Environmental Science & Technology

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Fullerene-Enhanced Accumulation of *p,p′*-DDE in Agricultural Crop Species

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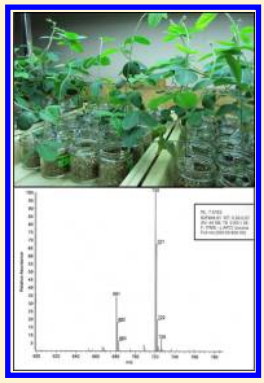
Supporting Information

ABSTRACT: The effect of C_{60} fullerene exposure on the accumulation of dichlorodiphenyldichloroethylene (*p,p′*-DDE; DDT metabolite) by *Cucurbita pepo* L. (zucchini), *Glycine max* L. (soybean), and *Solanum lycopersicum* L. (tomato) was determined. The plants were grown in 125 mL jars of vermiculite amended with 0 or 40 mg of C_{60} fullerenes. Prior to planting, the jars were amended with 40 mL solution containing 100 ng/mL of *p,p′*-DDE with 0 or 100 mg/L humic acid. During three weeks of growth, plants were watered with the same *p,p′*-DDE containing solutions. Total shoot *p,p′*-DDE levels in nonfullerene exposed tomato, soybean, and zucchini were 26.9, 131, and 675 ng, respectively; total root DDE content for the three plants was 402, 5970, and 5830 ng, respectively. Fullerenes increased the shoot *p,p′*-DDE content of zucchini by 29%; contaminant levels in soybean shoots were decreased by 48% but tomato shoot content was unaffected. The root and total plant *p,p′*-DDE content of all three species was significantly increased by fullerene exposure; enhanced contaminant uptake ranged from 30 to 65%. Humic acid, regardless of fullerene presence or plant type, significantly decreased the *p,p′*-DDE uptake. Fullerenes were detected in the roots of all plants but were not detected in plant shoots in the initial study. In a follow up study with zucchini designed to maximize biomass for extraction, over half the analyzed stems contained fullerenes at 60.5 to 4490 ng/g. These findings show that the carbon-based nanomaterials may significantly alter the accumulation and potentially the toxicity of cocontaminants in agricultural systems.



INTRODUCTION

The development and utilization of engineered nanomaterials (NMs), substances with at least one dimension less than 100 nm, has increased dramatically in the past decade. As an industry, nanotechnology is projected to reach $1 trillion by 2015.[1] This increased interest is driven by the unique chemical and physical properties that become evident at the nanometer scale and differentiate NMs from their corresponding bulk materials.[2] Many of these unique properties are not only the result of drastically increased surface area to volume ratios, which result in enhanced reactivity but also the ability to engineer specific characteristics onto the nanomaterials.[3] The resulting NMs may exhibit increased solubility, catalytic or conductive potential, and unique magnetic or optical properties.[4] As of March 2011, in excess of 1300 nanotechnology-containing products were commercially available, including electronics, optics, textiles, medical devices, cosmetics, food packaging, water treatment technology, fuel cells, catalysts, biosensors, agents for environmental remediation, and as components of pesticide/fertilizer formulations.[1,3,5,6]

The list of agricultural applications for nanotechnology that are currently being explored or utilized is extensive and includes crop improvement technologies, nanoemulsions, and preservatives in pesticides and fertilizers, as well as modified plastic packaging for agricultural commodities.[7−9] Corredor et al.[10] have been investigating the use of carbon-coated Fe nanoparticles (NPs) as carrier agents to deliver chemicals into specific plant tissues with the goal of deterring or eliminating pathogenic infection. The authors showed that when injected into pumpkin (*Cucurbita pepo* L.) the nanoparticles effectively penetrated plant tissues and were likely transported in vascular tissues away from the application site. Similarly, Liu et al.[11] demonstrated that carbon nanotubes could readily penetrate cell walls and membranes to deliver desired materials directly to plant organelles. Chinnamuthu and Boopathi[12] described the development of "nanocides"; a process being pursued by several

Received: May 17, 2012
Revised: July 25, 2012
Accepted: August 2, 2012
Published: August 2, 2012

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major agrochemical corporations in which active ingredient encapsulation within various nanomaterials yields both increased product stability and efficacy. DeRosa et al.[13] observed that the low efficiency of nitrogen utilization (30–50%) by crops could be enhanced by using carbon nanotubes or ZnO nanoparticles, both of which have been shown to penetrate plant root tissues, as nutrient delivery systems.

In recent years, there have been a number of studies measuring the toxicity of NMs on biota ranging from bacteria to mammals.[14−20] However, Card et al.[21] recently evaluated the available literature on the safety of food-related nanomaterials and noted that there is currently insufficient information to accurately assess the risks associated with exposure to nanomaterials in food products. In addition, a thorough evaluation of the environmental fate and resulting in situ effects of these materials on biota and ecosystems has not been undertaken.[22] This lack of understanding confounds accurate assessment of NM exposure, hazard, and risk.[22] One area of research with a notable lack of data is the impact of engineered nanomaterials on agricultural crops. This knowledge gap is of particular concern given the current use of NMs in pesticide and fertilizer formulations, and the fact that nanoparticle contamination of agricultural crops (i.e., the food chain) may represent a significant and uncharacterized pathway of human exposure to these materials.[23] The goal of the current study was to assess the impact of fullerene exposure on the uptake and translocation of a common agricultural contaminant, *p,p′*-DDE (persistent and estrogenic metabolite of DDT), by three crop species.

