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Multiwalled Carbon Nanotubes and C_{60} Fullerenes Differentially Impact the Accumulation of Weathered Pesticides in Four Agricultural Plants

Roberto De La Torre-Roche,[†] Joseph Hawthorne,[†] Yingqing Deng,[‡] Baoshan Xing,[‡] Wenjun Cai,[§] Lee A. Newman,[§] Qiang Wang,[∥] Xingmao Ma,[∥] Helmi Hamdi,[⊥] and Jason C. White*,[†]

[†]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, Connecticut 06504, United States

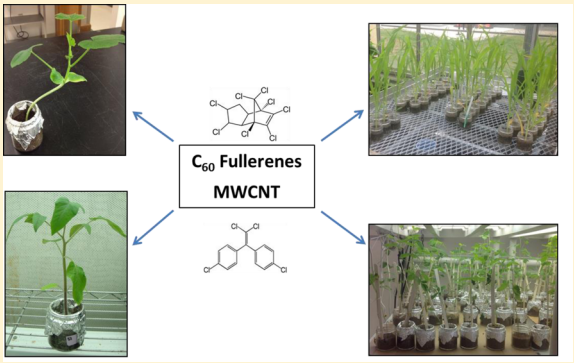
[‡]Stockbridge School of Agriculture, University of Massachusetts, 80 Campus Center Way, Amherst, Massachusetts 01003, United States

[§]Department of Environmental and Forest Biology, SUNY - College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, New York 13210, United States

[∥]Department of Civil and Environmental Engineering, Southern Illinois University Carbondale, Carbondale, Illinois 62901, United States

[⊥]Water Research and Technology Center, University of Carthage, P.O. Box 273, Soliman 8020, Tunisia

ABSTRACT: The effect of multiwalled carbon nanotubes (MWCNT) or C_{60} fullerenes on the uptake of weathered chlordane or DDx (DDT + metabolites) by *Cucurbita pepo* (zucchini), *Zea mays* (corn), *Solanum lycopersicum* (tomato), and *Glycine max* (soybean) was investigated. The plants were grown in 50 g of soil with weathered chlordane (2150 ng/g) and DDx (118 ng/g) that was amended with 0, 500, 1000, or 5000 mg/kg MWCNT or C_{60}. After 28 d, the root and shoot content of chlordane components and DDx was determined by GC-MS. Zucchini and tomato growth were unaffected by carbon nanomaterial coexposure, although C_{60} at 500 mg/kg reduced corn and soybean biomass by 36.5−45.0%. Total chlordane content ranged from 1490 (tomato) to 4780 (zucchini) ng; DDx amounts ranged from 77.8 (corn) to 395 ng (zucchini). MWCNT coexposure decreased chlordane and DDx accumulation 21−80% across all crops, depending on species and nanotube concentration. Conversely, C_{60} had species- and contaminant-specific effects on pesticide uptake, ranging from complete suppression of DDx uptake (corn/tomato) to 34.9% increases in chlordane accumulation (tomato/soybean). The data show that pesticide accumulation varies greatly with crop species and carbon nanomaterial type/concentration. These findings have implications for food safety and for the use of engineered nanomaterials in agriculture.



INTRODUCTION

The field of nanotechnology continues to grow rapidly, achieving a projected market value of $3 trillion by 2020.[1] This increase in research, development, and implementation is driven by the widely known phenomena of unique chemical and physical properties becoming evident at the nanometer scale, effectively differentiating NMs from their corresponding bulk products. Engineered nanomaterials are currently used in a wide variety of commercially available products, including medical devices, plastics, textiles, cosmetics, and electronics.[2−5] Although many of the unique properties and enhanced reactivity result from the much higher surface area to volume ratios, the ability to engineer specific properties onto or into these substances has also become common. With such widespread and increasing usage, the release of engineered nanomaterials into the environment is inevitable. In spite of this, there is general agreement in the scientific community that our understanding of nanomaterial fate and effects in the environment is inadequate. This, along with size-specific exposure/toxicity/risk concerns and the general lack of a regulatory framework for nanomaterials in consumer products, has given rise to the disciplines of nanotoxicology and econanotoxicology in an effort to address these critical research needs.[3,6−9]

One area of notable concern is the use of engineered NPs in agriculture and food production/processing. Nanoscale-products in agriculture are generally designed to (i) obtain agricultural products more quickly and with high yield, which subsequently will lessen the use of water and energy, and (ii)

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produce less waste. This overall goal is to provide more benign, efficient, cost-effective, and sustainable agricultural practices and production. [10] However, the general lack of understanding regarding nanomaterial fate and effects in agricultural systems is troublesome given the potential for food chain contamination and for an uncharacterized pathway of human exposure.[4,11−13] Carbon nanomaterials have been the focus of much interest, largely because of unique physical and chemical properties that enable synthesis and manipulation to a degree not yet matched by inorganic nanostructures.[6,14] Miralles et al.[15] demonstrated that MWCNTs enhance alfalfa and wheat germination and root elongation, but that particle uptake and translocation was insignificant. Khodakovskaya and co-workers demonstrated that MWCNTs can increase the growth of tobacco cells and tomato plants by affecting expression genes that are essential for cell division and plant development.[13,16,17] However, a number of other investigators have demonstrated toxicity of carbon nanomaterials to a range of plant species[12,18−20]

