Uptake and Elimination Behaviors of Polyethyleneimine (PEI)-Coated Multi-Walled Carbon Nanotubes by *Eisenia foetida* and *Daphnia magna*

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ABSTRACT

Determining the effects of various surface coatings on carbon nanotubes (CNTs) is critical given the numerous expected applications of CNTs and their inevitable release into ecosystems. To explore the potential ecotoxicological effects of CNT surface modifications, we modified multi-walled carbon nanotubes (MWNTs) with polyethyleneimine (PEI) coatings. Uptake and elimination experiments for PEI-MWNTs with various surface charges spiked to soils revealed limited earthworm accumulation and ready elimination. Conversely, *Daphnia magna*, a filter-feeding aquatic invertebrate, accumulated substantial concentrations of PEI MWNTs and was only able to excrete them with algae feeding. This suggests that the availability of food in ecosystems will substantially affect the long-term fate of nanotubes ingested by daphnia. Uptake was quantified in both species using carbon-14 labeled nanotubes.

Keywords: nanotoxicology, single-walled carbon nanotubes, bioaccumulation, nano-environmental health and safety, ecotoxicology

INTRODUCTION

Carbon nanotubes (CNTs) have commercial potential in a range of applications, which stems from their numerous unique properties including their strength, adsorption capacity, and electrical conductivity[1]. There are two primary classes of carbon nanotubes: single-walled carbon nanotubes (SWNTs) composed of a single tube and multi-walled carbon nanotubes (MWNTs) composed of multiple concentric tubes. While a substantial research effort has focused on the physico-chemical properties and commercial applications of carbon nanotubes, investigations of their potential ecological risks have been conducted with substantially lesser frequency. Given the expected widespread use of carbon nanotubes in future applications/products, it is inevitable that CNTs will intentionally and unintentionally be released into the natural environment [2, 3].

One of the main analytical limitations of studies to investigate the environmental distribution and potential ecotoxicological risks of carbon nanotubes is their detection in environmentally relevant matrices such as soils or organisms [4, 5]. Unlike typical hydrophobic organic chemicals, as synthesized or commercially purchased CNTs are typically polydispersed with a broad range of lengths and diameters in each batch. Thus, chromatographic techniques that have been historically used to analyze the concentrations of organic chemicals in environmentally relevant matrices are not applicable with carbon nanotubes. Moreover, while many inorganic environmental pollutants can be analyzed using elemental analysis techniques such as atomic absorption spectroscopy or inductively-coupled plasma-mass spectrometry, CNTs are made predominately of carbon, thus it is not feasible to quantify them using elemental analysis when they are in a carbon matrix.

This has lead to the development of radioactively-labeled carbon nanotubes, which allows for their detection via the carbon-14 isotope [6-8]. To detect CNTs in complex environmental matrices, the samples can be combusted using a biological oxidizer, which promotes the complete oxidation of the samples at elevated temperatures under a stream of oxygen, and then the emitted carbon-14 dioxide is captured in liquid scintillation cocktail. In previous studies, I have synthesized radioactively-labeled carbon nanotubes using a modified chemical vapor deposition system with carbon-14 methane [6, 7]. These nanotubes have been used to quantify the uptake and elimination of unpurified, purified, and acid-treated (3:1 ratio of sulfuric to nitric acid) MWNTs by earthworms (*Eisenia foetida*) [6, 9], oligochaetes (*Lumbriculus variegatus*) [7, 9], and water fleas (*Daphnia magna*) [10].

This conference proceeding summarizes two papers recently published on the uptake and elimination of polyethyleneimine (PEI) coated MWNTs by earthworms and waters fleas [11, 12]. A previous publication describes the modication of MWNTs with PEI coatings to enhance aqueous stability and endow them with positive (MWNT-PEI), negative (MWNT-PEI-Suc), or neutral (MWNT-PEI-Ac) surface charges [13]. Additional characterization of the MWNTs with and without the PEI coatings (including thermogravimetric analysis, transmission electron microscopy, scanning electron microscopy, surface area analysis, and x-ray photoelectron spectroscopy analysis) has been conducted and described in previous papers [6, 7, 9, 10, 13-15]. In this conference proceeding, results are provided for uptake/accumulation and elimination in earthworms and daphnids. Detailed information about the experimental methods can be found in the published manuscripts [11, 12].

