality for MWCNTs that were stirred, sonicated, and sonicated with NOM prior to sediment amendment the sonicated MWCNTs were more toxic than the stirred nanotubes at a dose of 200 mg/g only 300 mg/g of sonicated MWCNTs, but not MWCNTs stirred or sonicated in NOM at the same concentration, caused significantly decreased survival 99 mg/g of MWCNTs did not cause elevated toxicity regardless of the dispersion method
Petersen et al. 2008 <sup>38b</sup>	MWCNTs and SWCNTs	oligochaete <i>Lumbriculus variegatus</i>	number of worms (28 days)	no change in the number of worms was observed for concentrations of 0.3 mg/g MWCNTs and 0.03 mg/g SWCNTs
Petersen et al. 2008 <sup>37b</sup>	MWCNTs and SWCNTs	earthworm <i>Eisenia fetida</i>	mortality (28 days)	increased mortality was not observed for concentrations of 0.3 mg/g of MWCNTs and 0.03 mg/g of SWCNTs
Petersen et al. 2009 <sup>136b</sup>	MWCNTs and SWCNTs	earthworm <i>Eisenia fetida</i>	mortality (28 days)	increased mortality was not observed for concentrations of 3 mg/g of SWCNTs or MWCNTs
Petersen et al. 2011 <sup>35b</sup>	functionalized MWCNTs	earthworm <i>Eisenia fetida</i>	mortality (28 days)	increased mortality was not observed for concentrations of 0.5 mg/g of MWCNTs
Scott-Forsmand et al. 2008 <sup>151</sup>	MWCNTs	earthworm <i>Eisenia veneta</i>	mortality, growth, cocoon production and hatchability (28 days for all end points)	mortality, growth, and hatchability were not impacted up to 495 mg/kg food

Table 3. Continued

reference <sup>a</sup>	type of CNTs	organism(s) tested	end points and duration	findings
Velzeboer et al. 2011 <sup>155</sup>	MWCNTs	benthic macroinvertebrate communities	numbers of organisms, taxa composition, Shannon index (3 months all end points)	cocoon production was reduced at concentrations above 37 mg/kg food increase in numbers of organisms with increasing MWCNT concentrations (MWCNT concentrations tested were 0.002, 0.02, 0.2, and 2 mg/g sediment dry weight) there was not a significant effect on community diversity or the Shannon index (biodiversity)

<sup>a</sup> Plant toxicity has been summarized in recent reviews<sup>157,158</sup> and is not covered in this table. <sup>b</sup> The purpose of these studies was not to assess mortality and thus standard methods to assess these end points were not employed.

species and increases for others after exposure to functionalized and nonfunctionalized SWCNTs<sup>146</sup> or decreased biomass for *Cucurbita pepo* (zucchini) after MWCNT exposure.<sup>160</sup> Beneficial effects of CNT exposure have also been reported and these include enhancing germination rates and shortening the germination time in tomato plants,<sup>148</sup> and increasing root elongation in onions and cucumbers after exposure to nonfunctionalized SWCNTs.<sup>146</sup> The effects of interactions with soil were not considered in any of these studies, and it is likely that presence of soil would likely decrease the observed effects as a result of CNT sorption/attachment interactions with soil particles.

**Ecotoxicological Effects in Aquatic Ecosystems.** The toxicity of CNTs has been investigated using water-only experiments with a broad range of aquatic organisms including fish,<sup>161–164</sup> algae,<sup>165,166</sup> daphnia,<sup>31,33,71,144,150,167,168</sup> estuarine copepods,<sup>169</sup> amphibian larvae,<sup>143,170–172</sup> protozoa,<sup>77</sup> and bacteria.<sup>73,93–95</sup> Given the apparent lack of CNT absorption across epithelial membranes, toxic effects from CNT exposure appear to occur primarily as a consequence of interactions with epithelial surfaces. Accumulation of CNTs on surfaces of organisms could impede swimming behavior as has been observed in *Daphnia magna* exposed to fullerenes.<sup>173</sup> Additionally, the observed toxicity for daphnia exposed to lipid-coated SWCNTs was suggested to result from CNT clumping and deposition in the organism intestines.<sup>168</sup> In a study in which CNTs were suspended using several methods, a greater degree of CNT aggregation was related to higher toxicity in *Ceriodaphnia dubia* suggesting that the greater degree of clumping of CNTs within the gut may have been related to toxicity.<sup>150</sup>