Agricultural systems are the recipients of a number of organic chemicals and inorganic amendments, and the interactions of engineered nanomaterials with these constituents is relatively unknown. The high surface area and hydrophobic nature of carbon nanomaterials make interactions with other organic and inorganic substances likely.[21,22] Potential interactions could be complex, including both food safety concerns through potentially increased contaminant accumulation and economic issues through reduced pesticide efficacy. For example, Ma and Wang[23] found that C_{60} increased the uptake of trichloroethylene by 82% in cottonwood (*Populus deltoides*). Similarly, De La Torre-Roche et al.[24] observed that C_{60} exposure significantly increased the accumulation of freshly added DDE in vermiculate by zucchini, tomato, and soybean.[24] Conversely, Kelsey and White[25] found that C_{60} exposure had minimal impact on the accumulation of weathered DDE in soil by pumpkin and earthworms. The goal of the current study was to assess the impact of MWCNTs and C_{60} fullerenes over a broad concentration range on the uptake and translocation of weathered chlordane and DDx (DDT + metabolites) from soil by zucchini, corn, tomato, and soybean.

EXPERIMENTAL SECTION

Chemicals and Reagents. Standards of *o,p′*-DDE, *p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, *cis*-chlordane, *trans*-chlordane, and *trans*-nonachlor were acquired from the Environmental Protection Agency (EPA) National Pesticide Standard Repository (Fort Meade, MD) or from Chem Service, Inc. (West Chester, PA). Multiwalled carbon nanotubes (MWCNT; 95% purity, 13−18 nm o.d., 5−10 nm i.d.; 10−30 μm length) and C_{60} fullerenes (99.0% purity) were obtained from Cheaptubes (Brattleboro, VT). To enable physical characterization of the materials, suspensions of each carbon nanomaterial in 10% Hoagland's solution at 500−5000 mg/L (see below) were prepared, shaken vigorously, and allowed to settle overnight. A portion of the supernatant was then analyzed for particle size and zeta potential (Malvern, Nano Series ZS90). The average particle size and zeta potential of C_{60} fullerenes in solution at 500−5000 mg/L ranged from 1450 to 1900 nm and −16.2 to −19.5 mV, respectively. The average particle size and zeta potential of MWCNT in solution at 500−1000 mg/L ranged from 3500 to 3900 nm and −15.1 to −16.0 mV, respectively; values at 5000 mg/L were 17 700 nm and −7.73 mV, respectively.

Soil and Plants. A sandy loam (69.4% sand, 22.0% silt, 8.6% clay; 4.3% organic matter; pH 5.9 [water]; cation exchange capacity 18.6 cmol/kg), containing weathered chlordane and DDx pesticide residues resulting from historical application, was collected from the Connecticut Agricultural Experiment Station (CAES).[26,27] The soil was air-dried for 2 d and sieved to 2 mm for homogeneity and to remove nonsoil debris. Because of the well-known but unpredictable breakdown of *p,p′*-DDT to *p,p′*-DDE/DDD in the GC inlet, total DDx was calculated as the sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE. The concentration of weathered *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, and DDx in the soil were 752 μg/kg (±27.9), 927 (±37.4) μg/kg, 445 μg/kg (±20.5), and 141 (±7.3) μg/kg, respectively. Seeds of *Cucurbita pepo* L. (zucchini; cv Costata Romanesco), *Glycine max* L. (soybean), *Solanum lycopersicum* L. (tomato, cv Brandywine), and *Zea mays* L. (corn, cv Golden Jubilee Hybrid) were obtained from Johnny's Selected Seeds (Albion, ME), the Southern Illinois University College of Agriculture, Park Seed (Greenwood, SC), and Ferry-Morse Seed Co. (Fulton, KY) respectively. Seeds were pregerminated in pro-mix potting soil or vermiculite for 3−7 days, depending on plant species, prior to transplant to soil for the exposure assay.

Exposure Assay. Clear 125-mL jars (Fisher Scientific, Pittsburgh PA) were amended with 50 g of dry soil mixed with 20 mL of vermiculite (to prevent soil compaction). Twenty five, 50, or 250 mg of MWCNTs or C_{60} were added to the soil replicates as a dry nanopowder to achieve initial exposure concentrations of 500, 1000, and 5000 mg/kg, respectively. This concentration may be high, although technologies for quantifying carbon nanomaterials in natural solids are not readily available, but will permit comparison to previous work.[24] In addition, high dose exposure may occur through accidental discharge or multiyear accumulation in the soil. Control replicates were prepared in the absence of carbon nanomaterials. The jars were capped and shaken vigorously to ensure thorough mixing of contents. Hoagland's solution (10%) was then added to each jar to achieve 50% of soil water capacity. A single 3- to 7-d-old pregerminated seedling was gently added to each jar; a total of 15 replicates were prepared for each treatment, including control. The plants, depending on the institution conducting the experiment, were incubated in a growth room or greenhouse under ambient light and temperature. Replicate jars were top watered as needed with 10% Hoagland's solution (to avoid depletion of nutrients from the soil) according to the above treatments for a 28-d growth period.