■ EXPERIMENTAL SECTION

Analytes and Plants. Crystalline *p,p′*-DDE and *o,p′*-DDE were acquired from the Environmental Protection Agency (EPA) National Pesticide Standard Repository (Fort Meade, MD). C_{60} fullerenes (99.5% purity) were obtained from MTR Limited (Cleveland, OH). Seeds of *Cucurbita pepo* L. (cv Costata Romanesco), *Glycine max* L., and *Solanum lycopersicum* L. (cv Brandywine) were obtained from Johnny's Selected Seeds (Albion, ME), the Southern Illinois University College of Agriculture at SIUC, and Park Seed (Greenwood, SC), respectively. Seeds of *C. pepo* and *G. max* were pregerminated in pro-mix potting soil for 3−5 days, depending on plant species, prior to transplanting to vermiculite for the exposure assay as described below. Because of the fragile nature of the *S. lycopersicum* seedlings, seeds were directly planted in vermiculite.

Exposure Assay. Clear 125 mL jars (Fisher Scientific, Pittsburgh PA) were amended with 12 g of dry vermiculite (approximately 80 mL) and 40 mg of C_{60} fullerenes. The jars were capped and shaken vigorously to ensure homogeneous mixing of the fullerenes. The stems of selected *C. pepo* and *G. max* plants were gently inserted through 5 cm glass collars to protect the lower stem from direct contact with DDE-containing solution and the seedlings were planted in the vermiculite (one plant per jar). The glass collars were covered with aluminum foil to protect the stems from heat damage (Figure 1 of the Supporting Information). The planted jars were then amended with 40 mL, of a Miracle-Gro nutrient solution (The Scotts Company, Marysville OH), yielding an initial fullerene concentration of 1000 mg/L, containing 0 or 100 ng/mL *p,p′*-DDE (DDE) and 0 or 100 mg/L humic acid (Fisher Scientific, Pittsburgh, PA). In total, there were eight treatments: 1. DDE alone; 2. DDE + fullerenes; 3. DDE + fullerenes + humic acid; 4. DDE + humic acid; 5. negative control (no DDE); 6. fullerenes (alone); 7. humic acid (alone); 8. fullerenes + humic acid. Twelve replicate plants were grown for each treatment. Because each species was grown at a separate institution, conditions were variable but not in a significant fashion. *C. pepo* plants were incubated in a greenhouse under ambient light at approximately 27 °C and were top watered as needed with the solutions according to the above treatments for a 19 day growth period. The *G. max* plants were incubated in a light cart in the laboratory with 4 T5 cool white fluorescent bulbs and the temperature ranged from 21 to 23 °C. The *G. max* plants were top watered as needed with the solutions prepared according to the above treatments for a 21 day growth period. *S. lycopersicum* plants were direct seeded into vermiculite and were grown for 21 days prior to DDE exposure. The *S. lycopersicum* were grown in the laboratory with 4 Daylight fluorescent bulbs and the temperature ranged from 21 to 23 °C. Because of direct seeding, a syringe was inserted through the vermiculite to the bottom of the jar and the seedlings were bottom watered over an additional 20 day growth period so as to avoid direct contact between the stems and the DDE-containing solutions.

The majority of plant tissues (roots, stems) harvested in the above-described experiment were to be used for DDE analysis. Tissues not used for DDE quantitation were used for fullerene analysis but were of limited quantity. As such, separate zucchini plants were grown under the fullerene-exposure treatments as described above so as to enable exclusive analysis of stem tissues for the presence of C_{60}. Because the dynamic nature of conditions within the vermiculite during plant growth and the difficulty in isolating the C_{60} in a relevant way with regard to plant exposure, the fullerenes were not characterized with regard to absorbance or aggregation.

Vegetation Extraction. At harvest, replicate plants were separated into roots and shoots and the mass of each tissue was measured. Prior to weighing, roots were rinsed in tap water to remove vermiculite. To determine the amount of *p,p′*-DDE in plant tissues, 4−8 g of wet biomass was added to 35 mL Teflon-lined screw-cap vials with 32 mm septa.[24] Each vial was amended with 10 mL (root) or 20 mL (shoot) of [1:2] 2-propanol:*n*-hexanes and 100 μL from a 10 mg/L *o,p′*-DDE in a 2,2,4-trimethylpentane stock as an internal standard. The vials were placed onto a hot block at 65 °C for 2.5 h. After cooling (5 min), the extracts were decanted through a funnel lined with glass wool and collected in 500 mL glass separatory funnels with Teflon stopcocks. An additional 10 mL (root) or 20 mL (shoot) of 2-propanol/hexane was used to rinse the extraction vials and the rinsate was then added to the separatory funnels. The extracts were amended with 100 mL of reverse osmosis (RO) water and 10 mL saturated sodium sulfate solution, and were then shaken vigorously for 5 s. After phase separation (∼2 min), the water was drawn off and an additional 50 mL RO H_2O and 10 mL saturated sodium sulfate solution was added to the separatory funnels. After phase separation and water removal, the hexane extracts were collected in 35 mL amber Teflon screw-cap vials containing 10 g granular anhydrous sodium sulfate. A 1 mL portion of the extract was removed for cleanup on 3 mL florisil cartridges (200 mg)(Alltech, Deerfield, IL) that had been preconditioned with 5 mL of petroleum ether. The 1 mL vegetation extract was loaded onto the cartridge and was eluted with 5 mL of hexane. The extract was collected in an 8 mL vial and the volume was reduced to 1 mL under nitrogen on a heating block at 35 °C. The samples were

then transferred to chromatography vials for storage at −4 °C until analysis.