The toxicity of aqueous CNT suspensions varies considerably among organisms and this variance could be a consequence of differences in the relative impact of CNT disturbance on epithelial surfaces among different organisms. Larvae of the amphibian *Ambystoma mexicanum* did not exhibit increased mortality or genotoxicity after exposure to DWCNTs at concentrations up to 1 g/L,<sup>170</sup> while *Daphnia magna* had a 96 h LC50 value of 2.48 mg MWCNTs/L.<sup>167</sup> Fish have been exposed to dispersed SWCNTs, and toxicity has been reported including changes in oxidative stress end points in various tissues and vascular changes in the brain.<sup>163</sup> The result of fish exposed to aqueous SWCNTs is consistent with disruption of gill function by accumulation of SWCNTs and physiological changes in fish to compensate for decreased gill function.<sup>163</sup> Decreased gill function leading to sublethal oxygen deprivation is consistent with subtle changes in oxidative stress indicators in tissues and dilation of blood vessels in the brain of the fish. In this study,<sup>163</sup> there was not a carbon black or other particle control used to distinguish effects of SWCNTs that are most likely executed at gill surfaces. Interestingly, fish exposed to rather high dietary levels of SWCNTs (500 mg/kg food) did not have any lesions in any tissues based on histological examination of all organ systems.<sup>164</sup> The only significant effect of the SWCNT exposure was a transient increase in lipid peroxidation observed on week four of the 6-week exposure, and this level of peroxidation did not differ from control levels at other time points. Taken together, results of CNT exposure in aquatic organisms suggest that there can be toxicological effects but that these effects are mediated at the surfaces of epithelia. Whether SWCNT aggregates generate effects on epithelial surfaces that are consequent to the nanosize of their constituent components is possible, but this has not been adequately demonstrated in the literature for multicellular organisms.



Surface charge and surface coatings will also likely influence CNT toxicity.<sup>33,150,174</sup> For example, derivitization of CNTs dramatically influenced toxicity in *Ceriodaphnia dubia*: positively charged functional groups were related to increases in acute toxicity, and hydrophilic functional groups reduced acute toxicity.<sup>150</sup> While positive functional groups on polyethyleneimine-grafted MWCNTs caused toxicity to cells,<sup>175</sup> there was not a clear relationship between MWCNT surface charge and 24 and 48 h immobilization of *D. magna* with the size of the coating seeming to exert a more important effect.<sup>33</sup> The mechanism for the changes in toxicity relative to functional group in CNTs is unknown, but could be related to the changes in CNT aggregation and subsequent effects on epithelial surface interactions. CNTs with particular functional groups that enhance toxicity may indeed cause elevated risks if they are present in water bodies at sufficiently high concentrations, and thus risk assessment for CNTs should take into consideration the functional groups on the CNTs. Clearly, there is a need for further assessment of the toxicity of derivatized CNTs to determine the characteristics that drive their toxicity. Nevertheless, interactions with NOM may change the surface chemistry sufficiently so that the initial surface chemistry of the nanotubes upon entrance into the environment is unimportant. This is an important topic for future research.

There are a number of potential artifacts that could explain some of the CNT ecotoxicity results (see this recent review for a more extensive discussion<sup>22</sup>). One is the release of toxic metals from the catalyst materials used to synthesize the CNTs.<sup>162,176,177</sup> The release of heavy metals and excess polymers if they are used to stabilize the nanotubes should be assessed by measuring the toxicity of the filtrate after nanotube removal.<sup>33</sup> Given the highly limited absorption of CNTs into organisms, researchers are encouraged to measure CNT concentrations in relevant tissues if toxic effects of the CNTs are observed in tissues for which substantial absorption would have to occur for an effect to be observed.