Soil Extraction and Pesticide Quantitation. Twelve replicate 3-g soil samples were weighed into 35-mL glass vials with PTFE-faced silicone rubber septa. Each replicate was amended with 15 mL of *n*-hexanes (95% *n*-hexanes, Ultra Resi-Analyzed, Brand-Nu laboratories, Meriden, CT) and 100 μL of *o,p′*- DDE (from a 10 μg/mL solution) as an internal standard. The vials were then placed on a hot plate block digester (SCP Science, Champlain, NY) at 65 °C for 4 h. After the samples were cooled, a 1-mL portion was transferred to an auto sampler vial, and stored at −4 °C until GC-MS analysis.

Vegetation Extraction. At harvest, replicate plants were separated into roots, stems, and leaves; the mass of each tissue was determined. Roots were rinsed in tap water and twice with distilled water to remove particulates before weighing. To quantify chlordane and DDx content, replicate tissues were extracted by the QuEChERS method (De La Torre et al.[28]).

Briefly, up to 15 g (wet mass) of tissue was added to a 50-mL centrifuge tube containing 15 mL of acetonitrile and 30 μL of *o,p′*- DDE (from a 10 μg/mL solution) was added as an internal standard. The tubes were placed on a wrist-action shaker for 10 min. Six grams of magnesium sulfate and 1.5 g of sodium acetate were added; the tubes were shaken for 30 min and were then centrifuged at 3000 rpm for 10 min. Separate 15-mL plastic centrifuge tubes were amended with 1.5 g of magnesium sulfate and 0.5 mg of primary secondary amine (PSA). Two mL of toluene was added to wet the powders and 10 mL of sample extract was transferred to each tube. The tubes were shaken by hand for 30 s and centrifuged at 3000 rpm for 10 min. Six mL of each extract was concentrated to 1 mL under nitrogen. The samples were transferred to chromatography vials for storage at −4 °C until analysis.

Chemical Analysis. A 1000 mg/L stock of DDx analytes, *cis*-chlordane, *trans*-chlordane, and *trans*-nonachlor in toluene was diluted to prepare calibration standards of 5−2000 ng/mL. Each calibration level was amended with 100 ng/mL *o,p′*-DDE (a DDT metabolite not detected in the soil) as an internal standard. The concentration of chlordanes and DDx in the vegetation was determined on an Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a 5973 mass selective detector (MSD). Two microliters of sample were injected into a hot splitless inlet in pulse analog mode at 250 °C with He as the carrier gas and then onto a RTX-5MS 30-m column with 0.25 mm ID (Restek, Bellefonte, PA). The GC oven initial temperature was 150 °C for 1 min, then ramped to 180 °C at a rate of 300 °C/min, and then ramped at a rate of 5 °C/min to a final temperature of 280 °C which was held for 8 min. After a 4-min solvent delay, the MSD detected analytes using scan mode at a mass to charge ratio (*m*/*z*) 70−430. For tissues where organochlorine residues were not detected, additional analysis was conducted on the more sensitive Agilent (Avondale, PA, USA) 6890 GC with a ^{63}Ni μ-electron capture detector (ECD). An RTX-5MS 30-m column with a 0.25 mm ID (Restek, Bellefonte, PA) was used with the following oven program: 100 °C initial temperature ramped at 20 °C/min to 200 °C, then ramped at 2.5 °C/min to 230 °C, then ramped at 25 °C/min to 280 °C for a hold time of 6.0 min. The injection port was maintained at 250 °C and a 1-μL pulse-splitless injection was used. The carrier gas over the column was hydrogen. The low level calibrations standards by GC-MS and GC-ECD analysis were 50 and 10 ng/mL, respectively.

Statistical Analysis. At harvest, organochlorine content was determined separately in the roots, stem, and leaves of each plant species as a function of nanomaterial treatment (type, concentration). To achieve sufficient biomass for extraction, random replicates were composited; a total of 7 replicates per treatment were extracted and analyzed. All values of chlordanes or DDx content in a given tissue are expressed on a wet weight basis. The total plant amount for a given contaminant was calculated as the sum of the individual tissue amounts. The limit of quantitation (LOQ) was determined by averaging the instrumental response of the lowest calibration standards in a given GC sequence. Samples with instrumental response less than this LOQ value were listed as a nondetect. A one-way ANOVA on ranks with Tukey's multiple comparison test was used to determine the statistical significance of differences in contaminant content and biomass among treatments.

RESULTS AND DISCUSSION

Effect of Carbon Nanomaterials on Plant Mass. After 28 d, total zucchini wet biomass at harvest did not differ significantly from unamended controls as a function of MWCNTs or C_{60} fullerenes treatment (Table 1). Similarly, zucchini net growth (final minus initial seedling mass) was unaffected by carbon nanomaterial exposure and ranged 6.0−6.9 g in the MWCNT experiment and 4.0−5.0 g in the C_{60} fullerenes trial. The growth of tomato was also largely unaffected by carbon nanomaterial exposure, with total plant biomass ranging 7.3−7.7 g and a net growth of 5.8−6.8 g. Only C_{60} fullerenes at 1000 mg/kg negatively impacted tomato growth, with a 10.7% reduction in overall biomass relative to controls and an 8.66% reduction in net growth. Corn achieved a net growth of 6.3−8.1 g across all MWCNT treatments, with a total biomass of 7.7−9.2 g. Interestingly, 5000 mg/kg MWCNTs increased total corn biomass and net growth by 15.0% and 24.6%, respectively. Notably, exposure of corn to C_{60} fullerenes had an entirely opposite effect (Table 1); reductions in biomass and net growth relative to controls were 44.6 and 53.9%, respectively. Soybean was particularly sensitive to carbon nanomaterial exposure, experiencing significant phytotoxicity under both MWCNT and C_{60} fullerene amendment. MWCNT exposure at 1000−5000 mg/kg reduced biomass by 19.2−26.9% and net growth by 29.8−31.9%. C_{60} fullerenes at all concentrations reduced soybean biomass and net growth by 25.0−40.4% and 27.7−42.6%, respectively.