To determine fullerene content of the root and shoot samples from the three plant types in the initial experiment, 1−3 g of wet biomass (dependent on plant species and tissue type) was added to 35 mL Teflon-lined screw-cap vials with 32 mm septa. The vials were amended 10 mL of toluene. The vials were placed onto a hot block at 65 °C for 2.5 h. After cooling (5 min), a portion of the extract was passed through a glass micro fiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) for particulate removal and the eluent was collected in a chromatography vial that was stored at −4 °C until analysis. For the zucchini plants grown separately for C_{60} analysis, only stem tissues were harvested and replicates were composited by treatment to enable greater biomass for extraction and analysis by an alternative method. Wet stem biomass (4−8 g) were added into microwave PFA-Teflon vessel liners. The stem tissue was amended with 30 mL of toluene (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA), and after securely covering and capping the vessels a rupture membrane was installed in the vent fitting, which was secured to the vessels. The tissues subsequently placed in a CEM MES-1000 microwave solvent extraction system (CEM Corporation, Mathews, NC, USA) using the following program: 100% power, 10 min ramp to 120 °C; 20 min hold time. After cooling, the toluene was decanted into a collection vessel and the remaining vegetation in the extraction vessels were rinsed once with 10 mL portion of toluene, and the solvents were combined with the original extracts. The volume of each extract was then reduced to 1 mL on a heating block under a flow of nitrogen. The extracts were then passed through a glass microfiber filters and transferred to chromatography vials for storage at 4 °C until analysis.

Lipid Peroxidation, K Leakage, and Total Ion Leakage. Lipid peroxidation and total ion leakage was determined for roots and shoots of all plant species according to Jambunathan.[25] Lipid peroxidation was measured by the TBARS assay and malondialdehyde, which is formed during the degradation of fatty acids, was determined as a function of treatment. Total ion leakage is an indirect measure of cell death and was measured by conductivity from treated and control tissues in reverse osmosis water, expressed as a function of the total amount released upon autoclaving the tissues.[25] An additional measure of cell membrane integrity is K leakage as measured by Navari-Izzo et al.[26] Treated and control tissues were shaken in 50 mL of deionized water and K in the solution was determined after acidification ICP-MS (Agilent 7500).

Chemical Analysis. A 1000 mg/L stock of *p,p′*-DDE in 2,2,4-trimethylpentane was created and diluted to calibration standards of 10−500 ng/mL. One hundred ng/mL *o,p*-DDE in 2,2,4-trimethylpentane was added to each calibration level as an internal standard. The concentration of *p,p′*-DDE in the vegetation samples was determined on an Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a ^{63}Ni microelectron capture detector (ECD) by internal standard calibration. An Equity-5 (Supelco, Bellefonte, PA, #28089-U) GC column (30 m × 0.25 mm × 0.25 μm) was used and the oven program was as follows: 75 °C initial temperature ramped at 20 °C/min to 217 °C, then ramped at 0.25 °C/min to 219 °C, then ramped at 15 °C/min to 280 °C with a final hold time of 2.0 min. The injection port was maintained at 250 °C and a 2 μL splitless injection was used. The carrier gas over the column was He. The ECD was maintained at 300 °C and the makeup gas to the detector was 5% CH_4 in Ar at 60 mL/min. The detection limit of DDE is approximately 1 ng/mL injected; internal standard recovery was 99.9 ± 16.5%.

The analysis of C_{60} fullerenes was conducted according to Wang et al.[27] A 1000 mg/L stock of C_{60} fullerenes in toluene was created and diluted to calibration standards of 10−10 000 ng/mL. The concentration of fullerenes in roots and shoots was determined on an Agilent 1100 series HPLC (Avondale, PA, USA) with diode array UV−vis spectrophotometer (332 nm) by external standard calibration. A 250 mm × 4.6 mm i.d. COSMOSIL Buckyprep column packed with 5 μm particles with a 10 mm × 4.6 mm guard column was used (Phenomenex, Torrance, CA) with an injection volume of 100 μL and a mobile phase (100% toluene) rate of 1.0 mL/min. Fullerene presence in select zucchini stems was confirmed by direct flow injection using a mass spectrometer (Thermo LTQ, Thermo Scientific, San Jose, CA). The instrument was operated in negative ion mode using atmospheric pressure chemical ionization (APCI), scanning from m/z = 500−800. The C-60 fullerenes were quantified using the sum of the molecular ions (M-, m/z = 720; M-(with 1 13C), m/z = 721; and M- (with 2 13C), m/z = 722). These ions are in the proper ratio as predicted by the natural abundance of carbon 13. The detection limit for fullerenes by was approximately 5 ng/mL injected LC-Vis and approximately 10 ng/mL injected by APCI.

Statistical Analysis. At harvest, 10 of the 12 replicate plants were extracted and DDE content was determined separately in the roots and shoots. To achieve sufficient biomass for extraction, select replicates were composited. For each *C. pepo* tissue, 5−7 replicates per treatment were extracted. For each *G. max* and *S. lycopersicum* tissue, 3 replicates per treatment were extracted. The remaining uncomposited tissues were analyzed for fullerene content. All values of DDE or fullerene content are expressed on a dry weight basis. A two-way ANOVA (factors were fullerenes and humic acid) followed by a Holm-Sidak Multiple Comparison Test was used to determine the statistical significance of differences in contaminant content among treatments.

RESULTS AND DISCUSSION

DDE Exposure and Plant Mass. During the 19 day growth period, replicate *C. pepo* plants were amended with 240−265 mL of solution, equating to a total DDE exposure of 29−32 μg. After 19 days, the total plant masses ranged from 7.1 to 9.1 g (wet mass) but did not differ significantly as a function of treatment (Supporting Information, Table 1). On average, 86−89% of the plant biomass was in the shoot compartment; this value did not differ significantly based on treatment type.

