

Effects of Polyethyleneimine-Mediated Functionalization of Multi-Walled Carbon Nanotubes on Earthworm Bioaccumulation and Sorption by Soils

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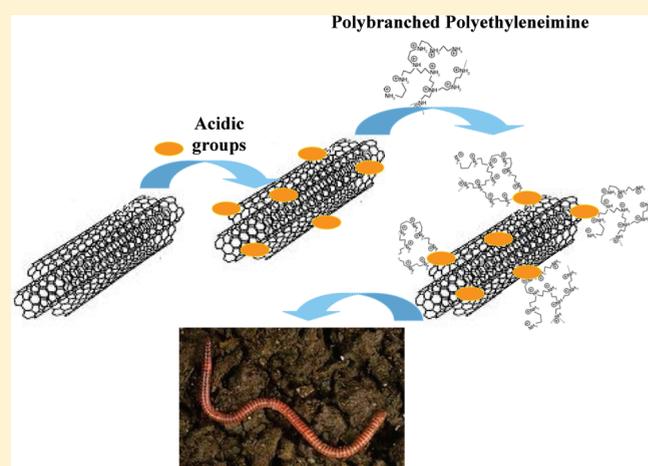
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S Supporting Information

ABSTRACT: Carbon nanotubes (CNTs) are often modified for different intended potential applications to enhance their aqueous stability or change properties such as surface charge. Such changes may also profoundly impact their environmental behaviors. Herein, we report the effects of modifying ¹⁴C-labeled multiwalled carbon nanotubes (MWCNTs) with polyethyleneimine (PEI) surface coatings to render them more stable in solution and to give them positive, negative, or neutral surface charges. These carbon nanotubes were used to test their sorption by soils and uptake and elimination behaviors by earthworms. Sorption results indicate nearly linear sorption isotherms for regular MWCNTs and non-linear isotherms for modified MWCNTs, indicating that the PEI coatings influenced MWCNT interactions with soils. Nevertheless, there were minimal differences in the sorption results among the different soils for each type of nanotube despite differences in the soil organic carbon and cation exchange capacities. Differences in uptake behaviors by earthworms were not apparent among different types of PEI-MWCNTs and MWCNTs with limited absorption into organism tissues consistently observed. Elimination patterns were well fit with an exponential decay model suggesting that the worms can readily eliminate any accumulated MWCNTs.



INTRODUCTION

Carbon nanotubes (CNTs) represent one of the most exciting classes of nanoparticles produced to date. Considerable interest arises from their remarkable structural, electrical, and chemical properties.¹ One of their most valuable characteristics is the ability to be functionalized either through modifying the ends or sidewalls of the nanotubes themselves or by grafting copolymers to the nanotubes.² Some specific nanotube applications that utilize this ability are CNT-peptide mediated assemblies of macromolecular structures,³ drug delivery mediators,^{4,5} and polymeric composites with stabilized-CNT reinforcing or conducting fillers.⁶

Certain environmental processes such as interactions with natural organic matter⁷ may also alter the surface chemistry of carbon nanotubes, and thus their environmental behaviors and the risks they may pose to humans or ecological receptors. Given the extensive masses of nanotubes already produced (e.g., 350 tons for the 2007/2008 year)⁸ and expected increased production in future years, understanding the effects different surface

characteristics of nanotubes may have on their environmental bioaccumulation behaviors and toxic effects is critical.

A profound challenge in determining the bioaccumulation potential of carbon nanotubes is an accurate quantification of their masses in environmentally relevant media (e.g., soil and sediment) and organisms. This challenge was recently reduced significantly by synthesis of carbon nanotubes radioactively labeled with the carbon-14 isotope.^{9–13} It has been determined using such radiolabeled nanotubes that earthworms,^{9,10} sediment-dwelling oligochaete *Lumbriculus variegatus*,^{9,11} and two estuarine invertebrates¹² do not accumulate purified nanotubes. One hypothesized cause for this was the minimal concentrations of carbon nanotubes in pore waters between soil or sediment particles. If this is the case, carbon nanotubes that remain

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suspended in water for extended time periods would be more available for uptake by ecological receptors, although the large size of the CNTs may be the major factor limiting absorption. Testing accumulation of nanotubes more stable in solution and investigating their sorption by soils and sediments may provide insights into the primary factors underlying the absence of observed accumulation.

Here, earthworms (*Eisenia fetida*) were exposed to multi-walled carbon nanotubes (MWCNTs) modified with polyethyleneimine (PEI) and differently adjusted to have positive, neutral, and negative surface charges, and the CNT bioaccumulation and elimination behaviors were studied. Earthworms are commonly used for testing the toxic effects of chemicals in terrestrial ecosystems because of their continual processing of soil and frequent consumption by vertebrate organisms.^{14–17} Modifying MWCNTs with PEI has been shown to allow them to remain suspended in solution for several months,¹⁸ which may potentially affect their uptake by earthworms but was recently not shown to impact their uptake and elimination behaviors with *Daphnia magna*.¹⁹ As described above, limited earthworm accumulation of MWCNTs may stem in large part from sorption of nanotubes to soils and sediments limiting their bioavailability. By comparing the sorption and accumulation behaviors with PEI-modified MWCNTs, we intended to directly test this hypothesis. Whereas sorption of MWCNTs has been recently studied using peat,²⁰ the results of this work provide the first sorption data for soils with MWCNTs having different surface charges and compare them with earthworm uptake behavior. Such information is important for modeling the environmental fate of MWCNTs and assessing the extent to which they remain in the pore water or interact with soil and sediment particles given that this distribution will likely cause differences in toxic effects observed.