RESULTS AND DISCUSSION

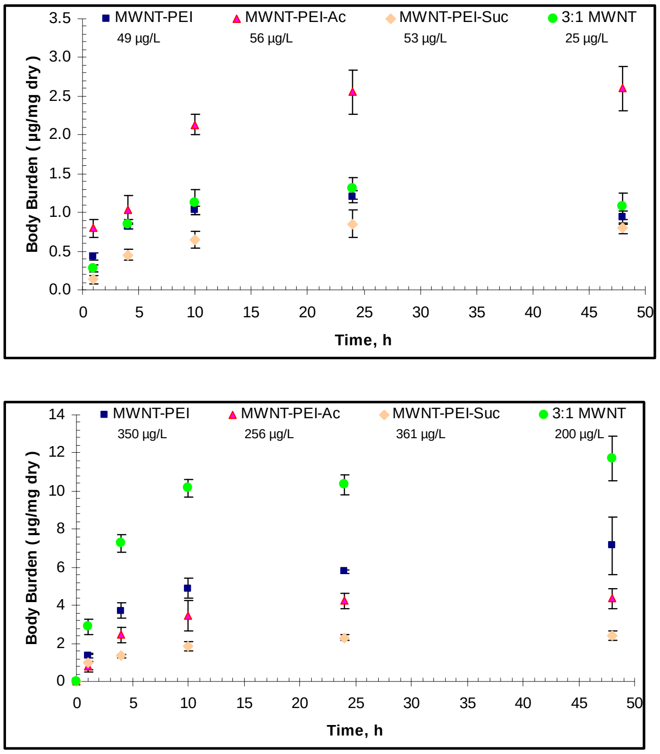

Figure 1: MWNT uptake by *Daphnia magna*. The aqueous concentrations ranged from 25 to 53 µg/L in the lower concentration range experiments (top) and from 200 to 361 µg/L in the highest levels tested (bottom) for 3:1 MWNTs, MWNT-PEI, MWNT-PEI-Ac, and MWNT-PEI-Suc. The initial concentrations vary based on a consistent radioactivity being used among the conditions. Mean and standard deviation values for the body burdens were calculated from triplicate samples. This data is reprinted with permission from [11].

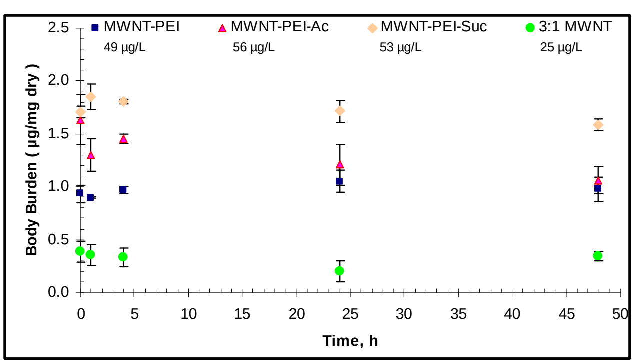

Figure 2a

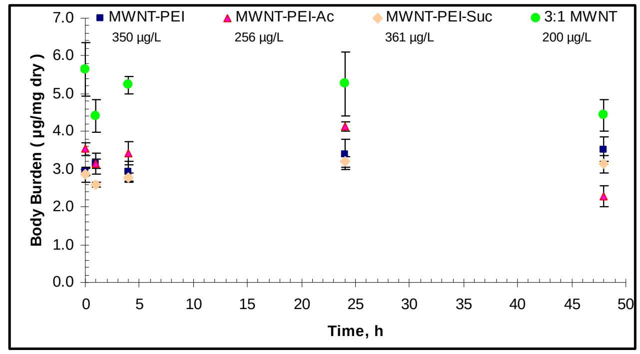

Figure 2b

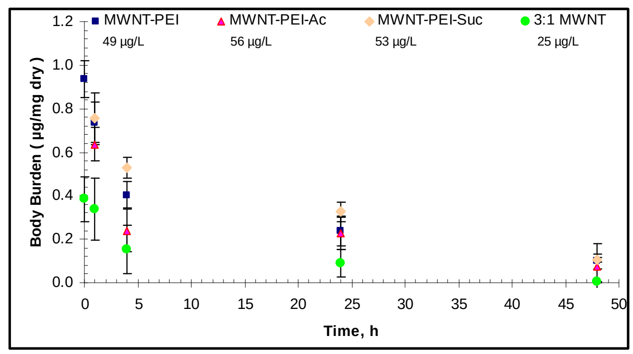

Figure 2c

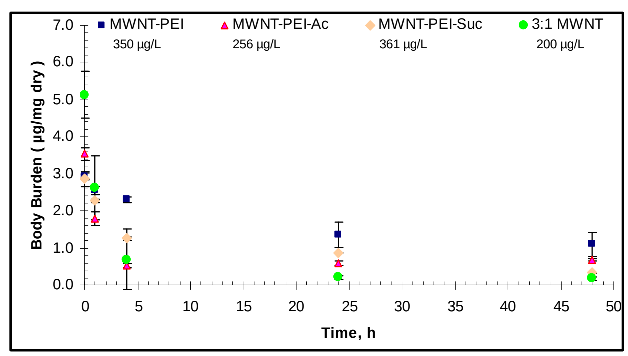

Figure 2d

Figure 2: MWNT elimination by *Daphnia magna*. The MWNT concentration for the 24 h uptake period before elimination was approximately 25 µg/L (a, c) or 250 µg/L (b, d) for 3:1 MWNTs, MWNT-PEI, MWNT-PEI-Ac, and MWNT-PEI-Suc. Elimination occurred either in the absence (a, b) or presence of algae (c, d). Mean and standard deviation values were calculated from triplicate samples. This data is reprinted with permission from [11].