G. max plants were exposed to 150−173 mL of solution during 21 days of growth or 18−21 μg of DDE. The total plant masses ranged 0.97−2.6 g (wet mass) and did not differ as a function treatment (Table 1 of the Supporting Information). The shoot mass of plants in the DDE treatment (1.4 g) was significantly greater than that of the DDE + fullerenes (1.2 g) and DDE + fullerenes + humic acid treatment (0.93 g). On average, 44−60% of the plant biomass was in the shoot compartment. Exposure to humic acid significantly increased the relative mass of the plant root compartment regardless of fullerene or DDE presence. The shoot mass ratio (shoot as a function of total mass) with and without humic acid exposure was 0.47 and 0.56, respectively (significantly different at p < 0.01, one-way ANOVA with Student−Newman−Keuls MCT).

Table 1. Concentration of Malondialdehyde (MDA) Produced by Plant Root and Shoots upon Exposure to DDE and C_{60} Fullerenes by the Thiobarbituric Acid (TBARS) Method[a]

plant	control	DDE	C60	DDE + C60
tomato				
shoot	1.23 A	1.75 B	1.34 A	1.82 B
root	1.32 A	1.39 A	1.70 B	1.68 B
soybean				
shoot	4.51 A	5.90 B	6.84 B	4.62 A
root	4.89 A	7.55 B	9.24 B	6.74 AB
zucchini				
shoot	1.90 A	1.83 A	1.66 A	1.88 A
root	10.3 A	9.09 A	9.56 A	11.9 A

[a]All values are $\times 10^{-4}$ mM. MDA is produced during the degradation of fatty acids. Plants were incubated under identical conditions to those used for DDE or C60 fullerene analysis. Within a plant type and tissue (rows), values followed by different letters are significantly different (one way ANOVA followed by a Student−Newman−Keuls multiple comparison test).

S. lycopersicum plants were exposed to approximately 120 mL of solution during 20 days of growth or 12 μg of DDE. The total plant masses ranged 0.97−2.2 g (wet mass); in general, biomass did not differ significantly as a function of treatment. The only significant differences were that the biomass for the DDE + fullerene + humic acid plants were significantly greater than that of the negative control and fullerene + humic acid plants (Table 1 of the Supporting Information). On average, 52−63% of the plant biomass was in the shoot compartment; this value did not differ significantly as a function of treatment.

The finding that DDE had no impact on plant growth is not surprising. White et al.[28−30] have shown that exposure to similar concentrations of DDE under hydroponic and soil conditions had no effect on plant growth. The literature on the phytotoxicity of carbon nanomaterials is mixed; observed effects often vary significantly with plant species, growing conditions, particle type, and concentration. Hawthorne et al.[31] reported no fullerene toxicity to *C. pepo* (squash) at 250−750 mg/L, with or without humic acid; however, Stampoulis et al.[32] observed a 30% reduction in *C. pepo* (zucchini) biomass upon exposure to 1000 mg/L single walled carbon nanotubes (SWCNTs). Ma and Wang[33] reported that 2−15 mg/L fullerenes had no impact on *Populus deltoides* B. growth. Conversely, Lin et al.[34] observed that *Oryza sativa* L. (rice) germination rates and biomass were reduced by 4.6−11% upon exposure to C_{70} and multiwalled carbon nanotubes under hydroponic conditions. Conversely, Khodakovskaya et al.[35] reported that carbon nanotubes at 10−40 mg/L penetrated *S. lycopersicum* (tomato) seeds and enhanced germination by promoting water uptake. There have been several studies noting the phytotoxicity of water-soluble fullerenes. Chen et al.[36] observed that $C_{60}(OH)_{20}$ effectively penetrated plant cell walls, subsequently accumulating outside the cell membrane and causing physical damage at concentrations as low as 10 mg/L. Similarly, Lui et al.[37] noted that exposure to $C_{70}(C(COOH)_2)_{4-8}$ at 20 mg/L resulted in shortened root length, as well as a range of physiological abnormalities at the cellular level.

Lipid Peroxidation, K Leakage, and Total Ion Leakage. The concentration of MDA, a measure of lipid degradation/peroxidation, determined in the control and treated tissues of the three plants is shown in Table 1. For both *S. lycopersicum* and *G. max*, exposure to C_{60} or DDE in specific instances caused significant increases in cell membrane damage experienced by both roots and shoots. However, zucchini tissues were unaffected by exposure. The release of total ions, as measured by conductivity, from the roots, and shoot of all tissues did not differ significantly as a function of treatment (Table 2 of the Supporting Information). Similarly, the release of K as a measure of cell membrane integrity was also largely unaffected by treatment (Table 3 of the Supporting Information). The exception was zucchini roots that were exposed to DDE; the release of K was significantly greater (4.3−9.2 times) than all other treatments. Little is known about the mechanisms of fullerene phytotoxicity; Sayes et al.[38] noted that C_{60} induced the formation of reactive oxygen species (ROS) and lipid peroxidation in human cells. Conversely, Chen et al.[34] noted that C_{60} fullerols compromised cell membrane integrity by physical interaction and not ROS production.