EXPERIMENTAL SECTION

Materials. Hyperbranched PEI ($M_w = 25\,000$), acetic anhydride, succinic anhydride, and all other high purity chemicals and solvents were obtained from Acros Organics (Geel, Belgium) and were used as received. Regenerated cellulose membranes (MWCO = 50 000) were acquired from Spectrum Laboratories (Rancho Domingues, CA).

PEI-Mediated Functionalization of MWCNTs. Carbon-14 labeled and nonlabeled MWCNTs were synthesized using catalytic chemical vapor deposition and purified with hydrochloric acid as described previously.^{10,11,13} The MWCNTs were then acid functionalized by treatment with $\text{HNO}_3/\text{H}_2\text{SO}_4$ ($v/v = 3:1$), filtered, and rinsed with boiling deionized water ($>18.2\text{ M}\Omega$). These 3:1 MWCNTs were then grafted with PEI as described previously¹⁸ by treating first with thionyl chloride to introduce acid chloride groups, and then reacted with PEI and triethylamine in anhydrous *N,N*-dimethylformamide (DMF) to form MWCNT-PEI. Subsequent reactions with acetic anhydride and succinic anhydride in DMSO produced acetylated MWCNT-PEI-Ac (Ac denotes acetyl groups) and carboxylated MWCNT-PEI-Suc (Suc denotes succinamic acid groups), respectively. For each type of PEI-grafted MWCNT, DMF, DMSO, and byproducts were removed by extensively dialysis against deionized water (6 times, 4 L) for 3 days. The purified nanomaterial was lyophilized and stored at 4 °C.

Each type of PEI-MWCNT has been characterized thoroughly previously.¹⁸ Transmission electron microscopy (TEM) images revealed this process did not change the morphology of the

nanotubes and nanotube aggregation was not observed. The covalent linkages between MWCNTs and PEI were confirmed qualitatively by ^1H NMR spectroscopy. 3:1 MWCNTs were previously found to have an average length of 407 nm with a broad length distribution as determined by scanning electron microscopy¹³ (Figure S1 of the Supporting Information), whereas TEM revealed diameters generally between 30 and 70 nm. The percentage of the total MWCNT composite mass that was attributable to the surface coatings was determined using biological oxidation (OX-500, R. J. Harvey Instruments Company) and comparing the measured radioactivities to those of the 3:1 MWCNTs (the specific radioactivity of the 3:1 MWCNTs was 0.1 mCi/g). The percentages were $24 \pm 1\%$, $30 \pm 2\%$, and $38 \pm 4\%$ ($n = 4$; errors always represent standard deviations) for the PEI-MWCNT, PEI-MWCNT-Ac, and PEI-MWCNT-Suc, respectively. The carbon purity of the 3:1 MWCNTs was previously determined by thermal gravimetric analysis (TGA) to be 99.9 ± 0.2 ($n = 3$),¹³ demonstrating almost complete removal of catalyst materials. The electrophoretic mobility of each type of MWCNT was tested using a Malvern Zetasizer Nano ZS model ZEN 3600 (Worcestershire, U.K.) using 10 mM phosphate buffer adjusted to pH 6 and a MWCNT concentration of 5 $\mu\text{g}/\text{mL}$. The electrophoretic mobilities were (-1.71 ± 0.09 , 1.32 ± 0.09 , 0.03 ± 0.03 , and -0.78 ± 0.04) ($10^{-8}\text{ m}^2\text{ V}^{-1}\text{ s}^{-1}$) for the 3:1 MWCNTs, MWCNT-PEI, MWCNT-PEI-Ac, and MWCNT-PEI-Suc, respectively. In a previous study, sonicating 3:1 MWCNTs for 6 h without an ice–water bath only caused a slight increase in the MWCNT oxygen content as determined by X-ray photoelectron spectroscopy (from 7.4 to 8.6%).²⁰ As such, sonicating the nanotubes in an ice–water bath, which has previously been shown to reduce nanotube damage,²¹ for 30 min is not expected to substantially impact the 3:1 MWCNTs tested here.

Soils. Soils, collected from Chelsea (MI), Rochester Hills (MI), and the North Campus of the University of Michigan (Ann Arbor, MI) referred to here respectively as “Chelsea”, “R-Hills”, and “NC” soils, were each air-dried and passed through 2 mm mesh sieves. Their respective organic carbon fractions were determined as $5.7 \pm 0.4\%$, $1.6 \pm 0.4\%$, and $3.9 \pm 0.1\%$ ($n = 4$; LECO CS 200-series). This variability in the organic carbon fractions and the use of these soils in previous experiments,^{9,10} thus facilitating direct comparisons between this and prior studies, were the reasons that these soils were selected. Cation exchange capacities for the Chelsea, R-Hills, and NC soils measured using a modified ammonium acetate total exchange capacity method²² were determined to be 0.418 ± 0.03 , 0.34 ± 0.05 , and 0.120 ± 0.001 meq/g ($n = 3$), respectively. Triplicate 6 g soil samples were equilibrated under conditions of constant horizontal mixing for 24 h with 45 mL of a 1 mol/L ammonium acetate solution with a pH adjusted to 7. A 5 mL aliquot of clarified supernatant was collected after processing the vials at low-speed centrifugation (10 g for five minutes). Inductively coupled plasma-mass spectrometry (ICP-MS) (PerkinElmer ELAN ICP-MS DRC 6000, Waltham, MA) was used to analyze extracted cations (Mg, Ca, Na, K, Fe, and Al), and cation concentrations were summed to yield the CEC values.