Table 1. Impact of MWCNT (Top) or C_{60} Fullerene (Bottom) Exposure on Total Plant Wet Biomass[a]

MWCNT	zucchini		corn		tomato		soybean	
control	8.5	A	8.0	A	7.5	A	5.2	A
500 mg/kg	7.7	A	8.0	A	7.7	A	5.0	A
1000 mg/kg	8.8	A	7.7	A	7.3	A	4.2	B
5000 mg/kg	8.4	A	9.2	B	7.3	A	3.8	B
Fullerenes	**zucchini**		**corn**		**tomato**		**soybean**	
control	5.4	A	8.0	A	7.5	A	5.2	A
500 mg/kg	5.7	A	4.4	B	7.2	A	3.3	B
1000 mg/kg	6.3	A	4.9	B	6.7	B	3.1	B
5000 mg/kg	5.9	A	4.7	B	7.6	A	3.9	B

[a]Each value is the average of 15 replicates. Within a plant and nanomaterial type (column), values followed by different letters are significantly different (one-way ANOVA on ranks with a Tukey multiple comparison test).

The literature on the phytotoxicity of carbon nanomaterials is not robust; published data are rather conflicted, with observed effects often varying considerably with plant species, growing conditions, particle type, and concentration. Studies with tomato grown in MWCNT-amended agar medium revealed enhanced germination rates and growth, presumably by affecting the expression of genes that facilitate cell division and development.[17] Alfalfa and wheat experienced enhanced germination and root elongation under 2560 mg/kg of MWCNT exposure.[15] Similarly, MWCNTs induced growth enhancement in tobacco cells.[16] Alternatively, Ma and Wang[23] reported that 2−15 mg/L fullerenes had no impact on cottonwood growth. These findings are in agreement with the results of De la Torre-Roche et al.[24] and Hawthorne et al.,[29]