DDE and Fullerene Content. No DDE was found in nonexposed plants. Conversely, the tissues of all plants exposed to the contaminant had measurable quantities of DDE. For *C. pepo*, the shoot and root DDE concentrations ranged from 990 to 1890 ng/g and 38 900−47 700 ng/g, respectively. For *G. max*, the shoot and root DDE concentrations ranged from 76.0 to 449 ng/g and 3080−9500 ng/g, respectively. For *S. lycopersicum*, the shoot and root DDE concentrations ranged from 69.7 to 141 and 2710−9310 ng/g, respectively. Direct comparison of DDE concentrations between treatments or across plants is confounded by differential exposure due to variability in watering volume (a function of plant mass and transpiration). As such, average DDE concentrations were converted to absolute amounts (ng) by multiplying by dry mass within plant species and were normalized based on volume of exposure/watering solution.

The DDE content of *C. pepo*, *G. max*, and *S. lycopersicum* shoots and roots, as well as total plant levels, are shown in Figures 1−3. For *C. pepo*, the presence of fullerenes significantly increased DDE tissue content; shoot, root, and whole plant contaminant levels were increased by 29.0, 30.5, and 30.4% respectively in the presence of the nanomaterial (Figure 1). Although humic acid significantly increased DDE uptake in the shoots in the absence of fullerenes, humic acid generally suppressed uptake of the organochlorine. The *C. pepo* root and whole plant DDE content were reduced by 20.2−43.1% (reductions significant at $p < 0.05$) upon exposure to humic acid.

For *G. max* (Figure 2), the presence of fullerenes decreased DDE shoot content by 48.0% (significant at $p < 0.05$) but the root and whole plant contaminant levels were increased by 47.7 and 45.7%, respectively (significant at $p < 0.05$). The presence of humic acid, regardless of fullerene presence or tissue type, significantly decreased soybean DDE content. The soybean shoot DDE levels were reduced by more than 80% upon humic acid amendment (significant at $p < 0.05$), whereas the root and whole plant contaminant levels were decreased by 28.5−31.2%, respectively (significant at $p < 0.05$).

For *S. lycopersicum*, fullerene exposure had no impact on the shoot DDE content but root and whole plant contaminant levels were significantly ($p < 0.05$) increased by 64.5 and 62.1%, respectively (Figure 3). Similar to *G. max*, humic acid exposure significantly ($p < 0.05$) decreased DDE uptake regardless of fullerene presence or tissue type. The reductions in shoot contaminant levels ranged from 27.3 to 36.9%, whereas root

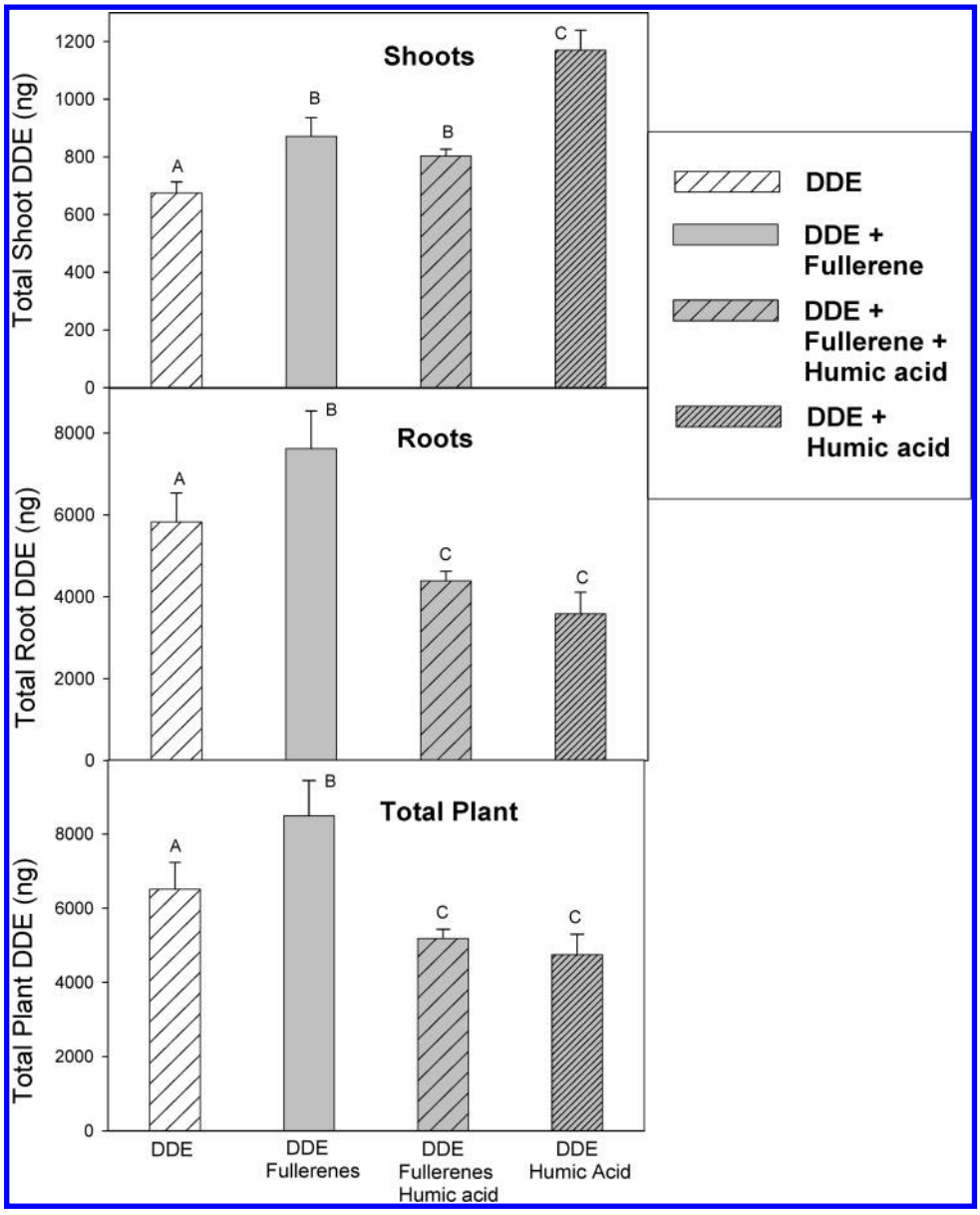

Figure 1. Effect of fullerene and/or humic acid exposure on the shoot, root or total plant content of DDE in *C. pepo*. Within a tissue type (panel), bars with different letters above them are significantly different (two-way ANOVA with Holm-Sidak multiple comparison test).

and whole plant contaminant levels were decreased by 39.4–72.2%.