Phase Distribution Experiments with Soils. Phase distribution experiments were carried out in deionized water for PEI-grafted and 3:1 MWCNTs for R-Hills, NC soils, and Chelsea soils by single solute sorption isotherms obtained using a batch equilibration technique at room temperature (21 ± 1 °C). MWCNTs differ from typical hydrophobic organic chemicals

(HOCs) in that their interactions with soils may be from attachment or sorption and that dissolved organic matter released from the soils may influence sorption behaviors. The use of the term sorption may therefore not accurately indicate that complicated interactions occur, which likely differ substantially from those for typical HOCs. Stock solutions of stabilized nanotubes were prepared by resuspending lyophilized nanomaterials in deionized water and tested over a range of concentrations that provided liquid-phase ^{14}C concentrations above background levels after the sorption experiment exposure interval. These solutions were transferred to 25 mL centrifugal borosilicate vials containing NC, R-Hills, or Chelsea soil samples (25 to 30) mg and mixed via constant horizontal shaking for 7 days, after which period the vials were allowed to rest vertically for 6 h. Two 3 mL aliquots were then withdrawn at half the height of the liquid column from each vial and mixed with 4 mL of Instagel scintillation cocktail (PerkinElmer, Shelton, CT) for subsequent ^{14}C liquid scintillation counting (LSC; LS6500 liquid scintillation counter; Beckman, Fullerton, CA). Blank controls (without soil) were treated using the same conditions to account for any losses or settling of suspended nanomaterials, and unamended soils were tested to measure the liquid-phase total organic concentration (TOC; Shimadzu Model TOC-Vcsh) through the duration of the sorption experiments. Phase distribution profiles for the CNTs with three types of soils were each fit with the Freundlich isotherm model given in eq 1:

$$q_e = K_F C_e^n \quad (1)$$

where K_F is the Freundlich model capacity factor; and n is the Freundlich model site energy heterogeneity factor,²³ and q_e and C_e are the equilibrium solid-phase and aqueous phase solute concentrations expressed as $\mu\text{g/g}$ and $\mu\text{g/L}$, respectively. It is recognized though that the carbon nanotubes would likely interact with dissolved organic matter released from the soils and thus are not dissolved in the aqueous phase as expected for HOCs.

Bioaccumulation Experiments. Uptake of PEI-grafted MWCNTs by the earthworm *Eisenia fetida* was determined using modified standard procedures.²⁴ *E. fetida* were cultured as previously described.^{25,26} Lyophilized PEI-grafted MWCNTs were reconstituted in deionized water by sonication using a Cole Parmer ultrasonic homogenizer for 30 min in an ice–water bath. This nanotube suspension was then carefully transferred using a manual pipet in small volumes to soils while rotating each soil container as it received the solution. The volume of this solution added to the soils yielded a soil moisture content of 22.5% in each container and a nanotube loading of 0.5 mg MWCNT composite per gram soil. The container was then sealed and tumbled overnight at 30 rpm. After homogenization, the concentration and homogeneity of the MWCNT distributions in soil was assessed by combustion of multiple freeze-dried (1 g dry mass on average) soil samples using a biological oxidizer, and the radioactivity was determined using LSC. Standard deviations for at least triplicate samples measured for every condition tested were less than 6% of the mean, thus indicating a homogeneous nanotube distribution in the soils.

Uptake and elimination experiments were performed as previously described.²⁵ To initiate the uptake experiments, three adult worms with combined live masses between 1.2 and 2.0 g were transferred to moist (22.5% water) soil samples (30 g dry mass) in 100 mL glass jars. The jars were loosely closed with a cap to prevent worms from escaping while allowing air exchange and

were kept in the dark at 21 ± 2 °C. Three worms were added to each of triplicate containers for each data point. Worms were removed after 2, 7, 14, or 28 days, washed with deionized water, and transferred to wet filter paper in Petri dishes for 24 h in the dark to allow purging of gut contents. The worms were then rinsed with deionized water before being transferred to glass tubes, freeze-dried overnight, weighed, combusted in a biological oxidizer, and the radioactivity was determined using ^{14}C liquid scintillation counting. Bioaccumulation factors (BAFs) were calculated as the ratio of the concentration of PEI-modified MWCNT in the organism divided by that quantified in the soil. After 28 days of earthworm exposure, triplicate soil samples of 1 g dry mass were combusted using biological oxidation and the radioactivity determined using liquid scintillation counting.

Depuration experiments were also conducted to assess the rate at which nanotubes would be eliminated from the organisms. After exposure for 14 days to soils spiked with modified carbon nanotubes in NC soil, earthworms were transferred to containers with unamended soil. After 2, 6, and 9 days in the soil, the earthworm specimens were removed and treated as described above for quantification of residual ^{14}C .

RESULTS AND DISCUSSION

Phase Distribution with Soils. This data is the first published measurements of MWCNT sorption interactions with soils. Nonlinear regressions using the Freundlich model given in eq 1 were fit to the isotherms of each type of MWCNT as shown in Figure 1 and Figure S2 of the Supporting Information. The resulting model parameters, K and n , along with their 95% confidence intervals and coefficients of determination, R^2 , are presented in Table 1. Freundlich isotherms fit the data well across the range of concentrations tested.