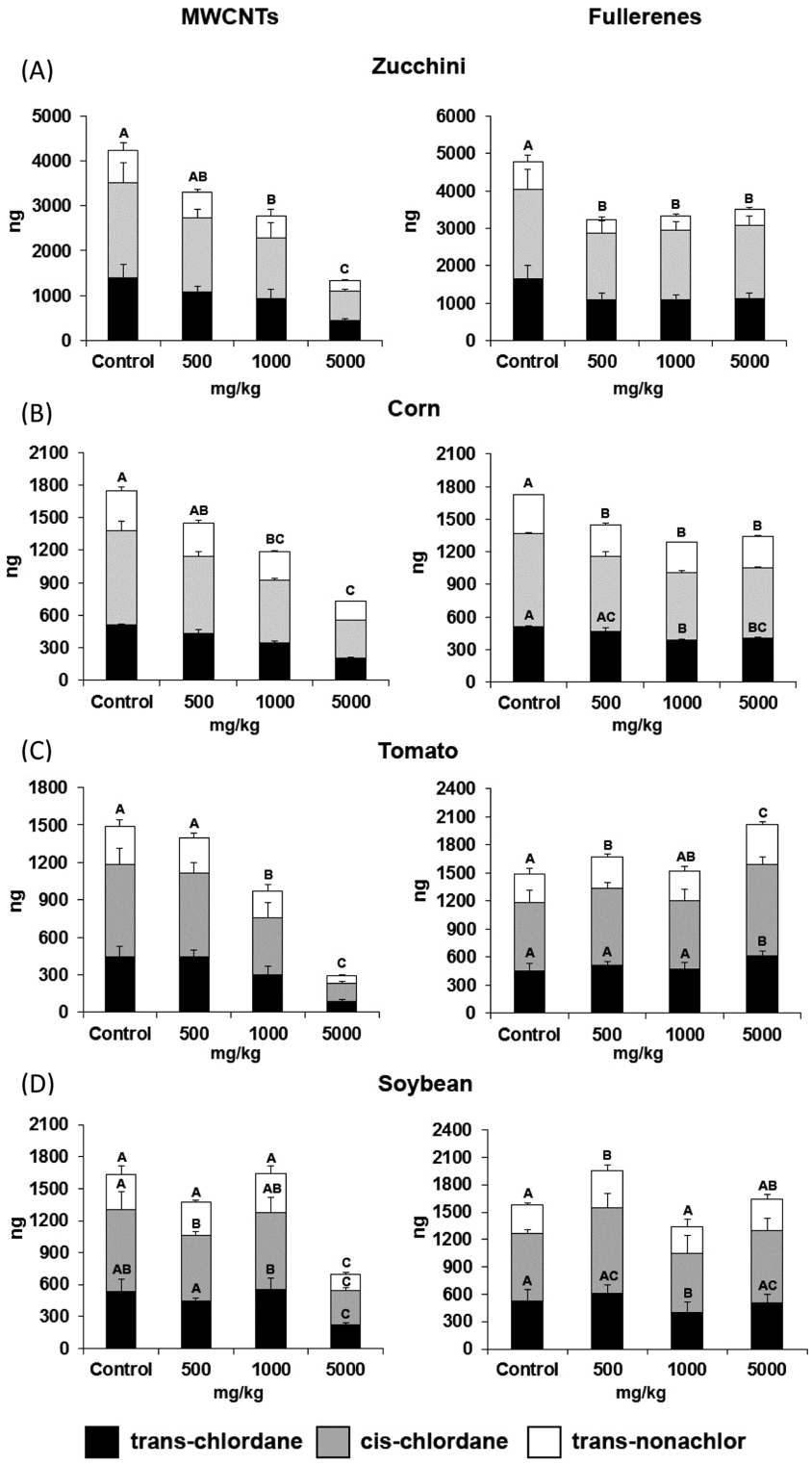

Figure 1. Total plant content of chlordane components in soil-grown zucchini, corn, tomato, and soybean coexposed to 0−5000 mg/kg MWCNT or C_{60} fullerene. Error bars are the standard error. Within a plant species and nanomaterial type, chlordane components with different letters are significantly different. If no letter is shown for a chlordane component, differences are of equivalent significance to those shown above the bar for total chlordane.

where no toxicity was reported upon exposure to C_{60} under hydroponic and vermiculite-based conditions. Conversely, others have reported MWCNT toxicity to *Arabidopsis* and rice cells.[30,31] Lin et al.[19] found that MWCNTs and C_{70} delayed rice flowering by up to 1 month; an effect attributed to the likely interference of carbon nanomaterials with nutrient and water uptake. Stampoulis et al.[18] observed a 30% reduction in zucchini biomass upon exposure to 1000 mg/L MWCNT under hydroponic conditions. These findings demonstrate the complex interactions of carbon nanomaterials with terrestrial plant species and highlight the need for further investigation.