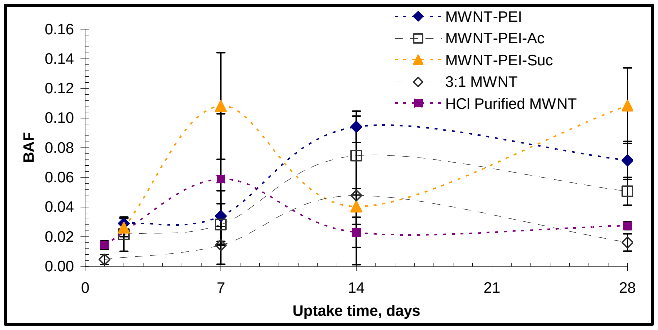

Figure 3a

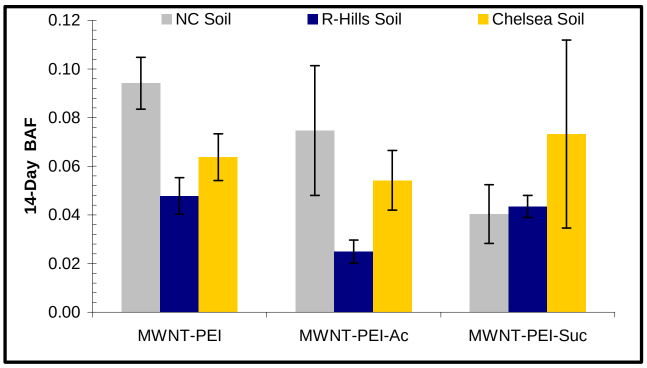

Figure 3b

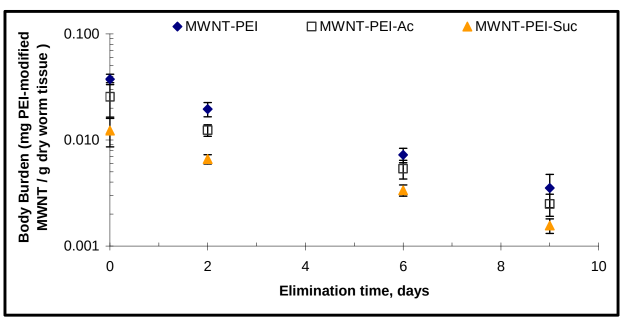

Figure 3c

Figure 3: Uptake and elimination of PEI-modified MWCNTs. (A) Bioaccumulation factors (BAFs = body concentration ÷ soil concentration); uptake by *E. foetida* of 0.3 mg/g HCl purified [6], 0.3 mg/ 3:1 MWCNTs [9], and 0.5 mg/g PEI-modified MWCNTs in NC soil systems. (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; body burdens indicate the concentration in the organism tissue. Note that the y-axis for part C used a logarithmic scale. Triplicate samples were tested for each data point in each figure, and error bars represent standard deviations of those measurements. This data is reprinted with permission from [12].

Overall, these results indicate that coating carbon nanotubes with PEI and giving them different surface charges does not appreciably impact their uptake behaviors by water fleas. For the uptake experiments with daphnia (see Figure 1), there were different trends in the uptake rates among the different types of MWNTs for the two different concentrations. This suggested that there was no definitive uptake trend for MWNTs without or with coatings with different surface charges. Similarly, surface coatings had no appreciable effect on the elimination behaviors of carbon nanotubes by the water fleas (see Figure 2). Daphnia required the presence of algae to enable elimination of the carbon nanotubes for each type of MWNT, and each type was eliminated at a first-order rate.

There also did not appear to be a trend in the uptake rates among the different types of MWNTs with regards to uptake by earthworms (see Figure 3 parts a and b). While there were larger bioaccumulation factor (BAF) values after 28 d for the PEI-coated MWNTs, these values had high uncertainties, thus limiting definitive comparisons among uptake of different types of MWNTs. However, the coatings did appear to impact MWNT elimination by earthworms (see Figure 3 part c). Earthworms did not appear to readily excrete MWNTs via first-order elimination kinetics in an earlier paper [6], but they did appear to eliminate the PEI-MWNTs in a manner that could be accurately modeled using a first-order elimination rate here [12], thus indicating that the PEI coating appeared to impact earthworm elimination.

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