The stability of the MWCNTs in solution and thus their potential for interactions with dissolved natural organic matter released from the soils is an important factor in the experiments described. Solution-phase TOC values during the 7 day sorption experiments were (5.4 ± 0.3 , 4.2 ± 0.4 , and 7.9 ± 0.6) mg/L ($n = 3$), for NC soil, R-Hills soil, and Chelsea soil, respectively (part a of Figure 2). The stability of suspensions of tested nanotubes at these dissolved organic matter (DOM) concentrations revealed that approximately $95 \pm 6\%$ ($n = 2$) of the initially added nanoparticles stayed in suspension for each type of MWCNTs at NC DOM concentrations representative of those found in the sorption experiments (part b of Figure 2). Similar results were also observed in DOM concentrations an order of magnitude smaller. These values were not substantially different from those without DOM, suggesting that the presence of soil DOM did not influence nanotube settling during the experiments. This result accords with a previous study with 3:1 MWCNTs and DOM released from peat, which also did not show an impact of DOM on the nanotube stability in the absence of additional sodium cations.²⁰

According to n values determined from the isotherms, 3:1 MWCNTs were more linearly sorbed to all three soils than PEI-modified MWCNTs, suggesting an important influence of the PEI coating. Linear sorption indicates that the overall energy of these interactions is uniform with increasing concentrations.^{27,28} After grafting with PEI, values of n for the modified MWCNTs are all less than one, suggesting that, as sorbate concentration increases, it is more difficult for additional loading thus indicating heterogeneous interaction energies. Linearity indices for the four

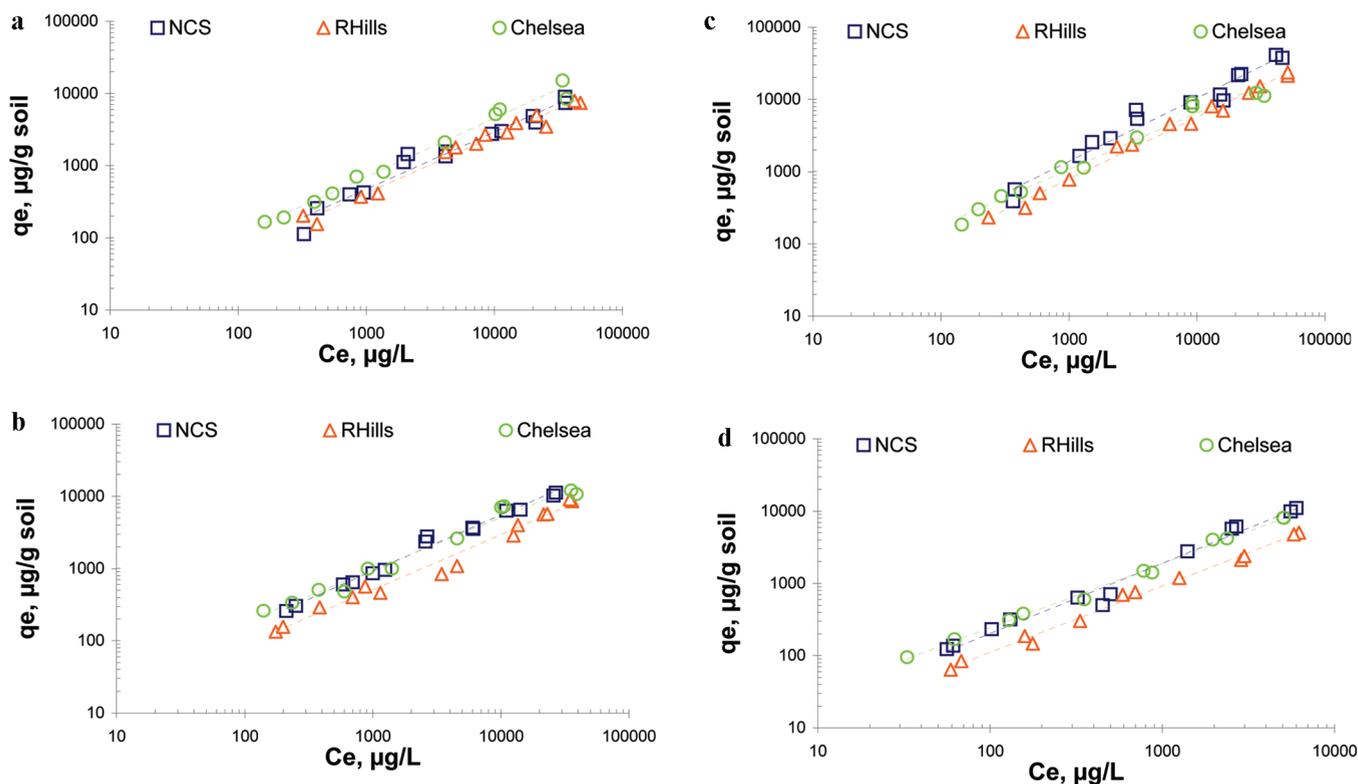


Figure 1. Sorption isotherms for a) MWCNT-PEI, b) MWCNT-PEI-Ac, c) MWCNT-PEI-Suc, and d) 3:1 MWCNTs in North Campus, Rochester Hills, and Chelsea soils. Data points represent individual measurements of equilibrium concentrations and dotted lines represent the Freundlich isotherm model fits of the sorption data.

Table 1. Parameters of MWCNTs Sorption Using a Freundlich Isotherm Model

| North Campus Soil | K | n | adjusted R^2 |
|----------------------|-------------------|-----------------|----------------|
| MWCNT | 2.31 ± 1.26^a | 0.97 ± 0.07 | 0.976 |
| MWCNT-PEI | 2.00 ± 1.52 | 0.79 ± 0.06 | 0.972 |
| MWCNT-PEI-Ac | 4.33 ± 0.91 | 0.78 ± 0.04 | 0.988 |
| MWCNT-PEI-Suc | 3.31 ± 1.98 | 0.87 ± 0.08 | 0.959 |
| Rochester Hills Soil | K | n | adjusted R^2 |
| MWCNT | 1.62 ± 0.90 | 0.92 ± 0.04 | 0.991 |
| MWCNT-PEI | 1.85 ± 1.38 | 0.78 ± 0.05 | 0.979 |
| MWCNT-PEI-Ac | 2.65 ± 0.95 | 0.76 ± 0.04 | 0.985 |
| MWCNT-PEI-Suc | 2.11 ± 1.02 | 0.86 ± 0.04 | 0.989 |
| Chelsea Soil | K | n | adjusted R^2 |
| MWCNT | 4.06 ± 0.85 | 0.89 ± 0.05 | 0.995 |
| MWCNT-PEI | 2.58 ± 1.49 | 0.81 ± 0.07 | 0.986 |
| MWCNT-PEI-Ac | 6.44 ± 3.04 | 0.72 ± 0.08 | 0.976 |
| MWCNT-PEI-Suc | 4.98 ± 2.16 | 0.78 ± 0.09 | 0.975 |