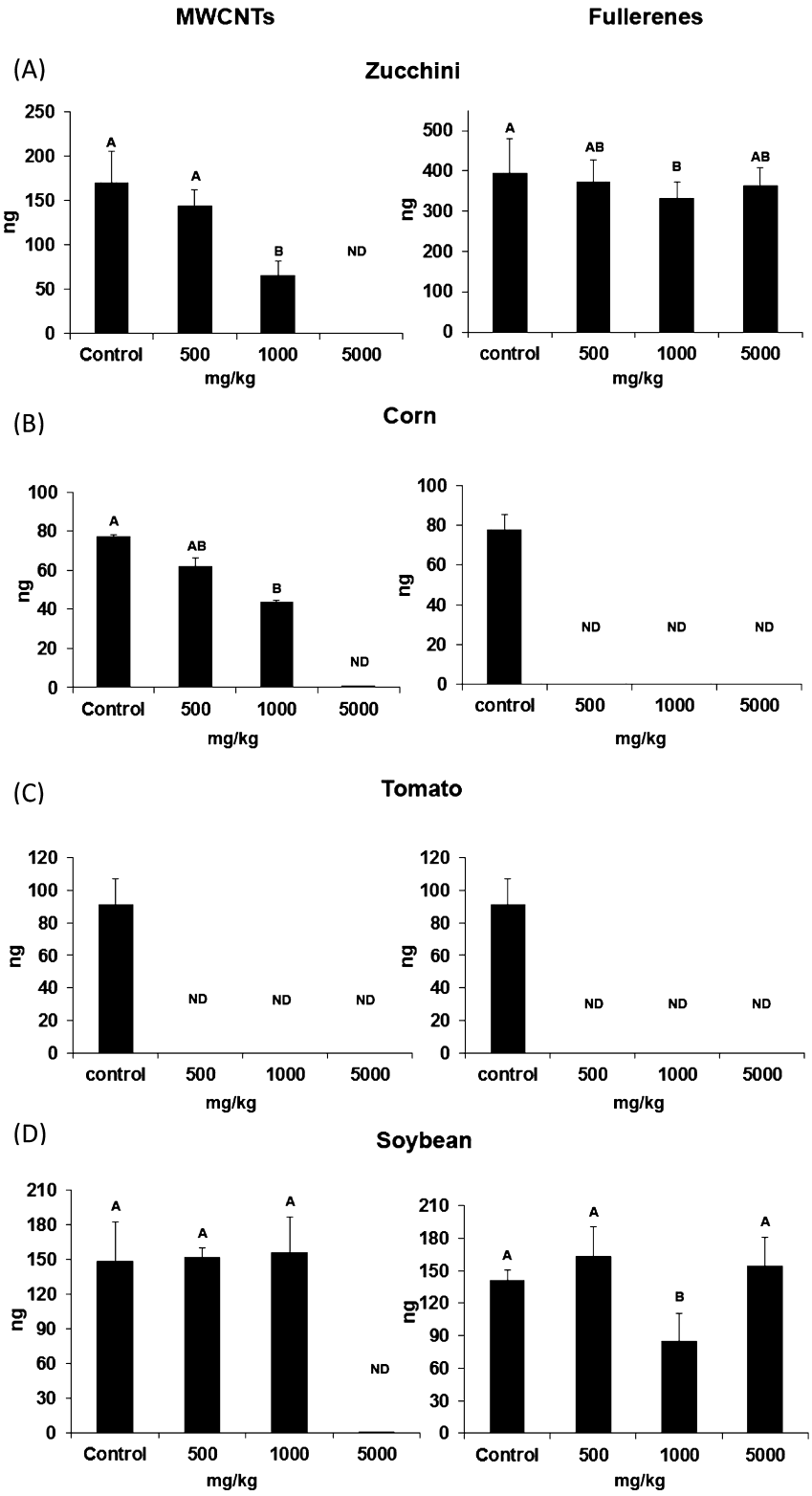

Figure 2. Total plant content of DDx (DDT + metabolites) in soil-grown zucchini, corn, tomato, and soybean coexposed to 0−5000 mg/kg MWCNT or C_{60} fullerene. Error bars are the standard error. Within a plant species and nanomaterial type, DDx with different letters are significantly different.

Effect of Carbon Nanomaterials on Pesticide Residue Content. For all plants, *cis*-chlordane was accumulated in the greatest amount, ranging from 737 (corn) to 2110 ng (zucchini). *Trans*-chlordane levels ranged from 448 (tomato) to 1410 ng (zucchini) and *trans*-nonachlor content ranged from 305 (tomato) to 714 ng (zucchini). The levels of the individual chlordane components in the plant tissues correlated well with the detected amounts in soil, indicating that residues did not selectively translocate. Total DDx content ranged from 77.8 (corn) to 169 ng (zucchini). These *in planta* values are in line

with the soil chlordane data, where *cis*-chlordane, *trans*-chlordane, and *trans*-nonachlor comprise 43.7, 35.4, and 21.0% of the total 2120 ng/g chlordane content, respectively. Total DDx levels in the soil are much lower at 141 ng/g. For zucchini, the chlordane and DDx content represents both root and shoot contaminant values; for the other species, detectable residues were found only in the root tissues. As mentioned above, corn and soybean growth were negatively impacted by carbon nanomaterial exposure; consequently, pesticide residue data are mass normalized to the control biomass for each plant species so as to enable direct comparison across plant type. The effects of carbon nanomaterials on pesticide residue uptake by the different plant species are shown in Figures 1 and 2; in general, MWCNT coexposure decreased accumulation of pesticide residues by crop species, whereas the effects of C_{60} fullerenes were more variable.

Multiwalled Carbon Nanotubes (MWCNT). The root contents of Σ-chlordane and DDx in the control zucchini were 2930 and 128 ng, respectively; levels in the shoot tissue were 1300 and 39 ng, respectively. Exposure to MWCNT significantly decreased organochlorine accumulation by zucchini; 500 mg/kg MWCNT resulted in a 21.7% decrease in Σ-chlordane in the total plant (Figure 1a). Reductions in contaminant uptake at 1000 and 5000 mg/kg were 33.7 and 68.3%, respectively. A similar trend was evident in DDx uptake upon MWCNT exposure (Figure 2a), although reductions at 500 mg/kg were nonsignificant, and at 5000 mg/kg DDX was not detectable in the plant tissues.

The root/total plant contents of Σ-chlordane and DDx in the control corn were 1750 and 77.8 ng, respectively. Similar to zucchini, MWCNT coexposure significantly decreased pesticide accumulation; 1000 and 5000 mg/kg MWCNT resulted in a 30.1 and 57.0% decreases in Σ-chlordane in plants (Figure 1b). Mass normalized DDx uptake in corn roots upon MWCNT at 1000 mg/kg exposure was reduced by 43%, and at 5000 mg/kg pesticide levels in the plant were not detectable (Figure 2b). The root/total plant contents of Σ-chlordane and DDx in the control tomato were 1490 and 91.0 ng, respectively. Similar to zucchini and corn, MWCNT coexposure at 500 mg/kg resulted in nonsignificant reductions in pesticide residue accumulation, but 1000 and 5000 mg/kg resulted in 30.3 and 80.0% decreases in Σ-chlordane content (Figure 1c). DDx uptake into tomato was completely suppressed by MWCNT exposure, regardless of nanotube concentration (Figure 2c). The mass normalized root/plant contents of Σ-chlordane and DDx in the control soybean were 1640 and 147 ng, respectively. Unlike other plants, MWCNT coexposure at 500−1000 mg/kg had no effect on Σ-chlordane or DDx accumulation. However, similar to all other species, 5000 mg/kg resulted in a 53.7% decrease in Σ-chlordane content and completely suppressed DDX uptake (Figures 1d and 2d).

C_{60} Fullerenes. The impact of C_{60} fullerenes on the uptake of weathered organochlorine residues by the different plant species is also shown in Figures 1 and 2. Unlike MWCNT coexposure, fullerenes had variable and inconsistent effects. The root contents of Σ-chlordane and DDx in the control zucchini were 2583 and 188 ng, respectively; levels in the shoot tissue were 2210 and 203 ng, respectively. Co-exposure to C_{60} fullerenes significantly decreased organochlorine accumulation by zucchini, although not in a concentration-dependent manner; 500, 1000, and 5000 mg/kg C_{60} resulted in 37.0, 35.0, and 30.7% decreases in total plant Σ-chlordane content (Figure 1e). DDx uptake by zucchini was unaffected by C_{60} exposure at 500 and 5000 mg/kg; a slight, yet statistically significant, decrease of 15% was observed at 1000 mg/kg fullerenes (Figure 2e).