## ■ CONCLUSIONS AND FUTURE WORK

One of the foremost limitations in all of the research areas discussed is detection and quantification of CNTs in complex environmental and biological media and polymer composites. In polymer/CNT nanocomposite research, this inability to detect and quantify sufficiently small quantities of CNTs has hindered the development of mechanistic and mathematical models to describe how various environmental parameters impact the release rates. Furthermore, this challenge partly explains why much past research on release due to fire has relied upon carbon nanofibers as a result of their more straightforward detection instead of CNTs. The lack of analytical methods for nanotube quantification has limited the number of studies investigating the direct interactions of CNTs with soil and sediment particles and the transport of CNTs through soils instead of porous media without organic carbon. Lastly, some ecotoxicological studies have posited effects in tissues other than those that would come into direct contact with the CNTs after ingestion or dermal contact. Because CNTs were not directly identified in these tissues and absorption into tissues has almost never been observed, it is possible that some toxic effects (e.g., brain pathologies<sup>163</sup>) could have been a result of alternative mechanisms, such as impaired gill functioning, as described above. Moreover, in many ecological studies in the aqueous phase in which CNTs could be quantified, that is still often not performed. There can be substantial CNT settling as a result of aggregation

and compaction in the digestive tract of organisms, and thus these concentrations need to be assessed whenever possible. A related limitation in many studies is a lack of CNT characterization prior to and during the studies. This partly stems from the challenge of quantifying changes in nanotube surface chemistry, aggregation state, and size in various environmental media. Techniques such as dynamic light scattering that are straightforward to perform provide limited information on polydisperse CNT mixtures, yet other approaches to assess nanotube size distributions such as SEM or TEM are time-consuming, challenging in media with other particles or high salt concentrations, and require sample drying.

One of the primary trends for future research related to the environmental transport of CNTs is moving from well-defined systems to more complex and environmentally relevant systems. For example, investigating heteroaggregation between CNTs and natural colloids is more experimentally challenging but critical for a full understanding of their environmental transport. Similarly, there is a need to investigate CNT transport in more complex porous media such as collectors with different mineralogical compositions and different types of soils. Testing interactions of CNTs with a broader range of soil and sediment components (i.e., different clays and different types of organic matter) is necessary to provide a mechanistic understanding and predictive models of these processes.

Overall, the ecological effects of CNTs in soils and sediments appear to be very limited even at unrealistically high concentrations (i.e., g/kg). Nevertheless, it is possible that more sensitive organisms may be identified in future research and that biomarkers may be found that are sensitive to and indicative of CNT contamination. Conversely, toxic effects have been observed at much lower concentrations in the aqueous phase (i.e., mg/L) although these values are still typically much larger than those that would be expected in the environment on average.<sup>9–11</sup> This suggests that CNT settling out of the aqueous phase and sorption to soils or sediments may reduce their potential toxic effects, while other processes such as bioturbation and scouring may increase the potential risks. Along these lines, assessing the ecotoxicological risks of CNTs to plants in hydroponic studies may substantially overestimate the risks CNTs may pose in soil ecosystems. Testing CNT toxicity to plants in soils is an important future research direction. The extent to which CNTs produce nanosize related ecotoxicological effects is another important topic. It seems that many of the effects observed are from gut clogging, and would thus not substantially differ from those observed if the organisms were exposed to suspended sediment or colloids at similar concentrations. As the capability of CNTs to be modified with various surface functional groups is utilized in consumer products, a related research effort should be to provide models to predict how certain surface functionalizations and coatings influence their toxicity, release pathways, and environmental fate.

## ■ ASSOCIATED CONTENT

§ **Supporting Information.** A table describing CNT characterization techniques and figures related to CNT release from CNT/polymer nanocomposites. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone (301)-975-8142; fax: (301)-330-3447; e-mail: Elijah.Petersen@nist.gov.

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