^a Adding or subtracting these values yields 95% confidence intervals.

types of MWCNTs rarely varied significantly among the three soils tested with 95% confidence intervals indicating that different soils may not necessarily strongly influence sorption patterns; rather the types of the MWCNTs play a dominant role with the sorption energy distribution being more homogeneous for 3:1

MWCNTs than the PEI-modified ones. It was not possible to fully differentiate between the effects of electrophoretic mobility and PEI coating on the n values. The largest n values were for the 3:1 MWCNTs, whereas the MWCNT-PEI-Suc, which also had a negative electrophoretic mobility value but which was only half as large as that for the 3:1 MWCNTs, had the second largest n values for NC and R-Hills soils, but not Chelsea soil. Thus, the negative charge of MWCNTs may influence the calculated n values in addition to the presence of a surface coating. Additionally, interactions between DOM and MWCNTs may change CNT electrophoretic mobilities and would likely coat the previously bare 3:1 MWCNTs thus potentially changing both parameters, a scenario that highlights the complexity of understanding sorption interactions for CNTs. Additional research utilizing a set of nanotubes with the same surface charge but different sizes of polymer coatings or a set of uncoated MWCNTs with different surface charges could help clarify the relative impacts of the surface coating versus electrophoretic mobility.

Statistically significant differences were rarely observed in sorption capacities for the different types of MWCNTs with and without PEI modifications or among the soils tested according to values of the distribution coefficients (K) measured. These results were unexpected given that the PEI modifications yield such different surface charges and that the surface coating was such a large fraction of the total nanotube composite mass. Such results may stem from soil DOM wrapping the carbon nanotubes or interacting with the PEI polymers. The soils tested also have different organic carbon fractions and cation exchange capacities. But these properties may not differ substantially

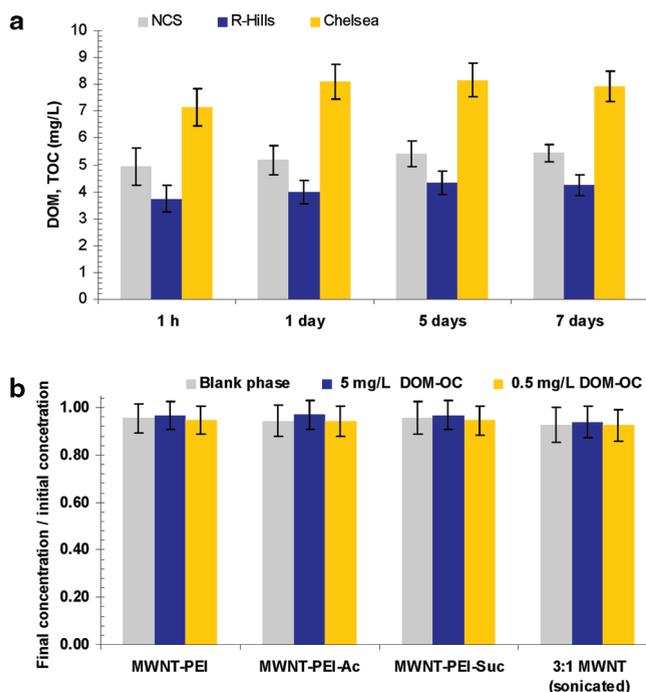


Figure 2. Dissolved organic matter release and impact on nanotube stability. a) Concentration profile of organic carbon releases from soils during sorption during 7 day equilibration. b) Stability of carbon nanotube solutions suspended in the presence of NC soil at DOM levels representative of those observed in the sorption experiments. All data represent mean values, and error bars represent standard deviations ($n = 3$ for (a) and $n = 2$ for (b)).

enough to impact the sorption interactions observed. A recent study has shown that peat does not sorb 3:1 MWCNTs without sodium cations added to solution.²⁰ It was believed that in the absence of added cations the DOM wrapping on the MWCNT surfaces inhibited MWCNT interactions with the solid surface, whereas cations caused a decrease in the surfaces charges of the MWCNTs and peat thus leading to sorption interactions. The insensitivity of the K values to the soil and MWCNT types observed here suggests that minimal sorption to soil organic matter may occur in the absence of added cations. Further experiments with individual soil and sediment components such as different mineral fractions and types of soil organic matter are needed to assess more definitively the relative affinities of CNTs for these various components. Additionally, unlike what would be typical for hydrophobic organic chemicals, changing aqueous conditions such as ionic strength or solution pH will likely strongly influence the sorption behaviors of the carbon nanotubes as was recently shown with sorption with peat.²⁰ This study provides an experimental method that could be utilized for such future experiments.