The MWCNT and C_{60} fullerenes experiments for both corn and tomato were conducted simultaneously and hence have the same controls within species but across nanomaterial; the corn root/total plant contents (mass normalized) of Σ-chlordane and DDx in the unamended corn was 1,750 and 77.8 ng, respectively. Similar to zucchini, C_{60} fullerene coexposure significantly decreased organochlorine accumulation but in a nonconcentration-dependent fashion; 500, 1000, and 5000 mg/kg C_{60} resulted in 15.7, 25.3, and 21.7% decreases in total plant Σ-chlordane levels (Figure 1f). Fullerene coexposure completed suppressed DDx uptake in corn roots, regardless of concentration (Figure 2f). As described in the MWNCT results above, the root/total plant contents of Σ-chlordane and DDx in the control tomato were 1490 and 91.0 ng, respectively. Fullerene exposure at 1000 mg/kg had no effect on Σ-chlordane levels; C_{60} at 500 and 5000 mg/g significantly *increased* tomato Σ-chlordane content by 12.3 and 34.9%, respectively (Figure 1g). Similar to corn, C_{60} fullerene coexposure completely suppressed DDx uptake by tomato (Figure 2g). The mass normalized root/plant contents of Σ-chlordane and DDx in the control soybean were 1581 and 141 ng, respectively. Fullerenes at 1000 and 5000 mg/kg had no effect on Σ-chlordane accumulation by soybean (Figure 1h). Similarly, 500 and 5000 mg/kg C_{60} had no impact on DDx uptake, but 1000 mg/kg resulted in a 39.5% decrease in contaminant content (Figure 2h).

Zucchini accumulation of chlordane components and DDX was 22.2−77.9% greater than other species and, notably, was the only species investigated with measurable organochlorine levels in the shoot tissue. These observations of differential organic contaminant accumulation as a function of plant species are in agreement with literature, where several studies have shown the unique capability of *Cucurbita pepo* to accumulate and translocate weathered pesticides under a range of growth conditions.[32−34] Notably, the mechanisms responsible for this ability are unknown. Interestingly, the DDx content of soybean roots was significantly higher than that of zucchini roots, although contaminant translocation in soybean was negligible.

The finding that MWCNT amendment consistently, and often in a concentration-dependent fashion, decreased pesticide bioavailability is not entirely surprising given the high sorption capacity of MWCNTs for polar and hydrophobic organic compounds (HOCs).[35−39] However, the data are somewhat mixed here as Chen et al.[40] showed that humic acid reduced the SWNTs absorption affinities of three nonionic compounds likely by mechanisms of molecular sieving, pore blockage, and competitive adsorption by humic substances. Notably, very little work has been done investigating the role of MWCNT−HOC interactions on chemical uptake and toxicity to biota; these reactions are potentially complex, with hypothesized mechanisms of reduced or enhanced bioavailability dependent on potential uptake of the MWCNT/contaminant complex. Miralles et al.[15] found that CNTs were absorbed onto the root surface of alfalfa and wheat, but uptake and translocation was not detected. Similar results were observed in rice, with the authors suggesting that the poor translocation was due to the large dimensional length of the material.[19] Conversely, MWCNTs were detected inside root and leaves from red spinach, wheat, and rapeseed.[41,42] In a hydroponic nanomaterial−contaminant interaction study, MWCNTs were ob-

served to pierce wheat root cell walls and enhance the transport of phenanthrene into the cellular cytoplasm.[43] Moreover, a study performed in MWCNT-amended soil revealed the presence of nanotubes in tomato roots and shoot tissues;[13,17] however, germination was directly in the MWCNT-media, somewhat confounding a determination of root to shoot translocation.

Several studies have shown that dissolved organic matter and specifically, humic acid, may interact with carbon nanomaterials in such a way as to promote dispersion and mobility in natural media.[35,44−46] One could predict such effects would maximize transfer of weathered organic contaminants from soil/solid sorption sites to a more available and mobile nanomaterial phase, effectively increasing the likelihood of physical interaction with, or contaminant transfer to, biota.[24] For example, Shen et al.[47] observed that polycyclic aromatic hydrocarbon (PAH) uptake by *Chironomus plumosus* from sediments was reduced at lower MWCNT amendment levels (less than 1.5%) but was actually increased at higher nanotube concentrations. Conversely, Xia et al.[48] showed that MWCNTs and other carbons consistently decreased perfluorochemical uptake by *C. plumosus*, with the magnitude of the decrease being a function of carbon type and surface area. Similarly, Petersen et al.[49] noted that MWCNTs decreased pyrene bioaccumulation from soil by earthworms in a concentration-dependent fashion; an effect described by the authors as similar to most "hard" carbons. Similarly, single-walled carbon nanotube (SWCNT) amendment to sediments decreased the availability of HOCs to *Streblospio benedicti*.[50] Although there have been no published studies involving MWCNT-amendment effects on contaminant fate with plants, our data showing consistent lower organochlorine content clearly suggest little nanotube availability/uptake and mobility.