No fullerenes were detected in *C. pepo* or *G. max* shoot samples. Fullerenes were detected in the *S. lycopersicum* shoots but this was determined to be surface contamination likely resulting from the transplanting procedure. Conversely, when robust quantities of zucchini stem biomass were available, C_{60} fullerenes were detected in over half of the tissues ranging from 60.5 to 4490 ng/g (dry weight). Fullerene presence was confirmed by mass spectrometry (Figure 2 of the Supporting Information); the reasons for the large variability in root to shoot translocation remain unknown but may be the result of the dynamic conditions within the growing media.

The fullerene content in and on *C. pepo* roots that were exposed to the nanomaterial was 33 700 ng/g but varied greatly among individual replicates (829–91 200 ng/g). Similarly, the fullerene content in and on *G. max* roots was 218 000 ng/g but varied greatly among individual replicates (68 200–290 000 ng/g). The fullerene content in and on *S. lycopersicum* roots was 1760 ng/g and also varied greatly among individual replicates (940–2550 ng/g). For all species, the root fullerene content did not differ as a function of treatment. However, the fullerene content, both as ng/g or as total root content (30 700 ng for *G. max*, 5410 ng for *C. pepo*; 110 ng for *S. lycopersicum*), differed significantly between the three species (one-way ANOVA with a Dunn's Multiple Comparison Test; $p < 0.01$). Given the dominance of nanomaterial adsorption to root surfaces, the significance of these findings is unclear.

C. pepo shoots accumulated 5.2–26 times more DDE in its shoots than did *G. max* or *S. lycopersicum*. The finding of differential DDE accumulation as a function of plant species is not surprising. White[29,39] and others[40] have shown that *C. pepo* possesses a unique ability to accumulate and translocate weathered POPs under hydroponic and soil-based conditions. The mechanism responsible for contaminant uptake is the topic of ongoing investigation.[30,4]