Accumulation Experiments. Earthworm accumulation experiments for up to 28 days were conducted to assess the extent to which surface coatings and different surface charges influence uptake behaviors (parts a and b of Figure 3). Interestingly, these results did not indicate substantial absorption of carbon nanotubes having PEI surface modifications, a result which mirrors previously published results with purified and 3:1 modified MWCNTs.^{9,10} The PEI-grafted MWCNTs did seem to have higher BAF values compared to the nonmodified MWCNTs, but standard deviations were consistently large, hindering definitive

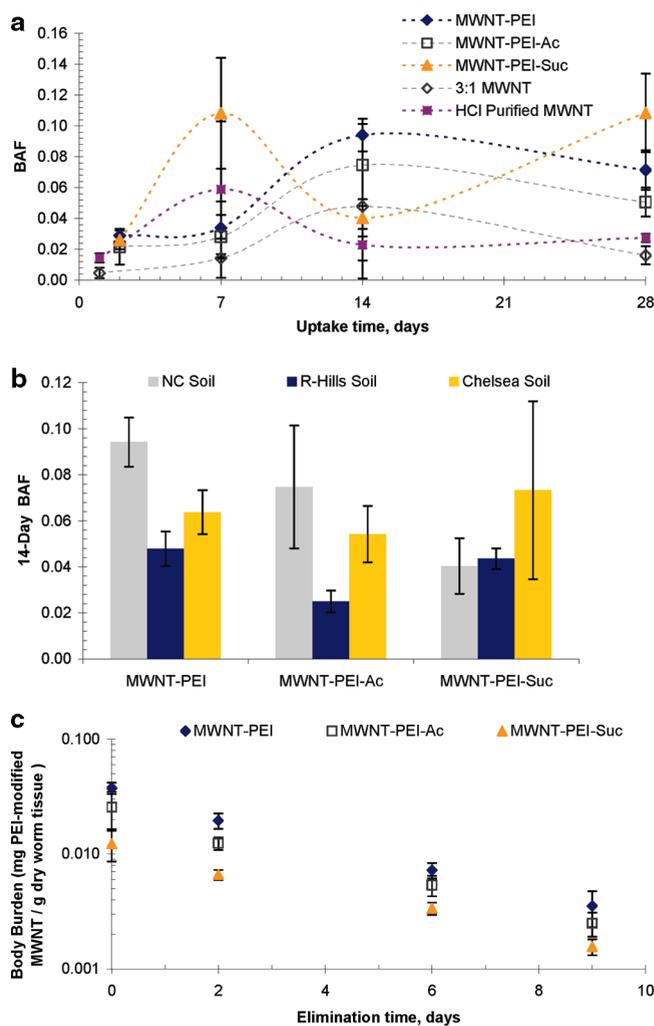


Figure 3. Uptake and elimination of PEI-modified MWCNTs. a) Bioaccumulation factors (BAFs) uptake by *E. fetida* of HCl purified and 3:1 MWCNTs (0.3 mg/g) and PEI-modified MWCNTs (0.5 mg/g) in NC soil systems. Data for the HCl purified and 3:1 MWCNTs are from ref 10 and ref 9, respectively. b) BAFs uptake by earthworms of PEI-modified MWCNTs (0.5 mg/g) after 14 days of exposure in RH and Chelsea soils. c) Elimination of PEI-modified MWCNTs (0.5 mg/g) after exposure for 14 days in NC soil. Triplicate samples were tested for each data point in each figure, and error bars represent standard deviations of those measurements.

conclusions about relative uptake rates. There was not a clear trend in the 14-d BAF values among the different types of PEI-modified MWCNTs obtained in three different soils (part b of Figure 3). Unlike behaviors for organic chemicals for which larger soil concentrations of organic carbon and especially hard organic carbons would be expected to significantly decrease accumulation,²⁹ there was no pattern in relative BAF values among different types of nanotubes in the three soils despite differences in the soil properties. Further, there appeared to be no trend during the 28 day accumulation period for any of the types of nanotubes. Instead, BAF values appeared randomly to increase or decrease during the duration of the experiment. This result is not expected to stem from substantially increased MWCNT sorption through the duration of the experiment, given that a pseudoequilibrium was reached after sorption for 7 days during preliminary experiments (data not shown). Additionally, these

Table 2. Elimination Rate Constants for PEI-Modified MWCNTs after a 14 Day Uptake Period

| North Campus Soil | elimination rate | 95% confidence | adjusted R^2 |
|-------------------|-----------------------|----------------|----------------|
| | constant (d^{-1}) | interval | |
| MWCNT-PEI | 0.263 | 0.227, 0.299 | 0.963 |
| MWCNT-PEI-Ac | 0.248 | 0.206, 0.290 | 0.944 |
| MWCNT-PEI-Suc | 0.217 | 0.184, 0.251 | 0.953 |

results cannot be explained by large differences in the distribution of the MWCNTs between the soil particles and soil pore water given the sorption results. Instead, this lack of a pattern resembles previous results for *E. fetida* for purified SWNTs, purified MWCNTs, and 3:1 MWCNTs,^{9,10} thus suggesting that the type of CNT or their surface coatings is not expected to impact the observed accumulation behaviors. The concentrations in earthworms remaining may be explained in large measure by soil remaining in its guts, although it is possible that there may be interactions between the MWCNTs and the gut epithelial cells. It was shown previously that earthworm BAF values for a nonbioaccumulating chemical would be 0.0315 ± 0.001 for earthworms allowed to purge their guts for 24 h.¹⁰ Given the high standards deviations, few of the BAF values shown are significantly larger than this value.

These results accord with recent results for uptake of 3:1 and PEI-grafted MWCNT by water flea *Daphnia magna*, which also did not indicate a trend among these various types of MWCNTs.¹⁹ Additionally, several studies have microscopically indicated that various organisms do not absorb MWCNTs,^{30,31} a result in agreement with the lack of absorption observed here. Importantly, these results together suggest that surface coatings, as would be expected for many applications of carbon nanotubes or after their interactions with DOM, would not lead to enhanced accumulation in organisms.