In contrast to MWCNTs, C_{60} fullerene effects on pesticide uptake were far less consistent. Although Σ chlordane uptake was reduced upon amendment with zucchini and corn, effects were modest relative to MWCNTs and were not concentration dependent. In addition, DDx uptake patterns were completely different, with zucchini levels being largely unaffected by C_{60} but contaminant accumulation in corn being completely suppressed. Importantly, Σ chlordane accumulation in soybean and tomato was either unaffected by C_{60} presence or actually significantly increased. Fullerenes, with high surface area and sorption potential, can interact with organic chemicals in ways quite similar to that described for MWCNT. It has been noted that stable aqueous fullerenes (nC_{60}) have great mobility and can significantly enhance the transport of HOCs through aqueous and solid environments by serving as a contaminant carrier.[22,51,52] Similarly to MWCNTs, humic acid can significantly promote fullerene dispersion, mobility, and HOC-enabled transport by preventing aggregation and reducing particle size.[21,52−54] Zhang et al.[22] found that nC_{60} facilitated the transport of a polychlorinated biphenyl (PCB) and phenanthrene through two sandy soil columns. Wang et al.[51] observed that the enhanced PCB mobility in the presence of nC_{60} in soil varied with synthesis conditions but was due to strong hysteresis that effectively prevented HOCs release or desorption. Wang et al.[21] also found that although modified NOM−nC_{60} have mobility similar to unmodified nanoparticles, their contaminant-mobilizing capabilities are significantly greater.

Unlike MWCNT, several studies have investigated the effects of fullerenes on contaminant toxicity and accumulation on a number of species. Park et al.[55] and Fang et al.[56] observed that the uptake of several organic compounds to invertebrate and vertebrate (zebra fish) species was significantly reduced in the presence of C_{60} fullerenes. However, Brausch et al.[57] reported that functionalized fullerenes significantly increased bifenthrin acute toxicity to *D. magna*. Alternatively, several groups have noted that the impact of fullerenes on organic contaminant toxicity to aquatic species was dependent on the organic chemical, with both increased and decreased toxicity and availability reported[58,59] Unfortunately, few studies on nanomaterial/cocontaminant interactions have focused on terrestrial plant species. Ma and Wang[23] showed that although hydroponic poplar (*Populus deltoides*) was unaffected physiologically by fullerene exposure, the carbon nanomaterial did increase the uptake of trichloroethylene by 26−82%. De la Torre-Roche et al.[24] showed that in DDE-amended vermiculite, C_{60} fullerenes amendment at 1000 mg/kg could significantly increase the root, and in some cases shoot, organochlorine content in zucchini, soybean, and tomato.[24] Importantly, liquid chromatography−mass spectrometry confirmed the presence of C_{60} in the roots of all species and the stems of zucchini. Similarly, Lin et al.[19] demonstrated the uptake and translocation (root to leaves) of NOM−C_{70} by rice plants. Chen et al. revealed that NOM−C_{70} particles crossed the plant cell membrane by both partitioning and endocytosis, but that while $C_{60}(OH)_{20}$ readily diffused through the plant cell, the fullerol was excluded by the plasma membrane. Last, in a soil-based study by Kelsey and White, the uptake of weathered DDE residues by pumpkin and earthworm species was largely unaffected by the presence of C_{60}.[25]

It is also worth noting that, in some cases, chlordane and DDx were differentially impacted by C_{60} coexposure. For example, in corn and tomato, C_{60} exposure induced modest reductions in chlordane accumulation but DDx uptake was reduced to nondetectable levels at all fullerene concentrations. This differential impact as a function of pesticide type could be the result of a number of factors, including the more than 10-fold difference in soil concentration. However, Mattina et al. did also note significant differences in the uptake of these two residues by cucurbits and speculated that molecular configuration may result in unique interactions with key membrane transport proteins that impact pollutant accumulation.[25] Conversely, the effects of C_{60} on pesticide uptake by soybean and zucchini, as well as MWCNT for all four plants, followed similar trends across both pesticides.

This research demonstrates that the bioaccumulation of weathered chlordane and DDx, well-known nondegradable, persistent, and estrogenic pollutants, by four food crops was suppressed in a dose-dependent fashion by the presence of MWCNTs. Conversely, the effects of C_{60} on pesticide bioaccumulation were species-specific. C_{60} inhibited the uptake of contaminants in zucchini and corn, but the addition of higher concentration of nanoparticles did not further decrease bioaccumulation. Moreover, for soybean and tomato, pesticide bioaccumulation was largely unaffected by the presence of C_{60}, and in some treatments uptake was increased by the nanomaterial. The increasing use of carbon nanomaterials in commercially available products such as agrichemicals (pesticides, fertilizers) has resulted in NM release into the environment, including agricultural systems. However, accurate exposure and risk assessment has been confounded by a poor understanding of NM fate and effects in the environment, including potentially significant interactions with organic and

inorganic cocontaminants. The current study demonstrates that the accumulation of weathered organochlorine pesticide residues varies greatly with both carbon nanomaterial type and concentration, as well as with crop species. The mechanisms responsible for these phenomena and the potential implications for food safety are a topic of ongoing intense investigation.

AUTHOR INFORMATION

Corresponding Author

*Phone: 203-974-8523; fax: 203-974-8502; e-mail: Jason.White@ct.gov.

Notes

The authors declare no competing financial interest.

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