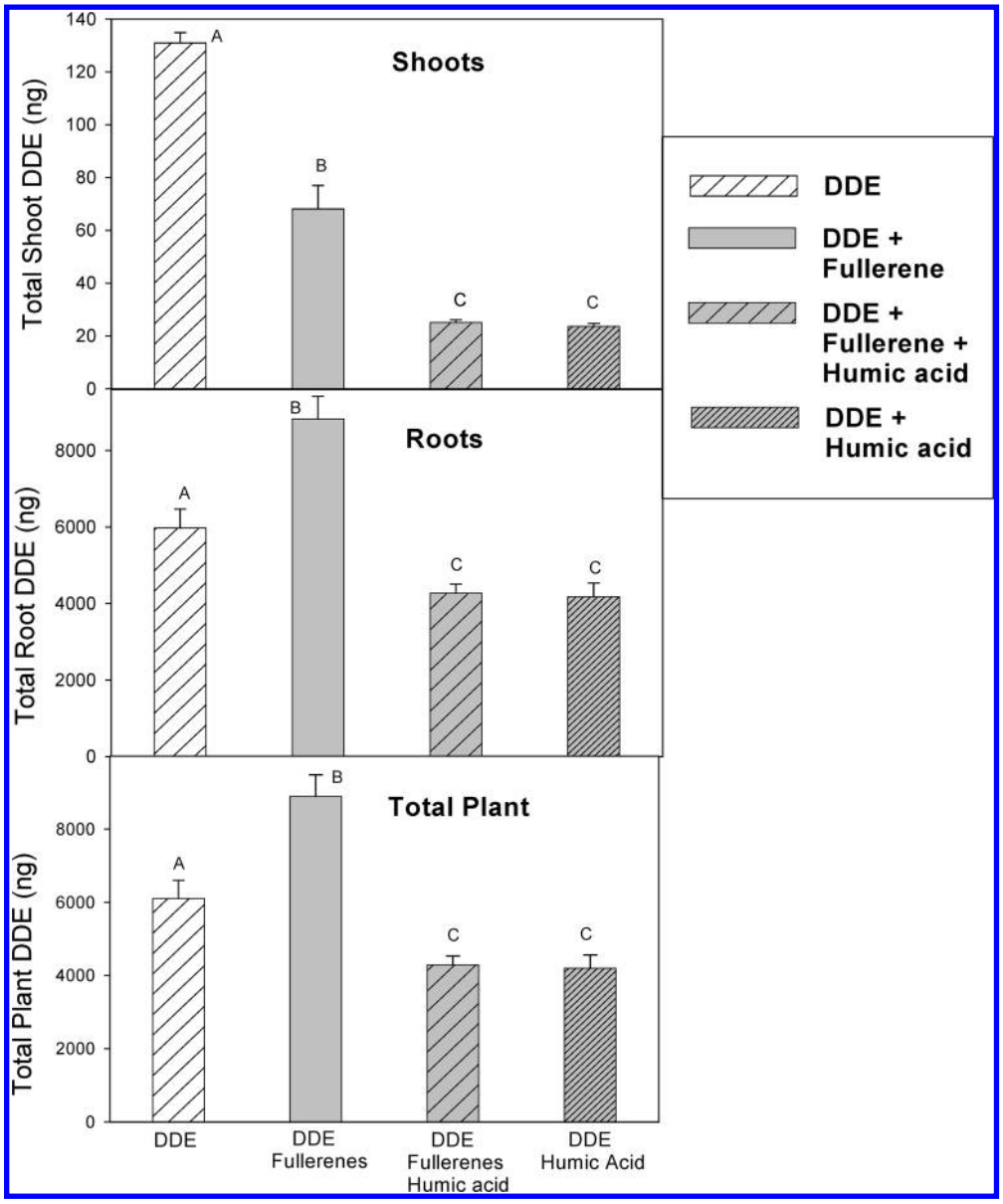

Figure 2. Effect of fullerene and/or humic acid exposure on the shoot, root, or total plant content of DDE in *G. max*. Within a tissue type (panel), bars with different letters above them are significantly different (two-way ANOVA with Holm-Sidak multiple comparison test).

The finding of consistently enhanced DDE content in the roots, as well as in the plant in general, with fullerene amendment is surprising. Many reports have shown that carbon-based amendments to soils and sediments significantly decrease contaminant availability. Rhodes et al.[42] observed that the mineralization of phenanthrene in four soils decreased by 99% upon addition of activated carbon at 1−5%. Gomez-Eyles et al.[43] showed that biochar amendments resulted in similar decreases in the bioavailability of polycyclic aromatic hydrocarbons to earthworms. The finding that humic acid decreased DDE uptake is not surprising. Landrum et al.[44] noted that the bioavailability of PAHs and PCBs to amphipods was significantly reduced in the presence of dissolved organic matter, largely through organic contaminant partitioning into this soluble organic phase. Black and McCarthy[45] described similar dissolved organic matter (DOM)-driven reductions in organic contaminant uptake (benzo[a]pyrene, tetrachlorobiphenyl) by fish. Conte et al.[46] actually demonstrated that humic acid washing of contaminated soils removed up to 90% of the weathered PAHs. Interestingly, Lin et al.[47] observed that humic acid significantly promoted carbon nanotube dispersion, which could theoretically increase plant interactions with the nanomaterial. However, in the current study, fullerene root content was decreased as a function of humic acid treatment.

A number of studies have also looked specifically at carbon nanomaterial−contaminant interactions. Chen et al.[48] noted that the sorption of benzene and toluene to single and multiwalled carbon nanotubes (SWCNT, MWCNT) was consistently strong but varied mechanistically, dependent both on sorbate and sorbent properties. Similarly, Apul et al.[49] observed that nanotube morphology was critical in measuring the rate and extent phenanthrene adsorption. Yang and Xing[50] measured the desorption of PAHs from carbon nanomaterials and observed significant desorption resistance from fullerenes, largely due to sorbent deformation upon sorbate binding, but no such desorption resistance from carbon nanotubes.