Elimination Experiments. Rates and extents to which earthworms purge accumulated PEI-modified MWCNTs were investigated (part c of Figure 3 and Table 2). Unlike previous results with purified MWCNTs and earthworms, which showed no consistent decreases with time,¹⁰ the results observed here resembled those for MWCNTs and oligochaete *Lumbriculus variegatus*.¹¹ The more consistent patterns observed here may stem from triplicate containers being tested for each time point in this study, whereas the earlier study utilized only a single data point per time point.¹⁰ Nevertheless, the discrepancy among the studies is unexpected and contrasts with similar uptake behaviors observed for various types of acid-modified and PEI-grafted MWCNTs and similar elimination results observed for 3:1 MWCNTs and the various types of PEI-grafted MWCNTs with *Daphnia magna*.¹⁹

No clear pattern in the depuration rates was observed among the different types of PEI-MWCNTs, and 95% confidence intervals indicate that elimination rate coefficients were not statistically different (Table 2). However, a consistent but not statistically significant pattern for the elimination rate constants was observed for the different types of PEI-modified MWCNTs, which showed that larger and more negatively charged MWCNTs were eliminated more slowly. Given that the grafted MWCNTs became larger and more negatively charged in tandem, it is not clear whether there were slight effects in the elimination rate constants as a result of either or both larger or more negatively charged MWCNTs.

Environmental Implications. Overall, these results indicate that coating of MWCNTs with polymers generally caused a more nonlinear sorption profile, as indicated by n values significantly less than one, whereas 3:1 MWCNTs were nearly linearly absorbed. Relatively minor differences in the sorption behaviors among soils with varying organic carbon concentration suggest that the soil type may not be as important as the MWCNT characteristics in predicting sorption behaviors. Similarly to a recent paper with *Daphnia magna*,¹⁹ the presence of polymers with different surface coatings did not impact MWCNT uptake by earthworms with small and highly variable BAF values observed. These results suggest that such surface coatings are unlikely to influence organism accumulation of MWCNTs. The lack of accumulation suggests that one mechanism for MWCNT toxicity may be through impacting organism digestive processes and tissues. Carbon nanoparticles aggregating in gut tracts has been previously observed and suggested as a toxicity mechanism for *Daphnia*,^{13,30,32} but it is unclear to what extent similar effects would be observed for earthworms given their physiological differences. In the absence of MWCNT absorption, toxic effects of CNTs in soils to earthworms could also be observed if nanotubes adsorb micronutrients rendering them nonbioavailable. The ready elimination of PEI-modified MWCNTs observed here suggests that toxic risks of soils contaminated with MWCNTs would be mitigated if earthworms are able to migrate to noncontaminated soils; this would be relevant for scenarios in which MWCNTs contamination occurs at elevated concentrations in isolated hot spots with noncontaminated areas nearby where the earthworms could purge any accumulated MWCNTs.

■ ASSOCIATED CONTENT

Supporting Information. Results for sorption isotherms and MWCNT size characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ REFERENCES

(1) Mauter, M. S.; Elimelech, M. Environmental applications of carbon-based nanomaterials. *Environ. Sci. Technol.* **2008**, *42* (16), 5843–5859.

- (2) Homenick, C. M.; Lawson, G.; Adronov, A. Polymer grafting of carbon nanotubes using living free-radical polymerization. *Polym. Rev.* **2007**, *47* (2), 265–290.
- (3) Dieckmann, G. R.; Dalton, A. B.; Johnson, P. A.; Razal, J.; Chen, J.; Giordano, G. M.; Munoz, E.; Musselman, I. H.; Baughman, R. H.; Draper, R. K. Controlled assembly of carbon nanotubes by designed amphiphilic peptide helices. *J. Am. Chem. Soc.* **2003**, *125* (7), 1770–1777.
- (4) Bianco, A.; Kostarelos, K.; Partidos, C. D.; Prato, M. Biomedical applications of functionalised carbon nanotubes. *Chem. Commun.* **2005**, *5*, 571–577.
- (5) Shi, X. Y.; Wang, S. H.; Shen, M. W.; Antwerp, M. E.; Chen, X. S.; Li, C.; Petersen, E. J.; Huang, Q. G.; Weber, W. J., Jr.; Baker, J. R. Multifunctional dendrimer-modified multiwalled carbon nanotubes: Synthesis, characterization, and in vitro cancer cell targeting and imaging. *Biomacromolecules* **2009**, *10* (7), 1744–1750.
- (6) Dalmás, F.; Chazeau, L.; Gauthier, C.; Masenelli-Varlot, K.; Dendievel, R.; Cavaillé, J. Y.; Forro, L. Multiwalled carbon nanotube/polymer nanocomposites: Processing and properties. *J. Polym. Sci., Part B: Polym. Phys.* **2005**, *43* (10), 1186–1197.
- (7) Hyung, H.; Fortner, J. D.; Hughes, J. B.; Kim, J. H. Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environ. Sci. Technol.* **2007**, *41* (1), 179–184.
- (8) Mueller, N. C.; Nowack, B. Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.* **2008**, *42* (12), 4447–4453.
- (9) Petersen, E. J.; Huang, Q. G.; Weber, W. J., Jr. Relevance of octanol-water distribution measurements to the potential ecological uptake of multi-walled carbon nanotubes. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1106–1112.
- (10) Petersen, E. J.; Huang, Q. G.; Weber, W. J., Jr. Bioaccumulation of radio-labeled carbon nanotubes by *Eisenia foetida*. *Environ. Sci. Technol.* **2008**, *42* (8), 3090–3095.
- (11) Petersen, E. J.; Huang, Q. G.; Weber, W. J., Jr. Ecological uptake and depuration of carbon nanotubes by *Lumbricus variegatus*. *Environ. Health Perspect.* **2008**, *116* (4), 496–500.
- (12) Ferguson, P. L.; Chandler, G. T.; Templeton, R. C.; Demarco, A.; Scrivens, W. A.; Englehart, B. A. Influence of sediment-amendment with single-walled carbon nanotubes and diesel soot on bioaccumulation of hydrophobic organic contaminants by benthic invertebrates. *Environ. Sci. Technol.* **2008**, *42* (10), 3879–3885.
- (13) Petersen, E. J.; Akkanen, J.; Kukkonen, J. V. K.; Weber, W. J., Jr. Biological uptake and depuration of carbon nanotubes by *Daphnia magna*. *Environ. Sci. Technol.* **2009**, *43* (8), 2969–2975.
- (14) Ma, W. C.; van Kleunen, A.; Immerzeel, J.; de Maagd, P. G. J. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. *Environ. Toxicol. Chem.* **1998**, *17* (9), 1730–1737.
- (15) Matscheko, N.; Lundstedt, S.; Svensson, L.; Harju, M.; Tysklind, M. Accumulation and elimination of 16 polycyclic aromatic compounds in the earthworm (*Eisenia fetida*). *Environ. Toxicol. Chem.* **2002**, *21* (8), 1724–1729.
- (16) Jager, T.; Sanchez, F. A. A.; Muijs, B.; van der Velde, E. G.; Posthuma, L. Toxicokinetics of polycyclic aromatic hydrocarbons in *Eisenia andrei* (Oligochaeta) using spiked soil. *Environ. Toxicol. Chem.* **2000**, *19* (4), 953–961.
- (17) Scott-Fordsmand, J. J.; Krogh, P. H.; Schaefer, M.; Johansen, A. The toxicity testing of double-walled nanotubes-contaminated food to *Eisenia veneta* earthworms. *Ecotox. Environ. Saf.* **2008**, *71* (3), 616–619.
- (18) Shen, M. W.; Wang, S. H.; Shi, X. Y.; Chen, X. S.; Huang, Q. G.; Petersen, E. J.; Pinto, R. A.; Baker, J. R.; Weber, W. J., Jr. Polyethyleneimine-mediated functionalization of multiwalled carbon nanotubes: synthesis, characterization, and in vitro toxicity assay. *J. Phys. Chem. C* **2009**, *113* (8), 3150–3156.
- (19) Petersen, E. J.; Pinto, R. A.; Mai, D. J.; Landrum, P. F.; Weber, W. J., Jr. Influence of polyethyleneimine graftings of multi-walled carbon nanotubes on their accumulation and elimination by and toxicity to *Daphnia magna*. *Environ. Sci. Technol.* **2011**, *45* (3), 1133–1138.
- (20) Zhang, L.; Petersen, E. J.; Huang, Q. G. Phase distribution of ¹⁴C-labeled multiwalled carbon nanotubes in aqueous systems containing model solids: Peat. *Environ. Sci. Technol.* **2011**, *45* (4), 1356–1362.
- (21) Heller, D. A.; Barone, P. W.; Strano, M. S. Sonication-induced changes in chiral distribution: A complication in the use of single-walled carbon nanotube fluorescence for determining species distribution. *Carbon* **2005**, *43* (3), 651–653.
- (22) Carter, M. R. *Soil Sampling and Methods*; CRC Press: Boca Raton, FL, 1993; p 173–176.
- (23) Weber, W. J., Jr.; DiGiano, F. A., *Process Dynamic in Environmental Systems*; Wiley Interscience: New York, 2001.
- (24) ASTM, *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Test with the Lumbricid Earthworm Eisenia foetida*; E1676–97. In Philadelphia, 1998.
- (25) Petersen, E. J.; Pinto, R. A.; Landrum, P. F.; Weber, W. J., Jr. Influence of carbon nanotubes on pyrene bioaccumulation from contaminated soils by earthworms. *Environ. Sci. Technol.* **2009**, *43* (11), 4181–4187.
- (26) Tang, J. X.; Petersen, E. J.; Huang, Q. G.; Weber, W. J., Jr. Development of engineered natural organic sorbents for environmental applications: 3. Reducing PAH mobility and bioavailability in contaminated soil and sediment systems. *Environ. Sci. Technol.* **2007**, *41* (8), 2901–2907.
- (27) Huang, W. L.; Weber, W. J., Jr. A distributed reactivity model for sorption by soils and sediments. 10. Relationships between desorption, hysteresis, and the chemical characteristics of organic domains. *Environ. Sci. Technol.* **1997**, *31* (9), 2562–2569.
- (28) Weber, W. J., Jr.; McGinley, P. M.; Katz, L. E. Sorption Phenomena in subsurface systems: Concepts, models and effects on contaminant fate and transport. *Water Res.* **1991**, *25* (5), 499–528.
- (29) Cornelissen, G.; Gustafsson, O. Prediction of large variation in biota to sediment accumulation factors due to concentration-dependent black carbon adsorption of planar hydrophobic organic compounds. *Environ. Toxicol. Chem.* **2005**, *24* (3), 495–498.
- (30) Edgington, A. J.; Roberts, A. P.; Taylor, L. M.; Alloy, M. M.; Reppert, J.; Rao, A. M.; Ma, J. D.; Klaine, S. J. The influence of natural organic matter on the toxicity of multiwalled carbon nanotubes. *Environ. Toxicol. Chem.* **2010**, *29* (11), 2511–2518.
- (31) Mouchet, F.; Landois, P.; Sarremejean, E.; Bernard, G.; Puech, P.; Pinelli, E.; Flahaut, E.; Gauthier, L. Characterisation and in vivo ecotoxicity evaluation of double-wall carbon nanotubes in larvae of the amphibian *Xenopus laevis*. *Aquat. Toxicol.* **2008**, *87* (2), 127–137.
- (32) Tervonen, K.; Waissi, G.; Petersen, E. J.; Akkanen, J.; Kukkonen, J. V. K. Analysis of fullerene-C₆₀ and kinetic measurements for its accumulation and depuration in *Daphnia magna*. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1072–1078.