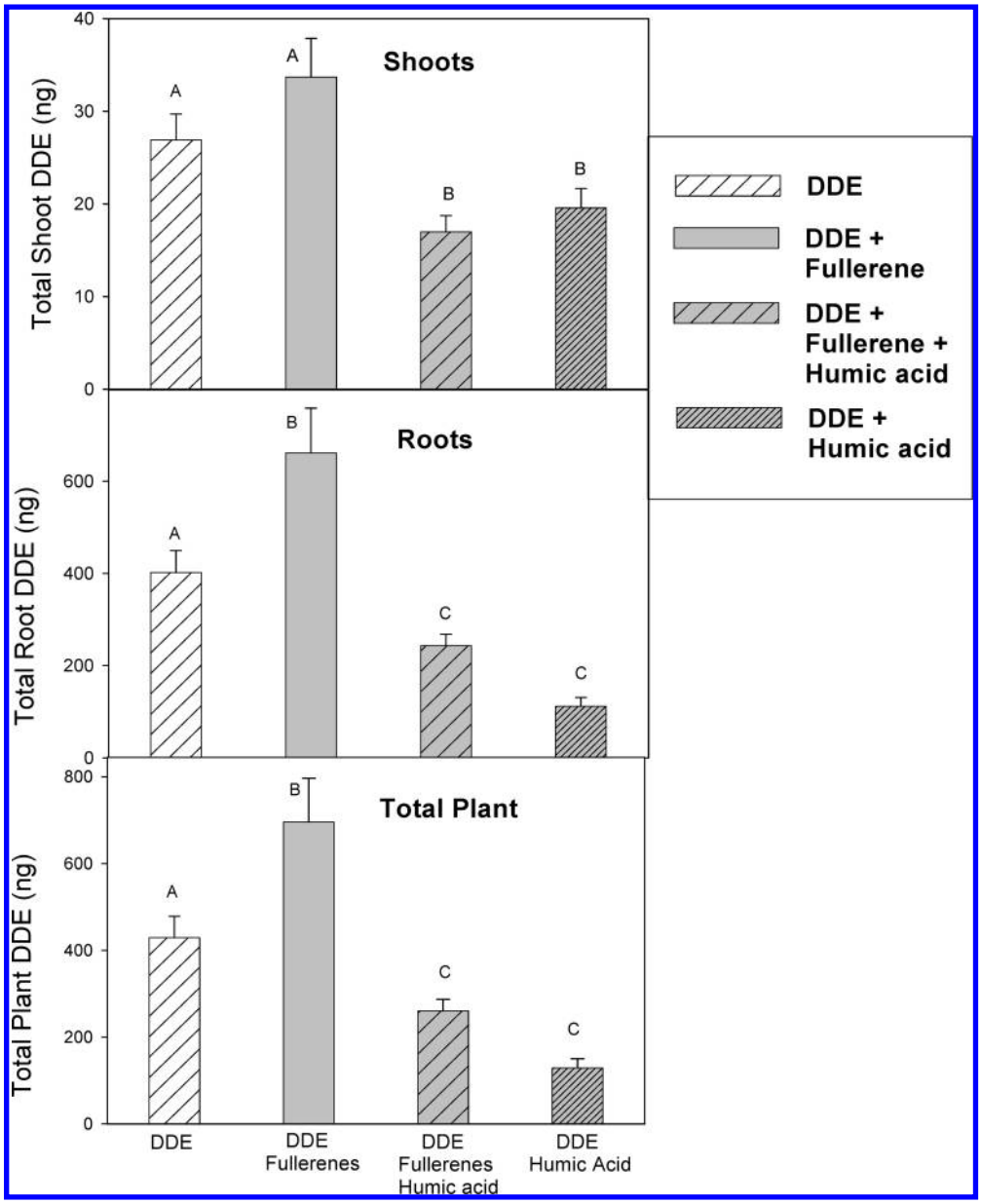

Figure 3. Effect of fullerene and/or humic acid exposure on the shoot, root, or total plant content of DDE in *S. lycopersicum*. Within a tissue type (panel), bars with different letters above them are significantly different (two-way ANOVA with Holm-Sidak multiple comparison test).

There are numerous recent studies evaluating the impact of carbon nanomaterial presence on organic contaminant uptake or toxicity but few consistent trends are evident in the data. Park et al.[51] observed the complete loss of 17α-ethinylestradiol bioavailability to zebra fish upon C_{60} amendment. Conversely, Baun et al.[52] measured the accumulation and toxicity of four organic compounds to an algal (*Pseudokirchneriella subcapitata*) and invertebrate species (*D. magna*) in the presence in C_{60}. Whereas phenanthrene toxicity and accumulation was increased in both species in the presence of the C_{60}, the opposite was observed for pentachlorophenol. Similarly, Fang et al.[53] showed that the uptake of tributylin by *D. magna* was significantly decreased upon fullerene addition but that dibutylin accumulation was unaffected. However, significantly greater quantities of both butylins were taken into the organism largely due to sorbate interaction with the nanomaterial. To further complicate matters, Hu et al.[54] observed that the bioaccumulation of the moderately hydrophobic hexachlorbenzene and hexachlorocylcylohexane in medaka was slightly increased by fullerene presence but the more hydrophobic PCBs and DDTs were significantly less available. The literature on the availability and toxicity of organic contaminants to plants in the presence of carbon nanomaterials is much more limited. Ma and Wang[33] 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%.

There are several possible mechanisms by which fullerenes could enhance contaminant uptake. First, if fullerene nanoparticles are accumulated by plants, hydrophobic cocontaminants might associate with the C_{60} and be taken into the roots. Khodakovskaya et al.[33] observed that carbon nanotubes readily penetrated tomato seeds promoting water uptake and increased germination rates. Similarly, Lin et al.[34] demonstrated the uptake and generational transfer of C_{70} by rice plants; interestingly, no such activity was observed for MWCNTs.

Conversely, Liu et al.[55] described an endocytosis-like process that resulted in SWCNT penetration of plant cell walls and membranes. Similarly, Chen et al.[36] demonstrated that C_{70}-natural organic matter (NOM) particles crossed the plant cell membrane by both partitioning and endocytosis, whereas $C_{60}(OH)_{20}$ were excluded from uptake. There were detectable levels of C_{60} in certain shoot tissues from the current study but the likelihood of this mechanism of enhancing DDE uptake in not known. A second possible mechanism may involve membrane damage resulting from fullerene exposure; our data is somewhat contradictory on this point. Although C_{60} and DDE exposure did significantly increase lipid peroxidation of both *S. lycopersicum* and *G. max*, no such effect was observed for *C. pepo*. In addition, measures of K leakage and cell death by total ion release were largely unaffected by nanoparticle exposure across all plant types. Liu et al.[37] used imaging techniques to demonstrate both hormonal and structural effects of fullerene exposure on *Arabidopsis* root tips. Chen et al.[36] described an accumulation of C_{60} fullerols between the plant cell wall and membrane that resulted in a measurable loss in membrane integrity. The authors hypothesized that, because fullerols are not known to induce reactive oxygen species formation (ROS), the loss in cell membrane integrity is likely the result of physical damage from direct contact with carbon nanomaterial aggregates. However, fullerenes similar to those used in the current study are known to induce ROS formation and lipid peroxidation in human cell lines.[38] The mechanism of fullerene-enhanced contaminant uptake remains of topic of intense investigation in our laboratories.

The large increase in nanomaterial use in consumer products has dramatically increased the likelihood of significant and potentially negative interactions with both humans and the environment. The current study demonstrates that the bioaccumulation of DDE, a persistent and estrogenic pollutant, into three food crops was significantly increased in the presence of C_{60} fullerenes. The effects of potentially enhanced pollutant levels within food crops, as well as the potential risk associated with food chain contamination by nanomaterials, is unknown but certainly the subject of ongoing investigation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank USDA-AFRI (#2011-67006-30181) for funding and Terri Arsenault, Dr. Walter Krol, and Dr. Brian Eitzer (CAES) for technical assistance.

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